Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)
CONTRIBUTIONS (SBTE) – PROCEEDINGS 2024

From the SBTE President

Dear friends of the Brazilian Society of Embryo Technology,

Again, I am honored to be the president of the current SBTE board. In another year leading our Society, we will seek to maintain the tradition and commitment to continue promoting scientific research and disseminating knowledge on reproduction generated in recent years. Last year, we performed a different event than the previous SBTE promotion, and, fortunately, our goals were achieved quite successfully. Several changes were made, from the style of the place where our meeting was performed to changes in the format of the event program. In 2023, we had the opportunity to meet friends in a commercial-style hotel. This year's event will have the traditional program with workshops and plenary sessions, presenting lectures in basic and applied areas in a resort (Tauá resort, Atibaia-SP) as is the SBTE tradition.

As a novelty, we will perform hands-on workshops (Doppler ultrasound, IVF, and semen analysis) before the SBTE meeting. Another innovation at our event this year is a discussion with renowned researchers from around the world who will present controversial topics about in vitro embryo production in cattle. This event will be called "The Great Meeting of the IVP."

Our meeting will take place in Atibaia-SP, initially with workshops (Open gate: beef and dairy cattle, Oocyte epigenetic maturation and totipotency, Male reproduction/ICSI in different species and Embryo cryopreservation) and then plenary sessions with presentations regarding zootechnical, economic and managerial metrics in livestock farming, the influence of public perception on management practices in the dairy and beef industry, reproduction of young animals, potential increases in fertility with the use of nanoparticles for gamete selection, pressing needs and recent advances to enhance in vitro embryo production, immune system and gestational success, how to mitigate pregnancy losses in embryo transfer programs and embryonic stem cells. Again, our Board of Directors is very motivated for this year's event, mainly due to the significant changes that aim to rescue a greater number of field and laboratory professionals and, therefore, to promote more in-depth discussions applied to the reality of our animal reproduction.

This event is proposed to be a milestone at SBTE, aiming at a healthy interaction between researchers and field technicians responsible for the scientific development of animal reproduction. Finally, I would like to thank everyone involved in the organization of our meeting, in particular the current board, the companies from our SBTE community, the research funding institutions (CNPq, CAPES, and FAPESP), all the national and international speakers and, above all, to all members of our Society and participants.

José Nelio de Sousa Sales
SBTE President
2023-2024

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CONTRIBUTIONS (SBTE) – PROCEEDINGS 2024

From SBTE Scientific Board

We started 2024 with renewed enthusiasm for the 37th Annual Meeting of the Brazilian Society of Embryo Technology. The meeting will take place between August 21st and 23rd at the 4-stars Tauá Hotel & Convention Atibaia, where the SBTE members, general attendees and their families can enjoy several outdoors sports, an amazing indoor water park and restaurants with outstanding cuisines.

This year we will have four workshops which will address advances in reproductive biotechnological practices in dairy and beef cattle, oocyte epigenetic maturation and totipotency, male reproduction and embryo cryopreservation. In addition, as a great innovation in our annual meeting, three hands-on workshops (Doppler ultrasound, in vitro embryo production and semen cryopreservation and analysis) will be offered the day before the SBTE main meeting and will be performed at the University of Sao Paulo (USP) in the campus located in Pirassununga and São Paulo.

At the end of the first day, after the workshops, we will have the official opening in the general plenary session, when the Young Scientist Award will be presented, in recognition of new researchers who stand out for their dedication to the scientific and technological development of reproductive biotechnologies in animals. In the following days, the plenary sessions will address a range of topics, from management, metrics and administration on a farm to the development and potential applications of embryonic stem cells in livestock. This year we will have a round table with researchers, practitioners and audience participation to debate critical topics for the efficiency of in vitro embryo production in cattle (don't miss it).

Poster presentations will be held in two sessions: the first session in the late afternoon of the first day, before the official opening, and the second in the late afternoon of the second day. This year, 232 abstracts were submitted, what highlights the importance of the SBTE meeting in disseminating science and technology to society. Remember that the poster sessions are an excellent opportunity to find high-level technical and scientific papers on different topics in reproductive biotechnology, in addition to get in touch and exchanging information with colleagues with different specializations, which certainly contributes to expanding our horizons of knowledge.

After the SBTE General Assembly, we will have the Celebration and Awards Dinner. During the dinner, the winners of the student competition, the best basic and applied science's studies, and the best practitioner's work will be announced. At the end, the "Assis Roberto De Bem" Outstanding Award will be presented, in recognition of the fundamental role of researchers and professionals in transforming knowledge into concrete results for the productive sector and society.

We are very thankful to the speakers and the reviewers/evaluators of manuscripts and abstracts for their significant contribution to the quality of the presentations and the papers presented. We also must thank the entire support team, who dedicated themselves to making this meeting yet another outstanding scientific event in the history of SBTE. Finally, we look forward to meeting our friends and colleagues at the Tauá Hotel & Convention and hope that in addition to the knowledge offered by the lectures, posters and debates, we may also be enriched by the technical-scientific exchange in a family-like meeting. Warm regards.

Guilherme Pugliesi
Luiz Sérgio de Almeida Camargo
Marcílio Nichi
SBTE Scientific Board
2023-2024

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

SBTE BOARD MEMBERS (2023/2024)

President: José Nélio de Sousa Sales (UFJF – Juiz de Fora, MG)

Vice-president: Roberto Sartori Filho (ESALQ/USP - Piracicaba, SP)

1st Secretary: Fabiana Fernandes Bressan (FZEA/USP - Pirassununga, SP)

2nd Secretary: Lindsay Unno Gimenes (FCAV/UNESP – Jaboticabal, SP)

1st Treasurer: Marcio de Oliveira Marques (GeraEmbryo Reprodução Bovina – Londrina, PR)

2nd Treasurer: Joanna Maria Gonçalves de Souza Fabjan (UFF – Niteroi, RJ)

Communications directors: Letícia Zoccolaro Oliveira (UFMG – Belo Horizonte, MG) and Joanna Maria Gonçalves de Souza Fabjan (UFF – Niteroi, RJ)

Scientific directors: Guilherme Pugliesi (FMVZ/USP – Pirassununga, SP), Marcílio Nichi (FMVZ/USP – São Paulo, SP) and Luiz Sergio de Almeida Camargo (EMBRAPA – Juiz de Fora, MG)

Business director: Gabriel Armond Crepaldi (ST Repro – Indaiatuba, SP)

Veterinary Practitioners Representative: Moises Máximo Rodrigues Júnior (MS Reprodução de bovinos - Campo Grande, MS)

SBTE ABSTRACT SESSION COORDINATORS

FTAI/AI: Gustavo Guerino Macedo, Luiz Francisco Machado Pfeifer, Ricarda Maria dos Santos

FTET/ET: Fábio Morotti

OPU-IVF: Luiz Gustavo Bruno Siqueira, Marcelo Demarchi Goissis

Folliculogenesis, oogenesis and superovulation: Bernardo Garziera Gasperin

Male physiology and semen technology: Maíra Bianchi Rodrigues Alves

Embryology, developmental biology and physiology of reproduction: Alexsandra Fernandes Pereira

Cloning, transgenesis and stem cells: Marcelo Demarchi Goissis

Supporting biotechnologies: Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics": Thais Rose dos Santos Hamilton

SBTE ABSTRACT REVIEWERS

Adriano Felipe Perez Siqueira
Alberto Lopes Gusmão
Alessandra Bridi
Alessandra Corallo Nicacio
Alexandre Henryli de Souza
Alexandre Rossetto Garcia
Alexsandra Fernandes Pereira
Alfredo Quites Antoniazzi
Álvaro de Miranda Alves
Alysson Jorge de Oliveira Sousa
Amanda Prudêncio Lemes
Amanda Zangirolamo Fonseca
Ana Carolina dos Santos Oliveira
Ana Carolina Pedrosa
Ana Paula Martini
Ana Rita Krause
Anderson Weiny Barbalho Silva
André Furugen Cesar de Andrade
André Luís Rios Rodrigues
André Maciel Crespilho
Andrei Guedes Fidelis
Angelo José Burla Dias
Anne Kemmer Souza
Anthony César de Souza Castilho
Antonia Debora Sales
Arnaldo Diniz Vieira
Audrey Bagon
Barbara Loureiro
Bernardo Garziera Gasperin
Breno Fernandes Barreto Sampaio
Bruno Campos de Carvalho
Bruno Freitas
Bruno Leonardo Ribeiro
Bruno Moura Monteiro
Bruno Rogerio Rui
Camila Bortoliero Costa
Camila Bruna de Lima
Camila Infantosi Vannucchi
Camilla Mota Mendes
Carine Corcini

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

Carla Fredrichsen Moya
Carlos Antônio De Carvalho Fernandes
Carlos Eduardo Ambrósio
Carlos Eduardo Leonardi
Carlos Frederico Martins
Carlos Henrique Lobo
Carolina Capobiango Romano Quintão
Christina Ramires Ferreira
Clara Ana Santos Monteiro
Clara Slade Oliveira
Claudia Maria Bertan Membrive
Cristiano Feltrin
Daniele Missio
Denilsa Pires Fernandes
Denis Vinicus Bonato
Edson Guimarães Lo Turco
Eduardo Schmitt
Eliane Vianna da Costa e Silva
Elis Lorenzetti
Elisa Santanna Monteiro da Silva
Elizangela Moreira
Érika Almeida Praxedes
Eriklis Nogueira
Evelyn Rabelo Andrade
Everton Pimentel Ferreira Lopes
Fabiana de Andrade Melo Sterza
Fabiana Fernandes Bressan
Fabiane Pereira De Moraes
Fabio Lucas Zito De Moraes
Fabio Morato Monteiro
Fábio Morotti
Fabíola Freitas Paula-Lopes
Felipe Perecin
Felipe Zandonadi Brandão
Fernanda Amarante Mendes de Oliveira
Fernanda Landin
Fernanda Patricia Gottardi
Fernando Caetano de Oliveira
Fernando Silveira Mesquita
Flávia Regina de Oliveira Barros
Flávio Vieira Meirelles
Gabriela Liberalino Lima
Gildas Mbemya Tetaping
Gilson Antonio Pessoa
Glaucio Lopes
Guilherme De Medeiros Bastos
Guilherme Fazan Rossi
Guilherme Pugliesi
Gustavo Desire Antunes Gastal
Gustavo Guerino Macedo
Gustavo Martins Gomes dos Santos
Gustavo Rodrigues Queiroz
Gustavo Zamberlam
Helena Fabiana Reis de Almeida Saraiva
Henderson Ayres
Hernan Baldassarre
Iago Matheus Rosa Leao
Ian Martin
Ines Cristina Giometti
Isabella Rio Feltrin
Ivan Bianchi
Ivo Pivato
Janaina Menegazzo Gheller
Janaina Torres Carreira
Jessica dos Santos Marques
Jessica Nora Drum
Joanna Maria Gonçalves Souza-Fabjan
João Carlos Pinheiro Ferreira
João Diego de Agostini Losano
João Henrique Moreira Viana
João Paulo Elsen Saut
José de Oliveira Carvalho
José F Cox
José Felipe Warmling Sprícigo
José Luiz Moraes Vasconcelos
José Nélio de Sousa Sales
José Roberto Viana Silva
José Victor Isola
Juliana Corrêa Borges Silva
Juliana Germano Ferst
Juliana Jales de Hollanda Celestino
Juliano Coelho da Silveira
Juliano Rodrigues Sangalli
Kaio César Simiano Tavares
Karina Goularte
Karina Gutierrez
Katia Cristina Silva Santos
Keila Moreira Maia
Ken Kawaoka Nagai
Larissa Berloffa Belardin
Larissa Zamparone Bergamo
Leonardo Franco Martins
Leonardo Vitorino Costa de Aquino
Letícia Zavdoski Garcia
Letícia Zoccolaro Oliveira
Lígia Garcia Mesquita
Lilian Katia Ximenes da Silva
Lindsay Unno Gimenes

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

Liza Margareth Medeiros de Carvalho Sousa
Lucas Hax
Lucas Melo Gonçalves
Luciana Rocha Faustino
Luciana Simões Rafagnin Marinho
Lucio Pereira Rauber
Luiz Francisco Machado Pfeifer
Luiz Gustavo Bruno Siqueira
Luiz Sérgio de Almeida Camargo
Maíra Bianchi Rodrigues Alves
Manoel Francisco Sá Filho
Mara Iolanda Batistella Rubin
Marcel Henrique Blank
Marcela Silva Cordeiro
Marcella Pecora Milazzotto
Marcelo Demarchi Goissis
Marcelo Fábio Gouveia Nogueira
Marcelo Marcondes Seneda
Marcelo Rezende Luz
Marcelo Tigre Moura
Marcilio Nichi
Marcio Nogueira Rodrigues
Marco Antonio Alvarenga
Marco Roberto Bourg Mello
Marcos Roberto Chiaratti
Margot Alves Nunes Dode
Maria Angélica Miglino
Maria Helena Tavares de Matos
Maria Valéria de Oliveira Santos
Mariana Groke Marques
Mariana Ianello Giassetti
Mariana Machado Neves
Mariani Farias Fiorenza
Maricy Apparicio Ferreira
Marilu Martins Gioso
Mário Binelli
Mateus José Sudano
Mayra Elena Ortiz D'Ávila Assumpção
Miller Pereira Palhão
Milton Maturana Filho
Moana Rodrigues França
Monique Tomazele Rovani
Moysés dos Santos Miranda
Nadja Gomes Alves
Naiara Zoccal Saraiva
Natália Avila Castro
Nathalia Covre da Silva
Nathalia Nogueira da Costa de Almeida
Patrícia Kubo Fontes
Paula Rodriguez Villamil
Paulo Bayard Dias Gonçalves
Phelipe Favaron
Priscila Chediek Dall'Acqua
Radan Elvis Matias de Oliveira
Rafael Bisinotto
Rafael Gianella Mondadori
Rafael Ulguim
Rafael Vilar Sampaio
Raphaela Gabrielle Brito Sousa
Raquel Varella Serapião
Renata Lançoni
Renata Simões
Ribrio Ivan Tavares Pereira Batista
Ricarda Maria dos Santos
Ricardo Perecin Nociti
Roberta Ferreira Leite
Roberto Sartori
Rodolfo Daniel Mingoti
Rodrigo da Silva Nunes Barreto
Rodrigo Rossi
Rogério Ferreira
Rogerio Fonseca Guimaraes Peres
Rubens Paes de Arruda
Rui Machado
Sabine Wohlres Viana
Sergio Farias Vargas Júnior
Simone do Socorro Damasceno Santos
Simone Maria Massami Kitamura Martins
Sony Dimas Bicudo
Thais Rose dos Santos Hamilton
Thaís Thatiane dos Santos Souza
Thales Ricardo Rigo Barreiros
Thiago Martins
Thomaz Lucia Jr
Tiago Henrique Camara De Bem
Valdevane Rocha Araujo
Vera Fernanda Hossepian de Lima
Vitor Braga Rissi
Wagner Rodrigues Garcia
Wanessa Blaschi
Weber Beringui Feitosa
Werner Giehl Glanzner
Willian Vaniel Alves Dos Reis
Wilma de Grava Kempinas

Animal Reproduction

SBTE BOARD MEMBERS

SBTE STAFF TEAM

Ana Karolyne Alves Miguel
Ana Luíza Müller Lopes
Gabrielle Ceragioli Damaceno
Laís Reis Carvalho
Leonardo Silva Fernandes do Vale
Lucas Araujo Lemos
Luma Canavessi Sartori
Matheus Pedroso Vicente
Mirela Balistrieri Dias
Rafael Luiz Stolf
Rafael Resende Rabelo Silva
Thiago Neder Lisboa

Animal Reproduction

SPONSORS SBTE

Axiota Saúde Animal
Biogenesis Bagó Saúde Animal
Ceva Saúde Animal
CPEX Embriões
GlobalGen Vet Science
IMV do Brasil
J.A. Saúde Animal
MSD Saúde Animal
NEOVET Equipamentos de Reprodução Animal Desenvolvido para Profissionais
Ourofino Saúde Animal
Spectrun Bio Engenharia Médica Hospitalar
TED Equipamentos para Reprodução Animal
União Química Farmacêutica Nacional
Universo do Embrião Reprodução Bovina
WTA Tecnologia para Reprodução Animal
Zoetis

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SPONSORS SBTE

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

1H NMR-based metabolomics for early fertility diagnosis in bovine females: insights and prospects for improved herd management

Alana Aparecida Amarilha Nobre[1], Paola Dias de Oliveira[1], Luciana Marçal Ravaglia[1], Gabrielle Terra Souza Netto[1], Willian Vaniel Alves dos Reis[1], Eliane Vianna da Costa e Silva[1], Glaucia Braz Alcantara[1], Gustavo Guerino Macedo[1]

[1]Universidade Federal de Mato Grosso do Sul (UFMS)

E-mail: alananobree@gmail.com

Proton nuclear magnetic resonance (1H NMR)-based metabolomics emerges as a tool to aid in understanding biological processes related to animal reproduction. This study aims to evaluate the metabolic profile and potential biological markers present in blood serum to correlate them with the fertility and/or gestation of bovine females. Nelore heifers (n = 50) underwent an 8-day P4/E2 based protocol with pregnancy diagnosis 30 days after AI (D40). Blood samples from the animals at D0, D10, and D40 were collected in tubes with clot activator from the median caudal vein/artery and then the serum was separated and stored at -80°C. Pregnant (n = 5) and non-pregnant (n = 5) females, all exhibiting the presence of corpus luteum in their respective ovaries at D0, had their serum separated for analysis. The samples were analyzed on a 11.75T Avance NEO 500 MHz 1H NMR spectrometer, using the cpmgpr1d pulse sequence, with TD = 64K, DS = 4, NS = 512, SW = 30 ppm, O1P at 4.7 ppm, and deuterated water as solvent. The 1H NMR data were analyzed by unsupervised (Principal Component Analysis, PCA) and supervised (Sparse Partial Least Squares - Discriminant Analysis, sPLS-DA) algorithms, using the online computational programs NMRProcFlow (INRA UMR 1332 BFP, Bordeaux Metabolomics Facility) and MetaboAnalyst 5.0. The sPLS-DA plots obtained by collection time indicated that at D0 there was a slight distinction between the animals that would later become pregnant, with emphasis on the metabolites leucine, valine, alanine, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and unsaturated lipids. On the other hand, at D10 (AI moment), there was no distinction between the two groups of animals. Finally, at D40, where confirmation of Nelore female pregnancy was already established, greater separation between pregnant and non-pregnant groups was observed due to the metabolites alanine, histidine, tyrosine, phenylalanine, LDL/VLDL, and unsaturated lipids. These findings offer promising avenues for improving bovine fertility assessment, highlighting the critical role of early diagnosis in optimizing herd management. The identification of key metabolites like alanine, LDL, and VLDL, not only in females which will be pregnant but also in those who became pregnant after 30 d of AI, implies a significant advancement in understanding bovine reproductive processes. These insights have the potential to change fertility assessment protocols, enhancing profitability and sustainability in the livestock industry. Leveraging this knowledge, producers can implement targeted nutritional management strategies to maximize reproductive efficiency, driving increased productivity and economic gains.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

A new cetrorelix-based follicular wave and ovulation synchronization protocol for fixed-time artificial insemination in cattle

Carlos Eduardo Porciuncula Leonardi[1], Gilson Antonio Pessoa[1], Gregg Patrick Adams[2], Jaswant Singh[2]

[1]Departamento de Clínica de Grandes Animais, Universidade Federal de Santa Maria (UFSM)
[2]Department of Veterinary Biomedical Science, University of Saskatchewan

E-mail: carlos.leonardi@ufsm.br

GnRH antagonists suppress secretion of gonadotropins and may be useful for breeding management in cattle. The objective of the study was to test the hypotheses that a single treatment with cetrorelix at random days of the estrous cycle in beef cattle will i) induce synchronous emergence of a new follicular wave and ovulation and ii) result in a pregnancy rate equal to that of an estradiol-based fixed-time insemination protocol. In Experiment 1, sexually mature Brangus beef heifers at random stages of the estrus cycle were given an intra-vaginal progesterone-releasing device (CIDR®; Zoetis, New Zealand; Day 0) and either 3 mg cetrorelix acetate im (GenScript, USA; Cetro, n = 19) or 2 mg estradiol benzoate im (Zoetis, EB, n = 19). On Day 8, the CIDR® was removed and heifers were given 500 µg cloprostenol im (MSD, Germany) and tail paint was applied for heat detection. The ovaries were examined daily by transrectal ultrasonography from Days 0 to 12. Heifers were given 250 µg gonadorelin im (MSD) and inseminated on Day 10, and again on Day 11 in heifers that had not yet ovulated. In Experiment 2, suckling Brangus beef cows (≥60 days postpartum) were given a CIDR® (Day 0) and either 3 mg cetrorelix acetate im (n = 106) or 2 mg estradiol benzoate im (n = 106). The CIDR® was removed on Day 8 and cows were given 500 µg cloprostenol and 400 IU eCG (Zoetis) im. Cows were given 250 µg gonadorelin im and inseminated once at 54 hr post-CIDR® removal. A subset of cows (n = 20 per group) was examined by ultrasonography on Days 8 and 10 to record the largest follicle. Pregnancy was diagnosed on Day 40 in both experiments. Data were compared by ANOVA for repeated-measures (ovarian endpoints) or GLIMMIX (ovulation and pregnancy rates). In Experiment 1, new follicular wave emergence was detected earlier in Cetro than EB group (59.3 ± 6.1h *vs* 92.2 ± 5.5h, P < 0.01), and the Cetro group had a larger dominant follicle than the EB group on Day 8 (13.4 ± 1.3 *vs* 11.2 ± 1.9 mm; P < 0.05) and Day 10 (11.9 ± 1.8 *vs* 9.8 ± 1.3 mm; P = 0.03). All heifers ovulated by Day 12. No difference was detected between Cetro and EB groups in the interval from CIDR® removal to ovulation (79.5 ± 17.9 *vs* 84.1 ± 16.9h; P = 0.44), in the proportion of heifers that displayed heat (84.2% *vs* 68.4%; P = 0.27), or the proportion diagnosed pregnant (78.9% *vs* 68.4%; P = 0.48). In Experiment 2, the Cetro group had a larger dominant follicle than the EB group on Day 8 (12.7 ± 0.6 *vs* 11.0 ± 0.3 mm; P < 0.01) and 10 (11.4 ± 0.5 *vs* 8.6 ± 0.4 mm; P = 0.02). No difference was detected between the Cetro and EB groups in the proportion of cows that displayed heat (79.2% *vs* 76.4%, P = 0.73), or were pregnant (42.6% *vs* 45.6%, P = 0.69). Our hypotheses were supported. We conclude that a cetrorelix-based synchronization and fixed-time insemination protocol is effective in beef heifers and postpartum cows and is a viable alternative to estradiol-based protocols in cattle. Research supported by NSERC and University of Saskatchewan Proof of Concept Fund.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Association between different times of pregnancy diagnosis with Doppler ultrasonography and the type of corpus luteum with conception rates, percentage of false positives and pregnancy losses in lactating dairy cows

Luiz Manoel Souza Simões[1,2], Laís Reis Carvalho[1], Lucas Araújo Lemos[1], Matheus Pedroso Vicente[1], Rafael Resende Rabelo Silva[1], Anna Carolina Vieira Rosa[2], José Nélio de Sousa Sales[3]

[1]Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras
[2]Prenhez Consultoria
[3]Departamento de Medicina Veterinária, Universidade Federal de Juiz de Fora

E-mail: luizmanoelss@hotmail.com

The objective of this study was to evaluate if different times (21-26 days after AI) of pregnancy diagnosis with Doppler ultrasonography (US) and type of corpus luteum (CL) affects the conception rate (P/AI), the percentage of false positives and pregnancy losses in lactating dairy cows submitted to TAI protocols. In the study, 229 Holstein and crossbred (Holstein X Jersey) dairy cows with body condition score of 2.78 ± 0.01 (scale of 1 - 5) and milk production of 32.9 ± 0.4 were used. On a random day of the estrous cycle (D0), cows received 2 mg of estradiol benzoate, 25 µg lecireline and an intravaginal device containing 1 g of progesterone (P4). After seven days (D7), cows received 500 µg of cloprostenol. On day 9 (D9), the P4 device was removed, and cows received 500 µg of cloprostenol and 1 mg of estradiol cypionate. All cows were inseminated 48 hours after the removal of the P4 device. On D21-26 post-AI, all cows were evaluated by transrectal Doppler US (DP50-Power) to estimate the CL blood perfusion (subjective evaluation of color signals on a scale of 0-100%, 5 points between degrees). Cows with an active CL (≥30% of blood perfusion) were considered pregnant and submitted to a confirmatory pregnancy diagnosis on D30 and D60. At the time of the ultrasound evaluations with Doppler mode, the cows were divided into two experimental groups according to the day on which the evaluation was carried out (Low group: between days 21-23 after AI and High group: between days 24-26 after AI). Furthermore, females were evaluated according to the amount of CL (1CL or 2CL) and the presence of the cavity (CLcav). Statistical analyses were performed by the GLIMMIX procedure of SAS. Cows evaluated with Doppler US at 21-23 days had a higher rate of active CLs [Low - 71.6% (48/67) and High 53.7% (87/162); P = 0.01], conception rate at 30 days [Low - 49.3% (33/67) and High 32.7 (53/162); P = 0.02] and pregnancy losses between 30 and 60 days [Low - 27.3 (9/33) and High - 7.4 (4/54); P = 0.01)] than cows evaluated at 24-26 days. However, there was no difference in the false positive rate between the different days of diagnosis using Doppler US [Low - 31.2 (15/48) *vs* High - 37.9 (33/87); P = 0.44]. Furthermore, the pregnancy rate at 30 days was higher in cows with one CL on Doppler diagnosis [1CL - 66.7% (58/87)^{a}; 2CL - 56.2% (9/16)^{ab} and CLcav - 25.0 (3/12)^{b}; P = 0.04]. The false positive rate was higher in cows with the presence of a cavitary CL in the Doppler diagnosis [1CL = 32.2% (28/87)^{b}; 2CL 43.8% (7/16)^{ab} and CLcav 75.0% (9/12)^{a}; P = 0.04]. It is concluded that early pregnant diagnostic cows (21 to 23 days after TAI), using Doppler US, have higher rates of functional CLs, conception at 30 days and pregnancy losses between 30 and 60. Furthermore, cows with a non-cavitary CL at diagnosis using Doppler US have a higher pregnancy rate and lower incidence of false positives compared to cows with a cavitary CL. Support: Santo Antonio da Estiva Farm and Prenhez Consultoria.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Body weight, pregnancy rate and gestational losses in early Nelore heifers under different supplementation strategies in feedlots

Felipe de Oliveira Pedro[1], Aldair Felix da Silva[1], Thais Ferreira Lima[1], Dauydison Antonio Gonzalez Cordeiro[1], João Victor Bezerra Machado[1], Wallery Caroliny Costa da Costa[1], Taynara dos Santos Santana[1], Fabiana de Andrade Melo Sterza[1], Silvio André Isler[1]

[1]Universidade Estadual de Mato Grosso do Sul

E-mail: felipedro2301@gmail.com

The aim of this study was to identify the supplementation strategy that would best contribute to weight gain and reproductive efficiency in early heifers. The study was conducted in Mato Grosso do Sul and included 60 weaned Nelore heifers. After rearing for 30 days on pasture, the heifers with an average age of 9.05 ± 0.42 months and weight of 259.3 ± 21.6 kg were introduced to the feedlot in collective stalls with free access to the feed and water *ad libitum*. The heifers were randomly divided into three groups in a completely randomized design considering the animal as the experimental unit: S0.5 (n = 20), S1.0 (n = 20) and STP (n = 20). The diet was composed of concentrate, an average of 80% total digestible nutrients, and silage (BRS Capiaçu) with 4.32% crude protein in the dry matter. STP (Stair-step nutrition) supplementation consisted of gradual increases in supply: 0.07% of body weight (BW), 0.15%, 0.3%, 0.5%, 1.0% and ending with 1.5% of BW. The concentrate of S0.5 and S1.0 consisted of 0.5% and 1.0% of BW, respectively. The experimental period was 130 days and, at the last 50 days of the experiment, the groups received a common diet of 1.5% of BW. Weight gain was monitored every 30 days on a Tru-test® automatic scale. After 55 days from the start of the experiment, the cyclicity induction protocol was carried out with the application of 1 mg of iP4 on D-24 and 1 mg of estradiol cypionate on D-12, followed by the start of the FTAI protocol on D0 (three managements in nine days), with random and standardized use of two bulls for AI. The heifers that did not reach a weight of 250 kg by the time of the cyclicity induction protocol were removed from the experiment. On the day of AI, estrus expression (ESCT: 1 to 3) and body condition score (BCS: 1 to 5) were observed, as well as the application of 1 mg of GnRH to all the heifers. Ten days after AI, one bull was inserted per group, with a male/female ratio of 1/12. Pregnancy diagnosis was performed by transrectal ultrasound 30 and 60 days after AI. Statistical analyses were carried out using the Proc MIXED and GLIMMIX procedures of SAS® considering an alpha of 5% significance and including in the model the effect of treatment and bull used in AI. The average weight at induction differed among groups ($p < 0.05$), being similar in the S1.0 and S0.5 groups (283 ± 35.70 kg and 280.2 ± 16.85 kg respectively) and lower in the STP group (250.5 ± 27.86 kg). The final weight was: S0,5% 359 ± 28,1 kg, S1.0% 382 ± 40.2 kg and STP 366 ± 29.2 kg ($p < 0.05$). The average daily gain (ADG) also varied among groups ($p < 0.05$), S1.0% (0.935 kg/d) was superior to the others, which did not differ from each other (S0.5% = 0.777 g/d and STP = 0.811 g/d). The estrus score did not differ among groups ($p > 0.05$), while the average BCS at AI was higher for S1.0 ($p < 0.05$) compared to the other groups (STP = 2.97 ± 0.25; S0.5 = 2.93 ± 0.30 and S1.0 = 3.20 ± 0.19). There were no differences for P/AI: STP = 56.3% (9/16), S0.5 = 40.0% (8/20) and S1.0 = 47.0% (8/17), final pregnancy rate: STP = 68.7% (10/16), S0.5 = 85.0% (17/20) and S1.0 = 88.2% (15/17) or gestational loss: STP = 12.5% (2/16), S0.5 = 10% (2/20) and S1.0 = 11% (2/17) ($p > 0.05$). Therefore, the supplementation protocols adopted showed satisfactory results. The S0.5 and S1.0 groups had better production performance. No significant difference was observed on reproductive traits, but, because of the number of heifers in each group, these results need to be confirmed.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Comparing estradiol-free protocols for timed artificial insemination and their impact on fertility of *Bos indicus* cows

Lucas Araújo Lemos[1], Laís Reis Carvalho[1], Matheus Pedroso Vicente[1], Leonardo Silva Fernandes do Vale[1], Luiz Manoel Souza Simões[1], Rafael Canela de Camargo[2], Rodolfo Daniel Mingoti[3], Lais Mendes Vieira[3], Henderson Ayres[3], José Nélio de Sousa Sales[4]

[1]Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras
[2]RC Multiplicação Genética
[3]Merck sharp & Dohme saúde animal
[4]Departamento de Medicina Veterinária, Universidade Federal de Juiz de Fora

E-mail: lucasaraujolemos6@hotmail.com

The objective was to evaluate the fertility of suckled *Bos indicus* Nelore cows pre-synchronized with progesterone and submitted to the TAI protocol based on GnRH. A total of 699 Nelore cows from two commercial farms (BCS = 2.62 ± 0.01) were randomly allocated into four experimental groups: Control (n = 170), GnRH/EC (n = 177), 2GnRH48 (n = 176) and 2GnRH54 (n = 176). Control group received the E2/P4-based TAI protocol, beginning (D0) with 2 mg of estradiol benzoate (EB; Fertilcare Sincronização®, MSD, Brazil) and an intravaginal progesterone (P4) device (Crestar® 1.0 g, MSD, Brazil). Eight days later (D8) 1 mg of estradiol cypionate (EC; Fertilcare Ovulação®, MSD, Brazil), 300 IU of eCG (Folligon®, MSD, Brazil), 530 µg of cloprostenol sodium ($PGF_{2\alpha}$; Ciosin®, MSD, São Paulo, Brazil) were administered concurrently to P4 devices withdrawal. The cows in the GnRH/EC, 2GnRH48, and 2GnRH54 groups received 200 µg of gonadorelin (GnRH; MSD, São Paulo, Brazil) on D0 and a were pre-synchronized with a P4 device seven days before the control group (D-7), which was retained until D8 (P4 for a duration of 15 days). P4 devices withdrawal were done on D8 and the GnRH/EC group received 1 mg of EC, 300 IU of eCG and 530 µg of $PGF_{2\alpha}$; 2GnRH48 and 2GnRH54 groups were treated 300 IU of eCG and 530 µg of $PGF_{2\alpha}$. TAI was performed 48h (Control, GnRH/EC and 2GnRH48) or 54h (2GnRH54) after P4 device withdrawal. The groups 2GnRH48 and 2GnRH54 received 100 µg of GnRH on TAI. Ultrasonographic exams were performed to follicular dynamic (D-7; D0; D8 and D10) and pregnancy diagnosis was carried out 30 days after TAI. Statistical analysis was performed by GLIMMIX of SAS (Initial model: variable = group + BCS on D0 + farm + interactions). Groups that received GnRH on D0 exhibited significantly greater numbers of follicles on both D0 (Control = 12.3 ± 0.3^{b}, GnRH/EC = 13.3 ± 0.3^{a}, 2GnRH48 = 13.2 ± 0.7^{a}, 2GnRH54 = 13.3 ± 0.3^{a}; P = 0.02) and D8 (Control = 11.9 ± 0.2^{b}, GnRH/EC = 13.3 ± 0.3^{a}, 2GnRH48 = 13.2 ± 0,3^{a}, 2GnRH54 = 13.4 ± 0,2^{a}; P = 0.001). However, on D10, the of the follicles were similar among groups (P = 0.71). There were no significant differences in cyclicity rates among the groups on D-7 (Control = 4.7%, GnRH/EC = 11.5%, 2GnRH48 = 12.4% and 2GnRH54 = 9.4%; P = 0.20) and D0 (Control = 10.0%, GnRH/EC = 8.5%, 2GnRH48 = 10.2% and 2GnRH54 = 11.4%; P = 0.91). The ovulation rate on D0 was greater in the GnRH groups (Control = 4.7%^{b}, GnRH/EC = 69.5%^{a}, 2GnRH48 = 69.9%^{a} and 2GnRH54 = 61.9%^{a}; P = 0.001). Conversely, the estrus rate was lower in cows that received GnRH on TAI (Control = 54.7%^{a}, GnRH/EC = 53.7%^{a}, 2GnRH48 = 35.7%^{b} and 2GnRH54 = 46.6%^{ab}; P = 0.001). Pregnancy-to-artificial insemination rate was lower in the GnRH/EC group (Control = 51.2%^{a}, GnRH/EC = 34.5%^{b}, 2GnRH48 = 38.6%^{ab} and 2GnRH54 = 40.3%^{ab}; P = 0.001) and there were no significant differences in cyclicity rates during pregnancy diagnosis (Control = 50.6%, GnRH/EC = 60.3%, 2GnRH48 = 53.7% and 2GnRH54 = 59.1%; P = 0.001. In conclusion, the GnRH/EC group was less effective in synchronizing ovulation in suckled *Bos indicus* cows.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Comparison of doses of buserelin acetate administered at the time of Presynch and at the start of an Ovsynch 56 protocol in Holstein cows

Luis Armando Contreras Méndez[1,2,3], Pablo Luna Nevárez[1], Pedro Alan López Castro[1], Abraham López Oliva[2], Eduardo de Jesús Mendoza Ramírez[2], Berenice Zúñiga Lovera[2], Lucas O. e Silva[4,5], Carlos Eduardo Consentini[6], Roberto Sartori[4,7]

[1]Instituto Tecnológico de Sonora
[2]Universidad Nacional Autónoma de México
[3]Departamento Técnico Virbac México, Virbac
[4]Luiz de Queiroz College of Agriculture, University of São Paulo
[5]Department of Animal Science, Texas A&M University
[6]GlobalGen Vet Science, [7]Department of Animal and Dairy Sciences, University of Wisconsin Madison

E-mail: luarcome@hotmail.com

The aim of this study was to evaluate reproductive outcomes of high-producing Holstein cows treated with single or double doses of buserelin acetate (GnRH, LiberActive®, Virbac Mexico), administrated 7d prior to and at the beginning (D0) of a timed-artificial insemination (TAI) protocol for first or further services. Multiparous (n = 599) and primiparous (n = 396) lactating cows (body condition score [BSC; 1-5 scale] = 3.0 ± 0.5), were randomly assigned into 2 groups, according to the GnRH dose administered im on D-7 and 0: single dose (S; 10 µg buserelin; n = 496) or double dose (D; 20 µg buserelin; n = 499). On D-7, cows received a first treatment with the respective GnRH treatment. On D0, the same treatment was repeated in all cows. Seven and eight days later, 150 µg D-cloprostenol (PGF, InducelActive®, Virbac Mexico) was given. Another treatment with 10 µg buserelin was administered in all cows 56 h after the first PGF and AI was performed 16 h after GnRH. On D8, cows received tail-chalk for detection of estrus. Ultrasound examinations were done on D-7, D0, and D7 to evaluate the presence of corpus luteum (CL), and 32 and 50d after AI to assess pregnancy per AI (P/AI) and pregnancy loss (PL). Statistical analyses were performed by PROC GLIMMIX of SAS 9.4 ($P \leq 0.05$). The presence of CL on D-7 was similar between S and D groups (79.4 *vs* 79.4%). GnRH treatment did not affect presence of CL on D0 (86.5 *vs* 87.0%) nor on D7 (92.9 *vs* 93.8%), respectively. Moreover, there were no differences between S and D groups in relation to expression of estrus (57.9 *vs* 55.9%), P/AI at 32d after AI (37.1 *vs* 34.7%), P/AI at 50d after AI (29.8 *vs* 27.5%), or PL (19.6 *vs* 20.8%). Regardless of treatment, presence of CL on D0 affected P/AI. Cows with CL (n = 863) on D0 had greater P/AI either 32 (37.5 *vs* 25.0%) or 50d after AI (30.0 *vs* 19.7%) than cows without CL. However, no effect was observed on PL (20.1 *vs* 21.2%; respectively). Likewise, presence of CL on D7 affected P/AI, regardless of treatment. Cows with CL on D7 (n = 926) had greater P/AI 32 (38.0 *vs* 6.1%) or 50d after AI (30.3 *vs* 6.1%) than cows without CL. In addition, there was an effect of expression of estrus on P/AI and PL, regardless of treatment. Cows expressing estrus (n = 566) had greater P/AI 32d post AI (40.3 *vs* 30.1%) and 50d post AI (33.6 *vs* 22.1%), and lower PL (16.7% *vs* 26.4) than those that did not express estrus. In conclusion, treatment with distinct doses of buserelin acetate (10 or 20 µg) 7 days prior to and at the beginning of a TAI protocol did not affect synchronization response or fertility in lactating Holstein cows. Moreover, regardless of treatment, presence of CL on D0 and D7, as well as expression of estrus were associated with greater fertility outcomes. Acknowledgements: Virbac México.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Conception rate and pregnancy loss in Holstein heifers inseminated with sexed semen after natural heat or fixed-time protocol

Beatriz Abrahão Bueno de Morais[1], Aron Goulart Doretto[1], Andrey Osvaldo Souza Ferro[1,2], Paulo de Tarso Lemos Borges[2], Davi França Jorge[2], Fábio Morotti[1,3], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina
[2]Policlínica Veterinária Pioneiros
[3]Universidade Norte do Paraná

E-mail: beatriz.abrahao@uel.br

The objective of this study was to evaluate the conception rate and pregnancy loss in dairy heifers who received sexed semen by artificial insemination (AI) or timed artificial insemination (TAI) program. Holstein heifers (n = 420) aged 13 to 15 months, with 360 to 400 kg of weight, body condition score 3.00 to 4.00 (3.2 ± 0.4), with average 2.2 ± 0.1 service/animal, regular vaccination schedule (CattleMaster®, Zoetis, Campinas, Brazil, and Bovi-Shield®, Zoetis), and from a single dairy farm were used in this study. Heifers that showed natural heat (n = 263) were identified by visual observation or electronic collar identification system (Allflex®, MSD, Joinville, Brazil) and inseminated 12 hours later with sexed-X semen from four bulls with known fertility containing 4 million sperm per straw. For timed AI, on a random day of the estrous cycle (D0), heifers (n = 157) received intravaginal insertion of a progesterone device (1.9 g, CIDR®, Zoetis) and intramuscular (i.m.) estradiol benzoate (2 mg, Gonadiol®, Zoetis). On D7 the animals received dinoprost tromethamine ($PGF_{2\alpha}$; 16.77 mg, i.m., Lutalyse®, Zoetis). On D8 the intravaginal device was removed and estradiol cypionate was injected (0.6 mg, i.m.; E.C.P®, Zoetis) being performed another dose of $PGF_{2\alpha}$. The TAI was performed on D10 following the same bulls and semen descriptions used in natural heat. Three technicians performed the inseminations and pregnancy diagnosis was made by ultrasound 30 and 60 days later. Data were analyzed using multivariate logistic regression and the model considered program (conventional AI and TAI), bull (A, B, C, and D), inseminator (I, II, and III), services, and main interactions. Data are presented as percentages; significance was considered $P \leq 0.05$ and tendency $P \leq 0.09$. The conception rate was influenced by the reproductive program at 30 days (P = 0.004; AI - 49.43% (130/263) *vs* TAI - 33.12% (52/157) and 60 days (P = 0.001; AI - 46.39% (122/263) *vs* TAI - 29.30% (46/157) and there was a tendency (P = 0.09 and P = 0.07) to be influenced by the bull. There was no effect of the inseminator (P = 0.65 and P = 0.84) and services (P = 0.19 and P = 0.13). Pregnancy loss was influenced by the program (P = 0.01), being higher in heifers that received TAI 11.54% (6/52) compared to those with natural heat 6.15% (8/130). The factors (program, bull, inseminator, and services) did not show interactions (P > 0.1) for conception rate or pregnancy loss. Under the conditions of the present study, the conception rate and pregnancy loss up to 60 days in heifers inseminated with sexed semen were influenced by the reproductive program.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Contrast of low and high reproductive performance on herd composition, productivity, and carbon footprint in dairy production system

Laís Ângelo de Abreu[1], Vanessa Romário de Paula[2], Bruno Campos de Carvalho[2], Virginia Mendonça Lourenço Benhami[2], Fernando Kenji Mori[1], Emanuele Almeida Gricio[1], Sofía Albertini[1], Lígia Mattos Rebeis[1], Alexandre Henryli de Souza[3], Pietro Sampaio Baruselli[1]

[1]University of Sao Paulo
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Gado de Corte)
[3]Cargill Animal Nutrition and Health (Minnesota – MN, EUA)

E-mail: laisabreu@usp.br

The Life Cycle Assessment (LCA), used to calculate the carbon footprint, has been used to map environmental impacts and support the development of technologies and the use of mitigation solutions for agricultural production. LCA studies of milk production in the tropics have shown that herd diet, genetics and fertility are factors that influence estimates of CO_2 equivalent emissions (CO_{2eq}). The objective of this study was to demonstrate the contrast of low and high reproductive performances, evaluated by the age at first calving (AFC) and calving interval (CI) index, on the herd composition, productivity, and CO_{2eq}. emissions of a dairy production system. The carbon footprint of milk production was estimated based on the LCA. The study followed ISO 14040 and ISO 14044 requirements. Open LCA 3.11.1 software was used for data modeling and estimation of CO_{2eq}. The frontier considered was cradle- to-farm-gate, comprising the stages of animal management, use of natural resources, energy, inputs and waste management, direct and indirect emissions. Data were collected on a farm located in the state of Minas Gerais, with 421 milking Holstein cows (n = 1037 animals in the herd), housed in a compost barn system. The AFC, CI, and the milk production averages were 24 and 14 months, and 32.0 liters of milk per day, respectively. The herd composition was classified as milking and dry cows, heifers (33-36, 29-32, 24-28, and 12-24 months), calves (0-12 months), and bulls. Twenty scenarios were modeled combining different CI (12, 13, 14, 15 or 16 months) and AFC (24, 28, 32 or 35 months), adjusting the herd structure and milk production. The adjusted for different CI of the herd had increasing lactation length while maintaining the number of milking cows. Also, it was considered that longer CI determined longer lactation lengths and the feed efficiency was adjusted for methane emission of lower productive cows. The carbon footprint was estimated for these scenarios and linear regression was performed to estimate the effect of AFC and CI on production, and CO_{2eq}/milk (corrected for fat and protein content). Statistical analyses were performed using the REG procedure of SAS© 9.4. It was observed that as AFC decreases, the quantity of calves and heifers required is also reduced, especially between the 24-28 months [AFC36 (n = 57), AFC32 (n = 56), AFC28 (n = 55), AFC24 (n = 0)]. Furthermore, the total of emissions for 1 kg of milk (CO_{2eq}/milk) was affected by CI and AFC (y = 0.0840 + 0.0216*CI + 0.0182*AFC, r^2 = 0.9971), being higher in the low-performance group (AFC36- CI16 = 1.07) than in the high-performance group (AFC24-CI12), in which the amount kg CO_{2eq}/milk produced was 0.786. In conclusion, the high efficiency and fertility (low AFC and CI) are relevant to improving productivity (14.0%) per liter of milk produced, being an opportunity for dairy production systems to be a part of ecosystem management and contributing to reducing the intensity of CO2eq. emissions (27%).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Cyclicity induction protocol with P4 device or injectable P4 (D-24) with or without estradiol cypionate treatment (D-12) in 14 - 24 months Nelore heifers

Igor Garcia Motta[1], Matheus de Azevedo Nunes[2], Bruna Lima Chechin Catussi[3], Emanuele Almeida Gricio[3], Bruno Silva do Espírito Santo[2], Carlos Henrique Pascotto[4], Bruno Gonzalez de Freitas[1], Pietro Sampaio Baruselli[3]

[1]Ourofino Saúde Animal
[2]Agropecuária Jacarezinho
[3]Animal Reproduction Department, School of Veterinary Medicine and Animal Science, University of São Paulo
[4]Reproconsult

E-mail: igor.mota@ourofino.com

The study aimed to assess the effectiveness of the cyclicity induction protocol in heifers using different sources of progesterone (P4; reused intravaginal device or injectable P4) 24 days (D-24) with or without estradiol cypionate (EC) 12 days (D-12) prior to the synchronization protocol for fixed-time artificial insemination (FTAI). A total of 626 Nelore (*Bos indicus*) heifers (aging 14 to 24 months, body weight (BW = 312.1 ± 1.04 kg, body condition score (BCS = 3.14 ± 0.01) from two commercial farms (469 Fazenda Novo Horizonte-MS and 157 Fazenda Rio Brilhante-MS) were used. On D-24, heifers were randomly divided into two groups: A) P4-Device (n = 318): received a P4 device previously utilized for 14 days (Sincrogest®, Ourofino); B) iP4 (n = 308): received 150 mg of iP4 (Sincrogest injetavel®, Ourofino). On D-12, cows of each group were randomly divided into two groups, resulting in: 1) P4-Device (n = 156): removal of P4 device; 2) P4-Device+EC (n = 162): removal of P4 device plus 1 mg of EC (SincroCP®, Ourofino); 3) iP4 (n = 157): no treatment; 4) iP4+EC (n = 151) received 1 mg of EC. On D0, an intravaginal P4 device, 2 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino) and 0.53 mg of sodic cloprostenol (PGF; Sincrocio®, Ourofino) were administrated. Also, the gynecological examination was performed by ultrasound (Mindray® DP-2200Vet) to analyze the presence of the corpus luteum (CL) on D0. On D7, heifers received 0.5 mg of EC, 0.53 mg of PGF, and 200 IU of eCG (SincroeCG® Ourofino), concurrent with P4 device removal. TAI was performed 48 hours later (D9) in both groups. Pregnancy diagnoses were evaluated by ultrasound after 30 days after TAI. Statistical analyses were performed using GLIMMIX of SAS 9.4. There was no interaction between P4 and EC groups for any variables, and data is presented by main factors. The presence of CL on D0 was similar between EC groups, but a tendency (P = 0.06) for P4 source was observed [P4-Device = 29.6% (94/318) *vs* iP4 = 36.4% (112/308)]. Treatment with iP4 source increased (P = 0.004) estrus rate at TAI [P4-Device = 36% (95/264) *vs* iP4 = 47.9% (124/259)]. Treatment with EC also presented a positive effect on estrus rate (P = 0.04) [Control = 37.8% (99/262) *vs* EC = 46% (120/261)]. The pregnancy rate did not differ between P4 source treatments [P4-Device = 43.1% (142/307) *vs* iP4 = 47.9% (137/318); P = 0.43] nor EC treatments [Control = 42.1% (147/312) *vs* EC = 42.2% (132/313); P = 0.23]. In conclusion, no differences were observed between P4 sources with respect to any variables. However, the treatment with iP4 on D-24 and EC on D-12 can improve the estrus rate in heifers undergoing fixed-time artificial insemination protocol, but no effect on pregnancy rate was observed.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

D-GnRH administration at the beginning of the TAI protocol in *Bos indicus* cows previously exposed to injectable progesterone

Rafael Resende Rabelo Silva[1], Laís Reis Carvalho[1], Matheus Pedroso Vicente[1], Lucas Araújo Lemos[1], Luiz Manoel Souza Simões[1], Igor Garcia Motta[2], Bruno Gonzalez de Freitas[2], José Nélio de Sousa Sales[3]

[1]Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras
[2]Ourofino Saúde Animal
[3]Departamento de Medicina Veterinária, Universidade Federal de Juiz de Fora

E-mail: rafaelresender@hotmail.com

The objective was to evaluate the use of D-GnRH (GnRH analogue) at the beginning of the ovulation synchronization protocol with no insertion of the intravaginal progesterone (P4) device on the fertility of *Bos indicus* cows pre-synchronized with injectable progesterone (IP4). On a random day of the estrous cycle (D-7), suckled *Bos indicus* cows (n = 355) with BCS of 3.2 ± 0.02 (scale of 1 - 5) received 150 mg of long-acting IP4 (Sincrogest Injetável®, Ourofino, Brazil). On day 0 (D0), cows were randomly distributed into two experimental groups (Control Group and D-GnRH Group). At the same time, cows in the control group (n = 178) received 2 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino, Brazil) and an intravaginal device containing 1 g of P4 (Sincrogest®, Ourofino, Brazil) and cows in the D-GnRH group (n = 177) received 1 mg of D-GnRH (Sincrorrelin®, Ourofino, Brazil). After eight days (D8), the P4 device was removed in the control group cows and 1.0 mg of estradiol cypionate (CE; SincroCP®, Ourofino, Brazil), 0.500 µg of cloprostenol sodium (Sincrocio®, Ourofino, Brazil) and 300 IU of eCG (SincroeCG®, Ourofino, Brazil) were administered in all animals in the experimental groups. TAI was performed 48h after administration of the ovulation inducer (EC; D10). Ultrasonographic exams were performed to follicular dynamic (D0 and D8) and pregnancy diagnosis was carried out 30 to 35 days after TAI. Statistical analyses were performed by GLIMMIX procedure of SAS. Follicular diameter (Control = 13.4 ± 0.3 and D-GnRH 13.9 ± 0.3; P = 0.37) and cyclicity rate (Control = 21.9% and D- GnRH 29.4%; P = 0.09) on D0 were similar between the experimental groups. Nevertheless, follicular diameter on day 8 (Control = 11.6 ± 0.2 and D-GnRH 12.9 ± 0.3; P = 0.003) and ovulation rate on D0 (Control = 2.3% and D-GnRH = 80.2%; P = 0.001) were greater in cows in the D-GnRH group. Pregnancy-to-artificial insemination rate (P/AI) was greater in cows of the Control Group (Control = 42.1% and D-GnRH = 28.2%; P = 0.001), but no difference was observed in cows of the D-GnRH group that ovulated or not on D0 (Ovulated = 26.4% and Not ovulated = 35.3%; P = 0.30). In conclusion, TAI protocols initiated with D-GnRH and without the insertion of the P4 device have lower fertility results in suckled *Bos indicus* cows pre-synchronized with IP4.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Economic viability of using long-acting progesterone post-TAI on conception rate and pregnancy loss in Nelore cows

Samuel Rodrigues Bonamichi do Couto[1], Thiago de Souza Vieira[1], Raphael Azevedo Lamim[1], Beatriz Alves Paulino[1], Nicolas Moreira Piedras Monnerat Caparelli[1], Bruna Gomes Santos[1], Greicy Sofia Maysonnave[1], Rondineli Pavezzi Barbero[1], Marco Roberto Bourg Mello[1]

[1]Departamento de Reprodução e Avaliação Animal, Instituto de Zootecnia, Universidade Federal Rural do Rio de Janeiro

E-mail: samuel_piquete@yahoo.com.br

The present study aimed to evaluate the technical and economic feasibility of long-acting progesterone (P4LA) supplementation in Nelore cows undergoing TAI, considering its effect on conception rates and pregnancy loss, as well as the costs of the treatment used. The hypothesis of this study is that the cost per pregnancy of Nelore cows supplemented with P4LA, 7 days after TAI, is lower than the cost per pregnancy of non-supplemented cows, in addition to obtaining heavier calves at weaning. To this end, 452 Nelore females presenting an average body condition score of 2.93 ± 0.66 were subjected to ovulation synchronization using a protocol of conventional hormonal therapy of three managements. After TAI (D0), animals were divided into two groups: Control (n = 234), which received saline solution, and P4LA (n = 218), which received 150 mg of P4LA (Sincrogest Injectável®, Ourofino). The drugs were administered intramuscularly in both groups on the seventh day after TAI (D7). Gestational diagnosis (GD) and pregnancy loss (PL) were evaluated with the aid of transrectal ultrasound (Mindray D2200 vet), respectively 30 and 60 days after insemination. Conception rate and pregnancy loss were compared using the Bioestat program with 5% significance. The cost per pregnancy at 30 days (CPP30) was calculated taking into account the cost of the protocol, based on the following formula [(number of cows submitted to the protocol)*(cost per protocol)]/(number of pregnant cows) being the P4LA group the cost of applying 150 mg of long-acting progesterone was added. The same equation described above was used to calculate the cost per pregnancy at 60 days (CPP60), where the number of pregnant females changed as a result of PL. The conception rates obtained at 30 days were 50% and 58% in the control and P4LA groups, respectively (P = 0.04). The PL at 60 days was 15.38% and 9.45% for the same groups (P = 0.07). The CPP30 was R$ 55.58 for the control group and R$ 55.35 for the P4LA group, while the CPP60 was R$ 65.69 for the control group and R$ 61.13 for the P4LA. However, an influence on the calf's weight at weaning was also observed, where averages of 203.5 and 215.1 kg were obtained in the offspring of the control and P4LA groups, respectively (P = 0.01). Thus, the kilogram of calf weaned per protocol cow was 72.2 for the control group and 95.7 for the P4LA group. Therefore, it is concluded that the use of PALA, 7 days after TAI, increases the economic and reproductive efficiency of Nelore females subjected to timed artificial insemination, reducing the cost per pregnancy and increasing the average weight of the calf at weaning.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effectiveness of recombinant eCG in follicular dynamics and pregnancy rate of Nelore heifers submitted to FTAI

Luciano Penteado da Silva[1], Lígia Mattos Rebeis[2], Sofía Albertini[2], Fernando Kenji Mori[2], Rafael Danelucci[3], Federico Randi[4], Pietro Sampaio Baruselli[2]

[1]Firmasa Tecnologia Para Pecuaria Ltda
[2]Animal Reproduction Department, School of Veterinary Medicine and Animal Science, University of São Paulo
[3]Costa Agropecuária
[4]Ceva Santé Animale

E-mail: luciano@firmasa.com.br

This study assessed the effectiveness of recombinant eCG (reCG; Foli Rec®, Ceva Santé Animale) treatment on the follicular dynamics and pregnancy rate in Nelore (*Bos indicus*) heifers. A total of 794 heifers aged 14 months, with body weight 332.9 ± 1.0 kg, and body condition score (BCS) of 3.6 ± 0.01 from one commercial farm (Fazenda Santa Edwiges - MT) were used. At the onset of the protocol (D0), each heifer received a 0.75 g P4 intravaginal device and 2 mg of EB i.m.. Seven days later (D7), simultaneous with the device removal, heifers received 150 µg of d-cloprostenol and 0.5 mg of estradiol cypionate i.m.. At this time, heifers were assigned to receive one of four treatments: 1. Control (n = 110): no treatment; 2. eCG_1.0 (n = 228): treatment with 1 ml (200 IU) of a conventional eCG; 3. reCG_0.7 (n = 229): treatment with 0.7 ml (49 IU) of Foli Rec® and, 4. reCG_1.0 (n = 227): treatment with 1.0 ml (70 IU) of Foli Rec®. All heifers were inseminated 48 hours later, and 10 µg of buserelin acetate was administered. A subgroup of heifers (n = 472) underwent an ultrasound on D7 to assess dominant follicle (DF) diameter and on D9 to evaluate DF diameter, growth daily rate, and early ovulation rate (ovulation before FTAI). P/AI was assessed by US 30 days post-insemination (D39). Statistical analyses were performed using GLIMMIX of SAS 9.4. The control group exhibited a lower follicular growth (mm) between P4 device removal and FTAI compared to eCG-treated groups (Control = 1.2 ± 0.15^{B}, eCG_1.0 = 2.1 ± 0.12^{A}, reCG_0.7 = 2.0 ± 0.11^{A}, reCG_1.0 = 2.0 ± 0.12^{A}; P < 0.001). Thereby, on D9, the eCG-treated groups had larger DF diameter than the control group (Control = 8.7 ± 0.27^{B}, eCG_1.0 = 9.8 ± 0.19^{A}, reCG_0.7 = 9.8 ± 0.19^{A}, reCG_1.0 = 9.7 ± 0.19^{A}; P = 0.003). No differences were observed between the reCG_0.7, Control and eCG_1.0 groups regarding early ovulation rate [Control = 5.9% (4/68)^{AB}, eCG_1.0 = 4.5% (6/134)^{A}, reCG_0.7 = 6.7% (9/135)^{AB}, reCG_1.0 = 11.9% (16/135)^{B}; P = 0.03]. No interaction early ovulation*treatment was observed for DF diameter at P4 device removal (P = 0.97). However, heifers with larger DF presented higher early ovulation rates [< 8 mm = 2.3% (6/262)^{A} *vs* ≥ 8 mm = 13.8% (29/210)^{B}; P = 0.0003]. Lastly, the treatments did not differ in the P/AI on D39 [Control = 35.5% (39/110), eCG_1.0 = 41.7% (95/228), reCG_0.7 = 44.5% (102/229), reCG_1.0 = 42.7% (97/227); P = 0.88]. In a subgroup of heifers (n = 467), no interaction of early ovulation*treatment was observed for P/AI (P = 0.97). However, the pregnancy rate was higher in heifers that did not show early ovulation [No Ovulated = 43.5% (188/432)^{A} *vs* Ovulated = 28.6% (10/35)^{B}; P = 0.0426]. In conclusion, the reCG_0.7 treatment presented higher follicular growth (mm), higher DF diameter at FTAI, and the same early ovulation rates compared to the Control group. Despite a 9% increase in P/AI in the reCG_0.7 group compared to the Control group, no statistical difference was verified.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effectiveness of two cyclicity induction protocols in 14- month-old heifers submitted to FTAI

João Paulo Silva Spezia[1], Sofía Albertini[2], Laís Ângelo de Abreu[2], Emanuele Almeida Gricio[2], Ana Carolina dos Santos Oliveira[2,3], Lígia Mattos Rebeis[2], Pietro Sampaio Baruselli[2]

[1]Spézia Consultoria
[2]Animal Reproduction Department, School of Veterinary Medicine and Animal Science, University of São Paulo
[3]Blumenau Regional University

E-mail: consultoria.spezia@gmail.com

The study aimed to assess the effectiveness of two cyclicity induction protocols in prepubertal heifers using injectable progesterone and estradiol cypionate before FTAI. A total of 1247 Nelore (*Bos indicus*) heifers, with an average of 14-month-old, body weight (BW) of 312.0 ± 24.5 kg, and a body condition score (BCS; 1 to 5 scale) of 3.2 ± 0.2, from a commercial farm (Mato Grosso, Brazil) were used. Forty-five days before FTAI (D-45), all heifers received 150 mg of injectable progesterone (IP4; Sincrogest injetável®, Ourofino). Twelve days later (D-33), 0.5 mg of estradiol cypionate (EC; SincroCP®, Ourofino) was administered. After 23 days (D-10), the females were randomly divided into the following experimental groups: 1) Control (n = 640): no treatment, and 2) Two-Induction (n = 611): received on D-13 150 mg of the same injectable progesterone. On D0, an intravaginal P4 device (Sincrogest®, Ourofino), 2 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino) and 0.530 mg of cloprostenol (PGF; Sincrocio®, Ourofino) were administered. Also, on D0, ultrasound examinations (US) were performed to assess the cyclicity rate and uterine score (scale of 1 to 3, 1 being the lowest score and 3 the highest). On D7, heifers received 0.5 mg of EC, 0.530 mg of PGF, and 200 IU of eCG (SincroeCG®, Ourofino), concomitant with P4 device removal. FTAI was performed 48 hours later (D9) in both groups. Pregnancy per artificial insemination (P/AI) was assessed 30 days after FTAI via US. The model used for statistical analysis was a binomial logistic model with a logit link function, fitted using PROC GLIMMIX in SAS 9.4, with a significance level of P ≤ 0,05. No effect of treatment was verified in the cyclicity rate on D0 [Two-Induction = 43.26% (263/608) *vs* Control = 46.48% (297/639); P = 0.25]. However, heifers from the Control group presented a higher proportion of uterine score 1 at D0 [Two-Induction = 3.38% (10/296) *vs* Control = 8.71% (25/287); P = 0.03]. Furthermore, heifers from the Two-Induction group presented a higher proportion of uterine score 3 at D0 [Two-Induction = 59.2% (360/608) *vs* Control = 51.4% (329/639); P = 0.003]. P/AI tended to be higher in the Two-Induction group [Two-Induction = 48.6% (296/608) *vs* Control = 44.9% (287/639); P = 0.09]. However, the P/AI tended to be greater in heifers with uterine score 1 when receiving two inductions [Two-Induction = 45.4% (10/22) *vs* Control = 29.1% (25/86); P = 0.07]. This effect of two inductions was not observed for P/AI in the heifers with uterine scores 2 [Two-Induction = 43.3% (98/226) *vs* Control = 39.2% (88/224); P = 0.18] and 3 [Two-Induction = 52.2% (188/360) *vs* Control = 52.8% (174/329); P = 0.43]. Furthermore, there was an effect of BCS, where heifers with a higher BCS had a greater P/AI [≤ 3 = 41.9% (220/524) *vs* > 3 = 50.2% (363/723); P = 0.004]. In conclusion, despite no differences in P/AI between treatments, the Two-Induction group presented a greater proportion of developed score 3 uterus and tended to increase the P/AI in score 1 uterus.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of calving interval on productivity and carbon footprint in high-producing cows

Bruno Campos de Carvalho[1], Laís Ângelo de Abreu[2], Vanessa Romário de Paula[1], Virginia Mendonça Lourenço Benhami[1], Fernando Kenji Mori[2], Emanuele Almeida Gricio[2], Sofía Albertini[2], Lígia Mattos Rebeis[2], Alexandre Henryli de Souza[3], Pietro Sampaio Baruselli[2]

[1]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Gado de Corte)
[2]University of Sao Paulo
[3]Cargill Animal Nutrition and Health (Minneapolis – MN, EUA)

E-mail: bruno.carvalho@embrapa.br

The demand for sustainable development has motivated the implementation of practices and technologies for agricultural production that aim to increase productivity while reducing global gas emissions. The Life Cycle Assessment (LCA), used to calculate the carbon footprint, has been used to map environmental impacts and support the development of technologies and the use of mitigation solutions. LCA studies of milk production in the tropics have shown that herd diet, genetics and fertility are factors that influence estimates of CO_2 equivalent emissions (CO_{2eq}). The fertility of the herd, evaluated by the calving interval index (CI), influences the productivity and carbon footprint of the herd. The objective of this study was to show the influence of CI on the results of milk production and CO_{2eq}. emissions in an operational system with different modeling scenarios. The carbon footprint of milk production was estimated based on the LCA. The study followed ISO 14040 and ISO 14044 requirements. Open LCA 3.11.1 software was used for data modeling and estimation of CO_{2eq}. from milk production. The frontier considered was cradle-to-farm-gate comprising the stages of animal management, use of natural resources, energy, inputs and waste management, direct and indirect emissions. Data were collected on a farm located in the state of Minas Gerais, with 200 milking Holstein cows (n = 795 animals in the herd), housed in a compost barn system, with CI average of 14 months and 34.5 liters of milk per day (considered as high producing in Brazil). The herd composition was adjusted for different CI, increasing lactation length while maintaining the number of milking cows. The carbon footprint was estimated for these scenarios and linear regressions were performed to estimate the effect of CI on production and CO_{2eq}/milk (corrected for fat and protein content) in the following groups: 1) CI-12 months (n = 818), 2) CI-13 months (n = 809), 3) CI-14 months (n = 795), 4) CI-15 months (n = 794), and 5) CI-16 months (n = 788). It was considered that longer CI determined longer lactation lengths and the feed efficiency was adjusted for methane emission of lower productive cows. Statistical analyses were performed using SAS©. The productivity (liters/cow/day) was directly affected by CI index [y = 52.74 – 1.30x, r^2 = 0.9991; CI-12 months (37.2 L), CI-13 months (35.8 L), CI-14 months (34.5 L), CI-15 months (33.2 L), and CI-16 months (32.0 L)]. The total of emissions for 1 kg of milk (CO_{2eq}/milk) was 0.8206 when the CI index was 14 months (average of studied farm). However, for the 12, 13, 15 or 16-month CI, the amount kg CO_{2eq}/milk produced was 0.7860 (reduction of 4.5%), 0.7908, 0.8437, and 0.8652 (y = 0.5254 + 0.0211x, r^2 = 0.9592; an increase of 5.5%) respectively. It is concluded that reproductive efficiency (lower CI index) is important to improve the productivity of high-producing dairy cows and to reduce the amount of CO_{2eq} per liter of milk produced.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of calving type on the reproductive efficiency of crossbred dairy cows

Nathália Ribeiro Resende[1], Natani Silva Reis[1], Luís Fernando Faria Coury[1], Ricarda Maria dos Santos[1]

[1]Faculdade de Medicina Veterinária (*FAMEV*), Universidade Federal de Uberlândia (*UFU*)

E-mail: nathalia.resende@ufu.br

Dystocia increases the risk of stillbirths and calf mortality in the first 30 days after birth, and can have impacts on reproductive and productive performance, with milk production and conception rate reduction, thereby increasing days open. The aim of this study was to evaluate the effect of calving type on the occurrence of retained placenta, calving to first AI interval, conception rate at first AI and days open of crossbred dairy cows. Data from 6808 calvings of crossbred dairy cows (Holstein x Gyr) was analyzed, from January 2015 to January 2024, on a commercial dairy farm in Minas Gerais State, Brazil. The parturition was classified as normal, assisted, abortion, twin, and stillbirth. Assisted calvings were considered when there was human interference during fetal release, while normal calvings did not require assistance. Abortions were considered when parturition occurred between 42 and 260 days of pregnancy and stillbirths were characterized by the calf's death before, during or immediately after calving. Every cow was evaluated for the occurrence of retained placenta (RP) and after a 40-day voluntary waiting period the cows were examined by ultrasound for evaluation of uterine and ovarian health and after that were submitted to the fixed-time artificial insemination protocol (FTAI). At 35 days after FTAI, the pregnancy was diagnosed by ultrasound exam. Data collected were incidence of RP, the calving to first AI interval, conception rate at the first AI after calving, and days open. Logistic regression was used to evaluate the effect of calving type on the incidence of RP and conception rate at the first AI, and analysis of variance was used to evaluate the effect of calving type on calving interval to first AI and days open, with MINITAB program. Statistical significance was defined as $P \leq 0.05$, and a tendency as $0.05 < P \leq 0.10$. From the 6808 calvings, 4.77% were classified as abortions, 4.36% assisted, 1.87% twins, 3.44% stillbirths and 85.56% normal calvings. There was an effect of the type of calving on the occurrence of RP ($P = 0.0001$), only 8.89% of cows with normal calvings had RP, while cows with twin calvings presented 30.71% of RP and with assisted calvings 29.29% of RP. Assisted calving cows tended to have long calving to the first AI interval ($P = 0.093$). The conception rate at first AI was not affected by the type of calving ($P = 0.662$) and cows with assisted calving presented a higher ($P = 0.004$) days open (151 days). In conclusion, cows with normal calving have lower incidence of retained placenta. Cows with assisted calving tends to have longer calving to first AI interval. The type of calving has no effect on the conception rate at first AI, however the days open is higher in cows with assisted calving.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of estradiol benzoate administration at the beginning of a super early resynchronization protocol (Resynch 14) on Nelore cows

Rafael Danelucci[1], Luciano Penteado da Silva[2], Sofía Albertini[3], Lígia Mattos Rebeis[3], Laís Ângelo de Abreu[3], Emanuele Almeida Gricio[3], Ana Carolina dos Santos Oliveira[3,4], Bruna Lima Chechin Catussi[3], Pietro Sampaio Baruselli[3]

[1]Costa Agropecuária
[2]Firmasa Tecnologia Para Pecuaria Ltda
[3]Animal Reproduction Department, School of Veterinary Medicine and Animal Science, University of São Paulo
[4]Blumenau Regional University

E-mail: danelucci@hotmail.com

This study aimed to verify the effect of administering 1 mg of estradiol benzoate (EB) at the time of intravaginal progesterone device insertion, 14 days after fixed-time artificial insemination (FTAI), on a super early resynchronization protocol (Resync 14) using color Doppler in Nelore cows. For this, 851 cows with a body condition score (BCS) of 3.10 ± 0.02 from 4 different herds were synchronized for FTAI. Fourteen days after FTAI (D14), concomitant with progesterone device (0.6 g) insertion, cows were allocated to one of two treatments: EB group (n = 433), which received 1 mg of EB, and Control group (n = 430), which received no further treatment. Progesterone devices were removed on D22, and cows underwent ultrasonography using color Doppler mode to assess corpus luteum (CL) perfusion on a scale from 0 (no perfusion) to 4 (highest perfusion). Cows with a perfusion rating of 2 to 4 were considered to have an active CL and were thus diagnosed as pregnant. Those with a perfusion rating of 0 to 1 were considered to have an inactive CL and were diagnosed as non-pregnant. Non-pregnant cows received 1 mg of estradiol cypionate (EC), 0.265 mg of cloprostenol sodium (PGF), and 300 IU of eCG. At this time, the dominant follicle (DF) diameter was also measured in non-pregnant cows. A second FTAI was conducted 48 hours later on D24. Pregnancy was confirmed in cows with an active CL on D22 using B Mode ultrasound on D30 to assess the false-positive rate (difference between P/AI at D22 and P/AI at D30). The pregnancy status of cows from the second FTAI was determined 30 days post-insemination (D60). The variables analyzed were DF diameter at D22, P/AI at D22 of the first FTAI, P/AI at D30 of the first FTAI, false-positive rate, and P/AI of the second FTAI. Statistical analysis of rate results was performed using the PROC GLIMMIX, and DF diameter analysis was performed using the PROC NPAR1WAY of SAS 9.4. There was a treatment effect on DF diameter. Cows of the Control group had a larger DF than the ones of the EB group (EB = 10.6 ± 0.16 mm *vs* Control = 11.3 ± 0.18 mm; P = 0.0057). No treatment effect was verified on P/AI at D22 [EB = 51.2% (216/422) *vs* Control = 55.5% (238/429); P = 0.2099]. Furthermore, there was also no treatment effect on P/AI at D30 [EB = 46.5% (192/413) *vs* Control = 49.0% (209/427); P = 0.4762]. The false-positive rate was similar between groups [EB = 11.1% (24/216) *vs* Control = 12.2% (29/238); P = 0.7222]. At the second FTAI, no effect on P/AI was observed between groups [EB = 41.2% (82/199) *vs* Control = 40.3% (77/191); P = 0.8579]. In conclusion, the treatment with 1 mg of EB at D14 (beginning of resynchronization protocol) did not affect the P/AI at the first FTAI. However, the treatment with 1 mg of EB at D14 reduced the diameter of the largest follicle on the day of P4 device removal (D22) but did not affect the P/AI at the second FTAI.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of exposing prepubertal *Bos indicus* heifers to one, two or no ovulation induction protocols prior to timed-AI on reproductive outcomes

Patrícia R. Cavalcanti[1], Rodrigo L. O. R. Alves[1], Lucas O. e Silva[1], Juan P. Acosta-Galíndez[1], Gustavo V. Barros[1], Carlos E. C. Consentini[1], Edgar J. Nagle[2], Roney A. Silva[2], Roberto Sartori[1]

[1]Luiz de Queiroz College of Agriculture, University of São Paulo
[2]Santa Vergínia Agro

E-mail: patriciacavalcanti@usp.br

Reproductive outcomes were evaluated in Nelore (*Bos indicus*) heifers submitted to ovulation induction protocols, based on progesterone (P4) and estradiol (E2). 1441 heifers (13.0 ± 0.02 mo old; 3.1 ± 0.01 BCS and 279.8 ± 0.7 kg of BW) were randomly assigned to 1 of 3 treatments prior to a timed-AI (TAI) protocol: CON (n = 486): no ovulation induction protocol; 1IND (n = 484): single ovulation induction protocol; or 2IND (n = 471): 2 ovulation induction protocols. On D-47, heifers from 2IND received an intravaginal P4 device (IVD; 2 g, previously used for 21 d), kept until D-40, when 0.5 mg E2 cypionate (EC) was given im. On D-19, heifers from 2IND and 1IND underwent the same protocol. On D0, all heifers were submitted to the same TAI protocol, starting with a new IVD (0.5 g), 0.5 mg cloprostenol (PGF) im, and 1.5 mg E2 benzoate im. On D7, IVD was removed 0.5 mg PGF, 0.5 mg EC, and 200 IU eCG were administered im, and tail chalk was used for estrus detection. TAI was performed 48h later. Blood samples were collected on D-47 and 0, to assess circulating P4 concentrations. Presence of CL was considered when P4 ≥ 1 ng/ml. Ultrasound was performed 30 and 65d after AI to assess pregnancy (P/AI) and pregnancy loss (PL). Statistical analyses were done by PROC GLIMMIX of SAS (P ≤ 0.05). For the analyses of effect of age and BW, heifers were classified in terciles: age (T1: < 14.1; T2: 14.2-15; T3: ≥ 15 mo old); BW (T1: < 281; T2: 281-314; T3: ≥ 315 kg). Mean circulating P4 on D-47 was similar among groups (0.3 ng/ml). However, on D0, 1IND had higher mean circulating P4, followed by 2IND, then CON (4.7 ± 0.1a vs 4.2 ± 0.1b vs 1.4 ± 0.1c ng/ml). A greater proportion of heifers from 1IND had P4 ≥ 1 ng/ml on D0, followed by 2IND, then CON (88a vs 80.5b vs 28.8c%). There was an effect of treatment on the expression of estrus (2IND: 66.7a vs 1IND: 67.1a vs CON: 57.4b%). Moreover, P/AI at 30 and 65d after AI was greater in 2IND than 1IND and CON (53.5a vs 44.2 b vs 46.5 b%; 49.8a vs 40.5 b vs 43.9b%, respectively). No differences were observed in PL (6.8 vs 7.6 vs 4.4%, respectively). Non-pregnant heifers from 2IND had a greater incidence of CL than CON, but without difference in relation to 1IND (85.4a vs 74.8b vs 80.8% ab) 30 d after AI. Regardless of treatment, age affected fertility: older heifers (T2 and T3) had greater P/AI 30 d after AI than T1 (42.9b vs 51.3a vs 49.3%a). However, no relationship was observed between BW and fertility (T1: 46.2 vs T2: 47.1 vs T3: 51%). In conclusion, implementing ovulation induction protocols prior to the AI protocol increased the presence of CL on D0 and the expression of estrus. The use of 2 induction protocols prior to AI resulted in greater P/AI and more cyclicity of non-pregnant heifers. Results from this study indicate that this strategy could be an optimized method for improving cyclicity and fertility of prepubertal Nelore heifers. Acknowledgements: Santa Vergínia Farm, #FAPESP2018/03798-7, CAPES, CNPq, GlobalGen vet science.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of GnRH applied at the beginning of the ovulation synchronization protocol on the ovarian dynamics and hemodynamics of Holstein cows submitted to pre-synchronization

Márcio Luiz Denck Tramontin[1], Bruno Argenton de Barros[1], Cecília Aparecida Spada[1], Letícia Obo Andreghetti[1], Francieli Gesleine Capote Bonato[1], Fábio Morotti[2], Marcelo Marcondes Seneda[2], Denis Vinicus Bonato[2]

[1]Universidade Paranaense (*UNIPAR*)
[2]Universidade Estadual de Londrina (*UEL*)

E-mail: marcioldtramontin@gmail.com

The objective of the study was to evaluate the effect of gonadotropin-releasing hormone (GnRH) on the dynamics and hemodynamics of Holstein cattle submitted to an ovulation synchronization (OS) protocol. Were uses 16 lactating females, multiparous, raised in a semi-extensive system and who underwent pre-synchronization that began with the insertion of a first-use intravaginal implant impregnated with 2 g of progesterone (P4) (Repro sync®, GlobalGen Vet Science, Jaboticabal, São Paulo, Brazil). On the seventh day, the intravaginal implant was removed, was applied intramuscularly 1 mg of estradiol cypionate (EC) (Cipion®, GlobalGen Vet Science) and 0.526 mg of cloprostenol sodium (CS) (Induscio®, GlobalGen Vet Science). After an interval of ten days, an ultrasound examination was performed (SonoScape, Model S8, Domed, Valinhos, Brazil) to quantify the number of *corpora lutea* (CLs) present in the ovaries and the OS protocol was started, in which each cow received intramuscularly 2 mg of estradiol benzoate (Syncrogen®, GlobalGen Vet Science) and a new device impregnated with P4. On the initial day of the OS protocol (D0), the cows had their blood samples collected for P4 measurement by chemiluminescence and were divided into 2 experimental groups. The control group (G1, n = 8) received intramuscularly 5 ml of 0.9% NaCl solution and the treated group (G2, n = 8) received 21 mcg of buserelin acetate (synthetic analogue of GnRH, Gonaxal®, Biogénesis Bagó, Buenos Aires, Argentina). On D7 of the OS protocol, the ovarian ultrasound examination was performed again for new quantification of CLs, blood samples were collected to measure P4 and the cows received 0.526 mg of CS. On the eighth day of the TAI protocol, the cows received 0.526 mg of CS, 1 mg of EC and the P4 implant was removed. On the D10, the ovulatory follicles (OFs) had their diameter measured and, using the Doppler function, the area of blood perfusion around the OFs was measured. The data were analyzed using the Shapiro-Wilk test regarding normality and compared using the T Test for independent samples, using the Minitab® statistical program. A P value ≤ 0.05 was adopted as significant and the results are presented as mean and standard error. On D0, the number of CLs was equal (P = 1.00) in both groups (1.12 ± 0.12) and there was no difference in the P4 level (G1 = 5.65 ± 0.63 *vs* G2 = 5.69 ± 0.62 ng/ml; P = 0.97). On D7, G2 had a greater number of CLs (2.25 ± 0.31 *vs* 1.12 ± 0.12; P = 0.005) and a higher level of P4 (12.22 ± 0.61 *vs* 9.38 ± 0, 34 ng/ml; P = 0.001). On D10 there was no difference in the diameter of the OFs (G1 = 12.90 ± 0.93 *vs* G2 = 12.49 ± 0.93 mm2; P = 0.76), but the OFs of G2 had a greater area of blood perfusion (16.74 ± 3.41 *vs* 7.52 ± 1.15; P = 0.031). The use of GnRH on D0 of the OS protocol in pre-synchronized cows resulted in an increase in the number of CLs and P4 level on D7 and, on D10, in OFs with a greater area of blood perfusion.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of hCG at the moment of TAI on ovulation rate and quality of CL of suckled *Bos indicus* cows submitted at ovulation synchronization protocol

Matheus Pedroso Vicente[1], Laís Reis Carvalho[1], Lucas Araújo Lemos[1], Leonardo Silva Fernandes do Vale[1], Ludmila Carvalho[1], Ketlyn kaylane Xavier[1], Rafael Resende Rabelo Silva[1], Luiz Manoel Souza Simões[1], Rodolfo Daniel Mingoti[2], José Nélio de Sousa Sales[3]

[1]Universidade Federal de Lavras (*Faculdade de Zootecnia e Medicina Veterinária - UFLA*)
[2]Merck sharp & Dohme Saúde Animal
[3]Universidade Federal de Juiz de Fora (*Departamento de Medicina Veterinária - UFJF*)

E-mail: matheus.vicenterepro@gmail.com

The objective was to evaluate the effect of hCG administration at the time of TAI on the ovulation rate and quality of the corpus luteum (CL) in suckled *Bos indicus* cows submitted to the ovulation synchronization protocol. In the study, 127 Nelore (*Bos indicus*) suckled cows at body condition score of 2.70 ± 0.01 (scale of 1 - 5) were used. On a random day of the estrous cycle (D0), cows received 2 mg of estradiol benzoate (EB; Fertilcare Sincronização®, MSD, Brazil) and an intravaginal device progesterone (P4; Crestar® IVG 1 g, MSD, Brazil). After eight days (D8), the P4 device were removed and 1.0 mg of estradiol cypionate (EC; Fertilcare Ovulação®, MSD, Brazil), 0.520 µg of cloprostenol sodium (PGF; Ciosin®, MSD, Brazil) and 300 IU of equine chorionic gonadotropin (eCG; Folligon®, MSD, Brazil) were administered. Furthermore, the tail head of was marked with chalk for evaluate estrus expression between D8 and D10. On day 10 (D10), cows that remained with the marking on the tail head were considered non-estrus. At the same time, cows were randomly distributed into three experimental groups (Control, GnRH and hCG groups). Cows in the control group (n = 42) received no additional treatment. Cows in the GnRH group (n = 44) received 100 µg of gonadorelin (Fertagyl®, MSD, Brazil) and cows hCG group received 1000 IU of hCG (Chorulon® 5000 UI, MSD, São Paulo, Brazil). Timed artificial insemination (TAI) was performed 48h after progesterone device withdrawal (D10). Ultrasound exams were performed every 12 hours for 96 hours or until ovulation. In addition, 12 days after TAI (D22), CL diameter and vascularization was measured using Doppler ultrasonography (scale 0 - 4). Statistical analyses were performed by GLIMMIX procedure of SAS. The ovulation rate at the end of the TAI protocol in cows that showed estrus was similar between the experimental groups [Control = 85.7% (18/21); GnRH = 81.8% (18/22) and hCG = 66.7% (14/21); P = 0.29]. However, cows that did not show estrus, the ovulation rate was greater in cows received hCG at the time of TAI [Control = 47.6% (10/21)^{b}, GnRH = 54.5% (12/22)^{b}, hCG = 85.0% (17/20)^{a}; P = 0.03]. The CL vascularization score [Control = 1.8 ± 0.2 (n = 21); GnRH = 2.2 ± 0.2 (n = 22) and hCG = 2.0 ± 0.2 (n = 21); P = 0.33] and CL diameter [Control = 20.1 ± 1.1 (n = 21); GnRH = 19.7 ± 0.1 (n = 22) and hCG = 17.8 ± 1.0 (n = 21); P = 0.13] was similar between the experimental groups in cows did not show estrus. In addition, CL vascularization score tended to be greater in cows (no show estrus) treated with hCG compared to cows that received GnRH [Control = 2.1 ± 0.3^{ab} (n = 21); GnRH = 1.6 ± 0.3^{b} (n = 22) and hCG = 2.4 ± 0.3^{a} (n = 20); P = 0.06]. It is concluded that the administration of hCG at the time of TAI increased the ovulation rate in *Bos indicus* cows that do not manifest estrus. Furthermore, there was a tendency towards a better CL vascularization score in these females.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of low dose hCG or GnRH at the time of TAI on conception rate and pregnancy loss in heifers

Fábio Morotti[1,2], Clodo Aldo Rodriguez Machuca[3], Miltón Robert Villalba Mostacedo[3], Saul Nicolas Romero Vargas[3], João Pedro Ferruzzi Virijosque[1], Aron Goulart Doretto[1], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Universidade Pitágoras Unopar Anhanguera
[3]Reproducción y Genética

E-mail: fabiomorotti@uel.br

We evaluated the effect of human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH) at the time of insemination on the conception rate and pregnancy loss from heifers in a timed artificial insemination (TAI) program. Nelore heifers (n = 678), with 19 to 28 months (24 ± 0.5), body condition score (BCS) 2.75 to 4.0 (3.28 ± 0.21), weight 275 to 495 kg (338 ± 39 kg), and regular vaccination schedule (Bioabortogen® and Bioleptogen®, Biogénesis, Brazil) were studied. On a random day of the estrous cycle (D0), the ovarian condition (anestrus: small follicles, transition: dominant follicle > 8mm, and cyclic: presence of CL) was evaluated and heifers received an intravaginal P4 device (1 g, Repro Neo®, Biogénesis) and intramuscular (i.m.) estradiol benzoate (2 mg, Bioestrogen®, Biogénesis). On D8 the device was removed, D-Cloprostenol (150 µg, Croniben®, Biogénesis), estradiol cypionate (1 mg, Croni-Cip®, Biogénesis), and eCG (200 IU, Ecegon®, Biogenesis) were applied, and the sacrococcygeal region was marked to evaluate the estrus expression. On D10, after evaluating estrus expression (high, low and absent), TAI was performed randomly into: Control (n = 172) conventional TAI, GnRH (n = 185) TAI + buserelin acetate/GnRH (10.5 µg, Gonaxal®, Biogénesis), hCG150 (n = 157) TAI + hCG (150 UI, Chorulon®, MSD, Brazil) and hCG300 (n = 164) TAI + hCG (300 IU, Chorulon®, MSD). Insemination was performed by a single technician with thawed semen from two bulls equally distributed among treatments. Pregnancy diagnosis was performed at 30 and 150 days. Pregnant heifers at 30 days were controlled to PL and non-pregnant were resynchronized for a second conventional TAI and 10-15 days later all heifers remained with the bull until the end of the breeding season. Data were analyzed by multivariate logistic regression including treatment, bull, batch of heifers, ovarian condition, estrus expression, weight, BCS, and all interactions ($P < 0.05$). Treatment did not influence ($P = 0.20$) the conception rate at 30 days [Control 54.6% (94), GnRH 56.2% (104), hCG150 53.5% (84) and hCG300 59.8% (98)], nor the bull ($P = 0.33$), BCS ($P = 0.28$), batch of heifers ($P = 0.55$), ovarian condition ($P = 0.69$) and estrus expression ($P = 0.12$). However, there was an interaction treatment*weight ($P = 0.02$). In general, heavier heifers (> 330 kg) had a higher conception rate ($P < 0.05$) than lighter heifers (< 330 kg), except in the hCG150 group, in which lighter heifers performed better. Pregnancy loss was similar ($P = 0.98$) among treatments [Control 14.9% (14), GnRH 14.4% (15), hCG150 15.5% (13) and hCG300 14.3% (14)] and no effect or interaction ($P > 0.1$) was observed. At the end of the season, treatment effect ($P = 0.04$) was observed [Control 85.5%^{b} (147), GnRH 91.9%^{a} (170), hCG150 91.1%^{a} (143) and hCG300 91.5%^{a} (150)], in addition to the effect of BCS ($P = 0.02$). However, no other effects or interactions ($P > 0.1$) were observed. In conclusion, a low dose of hCG or GnRH at the time of insemination did not improve conception at TAI or reduce pregnancy loss, but improved the pregnancy rate at the end of the breeding season in beef heifers.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of pre-synchronization with injectable progesterone and different types of GnRH on Day 0 of E2-P4 TAI protocol in Holstein dairy cows

Tiago Carneiro de Oliveira[1], Gabriel Matos do Couto[1], Bruna Lima Chechin Catussi[2], Emanuele Almeida Gricio[2], Igor Garcia Motta[3], Bruno Gonzalez de Freitas[3], Heni Falcão da Costa[3], Pietro Sampaio Baruselli[2]

[1]Fazenda São José
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Ourofino Saúde Animal

E-mail: tiagocarneiro_oliveira@gmail.com

This study evaluated the effects of pre-synchronization using long-acting injectable progesterone (IP4) seven days before the TAI protocol (D-7), along with two different GnRH (Buserelin or GnRH analog) at the onset of TAI protocol (D0), on reproductive parameters in Holstein cows. A total of 230 cows (109 multiparous and 121 primiparous) producing 40.0 ± 0.6L of milk and 61.2 ± 0.5 days in milk were randomly allocated into four groups in a 2x2 factorial based on cyclicity (80.3%). On D-7, cows were assigned to receive IP4 (n = 113): 300 mg of IP4 (Sincrogest Injetável®, Ourofino) or Control (n = 117): no treatment. On D0, all cows received an intravaginal P4 device (Sincrogest®, Ourofino) and 2 mg EB (Sincrodiol®, Ourofino), then were subdivided into Bus (n = 120; 10µg i.m. of Buserelin acetate; Sincroforte®, Ourofino) or A-GnRH (n = 110; 250 µg i.m. of GnRH analog; Sincrorrelin®, Ourofino), resulting in four groups: Cont-Bus (n = 61); Cont-A-GnRH (n = 56); IP4-Bus (n = 59) and IP4-A-GnRH (n = 54). On D7 and D8 cows received 0.53 mg cloprostenol (PGF; Sincrocio®, Ourofino). On D8, 1 mg of EC (SincroCP®, Ourofino) was administered with P4 device removal, followed by TAI 48 hours later. Ultrasound examinations (US) were performed in a subset of cows (n = 203) to evaluate the presence of CL on D-7 and D0, the largest follicle (LF) on D0, and ovulation rate (OR) to GnRH treatment on D7 (presence of new CL). Also, the OR at the end of protocol on D17 (presence of CL) and conception rate (CR) on D42 were assessed. Statistical analyses were conducted using PROC GLIMMIX of SAS 9.4. The data is presented as main effects when no IP4*GnRH interaction was found. An interaction between IP4 and CL on D-7 for LF on D0 was observed. Cows without CL on D-7 from the IP4 group exhibited larger LF compared to the control group (15.8 ± 1.0 mm *vs* 13.2 ± 0.9 mm respectively; P = 0.005). However, cows with CL on D-7 showed no differences in LF on D0 between groups. The presence of CL on D0 tended to be lower in the IP4 group compared to the control group [79.2% (80/101) *vs* 88.2% (90/102); P = 0.07]. OR after GnRH did not differ between IP4 groups (P = 0.46) but differed between GnRH groups. A-GnRH had a higher OR compared to Bus [70.3% (71/101) *vs* 38.2% (39/102); P = 0.0001]. The OR after the protocol was similar between IP4 groups [IP4 = 67.5% (75/111) *vs* Cont = 70.9% (78/110); P = 0.26] and GnRH groups [Bus = 67.2% (78/116) *vs* A-GnRH = 71.4% (75/105); P = 0.14). Moreover, an interaction IP4*GnRH was observed for CR [Cont-Bus = 16.4% (10/61)^{B}; Cont-A-GnRH = 35.7% (20/56)^{A}; IP4-Bus = 30.5% (18/59)^{AB}; IP4-A-GnRH = 25.9% (14/54)^{AB}; P = 0.04]. In conclusion, cows without CL receiving IP4 on D-7 had greater LF and a lower proportion of CL on D0. The administration of A-GnRH on D0 induced a greater OR. An interaction IP4*GnRH for CR was observed, in which Cont-A-GnRH had higher P/AI than Cont-Bus, while similar CR in IP4-Bus and IP4-A-GnRH was observed. Acknowledgments: Fazenda Bela Vista and Ourofino Animal Health.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of pre-synchronization with injectable progesterone on the conception rate in precocious and conventional primiparous cows in a timed artificial insemination program

Vitória Maria Valongo Marzolla[1], Fábio Morotti[1,2], Marcelo Marcondes Seneda[1], Carlos Henrique Lopes Pascotto[3], Gustavo Martins Gomes dos Santos[3]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Universidade Norte do Paraná (UNOPAR)
[3] Autônomo

E-mail: vitoria.marzolla@uel.br

This study evaluated the effect of pre-synchronization (pre-sync) with injectable progesterone (P4) on the conception rate of primiparous cows (precocious *vs* conventional) during the timed artificial insemination program. Nelore primiparous cows (precocious, 22 to 26 months, n = 251 and conventional, 32 to 36 months, n = 284), with BCS from 1.50 to 3.75 (2.66 ± 0.02), postpartum from 30 to 80 days (38.3 ± 0.62 days) were included in this study. The pre-sync was performed with 150 mg of injectable P4 (i.m.; Sincrogest®, Ourofino, Brazil) applied ten days (D-10) previously TAI protocol in 198 primiparous precocious and 142 conventional ones. The control groups (precocious; n = 53 and conventional; n = 142) did not receive injectable P4. Then, on D0 all the animals received an intravaginal P4 device (1 g, Sincrogest®) and injection of estradiol benzoate (2 mg, i.m., Sincrodiol®). On D8, the P4 device was removed and cloprostenol (0.5 mg, Sincrocio®), estradiol cypionate (1 mg, SincroCP®) and equine chorionic gonadotropin (300 IU, Sincro eCG®) were injected, in addition to painting the sacrococcygeal region to assess the estrus expression intensity. On D10, cows with absent estrus received buserelin acetate (0.010 mg, i.m., Sincroforte®). Two inseminators performed TAI with thawed semen from three bulls with known fertility. Pregnancy diagnosis was performed at 40 days. Data were analyzed by multivariate logistic regression. The model considered category (precocious and conventional), pre-sync (control and P4), estrus score (high, low and absent), bull (A, B and C) and inseminator (A and B), days postpartum, BCS, and main interactions ($P \leq 0.05$). The ranking between conception rates was analyzed using a 2x2 proportion test. The overall conception rate at TAI was 57.38% (307/535), being influenced by pre-sync ($P = 0.001$), category ($P < 0.0001$), and interaction pre-sync*category ($P < 0.0001$). Precocious primiparous treated with pre-sync showed a higher conception rate (53.03%^{b}; 105/198) compared to the control (43.40%^{c}; 23/53), but it was lower than conventional primiparous, regardless of whether treated (64.08%^{a}; 91/142) or not (61.97%^{a}; 88/142) with pre-sync. There was no influence of the bull ($P = 0.39$) and inseminator ($P = 0.12$), but there was an influence of the postpartum day ($P = 0.03$), BCS ($P = 0.002$) and estrus manifestation score ($P = 0.03$). However, none of these factors showed an interaction ($P > 0.1$) with the main effect of treatment. In general, females with high estrus expression had a higher ($P = 0.03$) conception rate (63.39%^{a}; 161/254) than cows with low expression (52.90%^{b}; 82/155) or those absent estrus (50.79%^{b}; 64/126). This same relationship was observed for both categories, and pre-sync did not influence ($P > 0.05$) this result. Under the conditions of the present study, pre-sync with injectable P4 showed an effective strategy to increase conception at TAI in precocious primiparous, but not in conventional primiparous.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of the bull on conception rate and pregnancy loss in high-producing dairy cows

Aron Goulart Doretto[1], Beatriz Abrahão Bueno de Morais[1], Andrey Osvaldo Souza Ferro[1,2], Davi França Jorge[2], Paulo de Tarso Lemos Borges[2], Marcelo Marcondes Seneda[1], Fábio Morotti[1,3]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Policlínica Veterinária Pioneiros
[3]Universidade Norte do Paraná (UNOPAR)

E-mail: aron.goulart.doretto@uel.br

This study aimed to evaluate the bull effect on the conception rate and pregnancy loss up to 60 days in high-producing dairy cows. Multiparous Holstein cows (n = 1658), with body condition score (BCS) 2.5 to 4.5 (3.06 ± 0.02), average of 31.50 ± 0.21 liters of milk, regular vaccination schedule against reproductive diseases (Fertiguard®, MSD, São Paulo, Brazil) from a single farm were studied. Cows that presented natural heat (n = 599) were identified by visual observation or electronic collar identification system (Allflex®, MSD) and inseminated 12 hours later with conventional semen. Cows (n = 1059) submitted to timed artificial insemination (TAI) protocol received on random day of the estrous cycle (D0 an intravaginal insertion of a P4 device (1.9 g; CIDR®, Zoetis, Campinas, Brazil), intramuscular (i.m.) application of gonadorelin acetate (50 µg; Ovalyse®, Zoetis) and estradiol benzoate (2 mg; Gonadiol®, Zoetis). On D7 the dinoprost tromethamine ($PGF_{2\alpha}$; 33.55 mg, i.m.; Lutalyse®, Zoetis) was applied. Then on D9, the intravaginal device was removed and estradiol cypionate (1 mg; i.m.; E.C.P®, Zoetis) was injected with another dose of $PGF_{2\alpha}$. The TAI was performed on D11 following the same semen description used in natural heat. Four technicians performed the inseminations with thawed semen from five bulls (I: n = 292; II: n =152; III: n = 315; IV: n = 423, and V: n = 476) with known fertility. Pregnancy diagnosis was made by ultrasound 30 and 60 days later. Data were analyzed by multivariate logistic regression. The model considered bull (I to V), insemination program (AI and TAI), inseminator, milk production, number of services, BCS, and main interactions ($P \leq 0.05$) The bull influenced the conception rate at 30 days [P = 0.02; I: 28.08%^{b} (82), II: 30.50%^{b} (129), III: 33.61%^{ab} (160), IV: 36.18%^{ab} (55), and V: 38.73%^{a} (122)] and 60 days [P = 0.004, I: 23.29%^{b} (68), II: 23.40%^{b} (99), III: 28.15%^{b} (134), IV: 26.32%^{b} (40), and V: 34.29%^{a} (108)]). The program (P = 0.25 and P = 0.36), inseminator (P = 0.01 and P = 0.007), services (P = 0.005 and P = 0.004), and milk production (P = 0.61 and P = 0.62) did not influence the conception rate at 30 and 60 days, respectively. The BCS affected conception at 30 days (P = 0.04), but not at 60 days (P = 0.23). Pregnancy loss was also associated with bull [P = 0.019; I:17.07%^{ab} (14/82), II: 23.26%^{b} (30/129), III: 16.25%^{ab} (26/160), IV: 27.27%^{b} (15/55), and V: 11.48%^{a} (14/122)]. Program (P = 0.49), inseminator (P = 0.95), services (P = 0.90), milk production (P = 0.92), and BCS (P = 0.21) did not influence pregnancy loss. No interaction effect (P > 0.1) was found for conception rates or pregnancy loss. In conclusion, this study supported the hypothesis that the bull is an important factor that influences the conception rate at 30 and 60 days of pregnancy and the occurrence of pregnancy loss in high-producing dairy cows. We reinforce the importance of choosing the bull to improve reproductive rates.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of the calving-FTAI interval on pregnancy rate and gestational losses in primiparous beef cows

Dauydison Antonio Gonzalez Cordeiro[1], Luana Gomes da Silva[2], João Victor Bezerra Machado[3], Marcio Santos[4], Taynara dos Santos Santana[3], Wallery Caroliny Costa da Costa[3], Luiz Carlos Louzada Ferreira[5], Eriklis Nogueira[6], Luiz Francisco Machado Pfeifer[6], Fabiana de Andrade Melo Sterza[3]

[1]Universidade Federal de Mato Grosso do Sul (*UFMS*)
[2]Lida Produtos e Serviços Agropecuários
[3]Universidade Estadual de Mato Grosso do Sul (*UEMS*)
[4]Universidade Anhanguera
[5]CiaAssessoria
[6]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA Gado de Corte)

E-mail: dauydisoncordeiro@gmail.com

The aim of this study was to evaluate the influence of the FTAI-calving interval, according to animal category, on the fertility of beef cows. Early Nelore primiparous cows (n = 879; 24 ± 3.3 months; BCS 2.75 ± 0.42), and conventional Nelore primiparous cows (n = 2370, 36 ± 4.6 months, BCC 3 ± 0.48) were enrolled. The average number of days after calving and the day of FTAI artificial insemination (PPP-AI) for Early Pos-Partum cows was 68.55 ± 22.87 days and for conventional primiparous cows it was 65.26 ± 21.32 days. Data from 6 reproductive seasons from commercial farms (2019-2023) were evaluated. Cyclicity was not considered in our study. On d-11, cows were treated with 1 mg of estradiol benzoate (Gonadiol®; Zoetis, SP, Brazil), i.m., and a first use, intravaginal progesterone-releasing device containing 1.0 g of progesterone (P4) was inserted (DIB®; Zoetis, SP, Brazil). On d-2 the P4 device was removed followed by the i.m. injection of 12.5 mg of PGF (Lutalyse®; Zoetis, SP, Brazil), 0.6 mg of estradiol cypionate (ECP®, Zoetis, SP, Brazil), and 300 IU of eCG (Novormon®, Zoetis, SP, Brazil). FTAI was performed on d-0. Pregnancy diagnosis was carried out at 32 and 120 days after AI. Optimal cut-off points for determining the relationship between the days PP and pregnancy status at first postpartum TAI were studied. The ROC curve analyses identified that the ideal interval between calving and TAI was 49.5 days PP for conventional primiparous cows (AUC = 0.54; P = 0.0004) and 56.5 days PP for early primiparous cows (area under the curve 0.53; P = 0.062). Therefore, in each animal category, the cows were separated into two groups: Early Primiparous - EEP (EEP, n = 584; PPP-AI 47.60 ± 6.0 average days), Late Pos- Partum - ELP (ELP, n = 295; PPP-AI 66.05 ± 11.65 average days), Conventional Primiparous: Early Pos-Partum - CEP (CEP, n = 699; PPP-AI 42.65 ± 6.5 average days) and Late Pos- Partum - CLP (CLP, n = 1671; PPP-AI 63.96 ± 11.31 average days). Pregnancy by AI (P/AI) and gestational losses (GL) before and after the ideal cut-off point were calculated by logistic regression, and the variables farm, reproductive season and BCS were included in the model. The P/IA of early cows was 50.68% (296/584) and 59.66% (176/295) for EEP and ELP, respectively (P = 0.014). The GL was 13.85% (41/295) for EEP cows and 9.65% (17/176) for ELP cows (P > 0.05). The P/AI was 47.5% (332/699) and 55.95% (935/1671) for CEP and CLP, respectively (P < 0.0001). The GL for CEP cows was 10.30% (34/330) and 10.18% (86/930) for CLP (P > 0.05). This study highlighted the importance of specific management strategies, in terms of calving-TAI interval, for early and conventional primiparous cows. So, we conclude that the interval between calving-FTAI interval for early primiparous cows (57 days PPP) should be 7 days longer than for conventional primiparous cows (50 days PPP), with significant impacts on the conception rate.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effect of trace mineral supplementation on Fixed Time Artificial Insemination conception rates in primiparous Nelore cows

João Victor da Silva Teodoro[1], Guilherme Felipe dos Santos[1,2], Álvaro Fabrício Freitas Sales[3], Gustavo Azevedo de Mello[3], Julio Prado[4], Marcello Guadagnini[1], José Luiz Moraes Vasconcelos[5]

[1]Axiota Animal Health
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Lageado Biotecnologia e Pecuária
[4]Axiota Animal Health
[5]Universidade Estadual Paulista Júlio de Mesquita Filho (*Instituto de Biociência - UNESP*)

E-mail: jteodoro@axiota.com

This study aimed to assess the impact of administering a single dose of a combination injectable trace mineral product (TM) on the conception rate at first insemination, within a fixed-time artificial insemination (FTAI) protocol, in primiparous Nelore cows. A total of 745 cows with a mean body condition score (BCS) of 2.93 ± 0.28 were included in the study. Cows received 2 ml of EB (Gonadiol®; Zoetis) and an intravaginal progesterone device (CIDR®; Zoetis) on day 0, and were then randomized into two groups: TM group (n = 366), receiving 1 ml/100 kg SC of Multimin® 90 (Axiota Animal Health, USA), containing zinc (60 mg/ml as zinc oxide), manganese (10 mg/ml as manganese carbonate), copper (15 mg/ml as copper carbonate), and selenium (5 mg/ml as sodium selenite) bioavailable; and an untreated control group (n = 379; CTRL). Additionally, on day 7, all cows received 200 IU of eCG (Novormon®; Zoetis), followed by 0.5 ml of EC (E.C.P®; Zoetis), 2.5 ml of PGF (Lutalyse®; Zoetis), and another 200 IU of eCG and CIDR® removal on day 9. Insemination was performed 48 hours after CIDR® removal (day 11), and conception checks were performed via ultrasonography on day 42. Statistical analyses were conducted using JMP 17 (SAS). Conception risk was calculated using contingency analysis and a logistic regression model with pregnancy check outcome (pregnant/not pregnant) as independent variable and insemination batch, treatment, BCS and treatment by BCS interaction as fixed dependent variables. BCS and insemination batch did not differ among treatment groups. Overall, the TM group had a conception rate of 46.4% and the CTRL group 42.5% (P = 0.27). As treatment by BCS interaction had a p-value < 0.20 and previous studies have shown a different effect of the product according to BCS, it was decided to calculate the association among treatment and pregnancy risk by BCS category (< 3 and ≥ 3). In primiparous Nelore cows with a body condition score (BCS) of 3 or higher (n = 400), there was no significant difference in conception rates between TM group and the CTRL group (P > 0.05). Conversely, in cows with a BCS of less than 3 (n = 345), the TM group exhibited a tendency for a higher conception rate (P = 0.07), compared to the control group, with 1.51 (95% C.I. 0.96-2.38) greater odds of conception compared to controls. When compared with contingency analysis and Fisher's Exact Test the TM group (n = 166) achieved a conception rate of 39.3%, whereas the control group (n = 177) attained a conception rate of 30.5% (P = 0.05). These data suggest that TM injection before insemination may enhance fertility outcomes in primiparous Nelore cows with low BCS, highlighting its potential role in improving reproductive performance under certain conditions.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Effects of eCG treatment on ovarian function and estrus expression in prepubertal Brangus heifers

Sergio Farias Vargas Júnior[1], Bárbara Luana Wollmeister[2], Gabriele Cristine Guarnieri[2], Gabriella dos Santos Velho[2], Rafael Gianella Mondadori[1], Bernardo Garziera Gasperin[1], Fernando Caetano de Oliveira[2], André Gustavo Cabrera Dalto[2], Monique Tomazele Rovani[2]

[1]Universidade Federal de Pelotas (*UFPel*)
[2]Universidade Federal do Rio Grande do Sul (*UFRS*)

E-mail: sergiofvjunior@gmail.com

The objective of the present study was to evaluate the effects of eCG on follicular growth, luteal function, and estrus expression in 14 months-old Brangus heifers. The heifers (n = 34) were subjected to the following hormonal protocol: on D-9, all heifers received a second-use intravaginal device (IVD; 1 g P4; Sincrogest®, Ouro Fino, Cravinhos, Brazil) and 2 mg of intramuscular (IM) estradiol benzoate (RIC-BE®, Agener União, São Paulo, Brazil). On D-2, the heifers were allocated into two groups: Control (n = 20; mean BCS = 3 ± 0.11; Body Weight = 305 ± 6.4 kg) and eCG Group (n = 14; mean BCS = 3 ± 0.13; BW = 300.5 ± 4.7 kg). The IVD was removed on D-2 along with the administration of 526 µg of cloprostenol sodium IM (Estron®, Agener União, São Paulo, Brazil). All the heifers were marked with tail chalk (Bovipaint®) at the base of the tail to monitor estrus. The eCG group also received 300 IU eCG IM (Novormon®, Zoetis, Campinas, Brazil). Measurements of dominant follicle (DF) diameter were taken through ultrasonography on D-2 and D0. Six heifers that did not show follicular growth (5 from Control, and 1 from eCG group) were excluded. Estrus was observed for four days and the heifers that showed estrus were inseminated. Blood samples were collected nine days after IVD removal for P4 concentration analysis. Statistical analysis was conducted using ANOVA and logistic regression (JMP Statistical Discovery LLC). As expected, the diameter of the DF on D-2 (Control = 7.00 ± 0.44 mm; n = 15; eCG = 6.89 ± 0.47 mm; n = 13) was smaller (P = 0.002) than on D0 (Control = 8.35 ± 0.44 mm; eCG = 9.18 ± 0.47 mm), regardless of the group. There was no difference in the DF diameter between groups, and there was no interaction between group and day (P = 0.44 and P = 0.32, respectively). There was no statistical difference in ovulation rate (P = 0.26; Control = 66.6%; eCG = 84.6), CL diameter (P = 0.55; Control = 13.1 ± 1.7 mm; eCG = 14.6 ± 1.9 mm) and serum P4 concentration in heifers that had CL (P = 0.40; Control = 5.1 ± 1.2 ng/mL; eCG = 6.1 ± 1.2 ng/mL). However, eCG treatment tended (P = 0.07) to increase estrus expression (53% *vs* 84%, for Control and eCG groups, respectively). Conception rate 40 days after AI did not differ between groups (P = 0.46), being observed 37.5% (3/8) and 54.5% (6/11) for Control and eCG groups, respectively. Pregnancy rate in Control and eCG groups was 20% (3/15) and 46% (6/13), respectively (P = 0.13). In conclusion, the preliminary results suggest that eCG treatment at IVD removal does not affect pre-ovulatory follicle diameter, ovulation and luteal function. However, eCG treatment tends to increase estrus manifestation. Future studies will be conducted to evaluate fertility in a larger number of animals and the effect of administering eCG two days before IVD removal in prepubertal heifers. Acknowledgments: FAPERGS, CNPq and CAPES for financial support.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Efficacy of a half dose of Ciosin® in E2/P4-based Timed Artificial Insemination protocol of Nelore suckled cows

Fernando Anisio de Oliveira[1], Thiago de Araújo Guimarães[2], Lais Mendes Vieira[2], Henderson Ayres[2], Rodolfo Daniel Mingoti[2]

[1]Fazenda Fazenda Santa Neide (Pantanal Paiaguás)
[2]Merck sharp & Dohme saúde animal

E-mail: fernando.anisio@hotmail.com

The objective of this study was to evaluate the impact of half dose of cloprostenol sodium (Ciosin®) on the pregnancy rate of lactating Nelore (*Bos indicus*) cows undergoing the E2/P4-based TAI protocol. The study consisted of a total of 1594 Nelore cows located in Coxim/MS - Brazil. The study utilized the most commonly employed E2/P4-based TAI protocol as per the farm management. At the start of the TAI protocol (D0), the animals received an administration of 2 mg of estradiol benzoate (Fertilcare Sincronização®, MSD, Brazil) and an intravaginal progesterone (P4) device (0.6 g of P4; Fertilcare 600®, MSD, São Paulo, Brazil). The withdrawal of P4 devices was performed seven or eight days later, while concurrently administering 1 mg of estradiol cypionate (Fertilcare Ovulação®, MSD, São Paulo, Brazil), and 300 IU of eCG (Folligon®, MSD, São Paulo, Brazil). Subsequently, the animals were randomly assigned to two distinct experimental groups: a control group and a treatment group. The control group received a standard dose of 2 ml equivalent to 500 µg cloprostenol sodium (Ciosin®, MSD, São Paulo, Brazil). On the other hand, the treatment group received an identical TAI protocol, but with a half dose of 1 ml equivalent to 250 µg of cloprostenol sodium. All treatments were performed at the moment of P4 device withdrawal. All injections were given intramuscularly (i.m.). All artificial inseminations were performed 48 hours after the withdrawal of the P4 device. Pregnancy check was conducted using an ultrasonographic examination 30 days after TAI. Descriptive statistics and inference were performed using SAS/STAT 9.4 (Inst. Inc., Cary, NC) included Freq and GLIMMIX procedures. The statistical analysis aimed to investigate interactions between all variables and treatment. No significant interaction was found between the variables and Treated [e.g.: Category*Treated, P = 0.86 and Protocol type*Treated, P = 0.71)]. Thus, it was possible to proceed with the analysis of main effects using the complete dataset, indicating that the effect of one variable is not modified by the level of another variable. Confirming our initial hypothesis, P/AI between treated and control groups were similar [Control = 63.6% (508 /1594) and Treated = 64.7% (514/795); P = 0.70]. No significant secondary effects were observed for pregnancy per artificial insemination (P/AI) within the study across different categories [Primiparous = 62.4%, (212/340); Multiparous = 64.6%, (810/1254); P = 0.69); and Protocol Type [0-7-9d = 65,2% (195/299); 0-8-10d = 63,9% (897/1295), P = 0.65). Similarly, no significant effects were found for sire semen (P = 0.62) and AI technician (P = 0.60). The findings of this study support the hypothesis that the dose of Ciosin® can be reduced to 1 ml equivalent to 250 µg of cloprostenol sodium in E2/P4-based timed artificial insemination protocol of Nelore suckled cows. Acknowledgments: Fazenda Santa Neide, Coxim-MS Pantanal Paiaguás.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Efficacy of the treatment of estradiol benzoate (Sincrodiol®) at the beginning of the ovulation synchronization protocol in beef cows

Bruno Gonzalez de Freitas[1], Heni Falcão da Costa[1], Igor Garcia Motta[1,2], Bruna Lima Chechin Catussi[2], Laís Ângelo de Abreu[2], Emanuele Almeida Gricio[2], Igor Renan Honorato Gatto[1], Pietro Sampaio Baruselli[2]

[1]Ourofino Saúde Animal LTDA
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: bruno.freitas@ourofino.com

This study aimed to evaluate the efficiency of estradiol benzoate treatment (EB; Sincrodiol®, Ourofino) in cows synchronized with P4 device for TAI. A total of 36 Nelore (*Bos indicus*) cows (aging 55 ± 9 months, body weight (BW) = 448 ± 93 kg, body condition score (BCS) between 3 and 3.25 and without CL, ensured by two transrectal ultrasonography examinations at D-7 and D0) were used. At the beginning of the FTAI protocol (D0), cows were homogeneously distributed according to age, BW, and BCS into two groups: A) Control (n = 18): no treatment on D0; B) EB-Treatment (n = 18): received 2 mg of EB on D0. Also, on D0, all animals received an intravaginal progesterone device (P4; Sincrogest®, Ourofino), which was removed after eight days (D8). Animals underwent daily transrectal ultrasonographic evaluation (D0 to D8) to monitor the emergence of a new follicular wave and for monitoring the growth of the dominant follicle. Statistical analyses were performed using GLIMMIX of SAS 9.4. Animals treated with 2 mg of Sincrodiol® showed a higher (P = 0.03) rate of synchronization of new follicular wave [Control = 50% (9/18) *vs* EB-Treatment = 89% (16/18)] with less dispersion (Bartlett Test; P < 0,05). The interval from D0 and the emergence of the new follicular wave was shorter in treatment group than in control group [Control = 4.0 ± 0.30 days *vs* EB-Treatment = 3.2 ± 0.22 days; P < 0.05]. On D8, a smaller diameter of the dominant follicle was observed in the treated cows [Control = 12.6 ± 0.36 mm *vs* EB-Treatment = 11.2 ± 0.32 mm; P < 0.05]. In conclusion, treatment with 2 mg of Sincrodiol® combined with an intravaginal progesterone device at the beginning of the synchronization protocol is effective in inducing a synchronized new follicular wave emergence. Furthermore, EB treatment promotes anticipation of the day of the emergence of the new follicular growth wave. At the moment of P4 device removal, cows that had not received EB treatment presented the largest diameter of DF.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Exposure to injectable progesterone prior to protocol for the resynchronization of ovulation in the fertility of *Bos indicus* cows

Walter Antônio Gonçales Junior[1], Marcos Henrique Alcantara Colli[1], Willian Araujo Ruivo[1], Laís Reis Carvalho[3], Matheus Pedroso Vicente[2], Lucas Araújo Lemos[2], Luiz Manoel Souza Simões[2,3], Igor Garcia Motta[4], Bruno Gonzalez de Freitas[4], José Nélio de Sousa Sales[5]

[1]Reproconsult - Produção e Reproduçao Bovina (*Tapejara, Paraná, Brazil*)
[2]Universidade Federal de Lavras (*Faculdade de Zootecnia e Medicina Veterinária - UFL*)
[3]Prenhez Consultoria
[4]Ourofino Saúde Animal LTDA, [5]Universidade Federal de Juiz de Fora (*DMV*)

E-mail: walter.reproconsult@gmail.com

The objective was to evaluate the fertility of suckled *Bos indicus* cows exposed to injectable progesterone (IP4) before protocol for the resynchronization of ovulation. In the study, 420 Nelore (*Bos indicus*) suckled cows at body condition score of 2.62 ± 0.01 (scale of 1 - 5) were used. Initially, 815 Nelore (*Bos indicus*) suckled cows were submitted to the ovulation synchronization protocol. On a random day of the estrous cycle (D-10), cows received of estradiol benzoate (EB; Sincrodiol®, Ourofino, Brazil) and an intravaginal device containing 1 g of progesterone (P4; Sincrogest®, Ourofino, Brazil). After eight days (D-2), the P4 device were removed and 1 mg of estradiol cypionate (EC; SincroCP®, Ourofino, Brazil), 0.500 µg of cloprostenol sodium (PGF; Sincrocio®, Ourofino, Brazil) and 300 UI of equine chorionic gonadotropin (eCG; SincroeCG®, Ourofino, Brazil) were administered. Timed artificial insemination (TAI) was performed 48h after progesterone device withdrawal (D0). Twenty- two days after the TAI (D22) cows were divided into two experimental groups (Control and IP4 groups). In this moment, cows of IP4 group received 150 mg of injectable progesterone (Sincrogest® Injetável, Ouro Fino, Brazil) and cows in the control group received no additional treatment. After eight days (D30) pregnancy was diagnosed, and cows considered pregnant (n = 395) were excluded from the study. Concomitantly, non-pregnant cows (n = 420) were resynchronized (D30: EB+P4; D38: EC+PGF+eCG; D40: TAI). Pregnancy diagnosis and cyclicity rate (presence of CL) were evaluated 30 days after the second TAI (D70). Statistical analyses were performed by GLIMMIX procedure of SAS by logistic regression. The estrous expression rate (Control = 56.7% and IP4 = 53.1; P = 0.72), pregnancy rate (Control = 47.7% and IP4 = 46.9%; P = 0.82) and cyclicity rate (D70; Control = 27.3% and IP4 = 36.1; P = 0.08) were similar between the experimental groups. It is concluded that the use of IP4 before protocol for the resynchronization of ovulation does not increase fertility in *Bos indicus* beef cows.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Factors associated with gestation length and adjusted weaning weight in a Nelore cow-calf system using Nelore or Angus sires

Ana Luíza Müller Lopes[1], Lucas O. e Silva[1,2], Rodrigo L. O. R. Alves[1], Otacílio Jarcem Escobar Junior[3], Hugo Savioli Marques[3], Rogério Fonseca Guimarães Peres[4], Roberto Sartori[1,5]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Texas A&M University (*Department of Animal Science*)
[3]Agropecuária Nelore Paranã
[4]R2 Pecuária Inteligente e Gestão
[5]University of Wisconsin Madison (*Department of Animal and Dairy Sciences*)

E-mail: anamuller@usp.br

The aim was to evaluate retrospective data from a calving season of a commercial cow-calf operation in Goiás, Brazil. Data from 10530 calves, from Nelore (*Bos indicus*) cows submitted to timed-artificial insemination (TAI) protocols using Nelore or Angus sires, were evaluated. The study evaluated gestation length (GL) and calf weaning weight (WW) adjusted at 210d. For GL analyses, factors considered were: cow parity, calf sex, and sire breed. For WW analyses, factors considered were: month of birth, calf sex, cow parity, sire breed, and calf weaning age. For all analyses, farm and sire were used as random effects. Statistical analyses were done by PROC GLIMMIX of SAS ($P \leq 0.05$). The GL of cows that conceived male (M; n = 3707) and female (F; n = 3811) calves from Nelore sires (NE) was longer (294.8 ± 0.1 *vs* 290.3 ± 0.2d) than cows conceiving from Angus sires (F1; M: n = 1559; F: n = 1453). For both sire breeds, cows carrying M calves had longer GL than F (NE: 295.7 ± 0.1 *vs* 293.9 ± 0.1d; F1: 290.9 ± 0.2 *vs* 289.7 ± 0.2d). There was a parity effect in GL. In general, for both sire breeds, precocious heifers had shorter GL than the other parity groups. Regarding WW, an effect of month of birth was observed for both sire breeds, in which earlier-born calves were heavier at weaning than those born later. NE M born in Sep (229.6 ± 1.0 kg) and Oct (219.1 ± 1.0 kg) had the greatest WW, followed by those that were born in Nov (211.9 ± 0.1 kg) and Dec (211.0 ± 1.1 kg). NE M calves that were born in Jan (195.8 ± 2.3 kg) and Feb (190.8 ± 3.8 kg) had the lowest WW. The same effect was observed for NE F (207.7 ± 0.8; 202.1 ± 0.9; 197.9 ± 0.8; 193.9 ± 1.0; 183.1 ± 2.1; 182.7 ± 3.5 kg, from Sep to Feb, respectively). Whitin all months of birth NE M were heavier than F. Earlier-born M F1 calves were also heavier than those born later (Dec = 234.7 ± 1.6^{a} *vs* Jan = 222.4 ± 1.2^{b} *vs* Feb = 216.4 ± 1.5^{c} kg). F1 F that were born in Dec were heavier (229.8 ± 1.2 kg) than the ones born in Jan (217.9 ± 1.1 kg) and Feb (215.4 ± 1.4 kg), which did not differ between them. Moreover, for F1 calves, the effect of sex was observed only in calves born in Dec and Jan, but not in Feb. Regarding the effect of sire breed on WW, F1 were heavier than NE calves, regardless of sex (222.9 ± 1.0 *vs* 192.8 ± 1.6 kg, respectively). An effect of parity was observed on WW in NE calves. In general, M were heavier than the F from all parities. Multiparous (M = 225.4 ± 0.7 *vs* F = 209.9 ± 0.6 kg) and primiparous cows (M = 224.5 ± 1.2 *vs* F = 200.0 ± 1.1 kg) weaned heavier NE calves compared to precocious heifers and 24 mo old heifers or secondiparous cows. Similar effect was observed for F1 calves, however, without differences between calf sex (M = 227.7 ± 1.1 *vs* F = 226.7 ± 1.0 kg; M = 227.3 ± 2.0 *vs* F = 225.2 ± 1.6 kg) for multiparous and primiparous, respectively. This study provides relevant insights about the effects of month of birth, cow's parity, sire breed and calf sex on productive outcomes, highlighting the importance of measuring and considering these factors for optimizing cow-calf operations.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Factors that affect the pregnancy rate and pregnancy loss of super-precocious primiparous Nelore submitted to TAI

Bruno Silva do Espírito Santo[1], Bruna Lima Chechin Catussi[2], Laís Ângelo de Abreu[2], Emanuele Almeida Gricio[2], Lígia Mattos Rebeis[2], Sofía Albertini[2], Pietro Sampaio Baruselli[2]

[1]Agropecuária Jacarezinho
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP).*

E-mail: brunosilvamedvet@gmail.com

The objective of the present study was to evaluate factors that affect the pregnancy rate (P/AI) and pregnancy loss (PL) in 2-year-old Nelore primiparous cows submitted to TAI. A total of 3248 Nelore (*Bos indicus*) primiparous heifers (6240 FTAIs performed) from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. On day 0 (D0), intravaginal P4 device (CIDR®; Zoetis, Guarulhos, SP-Brazil) and 2 mg of EB (Gonadiol®, Zoetis), i.m were administrated. On D9, cows received 16.8 mg (2.5 ml) of dinoprost tromethamine (Lutalyse®; Zoetis), 300 IU of eCG (Novormon®; Zoetis) and 0.5 mg of EC (ECP®; Zoetis) i.m. The primiparous had the P4 device removed on D9, and TAI was performed 48h later (D11). Cows were painted with chalk on tailheads, and the removal of chalk at TAI was used as an indication of estrus. Pregnancy diagnoses were evaluated by ultrasound (Mindray® DP-2200Vet) after 30 days (PD) and at the end of the breeding season (120 days after AI). Statistical analyses were performed using GLIMMIX of SAS 9.4. Receiver operating Characteristic (ROC) curve analysis (MedCalc®) was used to select optimal cut-off points for the variables analyzed. The body weight (BW) of heifers on the prepartum and of primiparous on D0 (beginning of the FTAI protocol) influenced the P/AI [prepartum = linear effect (P = 0.05) and D0 = quadratic effect (P = 0.01), respectively]. The P/AI was also influenced by BCS on D0 (linear; P = 0.02). Furthermore, older primiparous demonstrated lower pregnancy losses (PL; P < 0.0001). The age cut-off points calculated by ROC curve was 26 months [< 26 = 12.0% (n = 1171) *vs* > 26 = 7.4% (n = 1236)]. The body weight of heifers at mating (BWM) had shown that lighter animals had lower pregnancy losses when primiparous (P = 0.03). The BWM cut-off points calculated by ROC curve were 350 kg [< 350 = 8.7% (n = 1870) *vs* > 350 = 11.7% (n = 580)]. The BW on D0 influenced the PL (linear effect; P = 0.03). Higher was BW lower was the PL. The BW on D0 cut-off points calculated by ROC curve were 416 kg [< 416 = 10.0% (n = 2357) *vs* > 416 = 3.26% (n = 119)]. Also, the days of gestation period (DGP) had an impact on PL (P = 0.02). The DGP cut-off points calculated by ROC curve were 294 days [< 294 days = 10.0% (n = 2110) *vs* > 294 days = 6.3% (n = 365)]. It was observed that the calves with a lower BW at weaning, the mother had a higher probability of PL (P = 0.01). The BW on D0 cut-off points calculated by ROC curve were 151 kg [< 151 kg = 17.0% (n = 208) *vs* > 151 kg = 10.7% (n = 1065)]. The pregnancy loss was not influenced by the BW on the prepartum (P = 0.13), calf sex (P = 0.13), calf birth at weight (P = 0.29), estrus expression (P = 0.17) and bull (P = 0.77). In conclusion, BW on the prepartum and on D0, and BCS on D0, impact the efficiency of P/AI. Furthermore, the pregnancy loss was influenced by age, BW on D0 and calves BW at weaning, and the days of gestation period in 2 years old Nelore primiparous.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Flaxseed oil-based supplement alters water consumption, progesterone concentration, and plasma fatty acid profile of beef heifers

Kendal L. Green[1], Madison Kovarna[1], Ethan Schlegel[2], Cody Wright[1], Ana Clara Baiao Menezes[1], Zachary Smith[1], Jessica Nora Drum[1]

[1]South Dakota State University
[2]New Generation Supplements

E-mail: kendal.green@sdstate.edu

Flaxseed oil contains high levels of Omega-3 fatty acids which have been shown to positively affect reproductive performance. The objective of this study was to determine the effects of a flaxseed oil-based supplement (FlaxLic®, New Generations, SD), rich in Omega-3, on reproductive parameters, feeding behavior, and lipid profile of beef heifers. Crossbred Angus heifers (n = 60, BW = 397 kg ± 5.2 kg) were blocked by BW and antral follicle count and randomly assigned to two supplementation treatments: free-choice commercial lose mineral supplement (CON; n = 30) or free-choice flaxseed oil-based supplement (FLAX; n = 30) for 7 weeks (w). Heifers were individually fed via the Insentec feeding system (Insentec RIC, Hokofarm, Marknesse, Netherlands), where basal diet (TMR) and supplements were provided in separate feeders to measure supplement intake and feeding behavior. Further, water intake and behavior we measured via Insentec water system. Individual intake was compiled over 24h for each animal. Body weight, transrectal ultrasonography, and blood samples (BS) were collected weekly. On w5, heifers were enrolled in a fixed-time artificial insemination (FTAI) 7-day co-synch standard protocol. Largest follicle diameter (LF) and CL presence were recorded weekly and on days of the protocol (d0, d7, and d9). Pregnancy diagnosis was performed 30d and 60d following AI. BS were analyzed for progesterone (P4) by radioimmunoassay (RIA) to determine puberty attainment before w5, and fatty-acid profile was determined via gas chromatography-flame ionization detection. Heifers with a CL or P4 ≥ 1.0 ng/ml were considered pubertal. Data were analyzed using SAS 9.4 with PROC or PROC GLIMMIX. Average daily gain (ADG) was affected by w ($P < 0.01$). Supplement intake was greater ($P < 0.01$) for FLAX (0.73 ± 0.03 kg/d) compared to CON (0.18 ± 0.02 kg/d) and from w2 to w8 ($P = 0.01$). Time spent consuming the supplements was greater for FLAX compared to CON heifers (62.65 ± 2.11 *vs* 0.10 ± 0.11 min/d, respectively) over the entire trial ($p<0.01$). Further, FLAX heifers consumed less water than CON heifers (32.2 ± 0.39 *vs* 33.93 ± 0.35 kg/d, respectively; $P = 0.03$) and from w2 to w7. LF was not different between groups on any of the days of the protocol (d0, d7, d9; $P > 0.20$). Additionally, P4 on d0 (2.21 ± 0.49 *vs* 1.24 ± 0.45 ng/ml; $P = 0.15$) and d7 (3.29 ± 0.61 *vs* 2.39 ± 0.49 ng/ml; $P = 0.25$), puberty attainment by w5 (69% *vs* 53%; $P = 0.24$), and pregnancy rates at both 32d (69% *vs* 60%; $P = 0.48$) and 60d (88% *vs* 83%; $P = 0.84$) were similar between groups. However, there was a tendency of lower P4 at AI on FLAX (0.46 ± 0.13 *vs* 0.77 ± 0.12 ng/ml; $P = 0.09$). In addition, concentrations of linoleic (60.11 ± 4.34 *vs* 40.35 ± 4.04 mg/ml; $P = 0.02$), αLinolenic (16.73 ± 0.26 *vs* 15.86 ± 0.23 mg/ml; $P = 0.03$) were greater and tended to be greater arachidonic acid (3.00 ± 0.24 *vs* 2.32 ± 0.19 mg/ml; $P = 0.10$) on w8 for FLAX compared to CON heifers. These findings evidence that the flaxseed oil-based supplement alters the plasma lipid profile and may change P4 concentration at AI. More studies should be conducted to explore how these alterations may affect embryo development further on.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Follicular dynamics, uterine evaluation, and pregnancy rate in Nelore cows treated with prostaglandin and injectable progesterone in early puerperium

Rafaela Schoma Cardoso[1], Mariana Moreira dos Anjos[1], Pamela Mara Celestino Soares[1], Juliane Heloiza Aparecida Antunes[1], Elikismei Denilson Araujo Kuiava[2], Luiz Francisco Machado Pfeifer[3], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]GestPec - Consultoria e Gestão Pecuária
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*).

E-mail: rafaela.schoma@uel.br

Our objective was to evaluate Nelore (*Bos indicus*) cows during the postpartum (PP) period in two experiments, involving the follicular dynamics, uterine diameter, uterine cytology, and the pregnancy rate in TAI. For the first study (n = 40), the animals were randomly distributed as follows: Control group (GC, n = 10): 2 ml IM injection of 0.9% NaCl; Prostaglandin group ($GPGF_{2\alpha}$, n = 10): 0.5 mg IM injection of cloprostenol (Sincrocio® Ourofino, Brazil); Progesterone group (GIP4, n = 10): 150 mg IM of injectable P4 (Sincrogest® Ourofino); Combined progesterone and prostaglandin group ($GIP4+PGF_{2\alpha}$, n = 10): 0.5 mg IM of cloprostenol + 150mg IM of injectable P4. Body condition score (2.64 ± 0.04, P = 0.75) and weight (401.5 ± 7.25, P = 0.32) were equally distributed among treatments. All treatments were performed 10 days after calving along with the uterine cytobrush evaluation. Thirty days after the treatments, all cows were subjected to another uterine cytobrush and a TAI protocol. Ovarian follicular dynamics and uterine evaluation were assessed daily by ultrasonography (SonoScape™ Model S8, Domed, Valinhos, Brazil) from D0 to D10 and every 12 hours from D10 until ovulation. In Experiment 2, primiparous precocious (n = 455), primiparous conventional (n = 775) and multiparous cows (n = 830) from the same farm were evaluated on day 22 ± 2 (M ± SD) PP, and equally distributed, treated and inseminated as in Experiment 1 (CG, n = 520; $GPGF_{2\alpha}$, n = 513; GIP4, n = 521; and $GIP4+PGF_{2\alpha}$, n = 506). The cows were timed AI on days 30.4 ± 3.2 PP and inseminated by two technicians. Semen from six bulls (two batch/bull) was distributed equally among the treatments. Continuous variables were analyzed by ANOVA and Tukey's test, and dichotomous variables by Fisher's Exact Test ($P < 0.05$). Logistic regression was used to analyze the effects of these factors on P/AI. The ovulation rate was lower ($P = 0.02$) in the control group (60%) in comparison to the treated groups (GP4: 100%, $GPGF_{2\alpha}$: 100%, and $GIP4+PGF_{2\alpha}$: 90%; no difference among treated groups $P > 0.05$). The uterine diameter was smaller at the FTAI protocol when compared to 10 days postpartum ($P < 0.05$), with no difference among all groups. The percentage of polymorphonuclear cells in the uterus did not differ ($P = 0.68$) among groups, however, was higher ($P = 0.03$) on D10 PP compared with D30 PP. No effects ($P > 0.05$) of inseminator, batch or category on P/AI were observed. However, there were effects of treatment ($P = 0.05$) and bull ($P = 0.05$) on P/AI. The GIP4+ $PGF_{2\alpha}$ group presented a greater P/AI (70%, 354/506) than the CG (62.5%, 325/520), $GPGF_{2\alpha}$ (64.9%, 332/513), GIP4 (63.5%, 330/521) groups ($P = 0.05$). We concluded that treatments with $PGF_{2\alpha}$, IP4, and the combination of both improved the response ovulation at TAI protocol and the association between IP4 plus $PGF_{2\alpha}$ increases pregnancy in the first TAI of Nelore cows during the postpartum period.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Gradual acclimation to repeated handling prior hormonal protocol as an alternative to reduce stress in beef cattle

Júlia Medeiros Cruz de Lima Martins[1], Eduardo Kenji Nunes Arashiro[2], Guilherme Pugliesi[3], Guilherme Nunes de Souza[1], Felipe Zandonadi Brandão[2]

[1]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)
[2]Universidade Federal Fluminense (UFF)
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: juliamclmartins@gmail.com

The aim of this study was to evaluate the effect of gradual acclimation to repeated handling of cow and calf before TAI hormonal protocol on stress and reproductive indices. Nelore cows (n = 116) with an average of 59.81 ± 17.49 days postpartum were divided into two groups. During the thirty days period prior to initiating the TAI protocol, females in the Treatment group (TG, n = 58) were subjected to gradual acclimation to repeated handling 30 days prior TAI protocol, which consisted of weekly visits to the corral and passage through the cattle chute avoiding physical aggression, shouting or manipulation. At squeezing chute individual containment of each animal was performed by closing the entrance and exit gates. The cows and their calves were then placed in a support paddock where they received positive reinforcement (feed with chopped grass and fresh water) before returning to pasture. The control group (CG, n = 58) only went to the corral for selection and then to start the TAI protocol. Cow weight and body condition score at the beginning of the TAI protocol were not influenced by gradual acclimation (466.48 ± 63.19 *vs* 467.70 ± 61.79 kg, respectively for CG and TG; P = 0.916). Time-fixed insemination was considered as Day 0 (D0). In half of the animals from each group, preovulatory follicle volume (at D0) and corpus luteum (CL) volume (at D7 and D16) were measured by the same technician using B-mode transrectal ultrasonography. At D0, D7, D16, and during animal selection, blood was collected via coccygeal vein puncture for cortisol measurement by solid-phase radioimmunoassay technique using commercial kits. Data was subjected to Kolmogorov-Smirnov test for normality assessment. Normal data was compared by ANOVA or t-test. Non-normal data was evaluated by Mann-Whitney test. The impact of gradual acclimation on pre-ovulatory follicular (0.83 ± 0.32 *vs* 1.06 ± 0.52 cm³, P = 0.107; respectively for CG and TG) and on CL volume on D7 (3.49 ± 1.55 *vs* 3.78 ± 1.73 cm³, P = 0.570; CG and TG, respectively) and D16 (3.42 ± 1.19 *vs* 3.31 ± 1.48 cm³, D16, P = 0.779; CG and TG, respectively) was not significant. Cortisol concentrations at the time of animal selection did not differ (35.47 ± 14.48 *vs* 37.54 ± 16.72 ng/ml, respectively for CG and TG; P = 0.595), however, TG showed lower cortisol concentration at D0 (15.92 ± 9.50 *vs* 30.89 ± 15.45 ng/ml, respectively for TG and CG; P ≤ 0.001). At D7 and D16, cortisol levels were similar between groups (at D7, 13.82 ± 8.98 *vs* 13.98 ± 7.88 ng/ml, P = 0.936; and at D16, 11.84 ± 8.21 *vs* 14.30 ± 12.66 ng/ml, P = 0.704, respectively for CG and TG). The gradual acclimation to repeated handling reduced animal stress at the beginning of the TAI protocol, however, no effect was observed on the volume of the follicle and corpus luteum.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Impact of body condition score (BCS) dynamics during postpartum on pregnancy losses

Sofía Albertini[1], Emanuele Almeida Gricio[1], Lígia Mattos Rebeis[1], Laís Ângelo de Abreu[1], Bruna Lima Chechin Catussi[1], Ana Carolina dos Santos Oliveira[1,2], Humberto Albertini[3], Agustín Chaparro[4], Luciano Penteado da Silva[5], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Fundação Universidade Regional de Blumenau (FURB)
[3]Estância Montanía S.A.
[4]Genfut S.A.
[5]Firmasa Tecnologia Para Pecuária Ltda

E-mail: salbertiniw@gmail.com

The present study analyzed the relationship between body condition score (BCS) dynamics (gained *vs* maintained and lost) and pregnancy losses (PL) in Brangus cows (*Bos taurus* x *Bos indicus*). A cohort of 1520 pregnant Brangus cows (30 days of pregnancy) bred through artificial insemination (FTAI) or clean-up bulls were used. Cows came from three different stations within the same commercial farm. The conception within the breeding season came from the dry and transitional period (lower quality and quantity of pastures; September to December) and the rainy period (better quality and quantity of pastures; January to April). Cows were monitored via transrectal ultrasound at three pivotal stages: Pregnancy Diagnosis 1 (PD1) at 30 days, Pregnancy Diagnosis 2 (PD2) at 60 days, and Pregnancy Diagnosis 3 (PD3) at 90 days post-conception. BCS evaluations were conducted during these stages to observe changes across three distinct moments: BCS changes from PD1 to PD2 (Moment 1, M1), BCS changes from PD2 to PD3 (Moment 2, M2), and BCS changes from PD1 to PD3 (Moment 3, M3). The study employed a longitudinal design using a binomial logistic model in SAS 9.4 to explore interactions between PL and BCS dynamics, considering factors like farm station, breeding method, and conception period. The results were presented as PL rates for each moment with statistical tests identifying both trends ($P > 0.05$ to $P < 0.10$) and significant effects ($P < 0.05$). There was no interaction of PL*BCS dynamics for any of the moments studied ($P = 0.7316$). The PL rate at M1 was 3.62% (55/1520). Cows that gained BCS at M1 tended to have fewer PL than cows that maintained or lost BCS [Gained = 1.62% (4/247) *vs* Maintained/Lost = 4.01% (51/1273); $P = 0.0760$]. The PL rate at M2 was 1.57% (23/1465). No effect of BCS dynamics was observed at M2 for PL [Gained = 0.66% (2/304) *vs* Maintained/Lost = 1.81% (21/1161); $P = 0.1687$]. The PL rate at M3 was 5.13% (78/1520). A significant effect was noted at M3, where cows that either maintained or lost BCS had higher PL [Gained = 1.65% (7/425) *vs* Maintained/Lost = 6.48% (71/1095); $P = 0.0012$]. Also, there was a tendency regarding the period of conception. Cows that got pregnant during the dry and transitional period tended to have higher PL during M3 [Dry/Transitional = 6.23% (58/931) *vs* Rainy = 3.40% (20/589); $P = 0.0727$]. These results indicate that the M3 (30D to 90D) is a critical window where BCS dynamics could decisively impact pregnancy outcomes. In conclusion, significant effects were found between BCS dynamics and PL rate between days 30 and 90 post-conception, highlighting nutritional management's critical impact on cattle's reproductive success.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Impact of buserelin acetate dosage on LH release and ovulatory response in Nelore (*Bos indicus*) cows

Heni Falcão da Costa[1], Bruna Lima Chechin Catussi[2], Emanuele Almeida Gricio[2], Sofía Albertini[2], Igor Garcia Motta[1], Bruno Gonzalez de Freitas[1], Ferdinando Nielsen de Almeida[1], Pietro Sampaio Baruselli[2]

[1]Ourofino Saúde Animal
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: heni.costa@ourofino.com

This study aimed to evaluate the effect of buserelin acetate (Sincroforte®, Ourofino, Cravinhos, Brazil), a GnRH analog dosage, on LH release and ovulatory rate in Nelore cows. A total of 45 non-lactating multiparous cows (BW= 446.6 ± 8.8 and BCS = 2.94 ± 0.02) were used. On D-15, cows presenting a corpus luteum (CL) received an intravaginal progesterone device (P4; Sincrogest®, Ourofino) and 2 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino). On D-7, all animals received 0.53 mg of cloprostenol sodium (Sincrocio®, Ourofino) and 300 IU of eCG (SincroeCG®, Ourofino), along with P4 device removal. The following day (D-6), cows received 1 mg of EB. On D0, ultrasound examinations (US) were performed to measure the CL and dominant follicle (DF) diameter, those with DF smaller than 7.5 mm were excluded from the experiment. Subsequently, they were assigned to one of three treatments: Control (n = 13), receiving no additional treatment; GnRH10 (n = 13), administered 10 µg of buserelin acetate and GnRH20 (n = 13), administered 20 µg of buserelin acetate. Blood samples were taken on D0 at 0, 1, 2, 3, and 4 hours after GnRH treatment to evaluate LH concentrations. Cows were also scanned on D1, D2, and D3 to determine the time of ovulation. The LH peak was considered the greatest concentration of LH. A repeated measurements analysis was performed. The effect of treatments on ovulatory response was assessed using the Fisher Exact Test, and continuous variables were analyzed using the PROC GLIMMIX of SAS 9.4. GnRH treatment, regardless of dose, induced ovulation during diestrus. There was a treatment effect, and Control had decreased ovulatory response compared to GnRH (P = 0.001), but GnRH10 did not differ from GnRH20 [Control = 0% (0/13)^{B}, GnRH10 = 46.2% (6/13)^{A}, GnRH20 = 76.9% (10/13)^{A}]. No difference was observed in CL diameter (mm) on D0 [Control = 14.55 ± 0.53, GnRH10 = 14.95 ± 0.67, GnRH20 = 14.65 ± 0.61; P = 0.89]. An interaction between time and treatment was observed regarding LH concentration (P = 0.001). On hour 0, no differences between treatments were observed (Control = 0.14 ± 0.01; GnRH10 = 0.14 ± 0.01; GnRH20 = 0.14 ± 0.01). However, differences were presented on hour 1 (Control = 0.15 ± 0.02^{B}; GnRH10 = 1.34 ± 0.39^{AB}; GnRH20 = 2.39 ± 0.71^{A}), hour 2 (Control = 0.12 ± 0.02^{B}; GnRH10 = 2.46 ± 0.69^{AB}; GnRH20 = 4.13 ± 1.09^{A}), hour 3 (Control = 0.13 ± 0.01^{B}; GnRH10 = 1.66 ± 0.55^{AB}; GnRH20 = 2.91 ± 0.79^{A}) and hour 4 (Control = 0.14 ± 0.01^{B}; GnRH10 = 1.34 ± 0.32^{AB}; GnRH20 = 1.19 ± 0.32^{A}). No difference in LH peak was found between the GnRH groups (GnRH10 = 2.65 ± 0.80; GnRH20 = 4.29 ± 1.08; P = 0.24). In conclusion, 10 and 20 µg doses of GnRH induced ovulation during diestrus, with no differences in LH peak.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Impact of the response to ovulation induction protocols before timed-AI on reproductive performance of precocious Nelore beef heifers

Amanda Guimarães da Silva[1], Pedro Antônio Pádua Andrade[2], Samuel Volpe Souza[1], Júlia Soares Gonçalves[3], Barbara Alexandre Melo[3], Rogério Fonseca Guimarães Peres[4], Rodolfo Daniel Mingoti[5], Igor Garcia Motta[6], Guilherme Pugliesi[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade Paulista (*UNIP*)
[3]Agropecuária Nelore Paranã
[4]R2 Pecuária Inteligente e Gestão
[5]Merck sharp & Dohme saúde animal
[6]Ourofino Saúde Animal

E-mail: amandaguimaraes@usp.br

We aimed to compare the reproductive performance between Nelore beef heifers with a positive or negative response to induction protocol with injectable progesterone (iP4) or with a corpus luteum (CL) before timed-AI (TAI) protocol. For this, 1144 Nelore heifers aged 10-17 months were used, which were randomly distributed into two treatments: 1P4 (150 mg of iP4 at D−12 [D0 = beginning of TAI protocol]; n = 571); and 2P4 (150 mg of iP4 on D-24 and D-12; n = 573). Heifers were submitted to an E2/P4-based protocol and TAI was performed at D9. Heifers had the uterus and ovaries evaluated on D-24, D-12, and D0 and were attributed scores of 1 (less developed) to 5 (more developed) for both structures. After the evaluation of ovarian structures on D0, heifers were classified according to the presence or absence of CL, before and after induction protocol in one of the following three groups: 1) With CL (heifers with CL before the treatment with iP4; n = 285); 2) Positive Response (POS; heifers without CL on D-24 but with CL after iP4 treatment; n = 103); 3) Negative response (NEG; heifers without CL in all the assessments; n = 756). The first pregnancy diagnosis (PD1) was performed 22 days after TAI using color-Doppler ultrasound, and heifers with an active CL (blood perfusion ≥ 30%) were considered pregnant. The PD2, PD3, and PD4 were performed on days 30-35, 59-61, and 100-120 after TAI, respectively. Data were analyzed by PROC MIXED and GLIMMIX of SAS. The response to induction was greater ($P < 0.05$) in the 2P4 group than in the 1P4, 13.6% [78/427] and 8.4% [48/432], respectively. The uterus score on D0 tended to be greater ($P = 0.1$) in 2P4 [3.7 ± 0.03] than in the 1P4 group [3.6 ± 0.03]. Also, the uterus score on D0 was greater ($P < 0.05$) in the CL [4.2 ± 0.04] and POS [4.1 ± 0.06] groups than in the NEG group [3.3 ± 0.02]. The P/AI at PD1 was greater ($P < 0.05$) in the 2P4 [40.6%] than in the 1P4 group [32.2%]. The P/AI at PD2, PD3, and PD4 tended to be greater ($P = 0.1$) in 2P4 [35%, 31.3% and 27.6%] than in 1P4 group [28.8%, 25.4% and 22.6%]. The P/AI at PD1 and PD2 was greater ($P < 0.05$) in the CL [50% and 44%] and POS [43% and 38%] groups than in the NEG group [30.5% and 26.6%]. At PD3 and PD4, the P/AI was greater ($P < 0.05$) in the CL group [41.1% and 36.5%] than in the NEG group [23% and 20%], while the POS group [31% and 29%] was intermediate. The pregnancy losses were not affected ($P > 0.1$) by treatment or response to the induction protocol. In conclusion, administering iP4 two moments before the TAI in precocious heifers improved the uterine score at the beginning of the TAI protocol, reflecting improvements in the P/AI. In addition, regardless of the induction strategy, heifers that responded positively to the induction protocol presented enhanced pregnancy rates, equivalent to those with CL before the induction protocol. Acknowledgements: Agropecuária Nelore Paranã, Ourofino Saúde Animal, CAPES, and FAPESP (grant number: 2023/11777-8).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Injectable progesterone and $PGF_{2\alpha}$ during early puerperium to increase the conception rate in Holstein cows – preliminary results

Pamela Mara Celestino Soares[1], Juliane Heloiza Aparecida Antunes[1], Gabriela Rodrigues de Paula[1], Rafaela Schoma Cardoso[1], Paulo de Tarso Lemos Borges[2], Bruno Santi Orsi Climeni[2], Ruyter França Carvalho[3], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Policlínica Veterinária Pioneiros
[3]Médico Veterinário autônomo

E-mail: pamela.mc.soares@uel.br

This study aimed to evaluate the effect of injectable progesterone (iP4), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or both of them on the conception rate (P/AI) in Holstein cows during the puerperal period. The experiment was conducted on a commercial dairy farm in Arapoti, Paraná, with Holstein cows (n = 284), being 105 primiparous and 179 multiparous at 12 ± 1.91 days postpartum (D-56). All animals were randomly distributed into one of four experimental groups: 1) Control (n = 72): 2 ml of 0.9% NaCl, i.m; 2) $PGF_{2\alpha}$ (n = 79): 0.526 mg of cloprostenol sodium, i.m. (Sincrocio®, Ourofino, Brazil); 3) iP4 (n = 72): 150 mg of injectable progesterone, i.m. (Sincrogest® , Ourofino, Brazil) and 4) $PGF_{2\alpha}$+ iP4 (n = 61): 0.526 mg of cloprostenol sodium (Sincrocio®, Ourofino, Brazil) + 150 mg of injectable P4, i.m. (Sincrogest® Injectable, Ourofino, Brazil). At the beginning of the timed artificial insemination protocol (D0), the voluntary waiting period (VWP) was 56 ± 12.2 days, body condition score (BCS) of 2.8 ± 0.27 and days in milk (DIM) of 78 ± 10.4 days at AI (D11). The FTAI protocol included the administration of 0.01 mg GnRH + 2 mg EB, i.m, and insertion of an intravaginal device 2 g of P4, (D0) and 0.5 mg of cloprostenol, i.m., (D7). Subsequently, the intravaginal devices were removed and 0.5 mg of cloprostenol + 1 mg of EC, i.m., was applied (D9). Forty-eight hours after removing the device, the AI was performed (D11). Thirty days after AI, pregnancy diagnosis was performed (D41). Females were evaluated for conception rate in the first service (1st FAI), non-pregnant cows (n = 74) were inseminated (D52) and subsequently evaluated (D82) regarding the conception rate at the second service (2nd FAI). The conception rate was analyzed using the logistic regression model and the effects of treatment, category, bull, service order, BCS, VWP, DIM, and main interactions were considered. In the presence of a significant effect, the ranking between conception rates was analyzed using a 2x2 proportion test ($P \leq 0.05$). The accumulated conception rate (1st FAI+2nd FAI) among the groups is presented below: Control (28/72 - 38.8%), $PGF_{2\alpha}$ (27/79 - 34.1%), iP4 (31/72 - 43%) and iP4 + $PGF_{2\alpha}$ (33/61 - 54.1%). Although there was a numerical difference in the conception rate, there was no statistical difference ($P = 0.16$) so far. The conception rate did not vary depending on the category ($P = 0.97$), number of services ($P = 0.71$), BCS ($P = 0.16$), VWP ($P = 0.08$), DIM ($P = 0.64$), but rather as a function of the bull ($P < 0.0001$). The experiment is still in progress and will be completed when the number of 200 animals per test group is reached.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Long-acting injectable progesterone in synchronizing ovulation in Girolando cows: preliminary results

Nicolas Moreira Piedras Monnerat Caparelli[1], Samuel Rodrigues Bonamichi do Couto[1], Mateus Gonçalves Costa[1], Beatriz Alves Paulino[1], Maria Eduarda de Melo[1], Mellyssa Sad Rodrigues Gomes[1], Raphael Azevedo Lamim[1], Rondineli Pavezzi Barbero[1], Marco Roberto Bourg Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro (*UFRRJ*)

E-mail: nicolascaparelli@ufrrj.br

This study evaluated the feasibility of using long-acting injectable progesterone (IP4) to replace intravaginal devices (ID) in timed artificial insemination (TAI) in dairy cows. To this end, 2 experiments were conducted. Only pasture-raised cows, with a body condition score (BCS) ≥ 2.5 and 30-60 days postpartum were included. The selected animals had an average BCS of 3 ± 0.06 and an average daily milk production of 15.2 kg. Experiment 1: 56 crossbred cows (Girolando) were divided into two treatments with the objective of evaluating the follicular dynamics: ID group (n = 30) and IP4 group (n = 26). In ID females, on D0, ID (1 g, Primer®, Tecnopec, Brazil) was inserted, associated with 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino Saúde Animal, Brazil). ID was removed after nine days and 500 µg of cloprostenol (Sincrocio®, Ouro Fino Saúde Animal, Brazil) + 300 IU of eCG (Folligon®, MSD Saúde Animal, Brazil) were applied. + 1.0 mg of estradiol cypionate (SincroCP®, Ouro Fino Saúde Animal, Brazil). IP4 group received 150 mg of injectable progesterone (Sincrogest® Injetável, Ouro Fino Saúde Animal, Brazil) replacing ID on D0. Except for the administration of 10 µg of buserelin acetate (Sincroforte®, Ourofino, Brazil) on D11, the other management procedures were similar to ID group. Ultrasonographic evaluations (Mindray DP10) of the ovaries were performed every 12 hours from D9 until 48 hours post-TAI to evaluate follicular growth and determine the following parameters: interval between cloprostenol application and ovulation (ICO-hours); diameter of the dominant follicle when applying cloprostenol (DFD9-mm); DF diameter at TAI (DF-TAI-mm); DF growth rate (FGR-mm/day); ovulation rate (%). Blood samples were collected on D9 and D11 for serum P4 measurement to determine P4 concentration on D9 and D11 (ng/ml). In experiment 2: 50 Girolando cows were used, divided into two groups ID (n = 26) and group IP4 (n = 24), with treatments being similar to experiment 1 but with the objective of evaluating the conception rate. The variables were compared using the Bioestat program with a significance of 5%. No significant difference was observed between treatments for the following variables: ICO (ID = 67.0 ± 5.8h; IP4 = 60.8 ± 14.3h; P = 0.49); DFD9 (DI = 9.9 ± 1.8 mm; IP4 = 9.8 ± 2.3 mm; P = 0.88); DF-TAI (ID = 12.4 ± 1.2 mm; IP4 = 12.3 ± 1.4 mm; P = 0.92); FGR (ID = 1.2 ± 0.5 mm/day; IP4 = 1.3 ± 0.7 mm/day; P = 0.83); P4 D9 concentration (ID = 6.04 ± 3.5, IP4 = 7.2 ± 4.8 ng/ml; P = 0.55) and ovulation rate (ID = 76.6.7%, IP4 = 80.7%; P = 0.35). Regarding the concentration of P4 at TAI, the IP4 group presented a higher concentration (ID = 0.57 ± 0.2; IP4 = 0.89 ± 0.3 ng/ml; P = 0.02). There was no difference in conception rates (ID = 38.4%, IP4 = 41.6%; P = 0.4). Under the conditions of the present study, the replacement of ID with IP4 associated with the GnRH analog at TAI, in dairy cows, did not alter follicular dynamics and seems to maintain similar conception rates, which must be confirmed in studies with a larger number of animals.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Physiology and fertility outcomes of lactating dairy cows submitted to different resynchronization programs initiated 25 days after AI

Rafael Luiz Stolf[2], Carlos Eduardo Consentini[1], Caroline Cervi Vandresen[2], Anna Rosa Rolim[3], Rodrigo S. Belli[3], Thiago N. Marcantonio[3], Roberto Sartori[4,2]

[1]GlobalGen vet science
[2]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[3]Fazenda Canto Porto
[4]University of Wisconsin Madison (*Department of Animal and Dairy Sciences*)

E-mail: rafaelstolf@usp.br

The study evaluated strategies for resynchronization protocols for timed-AI (TAI) initiated 25d after a prior insemination in lactating dairy cows. After the 1st TAI, 1231 cows from a dairy herd were allocated, according to parity, into 2 groups for resynchronization: GnRH (G; n = 595) or GnRH plus P4 device (G+P4; n = 636). On d25, all cows received 16.8 µg buserelin acetate (GnRH). Ultrasound assessment was performed on d32 for non-pregnant diagnostic (NPD). In the G-group, cows were further categorized based on the presence of CL. Cows with CL received 0.53 mg of cloprostenol (PGF) on d32, followed by a second PGF on d33, plus 1 mg estradiol cypionate (EC), 300 IU eCG and tail chalk for estrus evaluation. On d35, another TAI was performed. Otherwise, the G-group without a CL, initiated another TAI protocol, starting with a new P4 device (2 g) and 16.8 µg of GnRH. On d39, these cows received the first PGF, and 24h later the second PGF, EC, eCG were given with similar doses, concomitant with P4 withdrawal. TAI was performed on d42. In the G+P4 group, all cows started the resynchronization protocol on d25 with GnRH and P4 devices. On d32, in NPD (regardless of presence of CL), open cows received PGF. On d33, the P4 device was withdrawn, PGF, EC, eCG was given, tail chalk was applied for estrus detection and 48h later, on d35, cows were bred. After being assigned to an experimental group, cows followed the same resynchronization strategy up to the 4th service. Ultrasound evaluations were performed on d25 and d32 to record presence and number of CL, and ovulation after d25. Pregnancy per AI (P/AI) and pregnancy loss (PL; d30 to 60) of 1st AI were evaluated. Statistical analyses were done by logistic regression using PROC GLIMMIX (SAS 9.4; $P \leq 0.05$). Presence of CL for G and G+P4 groups on d25 (77.7 [206/265] *vs* 78.5% [257/308]) and d32 (85.2 [226/265] *vs* 83.4% [257/308]) did not differ, as well as ovulation after d25 (56.6 [150/265] *vs* 48.7% [150/308]) and expression of estrus (77.2 [180/233] *vs* 73.3% [231/315]). P/AI on d32 did not differ (43.7 [116/265] *vs* 40.2% [124/308] for G and G+P4, respectively). Cows that received the 1st AI or all prior services had similar fertility when submitted to G and G+P4, respectively (45.8 [151/329] *vs* 44.0% [148/336] and 47.7 [281/589] *vs* 44.3% [277/625]). PL after the 1st TAI did not differ between G and G+P4 (13.5 [20/148] *vs* 10.2% [15/146]), nor for the resynchronizations (15.2 [37/242] *vs* 10.1% [23/227]). In conclusion, the resynch programs with all cows re-inseminated at 35d interval (G+P4 group) or the resynch that selected cows to be inseminated on d35 or d42 (G group), with a targeted use of P4 implant resulted in similar fertility, without compromising fertility of prior AIs. These findings provide flexibility for farmers and technicians to choose the best approach based on their reproductive management preferences. Also, both programs effectively reduced the interval between AIs and enhanced service rate.
Acknowledgements: FAPESP 2018/03798-7.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Postpartum uterine health of superprecocious primiparous *Bos indicus* cows

Jéssica de Souza Andrade[1], Karen Lauanne Silva Lacouth[1], Renata Reis da Silva[2], Vanessa Lemos de Souza[4], Ingrid Pedraça Barbosa[1], Leonardo Silva Gomes[3], Samira Alves de Souza Silva[4], Gabrielly Cristina Santos Noleto[1], Orenci Severino Machado Júnior[3], Luiz Francisco Machado Pfeifer[2]

[1]Universidade Federal do Acre (UFAC)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[3]Faculdades Integradas Aparício Carvalho (FIMCA)
[4]Universidade Federal de Pelotas (UFPel)

E-mail: jessica_andrade_@hotmail.com

Fertility and uterine health during the postpartum period are crucial for achieving adequate reproductive performance in beef herds. However, superprecocious primiparous cows (PSP) present distinct characteristics such as sexual precocity and early body maturity, which influence their productive efficiency. This study aimed to evaluate postpartum uterine inflammation and fertility in superprecocious primiparous *Bos indicus* beef cows subjected to timed artificial insemination (TAI) protocols. Nelore PSP cows (*Bos indicus*, n = 268) were subjected to a conventional Estradiol-progesterone (E2-P4) based TAI protocol (D0, 2 mg of E2 benzoate and insertion of P4 intravaginal device; D8, removal of intravaginal P4 device, 150 µg of D-Cloprostenol PGF, 300 IU of eCG, and 1 mg of E2 cypionate; Day 10, TAI). Pregnancy diagnosis was performed by transrectal ultrasonography 30 days after TAI. The experimental groups were determined according to days postpartum (DPP) at the beginning of TAI program. For that purpose, optimal cut-off points for determining the relationship between the DPP and pregnancy status at 1st TAI were calculated using the receiver operating characteristic (ROC) curve. Therefore, cows were divided, as follows: 1) Early PSP (n = 59), cows between 25 and 31 DPP; 2) Late PSP (n = 209), cows between 32 and 60 DPP. On Day 0, before the start of the protocol, cows underwent cytological collection of uterine tissue using the cytobrush technique. The samples collected from uterine tissue were fixed on glass slides, stained with a Panotic kit, and subjected to a 200-cell counting under an optical microscope. After cytology sampling, the cytological brushes were individually stored in microtubes with RNAlater®. Total RNA was extracted from a subgroup of the samples (26 and 32 from Early PSP and late PSP, respectively), and gene expression analysis for IL-1β, IL-8, TNF, PGR, ESR1, ESR2, and PTCH2 was performed by quantitative real-time PCR (qPCR-RT). The proportion of PMN and gene expressions were analyzed by analysis of variance (ANOVA), and the means were compared between groups using Tukey's test. Pregnancy per AI (P/AI) was analyzed by Chi-square test. The P/AI was lower in Early PSP cows than in Late PSP cows ($P < 0.01$; 45.7%, 27/59, and 66%, 138/209, respectively). Early PSP cows had a higher proportion of PMN cells than Late PSP cows (22,1% and 5,2%, respectively; $P < 0.001$). The gene expression of IL-1β, IL-8, TNF, ESR1, ESR2, and PTCH2 was higher ($P < 0.05$) in Late PSP cows than in Early PSP cows. The PGR gene expression tended to be lower ($P = 0.08$) in Early PSP cows than in Late PSP cows. These results show that PSP cows subjected to early TAI protocols (< 31 DPP) have a higher proportion of PMN and lower P/AI than late IATF (≥ 32 DPP). Intriguingly, in a uterine inflammatory and receptivity perspective, cows from the Late group had higher expressions of pro- inflammatory cytokine genes (IL-1β, IL-8, and TNF), and of uterine receptivity genes (ESR1, ESR2, and PTCH2).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Relationship among nutrition, milk production, reproduction, and performance of high-producing dairy herds

Carlos E. C. Consentini[1], Paulo David Carvalho[2], Alexandre Henryli de Souza[3]

[1]GlobalGen vet science
[2]ST Genetics
[3]Cargill Animal Health and Nutrition

E-mail: consentinicarlos@gmail.com

Our objective was to determine relationships among nutrition, milk yield (MY) and reproductive performance (RP) in 48 high-producing Holstein dairy herds (MY: 38.9 ± 0.6 kg/d; range: 30.0-50.4). The MY, RP, and complete dietary information (e.g. high-production pens after 21 days in milk) was retrieved for 12 months. Dietary components (% of dry matter) were crude protein (CP), rumen degradable (RDP) and undegradable (RUP) protein, neutral detergent fiber (NDF), non-fiber carbohydrates (NFC), starch, and fat. The RP data were the proportion of timed-AI (TAI) use for 1st and all services, service rate (SR), overall pregnancy per AI (P/AI) and P/AI at the 1st service (P/IA-1st), 21-d pregnancy rate (PR), and proportion of cows pregnant by 150d in milk (PREG150). Simple correlations and potential interactions were studied with CORR and GLIMMIX of SAS 9.4. Statistical significance was considered when $P \leq 0.05$. Correlations are presented with the correlation coefficient r (positive or negative) in parentheses, while other results are otherwise indicated. Following are the average and range of RP data: 1) SR: 58.5% (39-73), 2) P/IA-1st: 39.7% (20-51), 3) P/AI: 36.1% (22-49), and 4) PR: 20.3% (10-42). Proportion of TAI use for 1st service was more relevant than TAI use for all services in SR (r = 0.53 *vs* 0.33) and PR (r = 0.28 *vs* 0.15). Interestingly, the P/IA-1st had the strongest correlation with PR (r = 0.72), greater than overall P/AI (r = 0.66) and SR (r = 0.54). Similarly, PREG150 was more correlated with P/IA-1st (r = 0.66) than with P/AI (r = 0.62) and SR (r = 0.48). The MY tended to correlate with SR (r = 0.27, P = 0.09), but have no correlation with P/AI-1st (r = 0.06), P/AI (r = -0.03), PR (r = 0.11) and PREG150 (r = 0.08). There were no detectable correlations between nutrition variables and MY: CP (r = 0.06), RDP (r = 0.04), RUP (r = 0.02), starch (r = -0.22), NFC (r = -0.15), NDF (r = 0.08), and fat (r = 0.23). There were no associations of CP, RDP, and RUP with any RP data. The strongest relationship was a decrease in RP with increasing dietary NFC, including negative correlation with P/AI (r = -0.48), P/AI-1st (r = -0.51), and PREG150 (r = -0.33). Starch also had a negative relationship with P/AI-1st (r = -0.35). Conversely, greater NDF was positively associated with P/AI-1st (r = 0.34), as well as fat content (r = 0.34). When NFC was divided in tertiles (< 40, 40 to 42.2, and > 42.2% NFC), the highest tertile had lower P/AI (39 *vs* 36 *vs* 31%), P/AI-1st (43 *vs* 40 *vs* 33%), and PREG150 (54 *vs* 53 *vs* 47%). In conclusion, farms must consider the 1st service as key for RP, since higher use of TAI and greater P/AI-1st markedly improved SR, PR and PREG150. A greater dietary NFC may compromise RP, whereas herds with greater NDF content may achieve high MY with positive effects on RP. Finally, the absence of a negative relationship between MY and RP confirms that high performance in productive and reproductive standpoints is achievable in well managed farms.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Relationship between ovarian condition at the beginning of the ovulation synchronization protocol with the conception rate in Nelore and Angus cows submitted to TAI

Iasmim Favero Beceiro[1], Luigi Carrer Filho[2], Eriko da Silva Santos[2], Murilo Rezende Figueira[2], Isadora Naomi Vieira Ogawa[2], Fábio Morotti[3,4]

[1]Universidade Estadual de Londrina
[2]Neopecuária
[3]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal, REPROA*)
[4]Universidade Norte do Paraná (*UNOPAR*)

E-mail: iasmim.favero.beceiro@uel.br

This study evaluated the relationship between ovarian condition at the beginning of the ovulation synchronization protocol and conception rate in beef cows submitted to the timed artificial insemination (TAI) program. A total of 639 multiparous cows (241 Nelore and 398 Angus), 40 to 70 days postpartum, and body condition scores (BCS) 2.75 to 4.25 (3.29 ± 0.02) were evaluated in this study. On a random day of the estrous cycle (D0) the cows were evaluated by transrectal ultrasound for ovarian condition and classified into: Anestrus - small follicles (n = 21), Dominant Follicle (DF) - at least one dominant follicle ≥ 8 mm (n = 316), and Corpus Luteum (CL) - at least one CL (n = 302) and then the TAI protocol began. The protocol consisted of the intravaginal insertion of a progesterone device (1.0 g Repro neo®, GlobalGen, Jaboticabal, Brazil) associated with intramuscular (i.m.) application of estradiol benzoate (2 mg, Syncrogen® GlobalGen). Only cows of the CL group received cloprostenol sodium (0.50 mg, Induscio®, GlobalGen) on the same day (D0). On the day of removal of the device (D7), all females receive painting at the base of the tail to evaluate the estrus demonstration and i.m. application of equine chorionic gonadotropin (300 IU, eCGen®, GlobalGen) and estradiol cypionate (1 mg, Cipion®, GlobalGen) and cloprostenol (0.50 mg, Induscio®, GlobalGen). The TAI was performed 48 hours after withdrawal of the device by three inseminators using thawed semen from three bulls with known fertility. All cows received buserelin acetate (8 µg, Maxrelin®, GlobalGen) concomitant to insemination. The pregnancy diagnosis was performed by transrectal ultrasonography 30 days later. Data were analyzed using a multivariate logistic regression model, including ovarian condition, breed, bull, inseminator, and BCS in the model (P ≤ 0.05). Cows in anestrus and with DF had similar conception rates (P > 0.1), then were included in the same analysis group (n = 337). The overall conception rate of the study was 60.88% (389/639) and was influenced by ovarian condition at the beginning of the protocol (P = 0.02), being higher in cows with CL (66.23%, 200/302) compared to those with DF (56.08%, 189/337), both for Nelore (P = 0.03, CL: 63.95% *vs* DF: 56.77%) and Angus cows (P = 0.01; CL: 67.13% *vs* DF: 55.49%). Breed (P = 0.77), BCS (P = 0.52), and bull (P = 0.28) did not influence conception rate, but there was an effect of inseminator (P = 0.04). However, no interaction effect was observed (P > 0.1) with the main effects. In conclusion, the conception rate at first TAI service was satisfactory in both Nelore and Angus cows. The evaluation of the ovarian condition at the beginning of the protocol allowed us to identify the presence of CL, which is associated with a higher conception rate at 30 days compared to females that only have a dominant follicle. This may represent an interesting strategy as it allows for a better indication of specific reproductive management depending on the ovarian condition.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Relationship between progesterone treatment in the postpartum period and variation in body condition score on the pregnancy rate of Nelore cows submitted to TAI

Mayrim Ariana Pereira Muñoz[1], Gabriel Henrique Dias Soares[2], João Pedro Gatti[1], Carlos Henrique Lopes Pascotto[3], Gustavo Martins Gomes dos Santos[3], Marcelo Marcondes Seneda[1], Fábio Morotti[1,2]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Universidade Norte do Paraná (*UNOPAR*)
[3]Médico Veterinário autônomo

E-mail: mayrimpereira@gmail.com

This study evaluated the relationship between the use of progesterone (P4) in the postpartum and the variation in body condition score (BCS) from calving to the start of the timed artificial insemination (TAI) protocol, on the pregnancy rate in Nelore cows. Multiparous cows (n = 3506), 15 to 90 days postpartum (40 ± 15), from three commercial beef cattle farms were studied. The BCS was assessed at calving (D0) and the start of the TAI protocol (D40). At 30 ± 15 days postpartum, the animals were randomly designated into the injectable progesterone group (GIP4: n = 1857), which received pre-synchronization (pre- sync) with 150 mg of injectable P4 i.m. (Sincrogest® Injetável, Ourofino, Cravinhos, Brazil), and the control group (GC: n = 1649), which did not receive pre-sync. On D40, an intravaginal P4 device (1 g, Sincrogest®, Ourofino) was inserted and 2 mg of estradiol benzoate was i.m. applied. At D48, the intravaginal device was removed and cloprostenol sodium (0.5 mg, Sincrocio®, Ourofino), estradiol cypionate (1 mg, SincroCP®, Ourofino) and equine chorionic gonadotrophin (300 IU, eCG-Sincro eCG®, Ourofino) were i.m. applied. In addition, the sacrococcygeal region was painted to evaluate the intensity of estrus expression. On D50, two inseminators performed TAI with semen from four bulls with known fertility. Pregnancy diagnosis was performed 30 days later using transrectal ultrasound. The BCS variation was defined between calving and the start of the TAI protocol as gaining score (positive variation from 0.25 to 0.50 points), maintaining score (variation = 0), or losing score (negative variation from 0.25 to 0.50 points). Data were analyzed by logistic regression model. Treatment (GIP4 and GC), BCS variation (Gaining, Maintaining and Losing), farm, bull, inseminator, days postpartum, estrus scores, and main interactions were considered in the model. In the presence of a significant effect, the ranking between the rates was analyzed using a 2x2 ratio test (significance $P \leq 0.05$ and tendency $P \geq 0.06$ and < 0.9). The overall pregnancy rate was 58.61% (2055/3506) and was influenced by the BCS variation ($P = 0.04$), estrus expression intensity ($P < 0.0001$), bull ($P = 0.009$), farm ($P = 0.06$; tendency), but not by the P4 treatment ($P = 0.17$), and inseminator ($P = 0.13$). However, an interaction effect ($P = 0.001$) was observed between pre-sync with injectable P4 and BCS variation. In cows that lost BCS from calving to the start of TAI protocol, pre-sync increased the pregnancy rate [60.57% (364/601)] compared to the control [55.77% (290/520) $P = 0.001$]. However, pre-sync did not improve the pregnancy rate in cows that maintained BCS [$P = 0.79$; GC 59.74% (417/698) *vs* GIP4 56.65% (528/932)] and those who gained BCS [$P = 0.37$; GC 62.18% (268/431) *vs* GIP4 58.02% (188/324)]. No other interactions were found ($P > 0.1$). In conclusion, the pre-sync with injectable P4 proved to be a promising strategy for improving the pregnancy rate in cows that lost BCS during the postpartum.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Replacing the intravaginal device with long-acting injectable progesterone does not alter the follicular dynamics of Nelore cows

Maria Eduarda de Melo[1], Nicolas Moreira Piedras Monnerat Caparelli[1], Samuel Rodrigues Bonamichi do Couto[1], Daniela Cristina Rocha de Freitas[1], Beatriz Alves Paulino[1], Mellyssa Sad Rodrigues Gomes[1], Raphael Azevedo Lamim[1], Rondineli Pavezzi Barbero[1], Marco Roberto Bourg Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro (*Departamento de Reprodução e Avaliação Animal - UFRRJ*)

E-mail: mariaemelo@ufrrj.br

The objective of this study was to evaluate the feasibility of using long-acting injectable progesterone (P4LA) to replace intravaginal devices (ID) in ovulation synchronization protocols in beef cows. For this experiment, 46 suckling Nelore cows, with a postpartum period between 30 and 60 days, were randomly divided into three treatments: ID group (n = 16), P4LA group (n = 14) and P4LA + GnRH group (n = 16). In ID females, on a random day of the estrous cycle (D = 0), a new intravaginal progesterone device (1 g, Primer®, Tecnopec, São Paulo, Brazil) was inserted, associated with 2 mg of estradiol benzoate (Sincrodiol®, Ouro Fino, São Paulo, Brazil). The device was removed 9 days later and, upon removal, 500 µg of cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) + 300 IU of eCG (Folligon®, MSD, São Paulo, Brazil) + 1.0 mg of estradiol cypionate (D9) (SincroCP®, Ouro Fino Saúde, São Paulo, Brazil) were applied. Two days after device removal (D11), Timed Artificial Insemination (TAI) was performed. P4LA females received 150 mg of injectable progesterone (Sincrogest LA®, Ouro Fino, São Paulo, Brazil) replacing the intravaginal device on D0. And the females in the P4LA + GnRH group received 150 mg of P4LA, replacing ID, on D0 + 10 µg of buserelin acetate (Sincroforte®, Ourofino, São Paulo, Brazil) at TAI. To measure progesterone, blood samples were collected on the D9 and D11. Ultrasonographic evaluations (Mindray DP10) were performed every 12 hours from D9, until 48 hours post-TAI. The parameters evaluated were: interval between cloprostenol application and ovulation (ICO- hours); diameter of the dominant follicle (DFD9-mm) when applying cloprostenol; DF diameter at TAI (DF TAI-mm); DF growth rate (FGR-mm/day); ovulation rate (OR%); progesterone concentration (P4) on D9 (P4D9-ng/ml) and at TAI (P4 TAI-ng/ml); conception rate (CR-%). The variables were compared using the Bioestat program with a significance of 5%. No significant difference was observed between treatments for the following variables: ICO (ID = 80.0 ± 13.3h; P4LA = 84.0 ± 16.0h; P4LA + GnRH = 72.0 ± 14.7h); DF TAI (ID = 10.1 ± 0.8 mm; P4LA = 11.1 ± 1.5 mm; P4LA + GnRH = 11.1 ± 1.6 mm); FGR (ID = 1.04 ± 0.4 mm/day; P4LA= 1.03 ± 0.3 mm/day; P4LA + GnRH= 1.13 ± 0.4 mm/day); DFD9 (ID = 7.5 ± 1.3 mm; P4LA = 8.9 ± 2.0 mm; P4LA + GnRH = 9.3 ± 1.9 mm); CR (ID = 43.8%; P4LA = 28,6%; P4LA + GnRH = 43,8%). However, the P4LA and P4LA+GnRH groups presented lower concentrations of P4 on D9 (P4D9, ID = 8.4 ± 3.1 ng/ml; P4LA = 5.3 ± 3.9 ng/ml; P4LA + GnRH = 4.2 ± 2.5 ng/ml; P = 0.03) and higher on D11 (P4 TAI, ID = 0.7 ± 0.2 ng/ml; P4LA = 1.4 ± 0.7 ng/ml; P4LA + GnRH = 0.9 ± 0.3 ng/ml; P = 0.04). The ovulation rate was lower in the P4LA group compared to the other groups (ID = 75%; P4LA = 50%; P4LA + GnRH = 81.2%; P = 0.035). It is concluded that the replacement of intravaginal devices with P4LA, concomitantly with the application of GnRH at the time of TAI, did not interfere with follicular dynamics in Nelore cows.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Reproductive efficiency of Nelore (*Bos indicus*) cows submitted to conventional or early resynchronization programs for timed-AI

Mirela Balistrieri Dias[1], Tainá Lorraine P. Azevedo[1], Paula Renata Cortat[1], Sofia Brandão Calil[1], Marcos Henrique Alcantara Colli[2], Lucas Oliveira e Silva[1], José Renato Gonçalves[3], Laisse Garcia de Lima[3], Roberto Sartori[1,4]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Reproconsult - Produção e Reprodução Bovina (*Tapejara, Paraná, Brazil*)
[3]Estação Experimental Hildegard Georgina Von Pritzelwiltz
[4]University of Wisconsin Madison (*Department of Animal and Dairy Sciences*)

E-mail: mirelabalistrieri@usp.br

The aims were to evaluate the reproductive efficiency of Nelore (*Bos indicus*) cows submitted to resynchronization programs for timed-AI (TAI) and to compare doses of estradiol cypionate (EC; 0.5 *vs* 1.0 mg) in the Rebreed21 (REB) program. Primiparous (n = 253) and multiparous (n = 645) cows were randomly assigned to 3 resynchronization groups: conventional (CON; n = 299), REB with 0.5 (REB0.5; n = 299) or 1.0 mg (REB1.0; n = 300) of EC. All cows received the same protocol for 1st TAI, starting on d-9 with an intravaginal progesterone device (P4; 0.5 g), 2.0 mg estradiol benzoate (EB) and 0.53 mg cloprostenol (PGF). On d-2, 0.53 mg PGF, 300 IU eCG and 1.0 mg EC were given, concomitant with P4 removal and tail chalk for estrus detection. On d0, 8.4 µg buserelin acetate (GnRH) was given and TAI was done. On d12, REB-groups received a P4 device (0.5 g), kept until d19, when 300 IU eCG, 0.5 (REB0.5) or 1.0 mg (REB1.0) EC were given and cows had their tails chalked. On d21, pregnancy diagnosis (PD) was performed in all groups by evaluating the CL blood perfusion using the Doppler-mode ultrasound, and non-pregnant cows in REB- groups received 8.4 µg GnRH and 2nd TAI was performed. On d33, PD was confirmed by checking embryo presence in all groups, and those cows in REB groups inseminated on d21 were resubmitted to the same REB protocol, receiving the 3rd TAI on d42. In all groups, cows diagnosed as pregnant at PD on d21, but non-pregnant on d33 were considered false- positives (FP) and were resubmitted to the same 1st TAI protocol on d33, receiving the 2nd TAI on d42. Thereby, on d42, the last TAI of a 42d-breeding season (BS) was performed. Cows in CON received up to 2 TAI, while cows in REB program received up to 3 TAI. Ultrasound B-mode was performed on d54 and 75, to assess PD, FP and pregnancy losses (PL). Statistical analyses were done by GLIMMIX of SAS ($P \leq 0.05$). On d21, REB1.0 tended to have greater P/AI at 1st TAI than CON (62.7A; 57.2AB; 52.8%B). After 21d of BS, REB1.0 and REB0.5 had greater pregnancy rate (PR) than CON (77.7a; 75.6a; 52.8%b). However, final PR did not differ among groups (84.7; 84.9; 79.9%; respectively), although in REB1.0 and REB0.5 the mean time for pregnancy was shorter than in CON (10.8a; 8.5a; 15.8b d, respectively). Expression of estrus, PR, FP and PL did not differ between REB0.5 and REB1.0. Regardless of EC dose, REB program promoted greater PR than CON after d1 (59.9 [599] *vs* 52.8% [299]), d21 (76.6 *vs* 52.84%), and d42 of the BS (84.8 *vs* 79.9%). There was no difference in FP (13.7 [n = 183] *vs* 7.5% [n = 388]), nor in PL (3.2 [n = 158] *vs* 3.1 [n = 359]) between CON and REB programs. In conclusion, regardless of EC dose, which had no effect on reproductive efficiency, the REB program anticipated and increased the number of pregnancies during a 42-d BS. These findings highlight the potential of this early resynchronization program to enhance reproductive efficiency of Nelore cows. Acknowledgements: FAPESP 2018/03798-7, CAPES, Figueira Farm, Reproconsult, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Reproductive outcomes of Nelore (*Bos indicus*) heifers submitted to different strategies to induce ovulation prior to the timed-artificial insemination protocol

Pedro Henrique Déo[1], Carlos Eduardo Cardoso Consentini[2], Pedro Vinícius Souza Alves[3], Alexandre Barbieri Prata[2], Mauro Meneguetti[2], Thiago Andrade da Silva[3], José Felipe Warmling Sprícigo[1], Leonardo de França e Melo[1]

[1]Universidade Federal de Goiás (*Escola de Veterinária e Zootecnia - UFG*)
[2]GlobalGen Vet Science
[3]Fazenda Nova Piratininga

E-mail: pedro_deo@discente.ufg.br

The study compared strategies to induce ovulation (so-called cyclicity induction protocols; CIP) prior to the timed-artificial insemination (TAI) protocol in 2,012 Nelore heifers (22.5 ± 2.05 mo and 316.1 ± 22.2 kg). At the beginning of the experiment (d-24), heifers were randomly assigned to one of four treatment groups: 1) Control (CON): no CIP treatment; 2) Injectable progesterone (P4; INJ): 150 mg of injectable P4 on d-24; 3) INJ + estradiol cypionate (INJ+EC): 150 mg of injectable P4 on d-24 and 0.6 mg of EC on d-12; and 4) Progesterone implant + EC (IMP+EC): new intravaginal P4 (0.5 g) implant on d-24, followed by 0.6 mg of EC and implant removal on d-12. The TAI protocol was similar for all groups and initiated (d0) with 2 mg of estradiol benzoate, 0.53 mg of cloprostenol sodium (PGF) and insertion of a new 0.5 g P4 implant. On d8, concomitant with P4 implant withdrawal, heifers received another PGF treatment, 0.6 mg of EC and 200 IU of eCG. The TAI was performed on d10 and all heifers received 8.4 µg of buserelin acetate. Presence of corpus luteum (CL) was evaluated on d-24 and d0, as well as expression of estrus at the time of AI. Pregnancy per AI (P/AI) was determined 30 d after AI. All hormones used in this study were from GlobalGen vet science, except the injectable P4 (Ourofino). Statistical analyses were performed using the GLIMMIX procedure (SAS 9.4; $P \leq 0.05$). On d-24, 39.7% (799/2012) of the heifers had CL. Regarding presence of CL on d0, for all heifers (CON = 55.8 [275/493]c; INJ = 74.0 [378/511]^{b}; INJ+EC = 69.5 [349/502]^{b}; IMP+EC = 80.6% [408/506]^{a}) or those without CL on d-24 (CON = 36.4 [106/291]^{c}; INJ = 66.0 [217/329]^{b}; INJ+EC = 63.5 [184/290]^{b}; IMP+EC = 76.2% [231/303]^{a}), IMP+EC promoted the greatest proportion of CL, while INJ and INJ+EC did not differ between each other, and CON group had the lowest proportion of CL. The same response was observed for expression of estrus on d10, which was greater for IMP+EC, intermediate for INJ and INJ+EC and lower for the CON group, considering all heifers (CON = 63.7 [314/493]^{c}; INJ = 71.2 [364/511]^{b}; INJ+EC = 71.9 [361/502]^{b}; IMP+EC = 85.2% [431/506]^{a}) or those without CL on d-24 (CON = 57.4 [167/291]^{c}; INJ = 68.4 [225/329]^{b}; INJ+EC = 68.3 [198/290]^{b}; IMP+EC = 87.1% [264/303]^{a}). Presence of CL on d-24 (56.8 [454/799] *vs* 49.4% [599/1.213]) and on d0 (56.4 [795/1.410] *vs* 42.9% [258/542]) was positively associated with P/AI, as well as expression of estrus (56.3 [827/1.470] *vs* 41.7% [226/542]). There were no differences among CIP on P/AI, although CON group had lower fertility for all heifers (CON = 47.9 [236/493]^{b}; INJ = 53.8 [275/511]^{a}; INJ+EC = 52.4 [263/502]^{a}; IMP+EC = 55.1% [279/506]^{a}) or those without CL on d-24 (CON = 41.1 [120/291]^{b}; INJ = 52.6 [173/329]^{a}; INJ+EC = 49.3 [143/290]^{a}; IMP+EC = 53.8% [163/303]^{a}). In summary, IMP+EC increased the presence of CL on d0 and expression of estrus on d10, although fertility was similar among CIP strategies and greater than CON heifers.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Reproductive outcomes of Nelore (*Bos indicus*) heifers submitted to ovulation induction protocols, prior to a timed-AI protocol based on injectable progesterone or intravaginal implant, associated or not with estradiol

Livia B. Lanza[1], Rodrigo L. O. R. Alves[1], Leuton Barbosa[2], Daniel Lucas M. Vilasboa[3], Luis Otávio P. Lima[3], Pedro C. Menocci[1], Roberto Sartori[1]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Agrobeef Commercial Representation Ltda.
[3]EBO Agropecuária SA, Fazenda São Luiz

E-mail: livialanza@usp.br

Reproductive outcomes were evaluated in Nelore (*Bos indicus*) heifers submitted to ovulation induction protocols, based on progesterone (P4) and estradiol (E2), prior to a timed-AI (TAI) protocol. On D-47 (D0 = onset of a TAI protocol), 626 heifers (12 ± 0.1 mo old, 276.6 ± 14.9 kg of body weight [BW]) were randomly assigned to 4 groups: 1) 2IVP4+EC (n = 150): on D-47, heifers received an intravaginal P4 device (IVP4; 0.5 g, 7d used), which was kept until D-40, when 0.5 mg E2 cypionate (EC) was given. The same protocol was initiated on D-19; 2) IVP4+EC (n = 156): on D-24, heifers received an IVP4 (0.5g, 7d used), removed on D-12, when 0.5 mg EC was given; 3) iP4+EC (n = 155): on D-24, heifers received 150 mg injectable P4 (iP4), and 0.5 mg EC on D-12; 4) iP4 (n = 165): on D-24, 150 mg iP4 was given. On D0, all heifers received the same TAI protocol, starting with an IVP4 (0.5 g), 0.5 mg cloprostenol (PGF) and 1.5 mg E2 benzoate. On D7, the IVP4 was removed and heifers received 0.5 mg PGF, 0.5 mg EC, 200 IU eCG, and tail chalk for estrus detection. On D9, 8.4 µg buserelin acetate was given, followed by TAI. Ultrasound was done on D-47, 0, and 40 for presence of CL and uterine score (UTS; 1: < 15 mm, 2: 15 to 20 mm; 3: > 20 mm). Also, pregnancy diagnosis (P/AI) was done on D40 and 70, and pregnancy loss (PL) was assessed. Logistic regression with PROC GLIMMIX was used for statistical analysis (SAS 9.4; P ≤ 0.05). For BW analyses, heifers were classified as < 310 or ≥ 310kg, considering BW on D0. On D-47, the presence of CL (3.8%) and UTS (2.2 ± 0.1) did not differ among treatments. The presence of CL on D0 was greater in 2IVP4+EC and IVP4+EC, than in iP4, but not differing from iP4+EC, (70.8^{a}, 66.5^{a}, 47.8^{b}, 57.8%^{ab}). Likewise, the UTS on D0 was lower in iP4 (2.7 ± 0.1) than other groups (2.9 ± 0.1). There was no effect of treatment on expression of estrus (93.3%), P/AI on D40 (2IVP4+EC: 37.3, IVP4+EC: 39.1, iP4+EC: 40.7, iP4: 40.0%), D70 (2IVP4+EC: 35.3, IVP4+EC, 36.5; iP4+EC: 37.4, iP4: 37.6%), presence of CL on D40 (34.5%) and PL (2IVP4+EC: 5.4, IVP4+EC: 6.6, iP4+EC: 7.9, iP4: 6.1%). Regardless of treatment, heifers with BW ≥ 310kg had greater incidence of CL on D0 (64.7 *vs* 56.1%) and D40 (39.2 *vs* 30.5%), and greater UTS on D0 (2.9 ± 0.1 *vs* 2.7 ± 0.1) than heifers with BW < 310kg. There was no effect of BW on P/AI or PL. Regardless of treatment, heifers with CL on D0 had greater UTS on D0 (3.0 ± 0.1 *vs* 2.6 ± 0.1), more CL on D40 (45.9 *vs* 20.8%), and greater P/AI on D40 (42.7 *vs* 34.6%) and 70 (40.3 *vs* 32.1%). In conclusion, association of P4 with E2 enhanced uterine development and induced more heifers to ovulate previously to the TAI protocol. Moreover, presence of CL on D0 was associated with greater UTS and P/AI. Despite that, the combination of P4 and E2 before a TAI protocol was not able to influence P/AI or PL. Finally, BW was positively associated to cyclicity and UTS on D0 in 12 mo old Nelore heifers, but no influence on P/AI was detected. Acknowledgements: FAPESP 2018/03798-7, CNPq, CAPES, EBO, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Reproductive outcomes of prepubertal Nelore (*Bos indicus*) heifers submitted or not to ovulation induction protocols prior to a timed-AI protocol

Giselly B. Kolling[1], Rodrigo L. O. R. Alves[1], Ulises Esaú Medina Álvarez[1], David Marinheiro Alves da Silva[1], Leandro Diniz da Cunha[2], Rafael Araújo Braz[2], Maria Eduarda Rocha e Silva[3], Rodrigo Brüner[3], Leonardo de França e Melo[4], Roberto Sartori[1,5]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Norte Agro Artificial Insemination
[3]Tulipa Agropecuária
[4]Universidade Federal de Goiás (*Escola de Veterinária e Zootecnia - UFG*)
[5]University of Wisconsin Madison (*Department of Animal and Dairy Sciences*)

E-mail: gisellykolling@usp.br

The aim was to evaluate reproductive outcomes of Nelore (*Bos indicus*) heifers submitted or not to ovulation induction protocols prior to a timed-AI (TAI) protocol. On D-42, 683 heifers (10.9 ± 1.3 mo old; 267.1 ± 41.1 kg of body weight [BW]) were randomly assigned to 2 groups: no treatment prior to D0 (CON; n = 344), or 2 ovulation induction protocols (2IND; n = 339). Heifers of 2IND received a 0.5 g intravaginal progesterone (P4) device, kept until D-35, when they were treated with 0.5 mg estradiol cypionate (EC; im). On D-21, heifers underwent the same protocol, with addition of 0.53 mg cloprostenol (PGF; im) along with P4 withdrawal, on D-14. All heifers received the same TAI protocol, starting on D0 with the insertion of a 0.5 g P4 device and 1.5 mg estradiol benzoate im. On D7, 0.5 mg PGF, 0.5 mg EC, and 200 IU eCG were given im, concomitant with P4 withdrawal and tail chalk for estrus evaluation. Moreover, On D9, 8.4µg buserelin acetate was given im, and TAI was performed. Ultrasound was performed on D-42, -21, and 0 to assess uterine diameter (UD) and ovarian dynamics, and on D40 and 70 to check for pregnancy (P/AI) and pregnancy loss (PL). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($P \leq 0.05$). For BW analyses, heifers were classified according to terciles of BW on D7 (T1: ≤ 268 [n = 220], T2: 269-305 [n = 218], and T3: ≥ 306 kg [n = 232]). Presence of corpus luteum (CL) on D-21 and 0, and UD on D0 were greater in 2IND (D-21: 60.2 *vs* 3.5%; D0: 62.2 *vs* 16.6%; UD: 13.9 ± 0.1 *vs* 13.6 ± 0.1 mm), as well as estrus (75.2 *vs* 66.3%), than CON. Heifers with CL on D0 had greater expression of estrus (81.0 *vs* 64.1%), P/AI on D40 (48.9 *vs* 31.6%) and 70 (46.3 *vs* 26.5%), and lower PL (5.3 *vs* 16%). Also, P/AI on D40 was greater in heifers expressing estrus (44.7% [n = 483] *vs* 23.0 [n = 200]) and D70 (40.8 *vs* 18.5%), but there was no detectable effect on PL with or without estrus (8.8 *vs* 19.6%). An interaction was observed between treatment and presence of CL on D0. In heifers without CL, 2IND resulted in lower P/AI than CON (CL: 16.9 *vs* 38.3%), but in heifers with CL there was no difference (CL: 46.5 *vs* 57.9%). Eventually, a subgroup of subfertile heifers may have been created after not responding to treatments for induction of ovulation. Because of that, P/AI on D40 was greater in CON than 2IND (41.6 *vs* 35.1%), but not different on D70 (35.8 *vs* 32.7%). No difference was detected on PL (14.0 *vs* 6.7%). Regardless of treatment, there was effect of BW tercile (T1, T2, and T3) on estrus (58.2^{b} *vs* 73.4^{a} *vs* $80.6\%^{a}$), P/AI on D40 (24.6^{b} *vs* 39.9^{a} *vs* $50.9\%^{a}$) and 70 (20.9^{b} *vs* 35.3^{ab} *vs* $46.6\%^{a}$) respectively, with no effect on PL. P/AI on D40 was linearly affected by BW and age, since the greater the BW and age, the greater was P/AI. In conclusion, although the ovulation induction protocol had increased presence of CL, UD and expression of estrus, it was not able to improve P/AI compared to untreated heifers. Acknowledgements: FAPESP 2018/03798-7, CAPES, Tulipa Agrop., Norte Agro, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Resynchronization protocol with a 28-day interval between FTAI in Nelore (*Bos indicus*) heifers and cows

Gabriel Cunha Cruz[1], David Bueno Lourenço[1], Paulo Patente de Almeida Brandão[2], Sofía Albertini[3], Laís Ângelo de Abreu[3], Pietro Sampaio Baruselli[3]

[1]Gabriel Cunha Reprodução Animal
[2]Nelore Katispera
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: gabriel@criafertil.com.br

This study aimed to evaluate the efficiency of an early resynchronization protocol on pregnancy rate and pregnancy losses of the first and second FTAI in Nelore heifers and cows. A total of 2138 females [heifers (n = 846) and cows (n = 1292)] were randomly distributed into one of the following groups: 1) Resynch18 (n = 1065): initiating the resynchronization protocol 18 days after the first FTAI (D18), and 2) Resynch28 (n = 1067): initiating the resynchronization protocol 28 days after the first FTAI (D28). On D18, females from the Resynch18 started the resynchronization protocol without a pregnancy diagnosis. Animals received an intravaginal P4 device (P4D; Reproneo®, GlobalGen) and an application of estradiol benzoate (EB; 1 mg for heifers and 2 mg for cows; Syncrogen®, GlobalGen). On D26, the P4D was removed, and pregnancy diagnosis (PD) by ultrasound mode B was performed. Non-pregnant females received 0.530 mg of cloprostenol (PGF; Induscio®, GlobalGen), eCG (200 IU in heifers and 300 IU in cows; eCGen®, GlobalGen), and 1 mg of estradiol cypionate (EC; Cipion®, GlobalGen). Animals were inseminated 48 hours later. Females from the Resynch28 group were scanned via ultrasound mode B on D28, and females diagnosed as non-pregnant started the resynchronization protocol receiving 0.530 mg of PGF (Induscio®, GlobalGen), a P4D, and 2 mg of EB. The P4D was removed after 8 days (D36), receiving 0.530 mg of PGF (Induscio®, GlobalGen), eCG (200 IU in heifers and 300 IU in cows; eCGen®, GlobalGen) and 1 mg of EC (Cipion®, GlobalGen). Animals were inseminated 48 hours later. At P4D removal, a subset of animals (n = 1093) was painted with chalk on their tailheads as an estrus indicator. PD was performed by US 30 and 60 days after FTAI. The model used for this work was a binomial logistic model with a logit link function fitted using PROC GLIMMIX in SAS 9.4, with a significance level of $P \leq 0,05$. There was no effect of treatment on P/IA of the first FTAI [Resynch18 = 43.0% (457/1073) *vs* Resynch28 = 41.0% (437/1065); P = 0.46], showing no effect of Resynch18 on the previous FTAI. Also, there was no effect of the resynchronization protocols on the P/AI of the second FTAI [Resynch18 = 44.5% (247/554) *vs* Resynch28 = 45.7% (259/566); P = 0.69]. Furthermore, the resynchronization protocol did not affect the pregnancy loss (PL) of the first FTAI. [Resynch18 = 6.0% (27/457) *vs* Resynch28 = 5.2% (23/437); P = 0.67]. Also, the PL of the second FTAI was similar between protocols [Resynch18 = 4.8% (12/247) *vs* Resynch28 = 6.1% (16/437); P = 0.51]. Furthermore, there was no effect observed for the estrus rate according to the resynchronization protocol [Resynch18 = 71.6% (387/540) *vs* Resynch28 = 68.0% (376/553); P = 0.18]. It is concluded that the early resynchronization protocol in Nelore females using estradiol benzoate on D18 presents the same efficiency as the conventional resynchronization protocol. However, the new Resynch18 reduces days between FTAI, improving the herd's reproductive efficiency.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Simple and low-cost protocols, using estradiol esters and prostaglandin $F_{2\alpha}$, to synchronize ovulation in Girolando cows

Beatriz Alves Paulino[1], Mariana Santos Dutra Okada[1,2], Nicolas Moreira Piedras Monnerat Caparelli[1], Maria Eduarda de Melo[1], Mellyssa Sad Rodrigues Gomes[1], Raphael Azevedo Lamim[1], Samuel Rodrigues Bonamichi do Couto[1], Joaquim Esquerdo Ferreira[2], Marco Roberto Bourg de Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro (*UFRRJ*)
[2]Centro Universitário de Valença (UNIFAA)

E-mail: bia.alves0799@gmail.com

The objective of the present study was to compare two short-term protocols for ovulation synchronization, using estradiol benzoate (EB) or estradiol cypionate (EC) as ovulation inducers and prostaglandin ($PGF_{2\alpha}$) in Girolando cows. To this end, two experiments were conducted, the first comparing the follicular dynamics of Girolando cows submitted to the two protocols and in experiment II, the conception rate was evaluated. In this study, only females that had a corpus luteum with a diameter ≥ 14 mm and at least one follicle with a diameter between 8 and 20 mm were selected. The animals were randomly distributed into two treatments: 1) Group EB, which received at the beginning of the protocol (D0), 500 µg of cloprostenol sodium (Sincronize®, Calbos, São José dos Pinhais, Paraná, Brazil) and, 24 hours later (D1), + 2 mg of EB (Estrogin®, BioFarm, Jaboticabal, São Paulo, Brazil), both intramuscularly (IM), with TAI performed 24 hours after EB application (D2); 2) EC Group, which received on D0, 500 µg of cloprostenol sodium + 2 mg of EC (ECP®, Zoetis, São Paulo, Brazil) simultaneously, both via IM, with TAI performed 48 hours after its application (D2). In experiment I, the follicular dynamics of 18 females subjected to two treatments (Group EB, n = 9; Group EC, n = 9) was monitored, where the animals were evaluated at 12-hour intervals, from D0 until the moment of ovulation. The following parameters were compared: Interval between PGF2α application and ovulation ($PGF_{2\alpha}$-OV); Interval between TAI and ovulation (TAI-OV); Dominant follicle growth rate (DFGR); Dominant follicle diameter on Day 0 (DFD0); Dominant follicle diameter on Day 2 (DFD2) and ovulation rate (OR). In experiment II, 172 females were subjected to both treatments (Group EB, n = 85 animals and Group EC, n = 87 animals) with the aim of comparing conception rates. Pregnancy was diagnosed 30 days after TAI using transrectal ultrasound. Quantitative variables were compared by ANOVA while the conception rate was evaluated by the binomial test with 5% significance. In experiment I, no significant difference (P > 0.05) was observed for any of the evaluated parameters: $PGF_{2\alpha}$-OV (EB = 69.42 ± 9.07h *vs* EC = 72.85 ± 18.14h; P = 0. 66); TAI- OV (EB = 21.42 ± 9.07h *vs* EC = 25.71 ± 16.50h; P = 0.56); DFGR (EB = 0.95 ± 0.31 mm/day *vs* EC 0.62 ± 0.51 mm/day; P = 0.11); DFD0 (EB = 9.79 ± 1.85 mm *vs* EC 10.61 ± 1.21 mm; P = 0.28); DFD2 (EB = 11.26 ± 2.22 mm *vs* EC = 11.71 ± 1.95 mm; P = 0.66); OR (EB = 77.8% *vs* EC = 77.8%; P = 1.00). In experiment II, conception rates of 27.1 and 33.3% were observed for the EB and EC groups, respectively (P = 0.37). It is concluded that estradiol benzoate and cypionate are equally efficient in short ovulation synchronization protocols in Girolando cows.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Temperament's impact on the cost per pregnancy and weaned calf of Nelore females subjected to Timed Artificial Insemination

Thiago de Souza Vieira[1], Mellyssa Sad Rodrigues Gomes[1], Samuel Rodrigues Bonamichi do Couto[1], Daniela Cristina Rocha de Freitas[1], Nicolas Moreira Piedras Monnerat Caparelli[1], Maria Eduarda de Melo[1], Raphael Azevedo Lamim[1], Greicy Sofia Maysonnave[1], Rondineli Pavezzi Barbero[1], Marco Roberto Bourg Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro (D*epartamento de Reprodução e Avaliação Animal - UFRRJ*)

E-mail: t.vieira95@hotmail.com

The present study aimed to evaluate the impact of temperament of Nelore females subjected to Timed Artificial Insemination (TAI) on the cost per pregnancy and weaned calf. To this end, 326 Nelore females were selected on a commercial farm located in the interior of the state of São Paulo, in the Southeast region of Brazil. The animals had an average body condition score of 2.93 ± 0.66 and were subjected to temperament assessment through visual observations of the animal in the containment chute and its exit speed, rating on a scale of 1 to 4. Based on the evaluations, the animals were classified as having calm temperament (CAL = score ≤ 2; n = 243) or excitable (EXC = temperament score > 2; n = 83). The females underwent ovulation synchronization protocol, where on a random day of the estrous cycle (D0), a new intravaginal progesterone device (1 g, Primer®, Tecnopec, São Paulo, Brazil) was inserted, associated with 2 mg of estradiol benzoate (Sincrodiol® Ouro Fino, São Paulo, Brazil). The device was removed eight days later and, upon removal, 500 µg of cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) + 300 IU of eCG (Folligon®, MSD, São Paulo, Brazil) + 1.0 mg of estradiol cypionate (D8) (SincroCP®, Ouro Fino Saúde, São Paulo, Brazil) were applied. Two days after device removal (D10), TAI was performed. Gestational diagnosis (GD) and pregnancy loss (PL) were assessed using transrectal ultrasound (Mindray D2200 vet), respectively 30 and 60 days after TAI. The cost per pregnancy at 30 days (CPP30) was calculated considering only the cost of the protocol, based on the following formula [(number of cows submitted to the protocol)*(cost per protocol + labor + semen)]/ (number of pregnant cows). The cost per pregnancy at 60 days (CPP60) was calculated after PL assessment using the same equation described above. Conception rate and pregnancy loss were compared using the Bioestat program with 5% significance. Conception rates at 30 days post TAI were 53% and 34% for the CAL and EXC groups, respectively (P = 0.003), resulting in a CPP30 of R$ 117.93 for the CAL group and R$ 184.15 for the EXC group. The PLs at 60 days for the CAL and EXC groups were 13.28% and 17.85%, respectively (P = 0.52), resulting in a CPP60 of R$ 136.00 for the CAL group and R$ 224.18 for the EXC group. Regarding the weight of calves at weaning, averages of 204.2 and 200.7 kg were observed in the offspring of the CAL and EXC groups, respectively (P = 0.3). In this way, as the main product sold in beef cattle farming is the calf, it was observed in terms of kilograms of calf weaned per cow: 77.5 kg for ADQ and 54.7 kg for EXC. It is concluded that the reactive temperament of females negatively influences conception rates, increasing the cost per pregnancy. Furthermore, if pregnancy losses are considered, the increase in cost per pregnancy is less profitable, in addition to producing less kg of weaned calf per cow, significantly impacting the profitability of the activity.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

The use of GnRH (Gonaxal®) at TAI improves P/AI and the reduction of its dose results in the same pregnancy efficiency as the conventional treatment for *Bos indicus* beef heifers.

Roberta Machado Ferreira[1], Alessandra Mitiko Issaka[2], Emerson Ubiratan Rodrigues Rodel[3], Reuel Luiz Gonçalves[2], João Paulo Mendes Lollato[2], Jose Nélio de Sousa Sales[4], Bruna Lima Chechin Catussi[5], Pietro Sampaio Baruselli[5]

[1]Repro Saran Consultoria Agropecuária e Reprodução Assistida Ltda
[2]Biogénesis Bagó Saúde Animal Ltda
[3]RW Assistência Veterinária
[4]Universidade Federal de Juiz de Fora (*DMV - UFJF*)
[5]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: robertinhavet@yahoo.com.br

It was already shown that using GnRH at TAI is an interesting strategy to improve P/AI in bovine. In the current study, we assessed if the reduction of dose of GnRH could be done in TAI protocols for Nelore heifers with the same efficiency in pregnancy outcomes as with the conventional dose. The study was done during the breeding seasons of 2021, 2022 and 2023 and assigned 694 *Bos indicus* (Nelore) heifers from 3 farms in SP and MT States, Brazil. Heifers were kept in pasture with mineral salt and water *ad libitum.* At D-30, they were treated with 150 mg P4 (Sincrogest® injetável; Ouro Fino Saúde Animal, Brazil) IM and, after 10 to 13 days, 0.5 mg estradiol cypionate (Croni-cip®; Biogénesis Bagó Saúde Animal, Brazil) was given IM to induce cyclicity. On D0, only heifers with a CL and/or uterine maturity were assigned for TAI and received a P4 intravaginal device plus 150 µg D-cloprostenol (Croniben®; Biogénesis Bagó) and 1 mg estradiol benzoate (Bioestrogen®, Biogénesis Bagó) IM. On D8, the device was removed and 150 µg D-cloprostenol, 200IU eCG (Ecegon®; Biogénesis Bagó) and 0.5mg estradiol cypionate were given IM. Heifers were painted with chalk on their tailheads, and removal of chalk was used as an indication of estrus. TAI was done 48h after device removal, concomitant with estrus determination. At that time, heifers that showed or not estrus were homogeneously allocated to receive different doses of buserelin acetate (GnRH; Gonaxal®; Biogénesis Bagó), as follow: Control (0 µg GnRH); Full dose (10.5 µg GnRH = 2.5 ml Gonaxal) or Reduced dose (8.4 µg GnRH = 2 ml Gonaxal). Semen of 7 Nelore and 2 Aberdeen Angus bulls was equally distributed between groups. Diagnosis of P/IA was done 35-50d after TAI. Data was analyzed by logistic regression (PROC GLIMMIX from SAS), creating orthogonal contrasts (C1: control *vs* GnRH; C2: doses of GnRH). Heifers had average 3.07 ± 0.01 BCS, 347.1 ± 3.12 kg and 63.4% of CL on D0. As expected, occurrence of estrus was similar among groups, because GnRH treatment was done after estrus evaluation: Control = 67.7% (157/232), Full Dose GnRH = 69.3% (158/228) and Reduced GnRH = 68.8% (161/234); C1 = 0.71 and C2 = 0.91. Also, heifers that showed estrus had greater P/AI [51.7% (246/476)] than those without estrus occurrence [36.4% (79/218); P = 0.0008], regardless of treatment group. As for P/AI, heifers receiving GnRH (regardless of dose) had greater P/AI than Control heifers (C1 = 0.047) and similar P/AI (C2 = 0.60) was achieved using full or reduce dose of GnRH [Control = 41.8% (92/232), Full dose = 48.5% (110/227) and Reduced dose = 50.4% (118/234)]. The use of full (10.5 µg) or reduced (8.4 µg) dose of buserelin acetate (GnRH; Gonaxal) at TAI equally improved P/AI of Nelore heifers, assuring pregnancy outcomes greater than for control heifers (without GnRH). In conclusion, the reduction of the conventional dose (from 10.5 for 8.4 µg) of buserelin acetate (2 ml Gonaxal) was efficient on improving P/AI in Nelore heifers. Credits: Farms Fazendinha, Cachoeira and Vera Cruz IV.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Treatment with 325 mg of recombinant bovine somatotropin (rbST) at the beginning of the FTAI protocol increases the pregnancy in Holstein heifers

Carlos Alberto Rodrigues[1], Lígia Mattos Rebeis[2], Leonardo Soares Locoselli[2], Marco Rogério Dalalio[3], Sofía Albertini[2], Pietro Sampaio Baruselli[2]

[1]Clínica Veterinária SAMVET de São Carlos Ltda
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Agener União Química

E-mail: carlos@samvet.com.br

This study evaluated the reproductive efficiency of Holstein heifers submitted to a FTAI using 1 or 2 prostaglandins (PGF) in the protocol and evaluated the use of 325 mg of recombinant bovine somatotropin (rbST) at the beginning of the protocol. A total of 874 Holstein heifers located in Descalvado, SP (Agrindus, Brazil) were used. This study was divided into two parts. In the first experiment, 433 Holstein heifers (16.6 ± 0.13 months) were divided into PGF1 (n = 207) and PGF2 (n = 226) groups to adjust one or two treatments with PGF on the protocol, used later in the rbST experiment (Exp 2). At D0, the females received a P4 device (Primer PR®, Agener União) device, 2 mg of estradiol benzoate (EB; RIC-BE®, Agener União), and 25 mg of lecirelin (GnRH; Tec-Relin®, Agener União). On D7, the PGF2 group received 0.530 mg of cloprostenol (PGF; Estron®, Agener União). On D8, the device was removed, and heifers received 0.53 0mg PGF, 0.5 mg of EC (Cipiotec®; Agener União), and tail painting to check estrus. After 48 hours, only animals that demonstrated estrus were inseminated. An ultrasound evaluation (US) was performed at D40 and D70 to confirm the pregnancy. Statistical analyses were performed using GLIMMIX in SAS 9.4. There was no treatment*replication interaction. There was no difference in estrus rate between groups [PGF1 = 84.5% (175/207) *vs* PGF2 = 88.9% (201/226); P = 0.76]. Also, there was no difference in the pregnancy rate on D40 [PGF1 = 40.2% (70/174) *vs* PGF2 = 33.3% (67/201), P = 0.41] and on D70 [PGF1 = 38.5% (67/174) *vs* PGF2 = 32.3% (65/201), P = 0.53]. Pregnancy loss did not differ between groups [PGF1 = 2.9% (2/67) *vs* PGF2 = 3.0% (2/65), P = 0.99]. Based on this result, in Exp 2, only one PGF (D8) dose was administered in the FTAI protocol. In study 2441 Holstein heifers (17.2 ± 0.40 months) were used. At the beginning of the protocol, the animals were divided into two groups: Control (CON; n = 222) and rbST (n = 219). Animals from the rbST group received 325 mg of rbST on D0. As in Exp 1, only animals that demonstrated estrus were inseminated 48 hours after P4 device removal. An ultrasound evaluation was performed on D40 and D70 to assess pregnancy and pregnancy loss. Statistical analyses were performed using GLIMMIX in SAS 9.4. There was no treatment*replication interaction. There was no difference in estrus rate between groups [CON = 84.2% (187/222) *vs* rbST = 85.4% (187/219); P = 0.77]. Animals that received rbST had a higher pregnancy rate at D40 [CON = 42.4% (79/186) *vs* rbST = 52.6% (98/186); P = 0.02] and D70 [CON = 40.9% (76/186) *vs* rbST = 50.5% (94/186); P = 0.03]. There was no difference between treatments in pregnancy loss [CON = 3.9% (3/76) *vs* rbST = 3.1% (3/94), P = 0.89]. In conclusion, administering 1 dose of PGF is sufficient to synchronize Holstein heifers undergoing an 8-day synchronization protocol. Furthermore, the administration of 325 mg rbST at the beginning of the synchronization protocol increases the pregnancy rate of Holstein heifers submitted to FTAI.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Use of injectable progesterone associated with hCG for synchronization/induction of estrus in Saanen goats in the transition and anoestrus reproductive seasons

Juliana Dantas Rodrigues Santos[1], Milena Luzório Simões[1], Ana Paula Pereira Schmidt[1], Sandra Ribeiro da Silva[1], Caio Cezar de Carvalho Cunha[1], Aline Matos Arrais[1], Brenda Barbosa Martins[1], Paulo Sérgio Cerqueira Rangel[2], Felipe Zandonadi Brandão[3]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - UFF*)
[2]Universidade Federal Fluminense (*Faculdade de Veterinária - UFF*)
[3]Universidade Federal Fluminense (*Setor de Reprodução Animal - UFF*)

E-mail: julianadantas_rodrigues@hotmail.com

Intravaginal devices impregnated with progesterone or analogues, commonly used in hormonal protocols for synchronization/induction of estrus in goats, can cause vaginitis, harm fertility as well as the female's welfare. This study evaluated a protocol based on the use of injectable progesterone (IP4) associated with hCG in Saanen goats in the transition (January) and anestrus (September). In both periods evaluated, 30 Saanen goats (transition period - bodyweight: 30.7 ± 2.2; age: 297.0 ± 12.0 days old; anestrus period - bodyweight: 38.2 ± 6.2; age: 361.0 ± 26.7 days old; mean ± SD kg) were divided into two groups: Gcontrol (n = 15) and Gtreated (n = 15). Gcontrol animals received a sponge impregnated with medroxyprogesterone acetate (60 mg; Progespon®, Zoetis, Campinas, Brazil), which remained in situ for six days, 24h before removal of the sponge, were administered eCG (200 IU i.m.; Novormon®; Zoetis, Campinas, Brazil) and cloprostenol sodium (0.24 mg i.m.; Estron®, Agener União, São Paulo, Brazil). Twenty-eight hours after withdrawn of the sponge, the females received lecirelin (25 µg i.m.; TEC-Relin®, Agener União, São Paulo, Brasil). Gtreated animals received IP4 (20 mg, i.m.; Progecio®, União Química, São Paulo, Brazil) on day 0 and hCG (100 IU, i.m.; Fertcor®, Ceva, Paulínia, Brazil) 24h later. The animals were subjected to natural mating every 12h from sponge removal and application of hCG. Follicular development was monitored by ultrasound assessments at the same times as mating. Data were analyzed with general linear model to verify interaction between treatment and reproductive season. Non-parametric data were analyzed by Mann-Whitney test and for parametric data, T-test was applied. Frequencies were assessed by Fisher's exact test. In the transition period, Gtreated had a longer application interval from IP4 to estrus (78.4 ± 3.8 *vs* 43.4 ± 4.0h, respectively; $P < 0.0001$) when compared to the control (from sponge removal to estrus). In the second period (anestrus), there were no differences between treatments for any variable analyzed. In the comparison between reproductive seasons, the anestrus period had a longer estrus duration in Gcontrol compared to Gcontrol in the transition period, (84.0 ± 11.7 *vs* 46.1 ± 5.7h, respectively; $P = 0.011$), both groups of transition season had larger pre-ovulatory follicle diameters in relation to the groups in anestrus (transition period, Gcontrol: 7.53 ± 0.22, Gtreated: 7.24 ± 0.21 *vs* anestrus, Gcontrol: 5.89 ± 0.24, Gtreated: 5.88 ± 0.18 mm, $P < 0.0001$). In conclusion, the protocol based on injectable progesterone and hCG is efficient for inducing synchronized estrus in Saanen goats in both reproductive seasons.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

IATF/IA

Use of recombinant human FSH in TAI protocols: Preliminary results

Rodrigo Martins de Moura[1], Aline Silva Mendes Teixeira[1], Luiz Gustavo Bruno Siqueira[2], Carlos Antônio de Carvalho Fernandes[3], Emanuel Doroteu[1], Ricardo Alamino Figueiredo[2], João Henrique Moreira Viana[2]

[1]Universidade de Brasília (UnB)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[3]Universidade Alfenas *(UNIFAL)*

E-mail: rmoura241@gmail.com

A number of timed artificial insemination (TAI) protocols, particularly those develop for cows under postpartum anestrus, use eCG to stimulate follicular growth and increase ovulation rate and the of the subsequent corpus luteum (CL). The current trend in animal reproduction, however, is to replace hormones obtained by purification from biological samples by synthetic analogues. Reference doses, however, still need to be determined for each hormone and protocol, aiming at stimulating follicle growth but avoiding double or multiple ovulations. In the current study we tested two doses of a recombinant human FSH (rhFSH) in an ovulation synchronization protocol. Cycling, non-lactating Nelore cows with an average 3.0 ± 0.1 BCS received the same TAI protocol (2 mg estradiol benzoate and 1 g progesterone implant on day 0, and implant removal, 0.5 mg cloprostenol sodium and 1 mg estradiol cypionate on day 8), and were randomly allocated into three groups, which received on day 8: a) no stimulus (control group, G0, n = 15); b) 3.5 mcg rhFSH (G3.5, n = 13); or c) 6.5 mcg rhFSH (G6.5, n = 15). The size of the largest follicle was measured by rectal ultrasonography at days 8 and 10, and the number of CL formed was evaluated on day 17. Data was analyzed using the Mixed model of the SAS software, and a P-value < 0.05 was considered as significant. There was no difference among groups G0, G3.5 or G6.5 in the of the dominant follicle at days 8 (9.3 ± 0.1, 9.4 ± 0.1 and 9.2 ± 0.1 cm; respectively; P > 0.05) or 10 (11.6 ± 0.1, 11.7 ± 0.1 and 11.5 ± 0.0 cm; respectively; P > 0.05), or on ovulation rate (86.7%, 92.3% and 100.0%, respectively; P > 0.05). However, we observed a greater incidence of codominance (0.0%^{a} and 0.0%^{a} *vs* 26.7%^{b}, P = 0.03) and average number of ovulations (0.9 ± 0.1^{b}, 0.9 ± 0.1^{ab} and 1.3 ± 0.2^{a}, P = 0.03) in the group G6.5, compared with G0. Our results suggest that the target dose of rhFSH to be used in TAI protocols should be lower than 6.5 mcg to avoid double or multiple ovulations and, consequently, double pregnancies.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Effect of corpus luteum area and blood perfusion on the conception rate in buffalo recipients

Julio Cesar Barboza da Silva, Alessandra Bridi[1], Amanda Guimarães da Silva[2], Rodrigo Camponogara Bohrer[3], Gabriela Lamberti Murphy[4], Walter Alexandre Bovi Binotti[5], Guilherme Pugliesi[2], Felipe Perecin[6]

[1]Universidade do Oeste de Santa Catarina (*UNOESC*)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Geneal
[4]Oklahoma State University (OU)
[5]Fazenda Sesmaria
[6]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*).

E-mail: julioveterinario@hotmail.com

The objective was to evaluate the relationship between the corpus luteum (CL) blood perfusion (CLBP) determined by Doppler ultrasound and CL area (CLAREA) on day of embryo transfer (ET) in bubaline species on the pregnancy diagnosis. We hypothesised that the pregnancy rate is positively associated with CLBP in bubaline females. For this, a retrospective analysis was performed using data from 64 heifers and 71 cows subjected to an E2/p4-based timed-ET protocol (D0=beginning of ET protocol). On D18, all females were evaluated by transrectal B-mode to estimate the CLAREA and with Doppler ultrasonography (MyLabDelta, Esaote) to estimate the CLBP (subjective evaluation of colour signals on a scale of 35–100%, 5 points between degrees). After the ultrasound evaluation, females were randomly splited to receive a vitrified embryo (VT) or a direct transfer embryo (DT). Females with CLBP ≤30% did not received an embryo. Pregnancy check was performed at 30 and 60 days of pregnancy. A receiver operating characteristic (ROC) analysis was performed to determine a cutoff point for classifying the CLBP in low (≤45%; n = 20 heifers and 49 cows) or high (>45%; n = 44 heifers and 86 cows) and for classifying the CLAREA in small (<1.96cm²; n= 28 heifers and 33 cows) or large (≥1.96 cm²; n= 36 heifers and 38 cows). The pregnancy rate was analysed by the PROC GLIMMIX considering the main effect of CLBP class, CLAREA class, embryo, parity order, and their interaction or by PROC LOGISTIC of SAS software. The ROC curve indicated that the CLBP was a better (P=0.05) predictor of pregnancy than CLAREA in bubaline females. The pregnancy rate at 30 and 60 days were not affected by CLAREA class, embryo, parity order, and their interaction (P>0.1). However, was observed that the pregnancy rate at 30 and 60 days were greater (P≤0.05) in females with a high CLBP [43.0%, 37/86 and 39.5%, 34/86] than females with a low CLBP [22.4%, 11/49 – no embryo/fetal loss]. Logistic regression analysis indicated a tendency (P=0.08) to a linear positive relationship between the CLBP on ET and the probability of pregnancy at 30 days. In conclusion, bubaline females with a CLBP >45% on D18 tended to be more likely to be pregnant at pregnancy diagnosis at 30 days. Also, CLBP at the time of ET proved to be a better predictor of pregnancy than CLAREA. Acknowledgement: Sesmaria ranch, ABS e Boheringer Ingelheim.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Effect of days postpartum on the pregnancy risk of *in vitro* produced embryos in crossbred zebu beef cows in Paraguay

Sebastian Cardozo[1], Felipe Mendoza[2], María Paz Benítez Mora[2], Leticia Garcia[3], Osvaldo Bogado Pascottini[4]

[1] Department of Boquerón- Paraguay, centro de Reproducción Animal
[2]Faculty of Veterinary Sciences, National University of Asunción
[3]
[4]School of Veterinary Medicine, University College Dublin, Belfield, Dublin , Ireland

E-mail: dr.sebastiancardozo@gmail.com

We aimed to evaluate the effect of days postpartum at the time of transfer of i*n vitro* produced embryos on the conception risk of crossbred zebu beef cows. A total of 2,176 clinically healthy postpartum crossbred zebu beef cows from a single pasture-based beef farm in the Chaco region of Paraguay were subjected to an estrous synchronization protocol. Briefly, on day -10, after evaluating the ovarian structures via transrectal ultrasonography (Mindray DP-2200®), intravaginal devices with 700 mg of progesterone (Dispocell®) and 2 mg of IM estradiol benzoate (Von Franken™) were administered. Eight days later, the progesterone devices were withdrawn, and 400 IU eCG (Ecegon®) and 0.250 mg D-cloprostenol (Dextrogenol®) were administered IM. Day 0 was considered the day of ovulation. On day 7, vitrified-warmed *in vitro* produced embryos were transferred into the uterine horn ipsilateral to the ovulation by a single operator. While the presence of a CL was determined via transrectal palpation, its diameter was not measured. Pregnancy was diagnosed via ultrasonography on day 30. Univariable logistic regression models were used to fit the effects of days postpartum at embryo transfer (<60 (n = 1,109) vs. ≥60 days (n = 1,067)), ovarian structure on day -10 (CL yes (n = 178) vs. CL no (n = 1,998)), and BCS on day -10 (<3.5 (n = 505) vs. ≥3.5 (n = 1,671); on a scale from 1 to 5) on the pregnancy risk, using the cow cohort synchronization batch as a random effect. Results: Days postpartum at embryo transfer ranged from 48 to 61 days, with an average (± standard deviation) of 59.5 ± 7.3 days. The pregnancy risk was 50.6 ± 0.2% in cows that received an embryo at <60 days postpartum, which was not different (P = 0.93) from cows that received an embryo at ≥60 days postpartum (50.8 ± 0.2%). Similarly, the presence of a CL at the beginning of the synchronization protocol did not influence pregnancy risk (CL yes, 53.2 ± 0.3% vs. CL no, 50.5 ± 0.1%; P = 0.49). However, the pregnancy risk of cows with a BCS <3.5 (46.5 ± 0.2%) was lower (P = 0.05) than that of cows with a BCS ≥3.5 (52.2 ± 0.1%). In well-managed, pasture-based crossbred zebu beef cows, embryo transfer can be performed as early as ~48 days postpartum without negatively affecting fertility.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Factors affecting efficiency of artificial insemination or transfer of *in vitro* produced embryos in dairy herds

Caroline Cervi Vandresen[1], Lucas O. e Silva[1,2], Ana Luíza Müller Lopes[1], Rafael Luiz Stolf[1], Guilherme Correa de Sousa Pontes[4], Roberto Sartori[1,3]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Department of Animal Science, Texas A&M University
[3]Department of Animal and Dairy Sciences University of Wisconsin Madison
[4]Rehagro, Belo Horizonte, MG, Brasil

E-mail: carolinecerviv@usp.br

The aim was to evaluate factors affecting reproductive efficiency of dairy herds submitted to artificial insemination (AI) or transfer of *in vitro* produced embryos (ET). Retrospective data from 10 herds using both strategies between 2018 and 2023 were analyzed. Data from heifers (AI=18,681, ET=5,341) and lactating dairy cows (AI=42,036, ET=9,120) were used. The data set used only sires and inseminators with at least 100 repetitions. Pregnancy per AI or ET (P/AI, P/ET; 28-40d) and pregnancy loss (PL; between first pregnancy diagnosis and calving) were evaluated by biotechnology (AI *vs* ET); type of service (in estrus [ES] or synchronization [SYNC]); and breed composition, according to the proportion of Holstein genetics (pure-bred [HO]; >½HO; ≤½HO). The effect of type of semen (sexed [SEX] vs conventional [CONV]) was evaluated only within AI. In addition, cows were evaluated according to parity (1 [PRI], 2 [SEC] or ≥3 [MUL]). Data were analyzed by GLIMMIX (SAS; P≤0.05). For all models, farm, sire, and inseminator were used as random effects. P/ET was greater than P/AI (41.4 *vs* 37.9%) in heifers. However, with ET, heifers had greater PL (35.7 *vs* 18.4%). Type of service did not affect P/AI or P/ET, but PL was greater in heifers submitted to SYNC than ES (26.2 *vs* 18.0%), regardless of biotechnology. In heifers served by AI, CONV semen had greater P/AI than SEX (40.1 *vs* 35.8%). Type of semen did not affect PL. There was no main effect of breed on fertility, but a tendency for interaction breed*biotechnology was observed: P/ET was greater than P/AI in ≤½HO (42.1 *vs* 36.5%) and HO (37.0 *vs* 35.7%), but not in >½HO (42.1 *vs* 39.6%). For both biotechnologies, PL was lower in ≤½HO than >½HO and HO (AI: 15.2^{b} *vs* 18.7^{a} *vs* 18.7%^{a}; ET: 30.4^{c} *vs* 38.2^{b} *vs* 42.3^{a}%). For cows, there was no difference between P/AI and P/ET (36.4 *vs* 33.9%), but PL was lower for AI than ET (34.2 *vs* 47.7%). Regardless of biotechnology, cows undergoing SYNC had greater fertility than ES (36.9 *vs* 32.8%). Moreover, PL was greater for cows receiving ET in ES than SYNC (56.1 *vs* 47.3%), without influence of type of service on PL for cows bred by AI (33.6 *vs* 34.4%). Although SEX semen achieved greater P/AI (38.3 *vs* 35.7%), it resulted in greater PL than CONV (37.3 *vs* 33.1%). Concerning parity, PRI had the greatest fertility (36.9^{a} *vs* 35.3^{b} *vs* 34.6^{c}%), and PL was lower in PRI than SEC and MUL (AI: 33.1^{b} *vs* 35.3^{a} *vs* 35.2^{a}%; ET: 46.0^{b} *vs* 49.1^{a} *vs* 50.4^{a}%). Regarding breed, ≤½HO had greater P/AI than >½HO and HO (41.9a vs 37.4b vs 31.5c%), but only differed from HO for P/ET (37.7a vs 35.1ab vs 32.5b%). PL was lower in ≤½HO, and greater in HO for AI (31.6^{c} *vs* 34.5^{b} *vs* 36.5^{a}%) and ET (40.6^{c} *vs* 45.9^{b} *vs* 50.6^{a}%). This study enhances the understanding of the use of AI vs ET in dairy cattle and highlights the impact of several factors on reproductive efficiency. Moreover, this study raises concerns especially related to how IFV is being used in dairy cattle operations. Ack: CAPES; Rehagro.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Flaxseed oil-based supplement alters circulating progesterone in beef cows during a superovulation protocol

Kendal Green[1], Franklin Even[1], Derrick Goldammer[1], Hunter Haberman[1], Riley Puck[1], Ethan Schlegel[2], Zachary Smith[1], Ana Clara Menezes[1], Jessica Nora Drum[1]

[1]South Dakota State University (*Department of Animal Science - DSU*)
[2]New Generation Supplements

E-mail: kendal.green@jacks.sdstate.edu

Flaxseed oil contains high levels of Omega-3 fatty acids which have been shown to positively affect reproductive performance. Our objective was to determine the effects of a flaxseed oil-based supplement (FlaxLic®, New Generations, SD) on embryo development *in vivo* in beef cows. Crossbred Angus cows (n=22, BW=690.7kg±46.2kg), selected by total follicle count (≥15), in a cross-over design, were randomly assigned to two supplementation treatments: free-choice commercial lose mineral supplement (CON; n=11) or flaxseed oil- based supplement (FLAX; n=11). Cows were supplemented for an average adaptation period of 35 days. During the day, cows were housed in individual stalls with a once-daily total mixed ration (TMR) consisting of corn silage and dried distiller grains plus soluble (DDGS) and ad libitum access to supplements and water. At night, cows were kept in group pens with ad libitum access to water and grass hay. Between replicates, cows had a washout period of 35 days. After the adaptation period, all cows were enrolled in a 7-day co-synch pre-synchronization protocol. After the pre-synchronization (7 days later) all cows started on a superovulation (SOV) protocol. On d0, GnRH (100mcg gonadorelin; Factrel, Zoetis, Parsippany, NJ) was administered and a P4 implant (1.38g, Eazi-Breed CIDR, InterAg, Hamilton, New Zealand) was inserted. A total of 300mg of FSH (FSH; Folltropin V, Vetoquinol, TX) was administered over 4.5 days starting on d3, twice a day 12hs apart in decreasing doses. PGF (25mcg dinoprost; Lutalyse HighCon, Zoetis, Parsippany, NJ) was given on d7 and d8. Concomitant with artificial insemination (AI) on d9, cows received 2 doses of GnRH. AI was performed on the morning and evening of d9, using 2 straws each time. Transrectal ultrasound ovarian scans were performed to analyze follicular development, ovulation rate, and CL presence on d7, d11, and d16 respectively. Blood samples were collected on d0, d7, d9, and d16 for P4. Embryos were flushed on d16-16.5 after AI using non-surgical standard techniques. Recovered embryos were staged and graded according to the International Embryo Technology Society (IETS). P4 was analyzed by radioimmunoassay (RIA, MP Biomedicals, Irvine, CA). Statistical analysis was performed using SAS 9.4. Comparing FLAX vs CONT, ovulation rate (71%±15% vs 65%±26%; p=0.87), CL count at collection (20.74±2.12 vs 20.53±2.41; p=0.82), the total number of embryos (4.06±6.3 vs 4.61±7.30; p=0.68) and P4 concentrations at the beginning of SOV (2.29±0.80 vs 1.69±0.30 ng/ml; p=0.56) were similar between groups. However, P4 at AI was significantly lower (0.23±0.06 vs 0.57±0.14 ng/ml; p=0.04) in FLAX cows and higher at collection day (17.08±2.09 vs 13.14±2.60 ng/ml; p=0.04). In conclusion, these findings did not show evidence that the flaxseed oil-based supplement affects early embryo development, however, it positively affected the endocrine environment during two key points of a SOV protocol.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Impact of Angus x Nelore recipient category on P/ET and pregnancy loss

Emanuele Almeida Gricio[1], Bruna Lima Chechin Catussi[1], Bruno Silva do Espirito Santo[2], Sofía Albertini[1], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Agropecuária Jacarezinho

E-mail: griciomanu@gmail.com

The objective of this study is to assess the factors affecting embryonic survival following fixed-time embryo transfer (FTET). In this retrospective study, a total data of 1,828 Angus x Nelore recipients (Heifers = 483; Primiparous = 850; Multiparous = 495), already vaccinated against reproductive diseases, with body condition score (BCS) 2.96±0,01 from a commercial farm (Agropecuária Jacarezinho, Brazil) were used. The analyzed variables were categories (heifer, primiparous, and multiparous), the embryo (fresh vs. vitrified), and BCS. The model used was a binomial logistic model with a logit link function, fitted using PROC GLIMMIX in SAS with a significance level of □≤0.05. No interaction between category and embryo were observed for P/TE at 30 days (P=0.30), 60 days (P=0.19), or pregnancy loss (P=0.47). Furthermore, no interaction between category and BCS were observed for P/TE at 30 days (P= 0.52), 60 days (P=0.67), or pregnancy loss (P=0.89). There was an effect of the recipient category on P/ET at 30 days [Multiparous: 56.2% (222/395)a vs. Primiparous: 49.2% (293/596)b vs. Heifers: 41.1% (175/426)c; P<.0001] and 60 days [Multiparous: 49.1% (192/391)a vs. Primiparous: 39.1% (229/586)b vs. Heifers: 31.5% (134/426)c; P<.0001]. The pregnancy loss was lower (P=0.0092) for multiparous (11.7%; 26/222)b compared to primiparous (19.0%; (54/284)a and heifers (23.4%; 41/175)a. The P/TE when FTET was performed with fresh embryos were higher compared with vitrified embryos: P/ET at 30 days [Fresh: 59.1% (300/508) vs. Vitrified: 42.9% (390/908); P<.0001] and 60 days [Fresh: 51.9% (263/507) vs. Vitrified: 32.6% (292/895); P=<.0001]. Furthermore, the pregnancy loss was lower when fresh embryos were transferred to recipients [Fresh: 12.00% (36/300) vs. Vitrified: 22.3% (85/381); P=0.0006]. In conclusion, multiparous recipients demonstrate better pregnancy outcomes and lower loss rates than primiparous and heifers. Although embryonic cryopreservation presents considerable logistical and commercial benefits, fresh embryos demonstrate higher P/TE and lower pregnancy loss when compared to vitrified embryos. These results highlight the effectiveness of using multiparous recipients and fresh embryos to improve TET success in Angus-Nelore recipients.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Influence of the site of embryo deposition of cryopreserved embryos by vitrification on conception rates in bovine females

Giovana Andrade Buzolim[1], Arthur Rodrigues Mader[2], Laura Souza Santiago[2], Leticia Prates Martins[3], Maria Isabela Azeredo Silva[4], Juliana de Souza Pinto Pieroni[1]

[1]Centro Universitário Católico do Tocantins (UniCatolólica)
[2]Mader Reprodução Animal
[3]Universidade de Brasília (UnB)
[4]Universidade de São Paulo (USP)

E-mail: giovana_buzolin@hotmail.com

For the embryo transfer technique, veterinarians usually deposit embryos in the cranial portion of the uterine horn ipsilateral to the corpus luteum. However, there is little evidence that the site of transfer influences the conception rate, especially for cryopreserved embryos. With this in mind, the aim of this study was to evaluate the influence of the site of placement of embryos produced *in vitro* and cryopreserved by vitrification on the conception rate of precocious recipients. The oocytes came from Nelore donors aspirated between June and September 2023 and the embryos were produced and vitrified by the same company. The transfers took place in December 2023 on a commercial property located in Tocantins. The recipients were 460 precocious Nelore heifers, induced on D-22 with a second-use 0.5 g single-dose progesterone intravaginal device (Repro one®, Globalgen Vet Science, Jaboticabal-SP, Brazil). The following protocol was used to synchronize ovulation: D-0 2 mg estradiol benzoate, IM (Syncrogen®, Globalgen Vet Science) + 0.5 mg prostaglandin, IM (Induscio®, Globalgen Vet Science) + 0.5 g progesterone intravaginal device (Repro one®, Globalgen Vet Science); on D-8, 0.5 mg prostaglandin, IM (Induscio®, Globalgen Vet Science) + 1 mg estradiol cypionate, IM (Cipion®, Globalgen Vet Science) + 300 IU eCG, IM (eCGen, Globalgen Vet Science) and removal of the progesterone implant. On D17, the recipients were assessed using transrectal ultrasound (SonoScape A5V) to identify the presence of the corpus luteum. The expanded blastocysts were devitrified in the farm's laboratory. The site of embryo deposition was determined according to the ease of uterine manipulation at the time of transfer, which was classified as cranial, referring to the proximity of the uterine-tubal junction (n=233), middle, indicating the middle third of the uterine horn (n=194) or caudal, located in the initial region of the uterine horn ipsilateral to the CL, just after the uterine body (n=33).The diagnosis of pregnancy was made by ultrasound 23 days after the date of transfer. The data was analyzed by logistic regression using Proc Glimmix, SAS. The conception rate was 46% (108/233) for transfers carried out in the cranial portion of the horn ipsilateral to the CL, 41% (80/194) for the middle portion and 36% (12/33) for the caudal portion (p=0.5546). It can be concluded, in this study, the site of transfer of embryos produced *in vitro* and cryopreserved by vitrification did not influence the conception rate of the precocious recipients when transferred in different locations of the uterine horn ipsilateral to the corpus luteum.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Injectable progesterone prior to the fixed-time embryo transfer (FTET) protocol increases the conception and pregnancy rate in Nelore cows

Moises Maximo Rodiruges Rodrigues Jr.[1], Wendell Amaral Nantes de Oliveira[1], Hugo Maranho Júnior[2], Bruno Gonzalez de Freitas[3], Luiz Manoel Souza Simões[4]

[1]MS Reprodução
[2]Agromarisa
[3]Ouro Fino Saúde Animal LTDA
[4]Prenhez Consultoria

E-mail: moises@msreproducao.com.br

The objective of this study was to evaluate the effect of pre-exposition of injectable progesterone (P4i) to the FTET protocol on the service rate, conception rate and pregnancy rate of *Bos indicus* (Nelore) embryo recipient cows. In the study, 476 suckled Nelore cows were divided into two experimental groups [Control (n =251) and P4i (n = 225) Groups] ten days before the start of the FTET protocol. In the Control group, cows received 2.0mg of estradiol benzoate (Gonadiol, Zoetis, Brazil) and an intravaginal device containing 1,9g of progesterone (CIDR, Zoetis, Brazil) on D0. On day 7 (D7), cows received 12.5 mg of Dinoprost (Lutalyse, Zoetis, Brazil). On D9, the device was removed and cows received 1.0 mg of estradiol cypionate (ECP, Zoetis, Brazil) and 300 IU of eCG (Novormon, Zoetis, Brazil). In the P4i group, cows received 150 mg of injectable progesterone (Sincrogest Injectável®, Ouro Fino, Brazil) ten days before the start of the FTET protocol (D-10) and were subjected to the same ovulation synchronization protocol as the Control group. On D17, the presence, and echogenicity of the CL were evaluated, with CLs classified as scores of 1-3(adapted from Thomson *et al.,* 2021) in both groups. FTET was performed on D17 using *in vitro* produced embryos classified as expanded blastocysts. Ultrasonography examinations were performed 23 days after FTET to diagnose pregnancy. Statistical analyses were performed by GLIMMIX procedure of SAS (version 9.3) and binomial variables were presented as percentages. Significant differences were indicated by a probability of $P < 0.05$. There was no difference in CL score on the day of FTET (Control - 1.22 ± 0.03 vs P4i - 1.17 ± 0.03; P=0.3). The service rate was similar between groups [Control - 81.7% (205/251) vs P4i - 84.4% (190/225); P=0.42]. However, the conception rate [Control - 36.6% (75/205) vs P4i - 47.9% (91/190); P=0.02] and pregnancy rate [Control – 29.9% (75/251) vs P4i – 40.4% (91/225); P=0.01] were higher in cows of P4i group. In conclusion, the use of injectable progesterone prior to the FTET protocol increases the conception and pregnancy rate in suckled Nelore cows. Support: Prenhez Consultoria em Pecuária and MS Reprodução.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

The effect of using hCG in recipient goats: Preliminary results

Isabela Rezende Pedrosa Neves[1], Luana Rangel Côrtes[2], Ribrio Ivan Tavares Pereira Batista[3], Mariana Garcia Kako Rodrigues[4], Manoel Carlos Couto Araujo[4], Jeferson Ferreira da Fonseca[5]

[1]Universidade Salgado de Oliveira (UNIVERSO)
[2]Fundação Universidade Federal de Viçosa (*UFLA*)
[3]Universidade Federal Fluminense (*UFF*)
[4]Universidade Federal de Juiz de Fora (*UFJF*)
[5]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*

E-mail: isabelapedrosa.vet@hotmail.com

This study checked the effect of hCG on pregnancy rate of recipient goats. Multiparous acyclic dairy goats (Alpine/Saanen) from second to third parity order (n=40) were subjected to synchronous estrus induction by use of intravaginal sponges (60 mg of medroxyprogesterone acetate; Progespon®, Zoetis, São Paulo, Brazil) for six days plus 200 IU of equine chorionic gonadotropin (eCG; Sincro-eCG 6000®; Ouro Fino, Cravinhos, Brazil) and 131.5 µg of cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, Brazil) i.m. 24 h before sponge removal. Estrus was monitored twice daily with fertile males (D0 = Day of onset of estrus). Goats in estrus were assigned to receive 0.3 mL of saline (G-Control, n=12, body condition scores 3.5 ± 0.1, 1 to 5 variation - Detweiler *et al*., Annual Goat Field Day 23:127-130, 2008) or 300 IU human chorionic gonadotropin (hCG, Ferticor®; Hertape- Calier do Brasil Ltda, São Paulo, Brazil) (G-hCG, n=15, body condition scores 3.5 ± 0.1) i.m. at the time of embryo transfer (D7). A total of 10 donor dairy goats (Alpine/Saanen) from second to forth parity order and with body condition score from 3.5 to 4.0 were subjected to superovulation and nonsurgical embryo collection seven days after estrous onset (Fonseca *et al.*, Biopreservation and Biobanking 20:493-501, 2022). Embryos were selected and equally assigned according developmental stage and quality to both groups and non- surgically transferred in pairs to the uterine horn ipsilateral do corpora lutea checked by B- mode and color Doppler transrectal ultrasonography transrectal ultrasonography (Moraes *et al.*, Small Ruminant Research 192:106215, 2020). Pregnancy rates were checked by transrectal ultrasonography 40 days post estrus (33 days after embryo transfer). Statistical analysis was performed using BioEstat 5.3 (Belém, Brazil). Pregnancy rates were compared by Fisher's exact test at 5% minimum level of significance. Overall pregnancy rate was 63.0% (17/27), being 58.3% (7/12) and 66.7% (10/15) for G-Control and G-hCG, respectively (P > 0.05). Preliminary results showed an interesting pregnancy rate obtained by non-surgical technique while hCG positive effect remained to be checked in large trials in dairy goats. Financial support: CNPq (4039092021-0 and 303727/2021-7) and FAPEMIG (BPD-00308-22 and APQ-00448-24).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

TETF/TE

Uterine tone: a neglected criterion for the selection of bovine embryo transfer recipients

Cristiano Rodrigues Borges Guimarães[1], Matheus Felipe Seber[1], José da Páscoa Nascimento Neto[2], Carlos Antônio de Carvalho Fernandes[3], Éder Pereira Campos Drumond Rodrigues[2], Bárbara Azevedo Pereira Torres[2], João Henrique Moreira Viana[4], Miller Pereira Palhão[2]

[1]Médico Veterinário autônomo
[2]Universidade Federal de Lavras (*DMV/FZMV*)
[3]Biotran Assessoria e Consultoria em Medicina Veterinária LTDA
[4]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*

E-mail: cristianorbguimaraes@gmail.com

This study examined how corpus luteum (CL) characteristics assessed through ultrasound (luteal tissue area and blood flow) or rectal palpation, uterine tone, and plasma progesterone (P4) concentration on Day 7 relate to pregnancy outcomes in bovine embryo recipients. Crossbred embryo recipients, aged 2 to 5 years with an average body condition score (BCS) of 3.5±0.5, were enrolled. On Day-11, animals were given an intravaginal P4 device (Prociclar, 0.75 g; Hertape Calier) and 2 mg estradiol benzoate (Benzoate HC; Hertape Calier). On Day -3, the P4 device was removed, and 150 mg of (D+) sodium cloprostenol (Veteglan Luteolítico, Hertape Calier) was administered. On Day-2, 1 mg of estradiol benzoate was used for ovulation synchronization. Ovulation was expected on Day 0. On Day 7, 163 recipients underwent ultrasonography and rectal palpation to assess ovaries and uterus. Subjective scores (1-3 scale) were assigned for CL, area and blood flow, and uterine tone (turgid, semi-flaccid, flaccid). Sonographic exams used a portable device with a 7.5 MHz rectal probe (Mindray M5, Shenzhen, China). Blood samples were collected for P4 analysis. Each recipient then received a grade I frozen-thawed *in vivo*-produced blastocyst, transferred to the uterine horn ipsilateral to the CL. On Day 35, recipients underwent pregnancy diagnosis (DG35) via ultrasonography. Results were compared with assigned scores for CL and uterine tone. Objective variables, such as luteal tissue area, CL blood flow area, and Day 7 P4 concentrations, were analyzed using the Glimmix procedure. A binary logistic regression model (Proc Logistic) investigated associations between pregnancy outcome (pregnant or non-pregnant) and factors like CL characteristics, uterine tone, and their interactions. Statistical significance was set at probabilities below 5%, with probabilities between 5% and 10% considered approaching significance. All analyses were performed using SAS Studio on the SAS on-demand platform. We found a significant ($p<0.02$) interaction between corpus luteum (CL) and uterine tone, indicating a higher likelihood of pregnancy for recipients bearing a large CL among those with turgid uterine tone. There was a gradual escalation in the likelihood of pregnancy observed in recipients exhibiting turgid uterine horns (0.08, 0.28, and 0.64). Nevertheless, for recipients with semi-flaccid uterine horns, the CL did not influence the probabilities of pregnancy (0.47, 0.48, and 0.48, for small, average, and large, respectively). Ultrasound assessment of the CL using B-mode and Doppler-mode did not significantly predict pregnancy rates on Day 35 ($p<0.6$ and $p<0.5$, respectively). However, logistic regression analysis suggested a trend towards a quadratic effect ($p<0.08$ and $p<0.06$), indicating that pregnancy probability varied with the area of luteal tissue and P4 concentrations, respectively. No significant ($p>0.05$) association was found between pregnancy probability and CL blood flow area. In summary, uterine tone before embryo transfer may indicate successful recipient synchronization. Elevated P4 levels, as assessed by CL, may counteract uterine contractility, reducing adverse effects. Moreover, CL may be more crucial than vascularization when evaluating recipients 7 days post-ovulation.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

3D Culture with CellFate® membrane associated with the addition of melatonin increases *in vitro* production of bovine embryos

Aline Santos da Silva Guarim[1], Adriano Felipe Mendes[2], Isabella Rio Feltrin[3], Lucas de Oliveira Bezerra[2], Laura Chuba Machado Rolniche[2], Maria Eduarda Rocha Pinto[2], Emily Silva do Nascimento[2], Janice Koepp[4], Guilherme Pugliesi[3], Claudia Maria Bertan Membrive[2]

[1]Universidade Estadual da Região Tocantina do Maranhão (*UEMASUL*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Instituto de Biociência - UNESP*)
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[4]Biocelltis Biotecnologia (*Florianópolis-SC*)

E-mail: alineguarim@gmail.com

We hypothesized that the use of a three-dimensional (3D) system with a bacterial nanocellulose membrane (CellFate®; Biocelltis S.A., Brazil) during *in vitro* oocyte maturation (IVM) and *in vitro* embryo culture (IVC), associated with the use of melatonin in IVM, increases *in vitro* embryo production (IVEP). This study aimed to compare the 2D and 3D systems during IVM and/or IVC with or without the addition of 10 µM melatonin (Sigma Aldrich, M5250; *) in IVM on the quantity and quality of blastocysts produced. From the ovaries of slaughtered bovine females, ovarian follicles between 2 and 8 mm in diameter were aspirated using a 10 mL syringe coupled with an 18G needle. Oocytes with homogeneous cytoplasm and multiple layers of *cumulus* cells were divided equally into six groups: MIV and CIV 2D, MIV 3D and CIV 2D, MIV 2D and CIV 3D, MIV and CIV 3D, MIV 2D* and CIV 3D, and MIV 3D* and CIV 3D. IVM was carried out in a medium for IVM containing 10% fetal bovine serum (FBS) in 4-well plates, with the respective treatments, in an incubator at 38.5°C in a humidified atmosphere and 5% CO_2 for 24 hours. After IVM, the oocytes were subjected to *in vitro* fertilization (IVF) with semen from a single Nelore bull for 18 hours (D0 = day of IVF). The IVC was carried out in a specific medium containing 2.5% SFB in 4-well plates for 9 days, containing the respective treatments. There were 8 replicates, totaling 137 oocytes per group. The cleavage (D3) and blastocyst rates (D7 and D9) were measured, as well as the quality of the hatched blastocysts (D9) by RT-qPCR, through the abundance of the transcripts OCT4 (POU class 5 homeobox 1), SOD2 (superoxide dismutase 2), GPX1 (glutathione peroxidase 1) and BAX (BCL2 associated X), in pools of 3 embryos. The embryonic development and abundance of transcripts was analyzed by ANOVA and contrasts between groups, considering the culture well and pool of embryos as the experimental units. At D3, the cleavage rate was higher in the MIV 3D* group containing added melatonin and CIV 3D than in the MIV and CIV 3D group (P = 0.0313) and the MIV and CIV 2D group (P = 0.0300). On D7, the early blastocyst rate was higher in the MIV 3D* and CIV 3D group than in the MIV and CIV 2D group (P = 0.0079). On D9, the expanded blastocyst rate was higher in the MIV 3D* and CIV 3D group than in the MIV and CIV 3D group (12.16% *vs*. 3.94% respectively; P = 0.0491). The overall blastocyst rate on D9 did not differ between the MIV 3D* and CIV 3D and MIV 3D and CIV 2D groups (30.43% and 30.26%, respectively); these had higher values (P = 0.0323) than the MIV and CIV 3D (14.02%), MIV 2D and CIV 3D (19.77%) and MIV and CIV 2D (18.40%) groups. There was no difference between the groups for the transcripts evaluated. In conclusion, the MIV 3D* and CIV 3D, and MIV 3D and CIV 2D groups result in a higher overall blastocyst rate at D9, without altering the expression of OCT4, GPX1, SOD2, and BAX in bovine embryos.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Age-Dependent DNA methylation variability in Nelore cattle donors: implications for reproductive technologies

João Vitor Alcantara da Silva[1], Aldcejam Martins da Fonseca Junior[1], Jessica Ispada[1], Rafael Herrera Alvarez[2], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*Centro de Ciências Naturais e Humanas - CCNH/UFACB)*
[2]Agência Paulista de Tecnologia dos Agronegócios (APTA)

E-mail: vitoralcantaraj@gmail.com

Achieving a satisfactory oocyte maturation is still an obstacle within the domain of animal reproductive technologies, due to the multifaceted series of events the oocyte experiences during this pivotal phase. Among these, epigenetic maturation stands out, notably DNA methylation, which coordinates the transcriptional apparatus of the oocyte towards the acquisition of meiotic competency. Despite the prolonged fertility exhibited by certain females, the age of the donor holds the potential to compromise this intricate epigenetic process. The objective of this work was mapping the DNA methylation profile of oocytes from cows of different ages (from 4 until 19 y.o.) to verify i) if and at which point the DNA methylation begins to change and ii) the homogeneity of the methylation pattern among animals from the same age range. For this purpose, OPU from the non-dominant follicles were conducted in 26 Nelore cows synchronized with conventional protocols, at different ages (4 to 19 years old). The *cumulus*-oocyte complexes were denuded, and the oocytes were fixed in 4% paraformaldehyde. Oocytes recovered from each animal separately were assessed for the levels of 5-methylcytosine by immunohistochemistry. Images were acquired by Image J software, for fluorescence intensity, adjusted by the number of pixels in the area. Pearson's correlation analysis for methylation levels *vs* age and Student's t-test considering $p<0.05$ were done using GraphPad Prism software. As expected, the correlation analysis revealed that DNA methylation increased through lifetime ($p = 0.0002$ and $r = 0.5902$). Still, up to 8 y.o. the animals had a more homogeneous DNA methylation profile than the older cows (Coefficient of variation = 56.05% and 65.53%, respectively). Despite the higher variation, the cows up to 8 y.o. presented lower levels of DNA methylation when compared with those from 10 to 19 y.o. ($p<0.0001$). Interestingly, a single animal of 6 years old exhibited an abnormal DNA methylation profile for its age (high level of oocyte DNA methylation), revealing that even in the younger population, the DNA methylation profile may be affecting the reproductive outcomes, highlighting the necessity for the introduction of epigenetic improvement technologies. In conclusion, age is a determinant factor for the DNA reprogramming in oocytes and may affect the reproductive performance of a bovine herd. Strategies for the optimization of cattle reproduction, especially the epigenetic improvement, may mitigate the possible negative impacts of age.

Acknowledgments: The work was funded by São Paulo Research Foundation (FAPESP) processes n. 2019/25982-7, 2019/22025-1, 2022/14472-0, 2023/10575-2 and 2018/07450-5 and UFABC.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Assessing activity of astaxanthin at different levels in the *in vitro* culture of bovine embryos by indirect evaluation of ROS

Yumi Camila Tsukamoto Taguti[1], Nelise Kasue Samecima Prosperute[1], Mayara Paula Paglione[1], Thalis de Melo Oliveira[1], Amanda Bianchini Rosa[1], Alan Rodrigo Panosso[2], Maíra Bianchi Rodrigues Alves[1], Lindsay Unno Gimenes[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (*FCAV/UNESP - Departamento de Patologia, Reprodução e Saúde única*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*FCAV/UNESP - Departamento de Ciências Exatas*)

E-mail: yumi.taguti@unesp.br

In vitro embryo production (IVEP) is a technique that allows greater reproductive use of genetically superior animals. One of its stages, *in vitro* culture, involves a series of factors that can generate oxidative stress, favoring the accumulation of reactive oxygen species (ROS). Excess of ROS is associated with poor embryo quality, therefore, to reduce these effects it is possible to supplement IVC with antioxidants, such as astaxanthin (AST), during culture. AST is a carotenoid antioxidant found in the red shells of crustaceans, salmon, and sea stars. The aim in this study was to verify the antioxidant activity of different doses of astaxanthin during IVC by indirect evaluation of ROS. For this purpose, oocytes from grades 1 to 3 were submitted to *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC). The day of IVF was considered day 0 (D0). On the day of culture (D1), the presumptive zygotes were randomly assigned to one of four treatments: CON (control - *in vitro* embryo culture medium, SOF, without AST, n = 61), AST 0.8 (0.8 nM of AST, n = 74), AST 3 (3nM of AST, n = 68), AST 13 (13 nM of AST, n = 58). Data regarding *in vitro* embryo production can be found in 4DRK abstract. The activity of astaxanthin was assessed in the embryos at three different times during IVC (D1, D4 – in which half of the medium was replaced by fresh medium, and D7) by evaluating total ROS with the CellROX Green Reagent probe (Thermo Fisher Scientific, Oregon, USA) by fluorescence intensity. For the statistical analysis, linear, quadratic, and cubic regressions were tested, followed by ANOVA and Tukey's multiple comparison test at a significance level of ≤ 5% (Rstudio 2023.12.1). None of the regression models tested were significant ($p>0.05$). Our results indicate that there was an interaction between time and treatment ($P=0.02$). CON (D1: 0.70 ± 0.06; D4: 0.73 ± 0.04; D7: 0.77 ± 0.04; $P>0.05$) and AST0.8 (D1: 0.70 ± 0.08; D4: 0.73 ± 0.02; D7: 0.77 ± 0.04; $P>0.05$) showed no differences over all IVC times. On the other hand, AST3 (D1: 0.64B ± 0.06; D4: 0.68B ± 0.03; D7: 0.80A ± 0.04; $P<0.05$) and AST13 treatments (D1: 0.78A ± 0.04; D4: 0.64B ± 0.02; D7:0.83A ± 0.02; $P<0.05$) varied along the time. Therefore, we conclude that AST3 was effective in reducing ROS activity on D1 and D4, while AST13 only on D4, however, due to a non-linear response to doses during IVC, it is possible that the need for this antioxidant may be modulated according to the stage of embryo development.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Assessing nuclear and cytoplasmatic configuration in prepubertal bovine oocytes after *in vitro* ou *in vivo* maturation

Venâncio Augusto Oliveira Silva[1], José Eduardo Vieira Chaves[1], Otávio Augusto Costa de Faria[2], Leticia Prates Martins[2], Pedro Henrique Déo[1], Gilmar Arantes Ataide Junior[1], Laryssa Ketelyn Lima Pimenta[1], Leonardo de França e Melo[1], Margot Alves Nunes Dode[3], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás (*Escola de Veterinária e Zootecnia - UFG*)
[2]Universidade de Brasília (*UNB*)
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)

E-mail: venancioaugusto@discente.ufg.br

Genetic improvement programs extensively investigate the reduction of generation intervals. Oocytes from prepubertal female animals are prioritized for multiplying high-quality animals before sexual maturity. This study aims to evaluate nuclear maturation, quantify lipid droplets, and assess mitochondrial activity in oocyte from Nelore females. Moreover, comparing prepubertal and pubertal stages undergoing *in vitro* (IVM) or *in vivo* (IFOT) maturation. A total of 33 calves (aged 6-8 months) and 19 multiparous cows (aged >36 months) underwent OPU sessions. Viable COCs followed either for IVM or IFOT. In summary, groups of 25 COC were loaded into microtubes with 400 µL of IVM medium, consisted of TCM-199 with Earl's salts (Gibco® BRL, ON, Canada) supplemented with 5% FBS, 0.01IU/mL FSH, 0.1 mg/mL L-glutamine, Cysteamine (0.1mM) and antibiotic (amikacin, 0.075 mg/mL) and covered with 300µL of silicone oil, incubated for 18 hours at 38.5°C and 5% CO2 in a portable incubator (Minitub®). For *in vivo* maturation, groups of 25 immature COCs from both donor categories were injected into the dominant follicle of pubertal synchronized heifer. On day 9, recipients presenting one dominant follicle ≥10 mm, 30h of P4 withdrawal, were selected. The IFOT was then performed, along with the application of 50µg of Lecirelin. After 18h of maturation, COCs were removed from IVM or retrieved by OPU from the recipients. Following, the COCs were denuded, fixed, and stained with Lacmoid (BEZ-IVM, n = 71, COW-IVM, n = 72, BEZ-IFOT, n = 88, and COW-IFOT, n = 83) to the assessment of the meiotic stages in a phase-contrast microscope (Nikon E200). Another subset (BEZ-IVM, n = 12, COW-IVM, n = 10, BEZ-IFOT, n = 21 and COW-IFOT, n = 11) was incubated, subsequently fixed and evaluated for lipid droplet area using microscopy fluorescence (New Orleans, USA) (Bodipy), with images quantified by ImageJ® (NIH, USA) and mitochondrial quantification. Mitochondrial evaluation used 400 nM of MitoTracker Deep Red fluorescent dye, with data presented as mean pixel values. The Chi-square test was used for the meiotic progression evaluation and ANOVA followed by Tukey's test for relative lipid area (%) and mitochondrial activity (number of pixels), respectively, with a significance level of 5%. The percentage of oocytes completing nuclear maturation was similar ($p>0.05$) between BEZ-IVM and COW-IVM groups (67.6% and 63.9%, respectively). However, BEZ-IFOT (47.7%) and COW-IFOT (47.0%) groups had lower rates ($p\leq0.05$) than *in vitro* groups. BEZ-IVM had a larger relative area occupied by lipid droplets ($p\leq0.05$) compared to other groups, which did not differ ($p>0.05$). Greater mitochondrial activity was observed in BEZ-IVM and COW-IVM oocytes compared to those matured *in vivo* ($p\leq0.05$), with no difference between *in vivo* groups ($p>0.05$). In Summary, using IFOT as an *in vivo* maturation system was effective, however, probably the maintenance time of COCs inside the follicle needs to be adjusted to reach similar patterns of MII stage oocytes.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Beta-hydroxybutyrate alters bovine preimplantation embryo development through transcriptional and epigenetic mechanisms

Juliano Rodrigues Sangalli[1], Ricardo Perecin Nociti[2], Marcos Roberto Chiaratti[3], Alessandra Bridi[1], Ramon César Botigelli[4], Dewison Ricardo Ambrizi[1], Felipe Perecin[1], Juliano Coelho da Silveira[1], Pablo Juan Ross[4], Flávio Vieira Meirelles[1]

[1]Universidade de São Paulo (*USP*)
[2]Université de Montreal
[3]Universidade Federal de São Carlos (*UFSCAR*
[4]University of California Davis

E-mail: julianors@usp.br

Developing embryos are susceptible to fluctuations in the nutrients and metabolites concentrations within the reproductive tract, which can lead to alterations in their developmental trajectory. Ketotic dairy cows have diminished fertility, and elevated levels of the ketone body beta-hydroxybutyrate (BHB) have been associated with poor embryonic development. We used an *in vitro* model based on either *in vitro* fertilization (IVF) or parthenogenesis to investigate the effects of BHB on the preimplantation bovine embryo development, epigenome, and transcriptome. To this end, we produced parthenotes or *in vitro* fertilized (IVF) embryos and supplemented the embryo culture medium with 6 mM BHB (Sigma cat# 298360), concentration found in the blood of cows suffering with severe ketosis, termed BHB-group. For the control group, we cultured the embryos in regular SOF medium, since BHB is water soluble and it does not need vehicle control. We analyzed the blastocysts formation rate, diameter, total number of cells, levels of H3K9 beta-hydroxybutyrylation (H3K9bhb), and transcriptional alterations. Data were tested for normality, and analyzed using the most adequate statistical test as follows. As a result, we observed that BHB reduced the blastocyst rates (Parthenotes: Control 49.78 ± 1.47% x BHB 47.34 ± 1.44%, p = 0.047 from chi-square test; IVF Control: 25.34 ± 1.35% x BHB: 21.33% ± 1.68%, p = 0.0049, chi-square), the diameter (Parthenotes: Control 189.1 ± 1.21 µM x BHB 182.7 ± 1.10 µM, p = 0.0005, from Mann-Whitney test; IVF Control: 173.5 ± 1.62 µM x BHB: 167.5 ± 1.76 µM, p = 0.0016, Mann-Whitney) and the total number of cells (Parthenotes: Control 131.4 ± 2.44 x BHB 122.1 ± 2.08, p = 0.0326, Mann-Whitney; IVF Control: 143.2 ± 4.145 x BHB: 128.9 ± 4.225, p = 0.0167, unpaired T-test) in both parthenotes and IVF embryos. Exposure to BHB for either 3 or 7 days greatly increased the H3K9bhb levels (measured by immunofluorescence) in parthenotes at the 8-cells (~3-fold, p<0.0001, Mann-Whitney) and blastocyst stages (~10-fold, p<0.0001, Mann-Whitney). Additionally, BHB treatment affected the expression of HDAC1, TET1, DNMT1 (p<0.05 Student T-test) in 8-cells parthenotes embryos, and KDM6B, NANOG and MTHFD2 (p<0.05 Student T-test) genes in parthenotes blastocysts. Additionally, culture of IVF embryos with BHB for 7 days dramatically (~6-fold, p<0.0001, Mann-Whitney) increased H3K9bhb in blastocysts. RNA-seq analysis of IVF blastocysts revealed that BHB modulated the expression of over 300 genes, which were involved in biological processes such as embryonic development, implantation, reproduction, proliferation, and metabolism. These findings provided valuable insights into the mechanisms through which BHB disrupts preimplantation embryonic development *in vitro*, and they offer explanations for the reduced competence observed in blastocysts developing *in vivo* in ketotic cows. Financial Support: FAPESP process # 2022/06581-4 and 2016/13416-9.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Characterization of lipid profile in *in vitro* matured domestic feline oocytes

Erlandia Márcia Vasconcelos[1], Gabriela Ramos Leal[1], Thais Gomes de Oliveira[1], Bruna Rios Coelho Alves[1], Ribrio Ivan Tavares Pereira Batista[1], Georgia Correa Atella[2], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária* - UFF)
[2]Universidade Federal do Rio de Janeiro (UFRJ)

E-mail: erlandiamvasconcelos@gmail.com

Forskolin (F), L-carnitine (L), and conjugated linoleic acid (C) are lipid modulators, known to reduce cellular lipid content. They have been used in oocyte *in vitro* maturation (IVM) to optimize cryopreservation of those gametes or their derived embryos, once excess lipid in oocytes or embryos jeopardize their cryosurvival. This study aimed to characterize the lipid profile and mRNA content of cat oocytes matured *in vitro* with or without those lipid modulators. Elective surgery-derived cat ovaries had COCs retrieved and allocated into three groups: immature (IM), matured without (M-CONT), or with lipid modulators (M-FLC [F = 100 µM, L = 0.5 mg/mL, and C = 100 µM]). COCs were matured in TCM-199 media for 28h and then either denuded and stored at -80ºC (lipid and RNA extraction), or fixed (lipid staining). High-performance thin-layer chromatography (HPTLC) analysis was conducted for lipid detection (n=12/group). Differential expression (n=10/group) of genes related to lipid metabolism (FABP3, FADS2, DGAT1, DGAT2, PLIN2) and apoptosis (BCL2) was performed by RT-qPCR, using the CT(2−ΔΔCt) method, after normalization with the endogenous controls (YWHAZ and ACTB1). Remaining oocytes (n=15/group) were stained with Nile Red and observed under epifluorescence microscopy. Data were submitted to ANOVA followed by Tukey-Kramer test. Results were considered significant when $P < 0.05$. Eight lipid species were detected in all three groups. Esterified sterol (a structural lipid that forms cell membranes) was more abundant ($P<0.01$) in M-CONT than IM oocytes, but found in M-FLC oocytes at intermediate concentrations, not differing from M-CONT and IM oocytes. Triacylglycerol (serves as energy storage) was higher ($P=0.01$) in M-FLC, compared with IM oocytes, but similar between M-FLC and M-CONT. Monoacylglycerol (forms triacylglycerol) was higher in both M-CONT ($P=0.02$) and M-FLC ($P<0.01$), compared to IM. Transcripts related to triacylglycerol formation (DGAT1) fatty acid transportation (FABP3) and lipid droplet assembling (PLIN2) were upregulated ($P<0.05$) in M-FLC oocytes, compared with IM. In M-CONT oocytes, DGAT1 was downregulated ($P<0.01$) and FABP3 upregulated compared with IM ($P<0.01$). The transcripts encoded by FABP3 were upregulated in M-FLC oocytes ($P<0.01$), comparing with M-CONT. No differences were detected in the amount of intracellular lipids in Nile Red-stained oocytes. This preliminary data indicates that, in cats, esterified sterol and monoacylglycerol increase during IVM of oocytes. This seems a possible strategy to accumulate energy for embryonic development. The use of lipid modulators during IVM seems to mitigate the increase of esterified sterol, and increases the mRNA abundance of FABP3, and this might impact cryosurvival due to interference in membrane fluidity and the traffic of lipids in IVM oocytes. However, this requires further elucidation. Financial support: CAPES (001), CNPq, FAPERJ, and WTA.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of administering 325 mg of recombinant bovine somatotropin (Posilac®) on the oocyte quality of holstein pregnant heifers and holstein dry cows undergoing ovum pick-up and *in vitro* embryo production

Leonardo Soares Locoselli[1], Carlos Alberto Rodrigues[2], Emanuele Almeida Gricio[1], Augusto de Castro Netto[3], Daniela Martins Paschoal[4], Beatriz Pereira Baldissarelli[4], Marcelo Henrique Santos Leite[4], Lígia Mattos Rebeis[1], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Clínica Veterinária SAMVET de São Carlos Ltda
[3]Fininvitro Inseminação Artificial De Animais Ltda
[4]AGRINDUS SA Empresa Agrícola Pastoril

E-mail: leonardo.locoselli@usp.br

We aimed to evaluate the effects of applying 325 mg of recombinant bovine somatotropin (rBST; Posilac®) on oocyte quality and the efficiency of *in vitro* embryo production (IVEP) in Holstein pregnant heifers (PH) and Holstein dry cows (DC). For this, three replicates were carried out: first replicate (FR), second replicate (SR) and third replicate (TR). The treated group (TG; treated with 325 mg of rBST) and the control group (CG; no treatment) were allocated with the same animals in all replicates. To eliminate the effect of the bull on the PIVE, some animals were excluded in certain replicates. The TG (FR= 10 DC and 14 PH; SR= 14 DC and 12 PH; TR= 14 DC and 10 PH) received 325 mg of rBST seven days before ovum pick-up (OPU). The CG received no treatment (FR= 10 DC and 13 PH; SR= 18 DC and 13 PH; TR= 17 DC and 13 PH). All donors received 0.1 mg of gonadorelin 4 days before OPU. After OPU, the *Cumulus*-oocyte complexes (COC's) were classified according to their morphology and sent to the laboratory for IVEP. Cleavage was evaluated 3 days after *in vitro* fertilization (IVF) and the production of blastocysts 7 days after IVF. The data was analyzed as a 2 x 2 factorial (two treatments and animal category) using Proc GLIMMIX using repeated measures. Interaction between replicate order and animal category was observed for number of COC's recovered (P <.0001). The DC treated group showed a higher recovery of total COC's over the time (FR = 12.60 ± 1.93; SR = 12.43 ± 1.01 and TR = 11.43 ± 1.23). However, in the DC control (FR = 9.50 ± 1.08; SR = 7.11 ± 0.69 and TR = 8.06 ± 0.85) and in the PH control (FR = 8.62 ± 1.30; SR = 7.46 ± 0.72 and TR = 6.31 ± 0.89) groups the number of COCs recovered was consistent across replicates, but at a lower overall count. Conversely, there was a decrease in the number of COC's recovered along replications in the treated PH group (FR = 8.21 ± 0.83; SR = 6.92 ± 0.75 and TR = 4.30 ± 0.40). Interaction between replicate order and animal category was observed for number of blastocysts produced (P <0.0001). There was an increase in the number of blastocysts produced along replications in the treated DC group (FR = 1.40 ± 0.52; SR = 3.14 ± 0.78 and TR = 3.71 ± 0.46). However, there was a decrease in the number of blastocysts produced along replications in the treated PH group (FR = 3.00 ± 0.42; SR = 0.92 ± 0.23 and TR = 0.40 ± 0.22). No differences were observed in the number of blastocysts produced among replicates in control DC (FR = 1.50 ± 0.54; SR = 1.72 ± 0.34 and TR = 2.24 ± 0.54) and control PH (FR = 1.92 ± 0.24; SR = 2.08 ± 0.45 and TR = 2.00 ± 0.30). Treatment with rBST increased the recovery of total COC's in dry cows, which was not observed in pregnant heifers. Throughout the three replicates, an increase in embryo production along the replicates was observed in dry cows, while in pregnant heifers there was a worsening. Acknowledges: FAPESP (2023/14748-9), Fazenda Santa Rita (Agrindus S/A) and Agener União (Posilac®).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of administration of different doses of rBST on oocyte quality and *in vitro* embryo production in young Nelore donors

Pâmella Alves Correia[1], Ricardo de La Rocque[2], Felipe Nakashima Camargo[2], Lígia Mattos Rebeis[3], Alysson Oliveira Resende[4], João Paulo Guimarães da Costa[5], Marco Rogério Dalalio[5], Pietro Sampaio Baruselli[3]

[1]ABS Pecplan
[2]Sino Agropecuária
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[4]Global embryo
[5]Agener União Química

E-mail: pamcorreia@gmail.com

This study evaluated the efficiency of administering 325 mg of recombinant bovine somatotropin (Posilac®) or 500 mg of recombinant bovine somatotropin (Lactotropin®) seven days before OPU (Ovum pick-up) on oocyte quality and *in vitro* embryo production in young Nelore donors. A total of 192 Nelore donors (BW = 295.9 ± 3.79 kg, age = 9.7 ± 0.5 months) located in Uberaba-MG (Sino Agropecuária, Brazil) were used in three replications (R; R1 = 58 animals, R2 = 79 animals, R3 = 55 animals). At the beginning of follicular synchronization (D0), animals received 150 mg of injectable progesterone (P4i; Progecio®, Agener União) and 1mg of estradiol benzoate (EB; RIC-BE®, Agener União). At this moment, the animals were divided into three groups: Control (n = 64; without rBST administration), bST325 [n = 64; administration of 325 mg of rBST (Posilac®, Agener União)], bST500 [n = 64; administration of 500 mg of rBST (Lactotropin®, Agener União)]. The donors were evaluated seven days later, and suitable females (donors with more than 10 follicles in the sum of two ovaries) were aspirated. Statistical analyses were performed using GLIMMIX in SAS 9.4. There was no treatment*replication interaction. There was a tendency to have more donors suitable for OPU in the bST-325 group [Control = 56.2% (36/64)B *vs.* bST325 = 75.0% (48/64)A *vs.* bST500 = 64.0% (41/64) AB; P = 0.06]. There was no difference between the groups in the number of oocytes recovered (Control = 25.7 ± 2.64 *vs.* bST325 = 33.8 ± 3.19 *vs.* bST500 = 29.8 ± 3.17; P = 0.19). There was no difference between the groups in the number of oocytes cultured (Control = 20.1 ± 2.21 *vs.* bST325 = 27.1 ± 2.54 *vs.* bST500 = 24.1 ± 2.48; P=0.14). There was no difference between the groups in the number of cleaved oocytes (Control=13.9±2.07 *vs*. bST325 = 18.5 ± 1.93 *vs.* bST325 = 17.8 ± 2.05; P=0.12). There was a tendency towards an improvement in the number of total embryos in the bST325 and bST500 groups when compared to the Control group (Control = 2.8 ± 0.69B *vs.* bST325 = 4.8 ± 0.76A *vs.* bST500 = 5.2 ± 0.99A; P=0.07). There was a tendency towards an improvement in the number of viable embryos in the bST-325 and bST-500 groups when compared to the Control group (Control = 2.4 ± 0.66B *vs.* bST325 = 4.0 ± 0.69A *vs*. bST500 = 4.9 ± 0.96A; P=0.08). In conclusion, using 325mg recombinant bovine somatotropin tended to increase the number of synchronized donors capable of OPU-Ovum pick-up and embryo production in young Nelore females.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of alpha lipoic acid in the *in vitro* embryo production in cattle

Flávia Aguiar Carrieri[1], Mariana Moreira dos Anjos[1], Deborah Nakayama Yokomizo[1], Gabriela Rodrigues de Paula[1], Pamela Mara Celestino Soares[1], Letícia Guiaro Alves[1], Camila Bortoliero Costa[1], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Laboratório de Biotecnologia da Reprodução Animal (REPROA/UEL)

E-mail: flavia.aguiar.carrieri@uel.br

Alpha lipoic acid (ALA) is a coenzyme of mitochondrial multienzyme complexes and acts to eliminate reactive oxygen species, metal chelation, and indirectly in the recycling of other antioxidants. The aim of this preliminary experiment was to evaluate different concentrations (0, 2.5, 5, 10 and 25 µM; based on literature) of ALA in the *in vitro* bovine embryo culture medium. Ovaries (n = 838) from female cattle obtained from a local slaughterhouse were transported to the laboratory in 0.9% NaCl saline solution at 36°C. Follicles (3 to 8 mm) were aspirated and grade I and II cumulus-oocyte complexes (n = 2,201) were selected and sent to IVM for 24 hours. Subsequently, fertilization was carried out with semen from a single bull. After 18 hours, the presumptive zygotes were denuded by successive pipetting, washed, and cultivated in SOF medium (Synthetic oviduct fluid; ABS Global Brazil®, Mogi Mirim, SP, Brazil) according to treatments (0 µM, n = 443; 2,5µM, n = 443; 5µM, n = 440; 10 µM, n = 435; 25 µM, n = 440 presumptive zygotes). The feeding was carried out with the replacement of 50% of the SOF medium on the third day of cultivation (D3; 1st feeding) with SOF medium and on the fifth day of cultivation (D5; 2nd feeding) with glucose SOF medium. The media used throughout the IVC stage was supplemented with ALA (CAS 1077-28-7, Sigma Chemical®, St. Louis, Missouri, USA) according to the pre-defined treatments (8 replicates/group). The embryo production process was carried out at a temperature of 38.5°C, an atmosphere of 5% CO2 and 95% humidity in air. Cleavage (cleaved structures/CCOs), blastocyst (blastocyst/CCOs) and hatching (hatched/blastocyst) rates were assessed on days 2, 7 and 9, respectively. Data were analyzed by ANOVA using a generalized linear model. Treatment was considered a fixed factor and routines a random factor. The Tukey test was used as a post hoc mean test ($P<0.05$). For descriptive analysis, data are presented as mean (M) and standard error (SE). The cleavage (77.11 ± 2.19; 73.82 ± 2.64; 77.21 ± 1.90; 77.48 ± 2.57 and 72.96 ± 4.99; P = 0.55), blastocyst (46.22 ± 2.83; 30.90 ± 3.88; 34.21 ± 3.45; 37.28 ± 4.66 and 34.32 ± 6.25, P = 0.16) and hatching (75.83 ± 5.20; 64.81 ± 2.69; 66.94 ± 3.26; 71.35 ± 3.44 and 71.01 ± 4.91; P = 0.32) did not differ between treatments (control, 2.5 µm, 5 µm, 10 µm and 25 µm ALA, respectively). The results indicated that ALA supplementation at the concentrations evaluated during IVC did not increase development rates, however, they suggest that it had no cytotoxic effect on the embryo. It is believed that further studies are needed to investigate the antioxidant action of ALA on embryonic metabolism when included in IVC.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of melatonin on *in vitro* maturation and culture media on blastocyst production in prepubertal heifers submitted to dietary supplementation

Lucas Costa de Faria[1], Isabella Rodrigues dos Santos[1], Otávio Augusto Costa de Faria[1], Leticia Prates Martins[1], Adriano Queiroz de Mesquita[2], Margot Alves Nunes Dode[2], Roberto Guimarães Júnior, Eduardo da Costa Eifert[2], Sônia Nair Báo[1], Carlos Frederico Martins[2]

[1]Universidade de Brasília (UnB)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)

E-mail: lucascdfaria@gmail.com

The present study assessed whether the addition of melatonin at oocyte maturation (IVM) and/or embryo culture *in vitro* (IVC) media would affect oocyte competence and, consequently embryo production in prepubertal heifers nutritionally supplemented post-weaning. For that, 11 pre-pubertal Nelore heifers were supplemented from 7 to 13 months old, with corn silage and energetic-protein concentrate. Following a confinement period, they received a diet consisting of 11 kg of corn silage (33.0% dry matter) and 1.8 kg of concentrate (33.4% crude protein, 72.9% neutral detergent fiber, 2.8 Mcal/kg metabolizable energy) daily. After 3 months, 8 heifers (10 months old) underwent ovum pick up (OPU), without previous hormonal synchronization, for 4 months. Control group oocytes were obtained from cows (n=5). All recovered oocytes were divided into 3 groups: 1) Pre pubertal oocytes (n=390) *in vitro* maturated with melatonin (10-9M); 2) Pre pubertal oocytes (n=362) maturated (10-9M) and cultured *in vitro* with melatonin (10-9M); Control group (n=237). Previous results of our group have shown the use of 10-9M embryo development *in vitro,* had better results when compared to other concentrations. All groups were exposed to high oxygen tension (20% O2) throughout development phases (IVM, IVF, and IVC). Blastocysts yield was assessed at the 7th day of culture (n° of blastocysts/n° of oocytes). By the end of experiment, they underwent a puberty induction protocol and were time fixed artificial inseminated (TFAI). Statistical analysis was performed using ANOVA and Tukey tests (P≤0.05). No significant difference was observed in oocyte quality between heifers and cows (P>0.05), underscoring the importance of the nutritional supplementation of prepubertal heifers and its effect on reproduction. Regarding embryo production, blastocysts yield on D7 was higher in the group with melatonin in IVM and IVC compared to the group with melatonin only in IVC (38.12% *vs* 25.38%; p<0.05). Control group had greater blastocysts yield when compared to melatonin only in IVM (41.66% *vs* 25.38%; P<0.05), but there was no difference when compared to melatonin in both IVM and IVC (41.66% *vs* 38.12%). By the end of the 13th month, all heifers were weighed and had medium weight of 290.82 ± 53.9 Kg. This weight allowed an efficient puberty induction protocol and 54% of conception rate, proving the concept of max exploration of the genetic potential of pre puberty Nellore heifers by the use of biotechnologies of reproduction as IVP or time fixed artificial insemination. Therefore, these results emphasize the importance of nutritional care of growing females and the use of melatonin for IVP in high oxygen tension system for prepubertal heifers. These results also emphasize the importance of using prepubertal heifers as an alternative to reduce the generation interval, making it possible to potentialize genetic enhancement of Brazilian herd. This research was supported by CAPES, FAPDF, and Embrapa.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of the addition of melatonin to embryo culture media on the production of bovine embryos under high or low oxygen tension

Isabella Rodrigues dos Santos Oliveira[1], Lucas Costa de Faria[1], Fabiana Lima Rodrigues[2], Adriano Queiroz de Mesquita[3], Júlia Carvalho de Melo[1], Carlos Frederico Martins[3], Sônia Nair Báo[1]

[1]Universidade de Brasília (UnB)
[2]Centro Universitário ICESP
[3]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)

E-mail: isabellarodrigues.mv@gmail.com

Melatonin has shown different benefits for cellular tissues and embryo culture system, such as: anti-apoptotic effects in the cells, modulates the expression of active substances, reduces calcium influx into the cells and attenuates the production of reactive oxygen species. Therefore, the aim of the present study was to investigate the effect of adding 10-9 M melatonin to *in vitro* culture (IVC) medium on blastocyst rate when cultured under high (20%) and low (5%) O2 tension. For this purpose, ovaries were collected from a slaughterhouse and transported to the laboratory for follicular puncture. The selected oocytes with homogeneous cytoplasm and at least three layers of *cumulus* cells were then subjected to i*n vitro* maturation (IVM) and *in vitro* fertilization (IVF) under high O2 tension. On the day of IVC, the putative zygotes were categorized into four groups: 1) High O2+Mel; 2) High O2-Mel; 3) Low O2+Mel; and 4) Low O2-Mel and cultured in synthetic oviductal fluid (SOF) with 2.5% fetal bovine serum. Means were compared by Tukey-Kramer test at 5% probability and quantitative data were expressed as mean ± standard deviation. No difference in cleavage rate was observed between all groups (p>0.05). Regarding *in vitro* embryo production, there were no differences between treatments in high O2 tension (41.25 ± 4.73% *vs* 37.41 ± 6.10%, respectively with and without melatonin; P>0.05) and in low O2 tension (34.48 ± 8.53% *vs* 30.34 ± 6.23%, respectively with and without melatonin; P>0.05). However, when the culture systems were compared, we observed that high O2 tension with melatonin produced more blastocyst than low O2 without melatonin (41.25 ± 4.73% *vs* 30.34 ± 6.23%), indicating that the presence of melatonin can influence the embryo development in low O2 tension culture system, despite being a system that produces fewer free radicals. Furthermore, the effect of melatonin on embryo quality still needs to be investigated in this study in future. This research was supported by CAPES, FAPDF and EMBRAPA.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of the moon cycle on the efficiency of an *in vitro* embyo production (IVEP) system in a large-scale commercial program

Hugo Hernandez Fonseca[1], Antonio Carlos Nogueira de Gois[2], Alessandro Magno Cambraia Esteves[3], Joao Lucas Lobo Alves Correia[3], Patricia Villamediana Monreal[4], Fernando P. Perea[5]

[1]Saint George's University
[2]Apoyar Biotech LTDA (Juiz de Fora, MG)
[3]BH Embriões LTDA (Belo Horizonte, MG)
[4]South Dakota (SD, USA)
[5]Universidad de Cuenca (*Facultad de Ciencias Agropecuarias - UCUENCA*)

E-mail: hfonsec1@sgu.edu

This retrospective study (2020-2022) aimed to assess the influence of the lunar cycle (LC) on the efficacy of an *in vitro* embryo production (IVEP) system. We utilized the lunar phase on the day of oocyte retrieval (Ovum Pick Up or OPU) as the reference value to measure the LC effect. A total of 101.548 viable oocytes (VO) were collected from Gyr donors after 6.714 OPU-sessions in 133 cattle farms in the state of Minas Gerais, Brazil. *In vitro* embryos were produced in a commercial laboratory, following protocols for such purposes. The LC was divided into 30 periods of 0.98 days each based on the percentage of moonlight, with periods 1-15 representing the crescent phase (CF) and periods 16-30 representing the waning phase (WF). Data were analyzed by the general linear model and the least square means test of SAS (year, month, farm, bull breed and OPU technician were also considered as independent variables). The number VO recovered from the OPU-sessions were significantly affected by the LC (P<0.0001), and ranged from 12.1 to 19.2 oocytes (average: 14.8 ± 0.8). There were several main peaks of oocytes collected above average on days 4 (16.6), 5 (19.2), 6 (17.2), 15 (16.7), 20 (17.8), 21 (16), 24 (16.2), 26 (16.8), 27 (16.8), 28 (15.5). The cleavage rate (CR; average 70.9 ± 2.2) varied substantially across the LC and ranged from 56.4 to 79.4% (P<0.0008). There were two major peaks below average in the CR, one at CF (day 12: 56.4%) and one at WF (day 20: 62%), and several others above average: days 2 (77.6%), 4 (78.1%), 13 (76.6%), 14 (75.9%), 24 (79.4%), 25 (78.3%). Blastocyst rate (BR) ranged from 21 to 31.8% throughout the LC (P = 0.0002) and averaged 26.7±1.7%. Whereas there were five major increases in BR in the CF [days 1 (28.7%), 2 (28.9%), 7 (31.4%), 9 (29.6%), 11 (28.2%)], eight took place in the WF [days 18 (29.7%), 19 (29.9%), 21 (27.7%), 24 (30.6%), 25 (31.8%), 26 (29.1%), 28 (30.8%), 30 (30.8%)]. Likewise, there was a significant effect of the moon on day-7 transferable embryos rate (TR; P<0.0001), which averaged 21.3±1.6%. Several peaks of TR above the average were observed through LC, three of them in CF [days 9 (24.6%), 11 (22.3%), 12 (22.7%) and seven in WF [days 18 (23.4%), 19 (22.8%), 21 (22.3%), 24 (25.6%), 25 (26.6%), 28 (24.4%), 30 (24.1%). These findings are consistent with our previous studies, which demonstrated greater *in vitro* blastocyst production (Hernandez-Fonseca et al., Reproduction, Fertility and Development 35:189-90, 2023) and pregnancy rates in *in vitro* embryo transfers (Perea et al., Reproduction, Fertility and Development 36:218, 2024) in the second half of the waning phase of the LC. In conclusion, to improve transferable embryos production it is recommended to perform OPU in donors in the second week of the waning phase of the lunar cycle. As multiple factors may affect PIVE, these results may be applicable to farms or circumstances where it is necessary to improve the production of high quality embryos.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effect of the quantity of COCs and the injection quality on embryo recovery after Intrafollicular Transfer of Immature Oocytes (IFIOT)

Ana Caroline Chaves Vall Nicolás[1], Otávio Augusto Costa de Faria[1], Marcelo Sant'Ana Borges[2], Nayara Ribeiro Kussano[3], Ivo Pivato[1], Carlos Frederico Martins[3], Margot Alves Nunes Dode[3]

[1]Universidade de Brasília (*UnB*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*UNESP*)
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)

E-mail: carolnicolas.7@hotmail.com

The Intrafollicular Transfer of Immature Oocytes (IFIOT) has emerged as an alternative for bovine embryo production. However, the technique still yields unsatisfactory results for commercial-scale implementation. This study aimed to evaluate whether the quantity of cumulus-oocyte complexes (COCs) injected into the preovulatory follicle, along with injection quality, impacts the final embryo recovery. Immature oocytes were obtained from slaughterhouse ovaries. Subsequently, twenty-two Nelore cows synchronized as previously described (Faria *et al*., Reproduction, Fertility and Development 33(5) 372-380, 2021) were subjected to IFIOT using a 27-gauge needle (Unisis®, Fukuoka – Japan) for the injection of either 25 (T25 treatment , n= 9) or 50 COCs (T50, n= 8), loaded in 10 µl of PBS medium. The injection quality was classified into grades 1 (n= 12) (injection into the center of the follicle and visualization of the entry of all structures) or 2 (n= 5) (injection into the periphery of the follicle and entry of the structures too fast (vortex) or too slow (almost imperceptible), more than one perforation in the follicle and no visualization of the entrance of the structures and/or reduction of the follicle after removing the needle). Immediately after IFIOT, ovulators females were artificially inseminated with a dose of conventional semen from a Nelore bull with known fertility data. Simultaneously, groups of 25 to 30 COCs were allocated for IVP. Nine days after IFIOT, uterine flushing of ovulators females was performed. The structures recovered were classified as zona pellucida, unfertilized oocyte, degenerated embryo, morula, early blastocyst, blastocyst or expanded blastocyst. The recovery rate of structures and the rate of embryos per treatment and injection quality grade were evaluated. Expanded blastocysts produced by IFIOT and IVP were evaluated for diameter and total number of cells. Data were analyzed by analysis of variance with mixed models (SAS, 9.4 Version). The recovery rate of structures after IFIOT was not affected (P>0.05) by the quantity of injected COCs (T25 - 21.69%; T50 - 19.83%) or the injection quality (grade 1 - 24.66%; grade 2 - 16.86%). Similarly, the recovery rate of embryos was also not affected by the quantity of COCs (T25 - 3.77%; T50 - 3.56%) or the injection quality (grade 1 - 6.83%; grade 2 - 0.50%). Furthermore, there was no interaction between the quantity of COCs and the injection quality for the analyzed parameters (P >0.05). From 178 COCs, 43 (24.2%) blastocysts were obtained in the IVP group. IFIOT and IVP embryos showed similar (P > 0.05) diameter (IFIOT - 175,0 µm; IVP – 179,1 µm) and number of cells (IFIOT – 124,2; IVP – 141,3). It can be concluded that IFIOT embryo recovery is not affected by injection quality or the quantity of injected COCs. Additionally, IFIOT and IVP embryos showed similar quality, based on the evaluated parameters. Financial Support: FAP-DF, Embrapa, CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Effects of intrafollicular transfer of immature oocytes in the bovine follicular environment – Preliminary results

Nayara Ribeiro Kussano[1], Otávio Augusto Costa de Faria[2], Laryssa Ketelyn Lima Pimenta[3], José Felipe Warmling Sprícigo[3], Margot Alves Nunes Dode[1]

[1]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)
[2]Universidade de Brasília (*UnB*)
[4]Universidade Federal de Goiás (*Departamento de Ciência Animal - UFG*)

E-mail: nayarakussano@hotmail.com

Intrafollicular transfer of immature oocytes (IFTIO) is a promising technique but the results obtained to date are not satisfactory for use on a commercial scale. Therefore, this study aimed to evaluate the impact of the injection and the number of oocytes injected into the follicle on the biochemical profile of the follicular fluid (FF). Animals were synchronized (Faria *et al*., Reproduction, Fertility and Development 33(5) 372-380, 2021) and 30 h after removal of the P4 implant, those that had follicles larger than 10 mm were used for the experiment. Groups of 25 and 50 COCs recovered from slaughterhouse ovaries were used for IFTIO. On the day of the experiment, animals were distributed into four treatments: 1. NIFTIO: FF was aspirated by OPU 18 hours after IFTIO time (n = 7); 2. IFTIOM: FF was aspirated by OPU 18 hours after IFTIO being performed only with PBS medium (n = 7); 3. IFTIO25: FF was aspirated by OPU 18 hours after IFTIO being performed with PBS medium containing 25 COCs (n = 8); 4. IFTIO 50: FF was aspirated by OPU 18 hours after IFTIO being performed with PBS medium containing 50 COCs (n=8). After recovered FF samples were centrifuged and stored at -80°C for posterior analysis. FF of all treatments were evaluated for hormone concentrations (P4, E2, Testosterone and Cortisol) by chemiluminescence, Glutathione (GPx) concentration by Glutathione Peroxidase Assay Kit (Cayman® Chemical®, MI, USA) and concentration of total antioxidant activity by iron reducing/antioxidant power assay (FRAP). Data were analyzed by ANOVA or Kruskal-Wallis test ($P<0.05$). Of the four hormones analyzed, only P4 showed a lower concentration ($P<0.05$) in the NIFTIO (107.06 ng/mL) compared to IFTIOM (172.37 ng/mL) which was similar to the other groups. As for E2, a higher concentration ($P<0.05$) was observed in NIFTIO (300.23ng/mL) compared to the IFTIOM (68.92 ng/mL), the others groups did not differ from each other ($P>0.05$). No changes were observed among all treatments on the levels of cortisol, which change from 5.56 to 9.21 ng/mL, and testosterone levels that varies between 9.96 and 23.15 ng/mL ($P>0.05$). Regarding to total antioxidant activity, no difference ($P>0.05$) between treatments were observed by FRAP analyses, but a higher GPx activity ($P<0.05$) was detected in IFTIO 25 group compared to IFTIO 50, being similar among the others groups ($P>0.05$). Even though some changes were observed among treatment in the biochemical parameters, there was no clear effect of the injection or the number of COCs on those changes. Therefore, more samples should be analyzed to better clarify this effect on the biochemical profile of the FF. Financial Support: FAP-DF, Embrapa, CAPES and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Enhanced bta-miR-20a delivery via lipoplexes: An alternative for transcript regulation in bovine *cumulus* cells

Matheus Andrade Chaves[1], Juliano Rodrigues Sangalli[2], Camila Azzolin de Souza[1], Gislaine dos Santos[1], Samantha Cristina de Pinho[2], Flávio Vieira Meirelles[2], Felipe Perecin[2], Juliano Coelho da Silveira[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: matheus.chaves@usp.br

In vitro fertilization technology continues to advance, yet there remains a need for innovative strategies to enhance the quality of embryos produced using this technology. In this regard, our study aims to examine the impact of enhancing the bta-miR-20a content into bovine cumulus cells (BCCs) cultivated *in vitro*. This miRNA was selected due to its role regulating PI3K/AKT member and the support of oocyte maturation. To achieve the delivery to *cumulus* cells we used its complexation with liposomes (LPs), forming the so-called lipoplexes (LPXs). LPXs were prepared using the bulk method, with the addition of scrambled miRNA (scr-miRNA) or bta-miR-20a mimics (miR-mimic) (Thermo Fisher Scientific, USA) to LPs generated through hydration of a dry lipid film. First, a blend of cholesterol, anionic, and cationic phospholipids (Avanti Polar Lipids, USA), all dissolved in ethanol solutions, was concentrated under vacuum (60°C, 1h), and then hydrated with 1x Tris-EDTA buffer at pH 8. The dispersions were subjected to tip ultrasonication (75 cycles, 60% amplitude) to form LPs. The scr-miRNA or miR-mimic were then added to the LPs, left on ice for 30 min, followed by heat bath for 42 s at 42 °C. LPXs were submitted to extrusion using 100 nm polycarbonate membranes. The formulation was optimized using the gel retardation assay technique, in which lipid to genetic material ratios ranging from 0.25:1 to 10:1 w/w were used to encapsulate 100 ng of the bioactive. LPXs were characterized by mean diameter, polydispersity index and zeta potential (ZetaSizer Nano ZS, Malvern), besides concentration by nanoparticle tracking analysis (NTA) (NanoSight 3000, Malvern). Morphology was imaged via transmission electron microscopy. Encapsulation efficiency (EE%) was analyzed by removing the free material by ultrafiltration (Amicon® 100kDa) followed by quantification via NanoDrop (Thermo Fisher Scientific). BCCs were obtained from 3 to 6 mm ovarian follicles and cultured in bottles containing supplemented α-MEM media. A total of 15,000 BCCs were incubated with LPXs containing either scr-miRNA or miR-mimic for 12 h, followed by evaluation of transcripts by RT-qPCR. Lipofectamine 3000 (L3000) containing an equivalent amount of scr-miRNA or miR-mimic was used for controls. All analyses were performed at least in triplicates. Empty LPs presented an average diameter of 135 nm and a zeta potential of 23 mV. An increase in diameter (250 nm) and a reduction in zeta potential were observed for miR-mimic-LPXs (5 mV). No changes in concentration were observed via NTA after complexation. LPXs exhibited spherical or cup-shaped morphology. The lipid:miRNA ratio was maintained at 5:1 w/w for which the LPXs showed an EE% of 77% for miR-mimic. qPCR analysis showed that bta-miR-20a expression using LPXs was superior for controls ($n = 6$, $p<0.05$). Additional tests will be conducted to verify the effect of bta-miR-20a-loaded LPXs on its target genes. FAPESP 21/066450, 22/014337, 22/012367.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Establishing an endometrial 3D co-culture model

Anna Eloísa Serafim Viana[1], Eduardo Ribes Martínez[2], Giuliana de Avila Ferronato[2], Thais Sayuri Imura Oshiro[3], Ricardo Perecin Nociti[1], Tiago Henrique Camara De Bem[1], Flávio Vieira Meirelles[1], Marcia de Almeida Monteiro Melo Ferraz[2]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*
[2]Ludwig Maximilians Universität München (*Faculty of Veterinary Medicine, Clinic of Ruminants - LMU*)
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: anna.eloisa@usp.br

For the pregnancy to be maintained, a suitable uterine environment which includes different cells, such as epithelium, stroma, immune cells, and endothelium is needed. Such complex communication is not achieved in 2D cultures, but by using a 3D co-culture, part of this dynamic communication can be re-created. Here, we validated the use of a decellularized endometrial extracellular matrix (dECM) for producing a 3D endometrium aiming to support embryo development. To isolate glands, bovine uteri were collected, dissected, digested (HBSS, 0.1% collagenase, 2.5% dispase, 1% antibiotic antimycotic (AA)) for 55 min, 1% DNAse was added for 5 min, glands were collected by using a 100 and 70 µm cell strainers and kept in culture media (DMEM F12 + 10% FBS + 1% AA) overnight at 5% CO2 and 38.5ºC. The flow though solution was used to isolate stromal cells. To prepare the hydrogel (10 mg/mL), the dECM ink (20 mg/mL) was mixed with 11% PBS 10X and 15 mM of HEPES, and the pH was neutralized using 6.1% NaOH 1M. PBS 1X was added together with the stromal cells (1×106 cells/mL) and the glands (0.02 v/v), then alginate was added (1%). After 45 min at 5% CO2 and 38.5ºC, crosslinking solution was added (11mM CaCl2, 10mM HEPES, 0.1U/mL Thrombin), incubated for 15 min, and gels were cultured at 5% CO2 and 38.5ºC in Ovi-ECS media (Santos et al., Theriogenology 173, 37–47, 2021). Hydrogels without cells were also prepared. In the 1st experiment, we tested the influence of hormonal stimulation on the gland spheroid area. Therefore, at D4 of culture (D0 of experiment), Ovi- ECS media was supplemented with 200 pg E2 + 4.5 ng P4; on D1 with 3 pg E2 + 4.5 ng P4; followed by 3 pg E2 + 7.5 ng P4 on D2 and 3, and 3 pg E2 + 15 ng P4 from D3 to 9. A control group without hormones was also evaluated. At each time point, cells were imaged (n=6 per group), and at D9 cells were fixed for immunocytochemistry (ICC) with cytokeratin and vimentin (n = 3 per group). In the 2nd experiment, 5 D7 bovine in vitro-produced blastocysts were added to the culture wells with or without cells and cultured until D9, a control 2D well without cells, was used. At D9, hatching was evaluated, and hydrogels were collected for ICC (n = 3 per group). Data were analyzed in R using a generalized linear mixed model and a Tukey post-hoc test (significant when $p<0.05$). The dECM allowed spheroid culture for over 13 days without the need of passaging, as seen for cells cultured in Matrigel. On days 4, 7, and 9, the spheroid area of hormonal-treated samples was inferior to the control, independent of the presence of embryos. Here we presented the use of dECM as a natural scaffold for creating a 3D endometrium, promoting cell viability and differentiation, supporting spheroid growth, and embryo development. The limitations include partial gel degradation, lack of angiogenesis, and absence of immune cells. Nevertheless, it holds significant promise for gaining a better understanding of maternal-embryonic communication and for drug screening.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Establishment of a long-term feline oviduct spheroid *in vitro* culture system

Bruna Ribeiro Xavier-Getirana[1], Maria Clara Cruz Morais[1], Paulo Sérgio Cerqueira Rangel[1], Agostinho Soares de Alcântara-Neto[2], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Faculdade de Veterinária - UFF*)
[2]Universidade Estadual do Ceará (*Faculdade de Educação e Ciências Integradas de Crateús - UFC*)

E-mail: brunaxavier@id.uff.br

Oviduct epithelial cell (OEC) culture is a valuable tool to comprehend the role of oviductal microenvironment, and to increase the efficiency of embryo IVP. Oviduct epithelial spheroids (OES) are tridimensional multicellular structures formed during OEC culture capable of maintaining their differentiation and function for longer periods compared to conventional monolayer culture. While OES have been recently described for bovine and swine species, characterization of feline OES (fOES) remains unexplored. Considering the relevance of the domestic cat as a model to preserve endangered felids, this study aimed to establish a long-term *in vitro* culture system for fOES production and assess their viability at different culture times. After elective ovariohysterectomy, oviducts from five domestic queens at follicular phase were dissected and washed in alcohol 70% followed by PBS. Oviduct lumens were scraped using a sterilized slide in a Petri dish. Cells were diluted in 10 mL of TCM 199 HEPES supplemented with 1% gentamycin and 0.04% BSA, vortexed for 30 s and kept for 10 min at 38°C. The supernatant was discarded, and the cell pellet was diluted and maintained again at the same conditions. The resultant pellet was then diluted (1:10) in TCM 199 HEPES supplemented with 1% gentamycin and 10% FCS (TCM-FCS). Then, 300 µL of OEC suspension were transferred to Petri dishes containing 2,700 µL of TCM-FCS and cultured for 14 d at 38.5 °C in 5% CO2 and humidified atmosphere. Half of the culture medium was renewed every 48 h. On D3, fOES were selected and transferred to a new Petri dish. On D3, D5, D7, and D14, a total of 15 fOES were randomly selected, subjectively assessed regarding ciliary beats, and images were captured to evaluate the fOES and their cell number using Image J software. Non-parametric data were analyzed by Kruskall-Wallis test followed by Dunn's post hoc; for parametric data, ANOVA followed by Tukey's post hoc was applied. Data are presented as mean ± SEM. Differences were considered significant when $P<0.05$. Cell count in D14 (54 ± 11.6) was lower than in D3 (88.7 ± 5.7) and D7 (128 ± 28.1), but was similar to D5 (75.5 ± 34.2). Diameter (µm) of fOES was higher in D3 (47.9 ± 3.3) than in D5 (25.3 ± 4.6) and D14 (29.9 ± 4.9), but did not differ from D7 (35.7 ± 4.7). The fOES presented a larger area (µm2) in D3 (1904.6 ± 221.3) than in D14 (611.9 ± 198.4), with no difference to D5 (512.7 ± 252.8) and D7 (883.4 ± 223.2). Number of cells per area was lower in D3 (0.03 ± 0.00) than in D5 (0.05 ± 0.01) and D14 (0.05 ± 0.01), but did not differ from D7 (0.04 ± 0.00). Preserved cells with ciliary beats were observed from D3 to D14. In conclusion, even with the size reduction and cell loss, fOES maintain their morphofunctional differentiation over time. The present study establishes, for the first time, an efficient long-term fOES culture system.

Acknowledgments: Capes (001), CNPq, and Faperj

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Evaluation of astaxanthin on bovine *in vitro* embryo culture

Mayara Paula Paglione[1], Yumi Camila Tsukamoto Taguti[1], Nelise Kasue Samecima Prosperute[1], Thalis de Melo Oliveira[1], Amanda Bianchini Rosa[1], Maíra Bianchi Rodrigues Alves[1], Lindsay Unno Gimenes[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (*Departamento de Patologia, Reprodução e Saúde única - UNESP*)

E-mail: mayara.paglione@unesp.br

In vitro embryo production (IVEP) is a technique that offers several advantages for genetic progress. However, *in vitro* environment can result in an increase in levels of reactive oxygen species (ROS), generating oxidative stress and, consequently, affecting the viability of embryo development. In this context, the aim of this study was to supplement different concentrations of astaxanthin (AST) during *in vitro* culture, in order to minimize oxidative damage and improve embryo production. Therefore, bovine ovaries from slaughterhouse were aspirated to obtain grade I to III oocytes. *In vitro* maturation and fertilization (IVM and IVF) were carried out according to laboratory procedures. On the day of *in vitro* culture (IVF was considered D0), the presumptive zygotes were assigned in one of four treatments, which consisted in supplementing SOF medium with: CON (control without AST), AST 0.8 (0.8 nM of AST), AST 3 (3 nM of AST), or AST 13 (13 nM of AST; n = 90 for each treatment). The chosen concentrations were tested in a previous study of our group (Aidar, 2023. Dissertação de mestrado, FCAV-UNESP. 66 f). On D4, 50% of the medium was replaced by fresh medium. Statistics were carried out using analysis of variance (Rstudio 2023.12.1), where the fixed effect was treatment and the variables were cleavage rate on D4 (cleaved embryos with 16 or more cells/total oocytes*100) and blastocyst rate on D7 (total blastocysts/total oocytes*100), at a 5% significance level. The results obtained indicated no effect of any dose of AST on the cleavage rate [(CON: 44.4% ± 7.0 (40/90); AST0.8: 44.4% ± 3.0 (40/90); AST3: 34.4% ± 4.0 (31/90); AST13: 26.7% ± 5.0 (24/90); P= 0.57)], nor on embryo development [(CON: 27.7% ± 4.0% (25/90); AST0.8: 28.8% ± 2.0 (26/90); AST3: 24.4% ± 3.0 (22/90); AST13: 27.7% ± 4.0 (25/90); P= 0.30)]. In summary, no effect was observed on the cleavage and blastocyst rate, leading to the conclusion that astaxanthin did not have any effect within the range of concentrations used in this study. Therefore, the results indicate that the activity of astaxanthin could not be assessed by morphological analysis.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Factors affecting *in vivo* and *in vitro* embryo transfer success in Holstein cows

Gilmar Arantes Ataide Junior[1], Roberta Reis Silva[1], Daniela Demetrio[2], Mayara Oliveira[2], Ricarda Maria dos Santos[3], Helena Tavares Dutra[1], Bruna Mion[4], Murilo Romulo Carvalho[5], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás (*Departamento de Ciência Animal - UFG*)
[2]RuAnn Genetics
[3]Universidade Federal de Uberlândia (Faculdade de Medicina Veterinária - *FAMEV/UFU*)
[4]Lactanet
[5]Boehringer-Ingelheim Animal Health

E-mail: gilmar.arantes2@discente.ufg.br

This study aimed to evaluate the factors affecting pregnancy rate (PR) and loss (PL) in Holstein cows submitted to Embryo transfer (ET) of *in vivo*-derived (IVD) and *in vitro*-produced embryos (IVP). Retrospective data from 11,626 ET was collected from a private dairy farm in (Riverdale, CA–USA) from 2019 to 2021. The animals were milked 3 times/d with an average production of 12,000 kg of milk in 305d. Donors were selected based on their official linear type classification and production in three or more completed lactations. Those in the IVD group were flushed on d6.5 after the first AI, while donors in the IVP group went through OPU, and their embryos were recovered on d7 after IVF. The ET was performed in heifers (>13,5 months of age and ≥360 kg) after natural estrus, and in dry and lactating cows (75-80 DIM) synchronized with Presynch+Ovsynch protocol, using either fresh or cryopreserved embryos. Pregnancy diagnosis was performed by measuring PAGs in serum (IDEXX, EUA) 35d after ET in heifers and 22d in cows; and confirmed by transrectal US at 90 and 220d after ET to assess late PL. Data was firstly analyzed considering PR and PL between IVD and IVP. Secondly, data was separated by embryo origen (IVP or IVD), considering effects of donor status, conservation methods, embryo stage [Mo/Bi(grouped), BL and BX] and quality [grades (G)1 or 2], the ET day after estrous and interactions. Data was analyzed by logistic regression using PROC GLIMMIX of SAS on Demand. IVD had higher (P<0.001) PR (45.9% [4392/9564] *vs* 54.3% [1119/2062]), and lower (P<0.001) PL (14.2% [159/1119] *vs* 19.4% [850/4392]) than IVP embryos, respectively. G1 resulted in higher (P<0.05) PR than G2 embryos for IVP (49.5% [3484/7037] *vs* 36.4% [837/2296]) and IVD (56.3% [966/1715] vs 44.1% [153/347]); and reduced PL (P≤0.05). For IVP embryos, there was an interaction (P<0.001) between ET day and the embryo conservation, resulting in higher PR of fresh IVP embryos transferred on d7 (47.9% [1439/3000]) and d8 (50% [1478/2953]) than cryopreserved embryos transferred on d7 (44.5% [429/964]) and 8 (37.7% [197/522]). BX (21.2% [468/2203]) and BL (19.3% [269/1395]) showed higher PL (P=0.02) than MO/BI (14.2% [113/794]). Higher PL (P=0.03) was observed from ET on d6 (26.5% [149/562]) compared to d7 (20.1% [376/1868]) or 8 (16.5% [280/1675]). On d8, increased PL (P=0.006) was observed after G2 ET (24.7% [198/801]) compared to G1 (14.8% [200/1349]). For IVD embryos, there was a tendency (P=0.083) for improved PR in dry cows (58% [625/1077]) than in heifers (48.6% [291/598]). Finally, G1 embryos tended to have lower PL (P=0.06, 13.5% [131/966]) than G2 (18.3% [28/153]). In summary, ET of IVD embryos yielded higher PR and lower PL. For IVP, frozen/thawed ET reduced PR on days 7 or 8. In IVD, ET from heifers donors reduced PR compared to dry cows. Despite compromising recipient performance, IVP ET enables donors to experience more reproductive cycles, enhancing genetic dissemination potential.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Forskolin supplementation during IVM does not reduce the intracellular lipid content of cat oocytes

Gabriela Ramos Leal[1], Bruna Ribeiro Xavier-Getirana[1], Ana Paula da Silva Cupello[1], Gicele Santos Apolinario[1], Erlandia Márcia Vasconcelos[1], Bruna Rios Coelho Alves[1], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - UFF*)

E-mail: gabivetleal@gmail.com

The domestic cat is a reliable reproductive experimental model for endangered wild felids. Cat oocytes have high concentrations of intracellular lipids and some studies indicate that oocytes lipid content increases during IVM. Excessive lipid accumulation in oocytes is detrimental to oocytes quality if they are cryopreserved. Forskolin has been widely used during IVM to suppress meiotic resumption in several species, since it regulates cAMP production by stimulating adenylate cyclase. Besides, this stimulation induces the activation of dependent protein kinases that lead to the activation of intracellular lipases via phosphorylation, increasing the intracellular β-oxidation. This mechanism provides forskolin a lipolytic action. However, the role of forskolin in cat oocytes is still not elucidated. This study aimed to evaluate the effect of forskolin during IVM on oocytes' nuclear maturation and intracellular lipid content. COCs were recovered from cat ovaries obtained from elective surgeries and selected based on cytoplasm homogeneity and cumulus cell layers. A total of four replicates were conducted (n = 125 COCs). COCs were allocated into two groups according to the IVM treatment: Control (0 mM forskolin) and FK100 (100 mM forskolin added to IVM medium). The base IVM media was TCM 199 supplemented with 0.02 IU/mL FSH/LH, 100 µM cysteamine, 2.2 g/L sodium bicarbonate, 3 mg/mL BSA, 0.25 mg/mL sodium pyruvate, 0.15 mg/mL L-glutamine, 0.6 mg/mL sodium lactate, and 0.055 mg/mL gentamicin. IVM occurred for 24 h at 38.5°C in maximum humidity. After 24 h of IVM most COCs (n = 94) were denuded with hyaluronidase, fixed in 4% paraformaldehyde, stained with Hoechst 33342, and evaluated under fluorescence microscopy for nuclear maturation assessment. The remaining oocytes were submitted to Nile Red staining for lipid content assessment (n = 31). The nuclear maturation rate was evaluated with Fisher's exact test, and the lipid content was analyzed using unpaired Student's t-test (mean ± SEM). Analyses were performed in GraphPad INSTAT software at a significance level of 5%. According to the results, 100 mM forskolin supplementation during IVM did not impact (P>0.05) the intracellular lipid content (Forskolin; 1.06 ± 0.36 *vs* Control; 1.00 ± 0.20) or the nuclear maturation rate (Forskolin; 37.3% *vs* Control; 44.7%). We concluded that 100 mM Forskolin did not suppress meiotic resumption and did not have a lipolytic effect during IVM of cat oocytes, which is the opposite pattern observed in other species. This result suggests that forskolin did not stimulate adenylate cyclase in cat oocytes, but further studies are needed to better understand its role in this species. Acknowledgments: CNPq, CAPES (code 001), and FAPERJ.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Influence of an ipsi-lateral or contra-lateral corpus luteum on *in vitro* embryo production in Nelore cows

Pedro Luiz Bignardi[1], Leticia Padovani da Silva[1], Marcelo Sant'Ana Borges[1], Marina de Oliveira Silva[1], Laura Fernanda Sechirolli da Silva[1], Alessandra Bridi[2,3], Tiago Henrique Camara De Bem, Juliano Coelho da Silveira[2], Maria Eugênia Zerlotti Mercadante[1], Fabio Morato Monteiro[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (*Instituto de Zootecnia - UNESP*)
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[3]Universidade do Oeste de Santa Catarina (UNOESC - Campus Xanxerê)

E-mail: pedro.bignardi@unesp.br

Progesterone (P4) plays a fundamental role in oogenesis, follicular growth, ovulation, and maternal support for gestation in mammals. Studies report that oocytes collected during diestrus, with high levels of progesterone, exhibit better quality compared to oocytes collected in other phases of the estrous cycle. The aim of the study was to evaluate the *in vitro* embryo production (IVEP) from oocytes originating from follicles with high endogenous progesterone concentration due to the presence of the corpus luteum and compare it with oocytes from contralateral follicles. Twelve cyclic and empty Nelore cows, approximately 7.9 ± 1.7 years old and body weight of 595.7 ± 34.4 kg were used. To obtain corpus luteum in a known diestrus phase, aspiration of all antral follicles was performed, and 150 µg of D-cloprostenol i.m. (Induscio®, GlobalGen Vet Science) was administered. OPU was performed on all cows after 14 days. An analysis of interaction between ipsilateral ovaries (ipOV: ovaries with CL group) and contralateral ovaries (contOV: group without corpus luteum presence) was conducted to evaluate the effect of corpus luteum presence in each ovary on IVEP rates. The data were analyzed using a generalized linear model with the GLM procedure of SAS®. Comparing the ipOV and contOV groups, no differences were observed in total COCs recovered (131 ± 4.5 *vs*. 149 ± 4.6, respectively; P = 0.77), total viable COCs recovered (48.0 ± 2.4% *vs.* 50.3 ± 2.2%, respectively; P = 0.63), cleavage rate (69.8 ± 9.2% *vs.* 65 ± 14.4%, respectively; P = 0.46), and blastocyst rate (24.1 ± 7.8% *vs.* 30.2 ± 7.9%, respectively; P = 0.47). In conclusion, oocytes recovered from ovaries with ipsilateral corpus luteum in the diestrus phase do not impact the effectiveness of in vitro embryo production in Nelore cows.
Acknowledgment: FAPESP (2017/50339-5), CAPES finance code 001, Vitrogen®, Cravinhos, SP, Brazil and Ouro Fino®, Cravinhos, SP, Brazil.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Intrafollicular transfer of immature oocytes (IFIOT) as an alternative for maturation of bovine oocytes

Otávio Augusto Costa de Faria[1], Nayara Ribeiro Kussano[2], Lucas Costa de Faria[1], Leticia Prates Martins[1], José Felipe Warmling Sprícigo[3], Margot Alves Nunes Dode[2]

[1]Universidade de Brasília (UnB)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[3]Universidade Federal de Goiás (*Departamento de Ciência Animal - UFG*)

E-mail: otavio.fariamv@gmail.com

Studies have demonstrated that oocytes matured in the pre-ovulatory follicle by intrafollicular transfer of immature oocytes (IFIOT) have nuclear maturation and lipid droplets accumulation similar to those matured *in vivo* (Faria *et al.*, Reproduction, Fertility and Development 33(5) 372-380, 2021). However, it is still unknown whether the ability to form an embryo and the amount of lipids remained similar to the *in vivo* matured after IVF and IVC. Therefore, the objective of this study was to assess whether IFIOT improves maturation and affects embryo production and quality. To do this, oocytes matured by IFIOT were compared with those matured *in vitro* (IVM) and with those matured *in vivo* after ovarian superstimulation (FSH). After selection, 25-30 COCs recovered from abattoir ovaries were allocated to the IVM (22 h) or were injected into a dominant follicle of a cow ovulator (diameter ≥10 mm) for IFIOT systems. For the FSH group donors were super stimulated with 100 mg of Folltropin (Folltropin-V; Vetoquinol). *In vivo* matured oocytes were recovered by OPU at 22 h after GnRH injection (i.m), which was performed at the moment of IFIOT and, in the other group, at 18 h after the last injection of FSH. *In vitro* and *in vivo* matured oocytes were then fertilized and cultured *in vitro.* On day 7 of culture, expanded blastocyst were either stained for analyses of mitochondrial activity, lipid quantification and total number of cells, using confocal microscopy, or cryopreserved by DT method to evaluate resistance to cryopreservation. After thawing, the embryos evaluated for expansion at 6 and 24 h of culture. Data were analyzed by Chi-square and ANOVA (GLIMMIX) tests. To date, nine replicates have been carried out. As expected FSH group showed a higher (P<0.05%) cleavage (n = 134/138, 97.10%) and blastocyst rate (n = 56/138, 40.58%) than the IVM (n = 377/454, 83.07% and n = 131/454, 28.85%) and IFIOT (n = 365/425, 85.88% and n = 127/425, 29.88%) groups, which, in turn, were similar between then (P>0.05). Embryos from the three groups were similar regarding the total cells number and mitochondrial activity. Conversely, the mean area occupied by lipids on embryo from IVM groups (n = 23, 12.9% ± 7.73) was higher (P<0.05%) than in embryos from FSH (n = 23, 5.81% ± 4.36) and IFIOT (n = 28, 5.32% ± 4.08) groups, which, by the way, were similar (P>0.05%). Even though embryos differ of intracellular lipids contend, they had similar response to cryopreservation evaluated by re-expansion, at 6 hours (IVM = 25/55, 45%; FSH = 15/31, 48%; IFIOT = 29/53, 55%; P>0.05%) and 24 hours (IVM =37/55, 67%; FSH = 19/31, 62%; IFIOT = 44/53, 83%; P = 0.0621) after thawing. Therefore, the results suggested that the effects of the maturation system can affect the IVF embryo production and their quality. Moreover, the IFIOT system provides embryos with similarities to those produced *in vivo*, regarding lipid content, and can be used as a tool for the maturation. Considering that lipids content may reflect embryo metabolism, maturation by IFIOT, brings new possibilities for reproductive technologies that require *in vitro* matured of oocytes. Acknowledgement: FAP-DF, Embrapa, CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

In vitro embryo production efficiency from Holstein, Gir and Girolando donor cows and sires in a tropical climate

Lorena Lima Firmino[1], João Pedro Araújo Campos[2], Renato Ribeiro de Lima[3], Felipe Costa Gonçalves[2], Clara Emmanuelly Mota Martins[1], Esther Abihail Fuente Arévalo[1], Letícia Rodrigues Faria[1], Nadja Gomes Alves[1]

[1]Universidade Federal de Lavras (*Faculdade de Zootecnia e Medicina Veterinária - UFLA*)
[2]Agroopegen Reprodução Bovina
[3]Universidade Federal de Lavras (*Departamento de Estatística - UFLA*)

E-mail: lorena.firmino2@estudante.ufla.br

The IVP plays a key role in the genetic advancement of dairy herds; however, many factors may interfere with the efficiency of the technique. This study aimed to identify the main factors affecting IVP in a commercial laboratory. The database analyzed included information on 934 IVP routines from Holstein (HO, n = 393), Gir (n= 156), and crossbred ¾ HO x Gir (n = 103) donor cows; as well as information about sires (HO = 45; Gir = 16; and crossbred ¾ HO x Gir = 4); the season of the year in which OPU was performed; the total number and the number of viable cumulus-oocyte complex (COCs) collected and matured; and the numbers of cleaved structures and blastocyst. All OPU sessions and all IVP routines were performed by a single technician, and female-sexed semen was used. Data were analyzed using generalized linear models, considering Poisson distribution and log-link function for count data, and binomial distribution and logit-link function for proportion data (SAS, versão 9.2). There were fewer (P<0.05) total and viable COCs collected from HO (12.6 ± 0.8 and 4.5 ± 0.4) than from Gir (18.1 ± 1.2 and 8.8 ± 0.8) and from crossbred (20.8 ± 1.6 and 10.5 ± 1.2) donor cows, but there was no difference (P>0.05) between Gir and crossbred donor cows. The percentage of viable COCs collected from HO donor cows in winter (42.1 ± 2.7%) was higher (P<0.05) than that in other seasons; however, the season did not affect (P>0.05) the percentage of viable COCs collected from Gir and crossbred donor cows (donor–genetic grouping by season interaction, P<0.05). Regarding the HO x HO embryos, the cleavage rate (CR) to total oocytes and the blastocysts rate (BLR) to cleaved structures did not differ (P>0.05) among seasons (44.8 ± 1.7 to 52.8 ± 2.2; and 18.1 ± 2.2 to 27.2 ± 2.8%, respectively). The CR of HO x Gir embryos was higher (P<0.05) in spring (58.3 ± 2.9%) than in winter (45.0 ± 2.5%), but the BLR did not differ (22.7 ± 10.0 *vs* 25.2 ± 3.0, P>0.05). The CR of embryos Gir x HO, Gir x Gir, and crossbred x HO, in general, was higher in autumn and winter than in spring and summer (P<0.05), nonetheless, the BLR was not influenced by the season of the year (P>0.05). The CR and the BLR crossbred x crossbred did not differ among seasons (P>0.05). The CR of crossbred and the BLR from Gir donor cows were reduced (P<0.05) by fertilization with semen from sires of the same genetic group as the donor, in the winter (sire-genetic grouping within the combination donor-genetic grouping × season of the year, P<0.05). Furthermore, the BLR from HO and Gir donor cows varied according to the individual sire used for fertilization, ranging from 0 to 73.3 ± 15.6% and 0 to 83.8 ± 18.1%, respectively. Therefore, the sire contribution should not be ruled out, due not only to the contribution of the breed of the sire chosen but also to the variability and performance of each sire used in fertilization. *Correspondence author: nadja@ufla.br. Acknowledgements: CNPq, FAPEMIG, Agroopegen.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

In vitro production of bovine embryos on cattle farms in Southwestern Paraná, Southern Brazil

Gabriela Gonçalves Fagundes[1], Matheus Ramos Rosin[1], Ederson Cichoski[2], Leandro Francisco Cichoski[2], Jackson Luiz Budel[2], Camila Keterine Gorzelanski Trenkel[1], Susamara de Souza da Silva[1], Adalgiza Pinto Neto[1]

[1]Universidade Federal da Fronteira Sul (UFFS)
[2]Sêminna Biotecnologia da Reprodução Animal

E-mail: gabriela.gf@estudante.uffs.edu.br

Given the diverse conditions and dimensions of the Brazilian territory, associated with breed, donor characteristics, cultivation conditions, seasonality and semen quality, the results of *In Vitro* Embryo Production (IVEP) in cattle present wide variations. Thus, from September/2023 to March/2024, bovine donors from 16 cattle farms in Southwestern Paraná, were subjected to Ultrasound-Guided Follicular Aspiration or "Ovum Pick-Up" (OPU), with the oocytes being aspirated, recovered, quantified, assessed (as viable, denuded or degenerated) and subjected to *In Vitro* Maturation (IVM) (TCM-199 + 90% Fetal Bovine Serum (FBS) + 22 IU/ml FSH + 50 IU/ml LH, CENATTE®), cultured in Petri dishes (drops covered with FUJIFILM Irvine Scientific®), in a controlled atmosphere (5.5% CO2 and 20% O2, saturated humidity and a stable temperature between 38 and 39°C). After 24 hours of IVM, the oocytes were subjected to IVF after semen preparation (Fert-TALP and 90% Percoll gradient, CENATTE®), in a controlled atmosphere (5% CO2, 95% air, 95% humidity and a stable temperature between 38.5 and 39°C) for 18 to 22 hours, after which the number of cleaved embryos was evaluated (D2). The cleaved embryos were maintained in an *in vitro* culture (a simple medium enriched with patassium + SOF, CENATTE® at 39°C, N2 atmosphere and saturated humidity), for 7 days, after which they were classified according to their development stage and then vitrified. The performance of the donors was evaluated by the average number of total, viable, cleaved oocytes (D1) and viable embryos (D7), considering the breed, temperature, rainfall rate, OPU/donor number and OPU/farm order. The data were processed (GraphPad Prism – Version 10.1), subjected to ANOVA and means were tested (Kruskal-Wallis – $p<0.05$). The 214 OPUs performed on donors from 16 cattle farms resulted in an average/OPU of 17.42 total oocytes, 10.33 viable oocytes, 12.39 cleaved embryos and 5.83 viable embryos. Nelore donors showed superior performance by OPU ($p<0.05$ – 26.58 total oocytes, 16.79 viable oocytes, 19.47 cleaved embryos and 9.47 viable embryos), when compared to breeds Braford (16,63; 9,43; 11,82 e 6,02), Brahman (13,92; 7,92; 10,83 e 5,67), Brangus (16,35; 9,09, 11,82 e 4,85), Hereford (13,15; 7,85; 9,15 e 2,5), Holstein-Friesian (17,46; 11,11; 10,94 e 4,31) and Tabapuã (14,28; 8,24; 10,88 e 6,86), which showed similar performance ($p>0.05$) for total, viable, cleaved oocytes and viable embryos, respectively. The atmospheric temperature at the time of OPU influenced the performance of the donors ($p<0.05$), with the average number of viable embryos being higher at a minimum temperature above 16.9°C and a maximum temperature above 27.8°C. The Rainfall influenced the number of cleaved and viable embryos ($p<0.05$), with the highest averages (11 cleaved and 4 viable embryos) observed with a normal precipitation index (up to 220 mm), compared to the rainy precipitation index. greater than 220 mm) the averages were 8 cleaved and 3 viable embryos. The number of OPUs/donor influenced the average number of total, viable and cleaved oocytes ($p<0.05$), considering the average interval of 16.4 days for each OPU. The order of OPU on the cattle farm influenced the average number of total oocytes ($p<0.05$) without showing direct correlation between them, and did not interfere with the average of viable, cleaved embryos and viable embryos ($p>0.05$). It is concluded that the performance of donors aiming at IVEP in cattle raised on farms in Southwestern Paraná are subject to the interference of factors similar to those observed in other regions of the country, which are capable of interfering with the research results to be found.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

In vitro production of embryos from Nelore cows (*Bos indicus*) treated with rBST

Ana Carolina dos Santos Oliveira[1,2], Emanuele Almeida Gricio[1], Sofía Albertini[1], Lígia Mattos Rebeis[1], Stella Morais[3], Luccas Scaglione Gomes[4], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Fundação Universidade Regional de Blumenau (FURB)
[3]Fazenda Segredo
[4]São Judas Tadeu University

E-mail: annakarollavet@yahoo.com.br

The success of *in vitro* embryo production (IVP) is related to the quantity and quality of the collected oocytes. The present study aimed to evaluate the efficiency of follicular aspiration (OPU) and IVP in Nelore cows (*Bos indicus*) treated with or without rBST. The experiment was conducted at Segredo farm, located in Bataguassu-MS, using two replicates. Donors aged 30 to 110 months were synchronized on day 0 (D-0) using 150 mg of injectable progesterone (P4; Sincrogest®, Ouro Fino, Cravinhos, SP) and 2 mg of estradiol benzoate (BE; Ric-BE®, Agener União Química, São Paulo, SP). Additionally, animals in the treated group received 325 mg of BST (Posilac®, Agener União Química, São Paulo, SP) also on D-0: Control (CT; n=27; no treatment) and rBST (rBST; 325mg of rBST; n=25). All cows underwent OPU on day 7 (D7). At the moment of OPU the number of follicles was assessed by US. Produced embryos were either transferred fresh to previously synchronized recipients or frozen. Statistical analyses were performed using the Glimmix procedure of SAS® (9.4). Results were expressed as mean and standard error. No interactions treatment*replicate were observed for any of the variables. There was a tendency for a decrease in the number of large (>8mm) follicles in the rbST group (CT = 1.23 ± 0.12 *vs* rbST = 0.8 ± 0.14; P = 0.06). However, no effect of treatment was observed for small (<6mm) follicles (CT = 34.2 ± 4.75 *vs* rbST = 34.5 ± 4.75). There was no difference between treatments in recovery rate (CT = 63.0 ± 3.3% *vs*. rbST = 63.4 ± 4.4%; P = 0.91), number of cleavages (CT = 13.8 ± 1.3 *vs*. rbST = 13.9 ± 1.5; P = 0.74), and number of blastocysts (CT = 6.44 ± 1.2 vs. rbST = 6.92 ± 1.5; P = 0.65). The P/TE was 53% (32/60) for the control group and 56% (35/62) for the rbST group (P = 0.72). It is concluded that in Nelore donor cows, rbST treatment does not influence embryo production and P/ET.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Metformin ameliorates bovine zygotes development

Wasim Al Shebli[1], Naiara Zoccal Saraiva[2], Carolina Capobiango Romano Quintão[2], Clara Slade Oliveira[2], Eliza Diniz de Souza[2], Maria Angélica Quintão Barros[3], Luiz Sérgio de Almeida Camargo[2]

[1]Fundação Universidade Federal de Viçosa (*UFV*)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)
[3]Gênese Biotecnologia em Reprodução Animal Ltda

E-mail: wasimalshible81@gmail.com

In vitro embryo production experiences some obstacles to its utilization in livestock system, like unsatisfactory yield besides suboptimal quality of resultant structures, owning that to many factors such as the cellular damages mediated by elevated reactive oxygen species (ROS) generation under *in vitro* conditions. The present work aimed to improve blastocyst outcome and viability via the addition of metformin (MET), which inhibits oxidants release from the respiratory chain. Cumulus-oocyte complexes (COCs) punctured from antral follicles (3-6 mm in diameter) of abattoir ovaries, were matured for 24 h, and then co- incubated in fertilization medium (20 µg/ml heparin) with sperm (2 million *per* ml) previously separated by mini-Percoll gradients. After 18 h presumptive zygotes were partially denuded, placed in culture media (synthetic oviductal fluid containing 3% fetal calf serum) supplemented with MET as following: 0 (MET0, n = 106), 0.05 (MET1, n = 107), 0.1 (MET2, n = 111) and 0.2 mM (MET3, n = 118), and cultured under 5% CO_2, 38.5°C and saturated humidity. At 48 and 168 h post fertilization, the cleavage and blastulation were verified (six replicates), and 5-6 blastocysts/group from four replicates were submitted to terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay (DeadEnd™ Fluorometric TUNEL System, Promega, Madison, USA) in order to evaluate the apoptosis index and total cell number. Data of cleavage and blastocyst rates and apoptosis index were analyzed by binary logistic regression (Proc Logistic) and total cell number was analyzed by linear mixed model (Proc Mixed) using the SAS Analytics software (version 9.1), considering the difference significant when $P \leq 0.05$. Cleavage rates were similar ($P > 0.05$) among MET0 (90.9 ± 2%), MET1 (88.4 ± 1.4%), MET2 (85.3 ± 3%) and MET3 (87.9 ± 2.8%). The highest blastocyst rate ($P < 0.05$) was found in MET1 (60.3 ± 2.5%) when compared to others (MET0 = 43.6 ± 7.3, MET2 = 30.4 ± 3.7 and MET3 = 48.1 ± 6.1%) whereas blastocyst rate in MET2 was lower ($P < 0.05$) than in MET3. No difference ($P > 0.05$) was found between MET0 and MET3. In MET0, the blastocyst total cell number (114.4 ± 8.1) did not differ ($P > 0.05$) from MET1 (106.2 ± 6.8) and MET3 (101.5 ± 5.4), but it was higher ($P < 0.05$) than in MET2 (90.2 ± 4.6). No difference ($P > 0.05$) was found among MET1, MET2 and MET3. Apoptosis occurrence showed no significant difference ($P > 0.05$) between the treatments (MET1 = 26.4 ± 1.9%, MET2 = 27.9 ± 1.9% and MET3 = 22.9 ± 2%); however, the MT0 presented a lower ($P < 0.05$) apoptosis index (24.5 ± 2.5%) than MET1 and MET2. In conclusion, metformin contributes to blastocyst production in a dose dependent manner. Dose of 0.1 mM metformin can be proposed as additive to embryo culture medium to enhance embryo production, although it does not reduce the apoptosis incidence. Financial support: CAPES, FAPEMIG and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Oocyte quality of lactating dairy cows submitted to feed restriction and heat stress

Laís Reis Carvalho[1], Lucas Araújo Lemos[1], Matheus Pedroso Vicente[1], Danielle dos Santos Cinelli Pinto[1], Luísa Oliveira Orlandi[1], Milena Carvalho Coelho[2], Maria de Fátima Ávila Pires[3], José Nélio de Sousa Sales[2], Bruno Campos de Carvalho[3]

[1]Universidade Federal de Lavras (*Faculdade de Zootecnia e Medicina Veterinária - UFLA*)
[2]Universidade Federal de Juiz de Fora (UFJF)
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)

E-mail: laiszoo23@gmail.com

The objective was to evaluate the effects of heat stress on oocyte quality in dairy cows previously submitted to feed restriction. In the study, 12 Girolando (*Bos taurus x Bos indicus*) cows, with 88.2 ± 2.7 days in milk, milk production of 25.3 ± 0.4 kg/day, during the dry season (Jul-Sep) were used. The experimental design aims to evaluate only the effects of heat stress, as heat-stressed cows consume less dry matter. In this context, the cows in the study would already have reduced feed consumption when they underwent heat stress. The cows were housed in a free-stall barn for seven days to monitor dry matter intake and adaptation, followed by feed restriction (15% of ad *libitum* intake) for eleven days (Control group). Four days before (D-4) the beginning of the period of feed restriction the emergence of the follicular wave was synchronized (2mg estradiol benzoate + P4 intravaginal device). After ten days (D6), the P4 device was removed and measurement and follicular fluid aspiration of the dominant follicle was performed, followed by resynchronization of the emergence of the follicular wave. After five days (D11), all visible follicles were counted and OPU was performed. At the end of the feed restriction period, the same cows were adapted to a climate chamber for four days and exposed to heat stress for eleven days [Heat Stress Group (HS)] (day THI = 85; night THI = 72). The same experimental design as the control group was carried out to synchronize wave emergence and follicular aspirations. The recovered oocytes were evaluated for quantity and quality. In addition, vaginal temperature was measured every 10 minutes throughout the experimental period using digital thermometers inserted intravaginally. Respiratory rate was evaluated three times a day (08, 12 and 16h) by visual inspection of respiratory movements during a period of 30 seconds. Statistical analyses were performed by SAS using the GLM and GLIMMIX procedures. Vaginal temperature and respiratory rate were greater in cows submitted to heat stress (Control = 38.8 ± 0.1 and HS = 39.1 ± 0.1 °C; P<0.0001; Control = 40.6 ± 1.9 and HS =59.9 ± 2.8 respiratory movements; P<0.0001, respectively). The follicular diameter ten days after the start of the ovulation synchronization protocol was similar between the experimental groups (Control = 17.8 ± 0.8 and HS = 17.4 ± 1.1 mm; P=0.72). Furthermore, there was no difference between treatments in the number of follicles aspirated (Control = 15.6 ± 2.4 and HS = 14.4 ± 2.5; P = 0.72), in the total number of oocytes recovered (Control = 6.6 ± 1.7 and HS = 7.0 ± 2.0; P=0.90), total of viable oocytes (Control = 6.6 ± 1.7 and HS = 7.0 ± 2.0; P=0.45) and the rate of viable oocytes [Control = 64.3% (54/84) and HS = 71.23% (52/73); P=0.35)]. It is concluded that exposure to acute heat stress did not affect the oocyte quality of lactating dairy cows when compared to cows previously submitted to restriction of feed intake. Support: Fapemig APQ 00049-2018.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Pregnancy rate and *in vitro* development of bovine embryos treated with TUDCA in culture media

Daniele Campos Araujo[1], Maria Helena Souza de Aguiar[1], Yeda Fumie Watanabe[2], André Lucio Fontana Goetten[1], Flávio Vieira Meirelles[3], Marcos Henrique Barreta[4], April Brooks[5], Vitor Braga Rissi[4]

[1]Universidade Federal de Santa Catarina (Departamento de Agricultura e Biodiversidade - *UFSC*)
[2]VITROGEN Biotecnologia LTDA.
[3]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[4]Universidade Federal de Santa Catarina (*UFSC*)
[5]Agrimark Genetics

E-mail: danivet9276@gmail.com

The global production of bovine embryos through *in vitro* fertilization (IVF) has surpassed those produced *in vivo.* This trend continues due to the rapid advancements in *in vitro* techniques. Despite these advances, *in vitro* embryo quality remains inferior to *in vivo* production, especially in cryotolerance and pregnancy rates. Malfunctions of the endoplasmic reticulum (ER) due to *in vitro* environment and manipulation stress can lead to protein folding inability, reactive oxygen species production, and activation of autophagy and apoptosis pathways. TUDCA (tauroursodeoxycholic acid) is a natural bile acid primarily found in bears and small quantities in humans. When used as an additive in culture media, it leads to a reduction in ER stress. This study aimed to assess the impact of treating *in vitro* embryos with TUDCA on cleavage and blastocyst rates, as well as the pregnancy rate following transfer to synchronized recipients. A total of 419 cows were subjected to follicular aspiration in the same laboratory as part of a commercial embryo production program. Of these, 184 were of dairy breeds (Holstein), and 235 were of beef breeds (11 breeds). Out of the 6,861 oocytes collected using the ovum pick-up (OPU) technique, 4,808 were matured and fertilized *in vitro.* After fertilization, the zygotes were divided into two groups: one with 100 µM TUDCA during the entire cultivation period (n = 2,386) and the control group without treatment (n = 2,422). The cleavage and blastocyst rates were assessed using ANOVA in the JMP-SAS software. Recipient cows underwent ultrasound examination 30-45 days after embryo transfer to determine pregnancy status. Pregnancy rates were assessed using the chi-square test using the JMP-SAS software. A total of 1,481 embryos were evaluated on days 7 and 9 after fertilization. This resulted in 536 fresh embryo transfers, 442 frozen embryos, and 503 embryos of lower quality or not ready to freeze on day 7, which were kept in the dishes and discarded on day 9. The group treated with TUDCA exhibited the highest cleavage rate at 72.8 ± 1.5%, while the control group rate was 66.7% ± 1.4 ($P<0.05$). The group treated with TUDCA had a 5% higher blastocyst rate per cultured oocyte compared to the control group: 34.7 ± 1.5% and 29.79 ± 1.4% respectively ($P<0.05$). In total, 536 embryos were produced and transferred fresh. Pregnancy status was evaluated in 448 cows, and 210 pregnancies were confirmed. The pregnancy rates were comparable between the group treated with TUDCA (47.4%) and the control group (46.3%) ($P = 0.8$). In our study, although the pregnancy rate did not show a statistically significant difference between groups, the TUDCA treatment resulted in the production of a high number of embryos.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Reactive oxygen species attenuation in vitrified-warmed bovine embryos

Viviane Luzia da Silva Feuchard[1], Juliana Horta Diniz[1], Carolina Capobiango Romano Quintão[2], Naiara Zoccal Saraiva[2], Clara Slade Oliveira[2], Leticia Zoccolaro Oliveira[1]

[1]Universidade Federal de Minas Gerais (*Departamento de Clínica e Cirurgia Veterinária - UFMG*)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)

E-mail: viviane.lfeuchard22@gmail.com

Vitrification is the most used methodology to cryopreserve *in vitro* produced (IVP) embryos. Still, despite providing an efficient protocol that allows the survival of 95% of IVF embryos and good pregnancy rates (40%) after vitrification and direct transfer in cattle (Oliveira *et al*., Cryobiology 97:222-225, 2020) it is still necessary to increase the quality of vitrified warmed. Hence, the aim of this study was to investigate the effects of modulating reactive oxygen species in vitrified bovine IVP embryos. Grade 1 blastocysts were selected and vitrified using a two-step protocol. Vitrification was performed in groups of five embryos and holding media was supplemented with 10% fetal calf serum. Selected embryos were transferred 200µl 7.5%DMSO +7.5% ethylenoglycol for 3 min. Next, washed in 200µl 16%DMSO +16% ethylenoglycol +0.5M sucrose media and placed in an open vitrification device in a 0.5 µl droplet. After 30s, the device was immersed in liquid nitrogen. We first compared ROS production in fresh and vitrified-warmed blastocysts and then we evaluated the effects of antioxidant supplementation (100 µM of 2-mercaptoethanol; BME) on ROS levels in vitrified-warmed blastocysts. At the end, we compared the development of fresh and vitrified-warmed blastocysts in the presence (100 µM BME) or absence (Control) of antioxidants. The oxidative index and total number of cells were compared by T-test and hatching rates with Fisher's Exact Test. Oxidative index was analyzed with Kruskall-Wallis and Dunn's Post Test, and the total number of cells using ANOVA and Tukey with 5% of significance (Minitab, 21.4.1.0). It was observed (n = 117 blastocysts obtained in two replicates, 52-64 per group) higher ROS production (Fresh: 50.75 ± 2.79 *vs* Vitrified: 81.21 ± 7.59; P<0.05) and lower cell number in vitrified compared to fresh embryos (Fresh: 132.79 ± 6.10 *vs* Vitrified: 105.02 ± 4.61; P<0.05). We also detected that antioxidant supplementation reduced the ROS levels (Vitrified: 38.24 ± 1.27 *vs.* Vitrified/BME: 33.54 ± 1.08; P<0.05) and increased the cell number in treated embryos (Vitrified: 100.65 ± 3.98 *vs*. Vitrified/BME: 112.95 ± 3.72; P<0.05). The BME neutralizes ROS levels by inducing the synthesis of intracellular glutathione, which occurs by reducing cystine to cysteine. Thus, the results demonstrated that 100 µM BME supplementation in the culture medium reduced ROS levels and increased the total number of cells in vitrified embryos. Although the embryo hatching rate did not differ (P>0.05) among embryos from the fresh, vitrified and vitrified/BME groups, the total cell numbers were higher (P<0.05) in vitrified embryos supplemented with BME (148.04 ± 7.26) than in vitrified embryos without BME (117.40 ± 5.50) but similar (P>0.05) to fresh embryos cultured with (163.19 ± 9.21) and without BME (158.04 ± 14.53). It was concluded that the vitrification and warming process increased ROS levels and its attenuation with BME antioxidant improved embryonic quality. Acknowledgment: CAPES and Embrapa.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

The amount of oocytes of each morphological quality grade does not interfere the bovine *in vitro* embryo development

Isabella Maran Pereira[1], Maria Lúcia Alves Mundim Bernardes[1], Márcia Carneiro Silveira[1], Cláudia Lima Verde Leal[2]

[1]Fertilife Fertilização *In Vitro* (*Pesquisa & Desenvolvimento, Centro Educacional, Goiânia - GO*)
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: isabellaa.maran@gmail.com

Once the literature establishes the oocytes' quality as a determining factor for embryo development (Ferronato *et al.*, PLoS ONE, 18:1-17, 2023), the aim of this study was to evaluate the relation between the number of different COCs grades in the development rates of *in vitro* produced embryos in Nelore bovine cattle, in the actual IVEP commercial chain, by data analysis of an IVEP company. COCs from Nelore donor cows (n = 299; Recanto da Serrinha, Guapó, Brazil) were aspirated, graded from I to III (I: homogeneous cytoplasm and more than three layers of cumulus cells; II: homogeneous cytoplasm and up to three layers of cumulus cells; III: homogeneous cytoplasm and one layer not equally distributed of cumulus cells), and *in vitro* fertilized with Nelore bulls' previously validated semen. The cleavage rates and media feeding were performed at 96 hours post insemination (hpi) and at 168 hpi the blastocysts were evaluated in both development and grading. Grades I and II blastocysts were loaded for embryo transfer to synchronized recipient cows and pregnancy diagnosis was performed after 30 days of embryo transfer. Statistical analysis was performed using the BioEstat 5.0 software by One-way ANOVA. Data were tested for normal distribution and variance homogeneity, and level of significance was set at $p<0.05$. The batches were stratified into classes, divided as quartiles, according to the number of COCs of quality grade I (0; 1 to 3; 4 to 6; or >7); grade II (0; 1 to 5; 6 to 10; or >11), and grade III (0; 1 to 15; 16 to 45; or >46) used for IVEP. The cleavage, blastocyst (total and according to grade) and pregnancy rates were retrospectively analyzed and IVEP endpoints contrasted among classes of COC grades I, II, and III. Except for the lower cleavage rates for the absence of grade III oocytes (38.0%, $p<0.01$) when compared to all other amounts (range 71.3 to 81.8%), there was no statistical difference for any of the parameters analyzed. Whereas the absence of grade III COCs lowers the cleavage rates, subsequent developmental rates are not affected, not seeming to indicate any relevance for this observation. Nevertheless, it is important to consider that only one IVP protocol was analyzed and there may be variations for other protocols and cattle breeds. We therefore conclude that the amount of COCs of each quality grade does not interfere in the final outcome of blastocyst development and pregnancy rates for Nelore IVEP in a commercial scenario.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

The influence of corpus luteum and follicles on the quality of oocytes in beef cattle

Letícia Guiaro Alves[1], Fernanda Amarante Mendes de Oliveira[1,2], Camila Bortoliero Costa[1], Gabrieli Dutra Gonçalves[1], Gabriela Rodrigues de Paula[1], Flávia Aguiar Carrieri[1], Pamela Mara Celestino Soares[1], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Laboratório de Biotecnologia da Reprodução Animal (REPROA/UEL)
[2]Instituto Federal de Educação Ciência e Tecnologia do Amazonas (*Departamento de Ensino, Pesquisa e Extensão - IFAM/CTBT*)

E-mail: leticia.guiaro@uel.br

The study aimed to investigate the influence of corpus luteum and at least one follicle ≥ 6 mm (established as follicular divergence) on the recovery and quality of oocytes. Ovaries (n = 172) were collected from a local slaughterhouse and transported in pairs to the laboratory to be organized into four experimental groups: G1 - initial CL and no follicle ≥ 6 mm (n = 36); G2 - initial CL and presence of follicle ≥ 6 mm (n = 40); G3 - mature CL and no follicle ≥ 6 mm (n = 49); G4 - mature CL and presence of follicle ≥ 6 mm (n = 47). The oocytes were collected by puncturing the follicles ≥2 mm with a syringe and needle. The COC was classified according to the presence of *cumulus* cells and the appearance of the oocyte, as follows: grade I more than 3 layers of *cumulus* cells and homogeneous ooplasm with fine granulations, grade II less than 3 layers of *cumulus* cells and ooplasm showing changes in color and granulation, grade III presence of at least one layer of *cumulus* cells or partially denuded and ooplasm showing changes in color and granulation, denuded without *cumulus* cells and expanded with expanded cumulus cells and irregular ooplasm with dark color. COC recovery rates (with different quality) and viable oocytes rate (relation between total recovered and COC grade I and II) were analyzed using the Chi-square test. In the presence of a significant effect (P<0.05), the ranking between rates was analyzed using a 2x2 proportion test. There was a significant difference among groups in terms of quality and viable COC rate. The G4 group presented a higher percentage of viable COC, being 37.3% (523/1402), differing from the other groups, being 31.4% for G1 (450/1434), 32.6% for G2 (405/1243) and 32.4% (550/1695) for G3 (P<0.004). The rates of COC grade I in groups G2 [16.81%, (209/1243)] and G4 [17.55% (243/1402)] differed from groups G1 [13.25% (190/1434)] and G3 [13.27% (225/1695)] (P<0.039). When comparing the rates of COC grade II, only the G2 group with 15.77% (196/1243) differed from the other groups with lower rates, such as G1 [18.13% (260/1434)]; G3 [19.17% (325/1695)] and G4 [19.76% (277/1402)] (P<0.039). Based on the results obtained, we can conclude that ovarian structures influenced the quality of the COC. Additional studies are needed to elucidate the mechanisms of CL and follicular divergence affecting the quality of COCs.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

The use of a microfluidic sperm sorting on bovine frozen-thawed semen for *in vitro* fertilization

Bruna Lopes Cardoso[1,2], Mayra Fernanda Alves Borges[2], Luiz Felipe Fachin Pazianoto[1,2], Luísa Anastácia Santos de Oliveira[2], Felipe Perecin[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]CPEX Embriões

E-mail: brunalopescardoso@gmail.com

In bovine, the frozen-thawed semen used in IVF is widely processed by the density-gradient protocol, usually using Percoll or Bovipure reagents. Through centrifugation, the diluents and cryoprotectants are removed, and the motile and immotile sperm cells are separated. In human assisted reproduction, the microfluidic sperm selection device ZyMōt® Fertility (DxNow Inc., Gaithersburg, MD, USA) has been used on IVF or ICSI cycles resulting in better outcomes. Herein, we compared the efficiency of the ZyMōt device for bovine semen preparation for IVF with the conventional density-gradient protocol. Frozen straws of Nelore bulls were thawed at 37°C for 30 seconds in a water bath and one straw of each bull was processed per method: ZyMōt or Percoll. For this study, only conventional semen straws (non-sexed sorted), from seven different bulls, were used. We performed sperm separation using ZyMōt Multi (850 µL) following the manufacturer's instructions. In brief, one straw with 250 µL of semen diluted in 600 µL of IVF medium (850 µL of semen sample) was loaded into the inlet of the device using a syringe, and 750 µL of the IVF medium was added on top of the membrane. The device was placed in a humidified incubator at 38.5°C for 30 min; then 500 µL of spermatozoa was recovered from the outlet with a syringe and centrifuged for 10 minutes at 300xg. For Percoll, one straw (250 µL) was deposit on the top of the gradient (90%/45%) and the samples were centrifuged twice, with the first centrifugation at 5000xg for 5 minutes, followed by washing in IVF medium and a second centrifugation at 100xg for 3 minutes. After centrifugation in both protocols, a pellet of 60 µL was retrieved and the donors' COCs (n=3.833) were randomly inseminated with 4 to 5 µL of ZyMōt- or Percoll-processed semen, with visual assessment of motility and concentration. The semen concentration was not measured in both protocols because the experiment followed the standard operating procedure that is routinely used. On day 7, embryos were evaluated and classified according to IETS guidelines. Cleavage, blastocyst (blastocyst/ oocytes cultured) and development (blastocysts/cleaved) rates were compared by Chi-Square Test ($\chi 2$) considering $P<0.05$ as significant level. Sperm separation with ZyMōt resulted in higher ($P<0.0001$) blastocyst and development rates [26.74% (526/1967) and 42.01% (526/1252)] compared with Percoll separation [21.01% (392/1866) and 31.51% (392/ 1244)]. Interestingly, no differences were found in the cleavage rate between the groups 66.67% (Percoll) and 63.65% (ZyMōt). The reasons leading to better IVF outcomes after ZyMōt sperm separation are still unclear. We report here preliminary favorable results of ZyMōt sperm separation in a commercial bovine IVF routine. Further investigation, with much larger samples, is required to determine the effects of bull, donor, breed, breeding, and semen type (conventional or sex-sorted) in IVF cycles with ZyMōt sperm separation.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Treatment of *in vitro* produced embryos with colony-stimulating factor 2 during culture in the presence of serum does not affect pregnancy rates after embryo transfer

André Lopes Cirino[1], João Gabriel Viana de Grázia[2], Rafael Augusto da Anunciação[3], Luany Alves Galvão Martinhão[4,5], Luiz Sérgio de Almeida Camargo[6], João Henrique Moreira Viana[6], Peter James Hansen[7], Luiz Gustavo Bruno Siqueira[6]

[1]Universidade Federal de Juiz de Fora (UFJF)
[2]Apoyar Biotech Ltda
[3]Universidade José do Rosário Vellano (UNIFENAS)
[4]Universidade de Brasília (*UnB)*
[5]Laboratório Norte Embryo
[6]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)
[7]University of Florida (*Department of Animal Sciences, D.H. Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute*)

E-mail: andre.lopes@estudante.ufjf.br

The cytokine colony-stimulating factor 2 (CSF2) is a maternally-derived molecule produced by the endometrium a few days after ovulation in cattle. Previous studies reported a possible role of CSF2 in developmental programming of the preimplantation embryo by altering properties of the blastocyst such as differentiation and pluripotency of blastomeres, gene expression, embryo elongation and secretion of interferon tau (Hansen *et al.*, Animal Reprod Sci, 149:59-66, 2014). More recently, however, we reported that actions of CSF2 on the early embryo may be dependent on whether or not serum was present in the culture medium (Amaral *et al*., Scientific Reports, 12:7503, 2022). Therefore, the objective of this study was to determine pregnancy outcomes after CSF2 supplementation of serum- containing medium from days 5 to 7 of *in vitro* culture (IVC). *In vitro* embryo production was performed using standard procedures of a commercial laboratory (Apoyar Biotech, Alta Floresta, MT). *Cumulus*-oocyte complexes (COCs) were collected from slaughterhouse ovaries (Nelore females) and submitted to *in vitro* maturation for 22 to 24h. Matured COCs were fertilized using Y-sorted sperm from Nelore sires of proven fertility. Embryo culture followed standard procedures in medium supplemented with 3% fetal bovine serum (FBS), in a humidified atmosphere of 5% CO2 and 5.5% O2. On Day 5 of IVC, zygotes were randomly allocated to receive either vehicle (Control group) or 10 ng/mL CSF2 (CSF2 group). A yeast- derived recombinant bovine CSF2 (Kingfisher Biotech, Inc., Saint Paul, MN, USA) was used. Recipients were synchronized for timed embryo transfer (ET) with an estradiol-progesterone based protocol and, on day 7 after anticipated estrus, eligible females (presence of a CL >2 cm^2 in area) were randomly assigned to receive an *in vitro* grade 1 expanded blastocyst from Control (n=173) or CSF2-treated medium (n=180). Pregnancy diagnosis was performed by ultrasonography on Day 23 ± 2 after ET and the presence of an embryo with a heartbeat indicated an ongoing pregnancy. Statistical analysis was performed using Fisher's exact test in a 2x2 contingency table (GraphPad Prism software) to examine for differences in pregnancy rates between the two groups. A *P*-value of <0.05 was considered statistical significance. Data are presented as percent of pregnant recipients relative to total transferred recipients. Pregnancy rates on days 30-32 of gestation did not differ among treatment groups (45.7% versus 48.9% for Control and CSF2 groups, respectively; *P*=0.59). In conclusion, addition of CSF2 to a serum-containing medium from days 5 to 7 of development during IVC did not affect pregnancy rates after embryo transfer. Future studies may investigate further the occurrence of embryonic losses in pregnancies from CSF2-treated embryos cultured in medium with or without serum. Acknowledgements: Financially supported by FAPEMIG (Project APQ-02126-21) and CNPq (INCT 406866/2022-8.)

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-FIV

Use of a sperm separation device as an alternative for sires with low outcomes using gradient selection for *in vitro* embryo production: preliminary results

Marcelo da Cunha Xavier[1], Maurício Antonio Silva Peixer[1], Luiz Gustavo Araújo Oliveira[1], Patricia Furtado Malard[2], Rodrigo Moura Martins[2], Rosangela Vieira Andrade[2], João Henrique Moreira Viana[3]

[1]BIO Reprodução Animal
[2]Universidade Católica de Brasília (*UCB*)
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)

E-mail: marcelo@biotecnologiaanimal.com.br

Sperm quality is an important factor affecting *in vitro* embryo production (IVEP) outcomes. The male factor is associated with significant differences in embryo rates, regardless of the oocyte donor used (Peixer *et al.* 2023). The selection of sires to be used in IVEP, however, is mainly based on their genetic merit or importance, rather than on sperm quality. Moreover, currently most IVEP laboratories prepare sperm for *in vitro* fertilization (IVF) using centrifugation in colloid density gradients, such as Percoll. Although a simple and straightforward procedure, this method has been associated with acrosome. The aim of this study was to evaluate the use of a sperm separation device (VetMotl, VetMotl inc., USA) to select viable sperm from sires with known records of low results after IVEP. Frozen-thawed sperm from Nelore sires (n=5) were used. The sperm of each bull was processed either by conventional centrifugation in a percoll gradient (45-90%) or diluted 1:1 in FERT-TALP medium and 850 µL were loaded in a separation chamber, which was then incubated during 30 min in a CO_2 incubator. The supernatant was then recovered from the upper part of the chamber, and evaluated for sperm concentration. Sperm processed by both methods were then co-incubated (aprox. 1x106 sptz/mL) with *cumulus*-oocyte complexes from the same donors for 20h. The presumptive zygotes were cultured in SOF medium, which was adjusted for production of embryos to be transferred as fresh (two sires) or frozen-thawed (four sires) by direct transfer (DT). Cleavage and blastocyst rates were compared by the Chi-squared method. Sperm preparation using the separation chamber resulted in greater cleavage rates (81.8% [63/77] *vs*. 41.2% [103/250] for the fresh protocol and 51.4% [227/442] *vs.* 42.3% [135/319] for the DT protocol, respectively; P<0.05) and blastocyst rates (44.2% [34/77] *vs.* 16.0% [40/250] for the fresh protocol and 17.2% [76/442] *vs.* 7.5% [24/319] for the DT protocol, respectively, P<0.001), compared with Percoll centrifugation. In all sires, the use of the sperm separation chamber resulted in increase on embryo rates (from +2.4% to +48.1%, on average +26.5 ± 7.1%). In summary, the preliminary results suggests that the use of a sperm separation chamber is an alternative to improve blastocyst rates from sires with previous records of low IVEP outcomes.

Acknowledgements: VetMotl inc. and CNPq INCT 406866/2022-8.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Effect of different concentrations and exposure periods of TNF-alpha in the viability of cumulus-oocyte complexes

Adriano Cavalcanti Cardoso[1], Thamiris Vieira Marsico[2], Kelly Annes[1], Andressa Minozzo Oliveira[1], Mateus José Sudano[1]

[1]Universidade Federal de São Carlos (*UFSCAR*)
[2]Universidade Federal do ABC (*UFACB)*

E-mail: adriano.cavalcanti@estudante.ufscar.br

Elucidating the effects of pro-inflammatory cytokines during oogenesis and folliculogenesis has proven to be a challenging task largely due to its pleiotropic nature. Follicular fluid (FF) concentration of tumor necrosis factor-alpha (TNF-α) is regulated by infection diseases and environmental stressors experienced by females. This study aims to investigate the effects that different concentrations of TNF-α plays on the viability of cumulus-oocyte complexes (COCs). Homogenous bovine oocytes with more than three layers of cumulus cells (CCs) were subjected to IVM medium supplemented with TNF-α at different concentrations and exposure times. The gametes were cultured and divided into groups (n = 25/group) according to the FF cytokine concentration documented in literature and added to the medium: oocytes cultured in medium containing 2.9 ng/mL (physiological concentration); in medium containing 2.0 ng/mL (Covid-19 concentration); in medium containing 200 ng/mL (cytotoxic effect); in the absence of the cytokine (control). Additionally, they were divided regarding the exposure time, which varied between 8, 16, and 24 hours. For statistical analysis, data were subjected to analysis of variance (ANOVA) and logistic regression with a significance level of 5% ($P < 0.05$). To analyze the expansion of COCs during IVM (n = 8/group), images were captured before and after IVM and the area of the COCs was measured. To assess cytoplasmic maturation, the pattern of oocytes cortical granules (CGs) distribution was analyzed. For the nuclear maturation, the extrusion of the first polar body was considered and oocytes were subjected to nuclear staining with Hoechst 33342 solution. Lastly, the proportion of fragmented DNA of oocytes and CCs was also evaluated utilizing the TUNEL assay. As result, it was observed that the exposure of oocytes to 200 ng/mL was able to reduce ($P < 0.05$) the rates of the first polar body extrusion by 30% compared to the control group, and increase ($P < 0.05$) the area of COCs by 4.15 times the original when exposed during the final 8 hours of IVM. In addition, interference in the distribution pattern of CGs was also observed ($P < 0.05$), indicating differences within the same treatment and distribution pattern. CCs treated with 200 ng/mL in the initial third of IVM exhibited higher rates (17%) of fragmented DNA ($P < 0.05$) compared to those treated with 2.0 ng/mL in the initial and final thirds (1%), suggesting potential susceptibility of CCs to substances contained in the medium. On the other hand, in the TUNEL of oocytes (n = 275), only seven exhibited fragmented DNA, highlighting the fact that these gametes are refractory to cell death, primarily due to the environment in which they reside and the protection provided by the CCs. Despite the low oocyte DNA fragmentation rate, we conclude that the stress caused by TNF-α supplementation at the tested concentrations was able to affect the quality of analyzed COCs.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Effect of rutin on the *in vitro* maturation of oocytes

Isabela Maria Lopes[1], Luana Kealy Pimentel de Oliveira[1], Sueli de Oliveira Lima[1], Andreza Mayara Carneiro Lima[1], Érika Karoline de Oliveira Aureliano[1], Damaris Raquel Pires dos Santos[1], Joãozito Liandro de Oliveira Junior[1], Mabel Freitas Cordeiro[1], Maria Helena Tavares de Matos[1], Edilson Soares Lopes Júnior[1]

[1]Universidade Federal do Vale do São Francisco (*Ciências Veterinárias no Semiárido - UNIVASF*)

E-mail: isaabelalopess@outlook.com

Rutin supplementation in preantral follicle culture medium (Lins *et al*., 2017) demonstrated effects on antioxidant defense mechanisms, increasing the expression of enzymes, including catalase (CAT) and superoxide dismutase (SOD), at the same time which restricts the activity of xanthine oxidase, in the generation of reactive oxygen species (ROS). This study aims to investigate the impact of rutin on IVM and its implications on IVP in sheep. Ovaries obtained from a local slaughterhouse were used. They were transported within 1 hour in a 0.9% NaCl solution containing 0.05 g of pentabiotic/L, at a temperature of 33°C to 34°C, in a thermos bottle. To collect the COC, a vacuum pump with a pressure of 20 mmHg, an 18G needle and recovery medium were used. After collection, the COCs were evaluated for their level of quality, and only those with Grades I and II were selected for the IVM. After classifying the oocytes, a total of 993 oocytes were selected and then divided into five maturation groups: CON-IVM (control) and the RUT0.1, RUT10, RUT20 and RUT40 groups, in which the oocytes were matured in the presence of different concentrations of rutin (0.1, 10, 20 and 40 µg/mL, respectively). Fifteen oocytes were matured for 24 hours in a culture incubator at 38.5°C in a humidified atmosphere with 5% CO_2. After maturation, they were evaluated for the expansion of cumulus cells, based on morphology. Chromatin configuration, intracellular glutathione (GSH) levels (ROS) and mitochondrial activity were evaluated through fluorescence. DNA fragmentation was evaluated by TUNEL assay. They were classified as: not expanded, partially expanded or fully expanded. Regarding the expansion rate of cumulus cells, the RUT10 group showed the highest total expansion rate compared to the other groups ($P < 0.05$). Regarding the rate of nuclear maturation, the chromatin configuration of 597 matured oocytes was observed. As for GVBD, the RUT10 group presented the lowest proportion of oocytes at this stage ($P < 0.05$) compared to all others. Regarding MI, the RUT20 and RUT40 groups showed significantly higher rates when compared to the RUT0.1 and RUT10 groups. Regarding the MII rate, the RUT0.1 and RUT10 groups were higher when compared to the other groups. The RUT10 group showed the highest levels of mitochondrial activity when compared to the others ($P < 0.05$). Regarding GSH levels, RUT10 group showed higher intracellular levels when compared to the control, although similar to the RUT0.1 group ($P > 0.05$). Regarding ROS levels, the groups with added rutin presented lower levels when compared to the CON group ($P < 0.05$). Subsequently, DNA fragmentation analysis showed that the RUT10 group had a lower proportion of fragmented oocytes compared to the control group. In conclusion, supplementation with 10 µg/mL of rutin positively influences the *in vitro* maturation of sheep oocytes, improving the levels of mitochondrial activity, glutathione peroxidase and reducing DNA damage in sheep oocytes.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Effects of hCG administered by different routes seven days after onset of estrus in synchronous estrus induced acyclic dairy goats

Shara Eliete Davi Silva[1], Luana Rangel Côrtes[1], Alice Lima Martins[1], Bruna Waddington de Freitas[1], Signety Ferreira Vieira[2], Paulo Sergio Cerqueira Rangel[1], Mariana Garcia Martins, Jeferson Ferreira da Fonseca[3], Felipe Zandonadi Brandão[4], Luiz Gustavo Bruno Siqueira[3]

[1]Universidade Federal de Viçosa (*UFV*)
[2]Universidade Federal de Campina Grande (*UFCG*)
[3]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)
[4]Universidade Federal Fluminense *(UFF*)

E-mail: saeliesilva@gmail.com

Considering important effects of hCG administration by intramuscular (i.m.) route on progesterone and pregnancy (Rodrigues *et al*., 2022), this study checked if intrauterine route could provide similar hCG serum levels as well as i.m. route in dairy goats during non-breeding season. Mature goats (n = 44; body weight of 56.5 ± 1.5 kg; body condition score of 3.0 ± 0.2) had synchronous estrus induced by intravaginal sponges (60 mg of medroxyprogesterone acetate; Progespon®, Zoetis, São Paulo, Brazil) for six days plus 200 IU of equine chorionic gonadotropin (eCG; Foli-rec 7000®; Zoovet, Santa Fé, Argentina) and 131.5 µg of cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, Brazil) i.m. 24 h before sponge removal. Estrus was checked, females bred with fertile males (D0 = Day of first mating) and CL checked by transrectal ultrasonography on D7. Goats with viables CLs (n = 35) were assigned to three groups: goats that received 300 IU of intrauterine transcervical hCG (hCG-IU; n = 12) or i.m. hCG (hCG-IM; n = 11) injections or 1 mL of saline solution (control; n = 12) i.m. on D7. Blood samples were collected from all animals via jugular vein puncture before hCG administration, 12 h after, and on days 8, 10, 13, 17, and 21 between 6-7 am. Serum was stored at -20°C and analyzed by solid-phase radioimmunoassay (RIA – hCG Kit, Irma Kit, Immunotech, Prague, Czech Republic). Statistical analysis (BioEstat 5.3, Belém, Brazil; IBM SPSS Statistics, version 19) used general linear model with repeated measures over time applied to data collected between days. Non-parametric data were analyzed by the Kruskall-Wallis test followed by Dunn's post hoc; for parametric data, ANOVA followed by Tukey's post hoc was applied. Frequencies were assessed by the Chi-square or Fisher's exact test. Four goats from the hCG-IU group were not considered in the study because cervical transposition was not possible. In all studied animal groups, mean circulating hCG concentrations did not differ ($P > 0.05$) on D7. In the hCG-IM group, hCG concentrations increased on D7.5, remaining so until D8 and decreased from D8 to D10, ($P < 0.05$) and thereafter, there was no difference ($P > 0.05$) between groups. Control goats tested positive for hCG. Pregnancy rates on D30 and 60 were similar ($P > 0.05$) between hCG-IM (91.0% or 10/11) and control (83.0% or 10/12) groups, and both groups were superior ($P < 0.05$) when compared to hCG-IU group (25.0% or 2/8). Intramuscular route should be considered as preferential for sustained increase in hCG levels over the measured days not disturbing pregnancy. The fact of control goats testing positive for hCG suggests the possibility of the cross-reactivity of RIA hCG antibodies with LH, r-eCG, or another unidentified molecule associated with early pregnancy in goats.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Enhancing Bovine Oocyte Development: Using SCD1 Enzyme for *In vitro* Maturation

Alexandre Marcos de Melo[1], Carolina Capobiango Romano Quintão[1], Clara Slade Oliveira[1], Wasim Al Shebli[1,2], Luiza Bastos Guimarães[1], Victor Felipe Sophia da Costa Neves[1], Luiz Sérgio de Almeida Camargo[1], Naiara Zoccal Saraiva[1]

[1]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA – Gado de Leite*)
[2]Universidade Federal de Viçosa (*UFV*)

E-mail: alexandremelo.cb@gmail.com

Stearoyl-CoA desaturase 1 (SCD1) is an important enzyme in lipid metabolism, influencing fatty acid composition and fat storage. Recent evidence suggests that the presence of SCD1 is crucial for oocyte competence and embryonic development. Unsaturated fatty acids (UFAs) play an important role in bovine fertility. Oleic acid [OA; C18:1 cis-9] is an example of a UFA with positive effects on oocyte competence, with its synthesis regulated by the enzyme SCD1. The aim of this study was to develop *in vitro* maturation (IVM) strategies to increase the availability of UFAs in bovine oocytes and to evaluate the effects of modulating SCD1 enzyme activity in an IVP system, specifically on the endogenous synthesis of OA during the IVM of bovine oocytes. For this purpose, oocytes obtained from slaughterhouse ovaries were subjected to IVM, with the base medium composed of TCM 199 buffered with sodium bicarbonate, FSH, hCG, estradiol, sodium pyruvate, amikacin, and 8 mg/mL BSA (FBS-free). To standardize IVM conditions with substrate (stearic acid; SA) and product (OA) of the SCD1 enzyme, the following groups were established: A - BSA control (8 mg/mL); B - ethanol control; C - 25 µM SA; D - 50 µM SA; E - 100 µM OA; and F - 200 µM OA. Oocytes were cultured in groups of up to 50 structures in 400 µL of IVM medium, in four-well plates, without addition of mineral oil, at 38.5°C and an atmosphere of 5% CO2 in air. After 24 h of IVM, oocytes were fertilized in TALP-IVF medium supplemented with BSA, heparin, penicillamine, hypotaurine, and epinephrine for a period of up to 24 h, and then the presumptive zygotes were cultured in modified SOF medium supplemented with 1.5% FBS at 38.5°C and 5% CO2 in air. Four replicates were performed, totaling approximately 100 oocytes per group, and cleavage and blastocyst production rates were evaluated after 48 h and 7 days of IVF, respectively. Analyses were performed using GraphPad Prism 10, with proportions evaluated by the Chi-square test ($\chi 2$). Regarding cleavage rates, there were no significant differences ($P > 0.05$) between groups (A: 75/92 - 81.5%; B: 75/95 - 78.9%; C: 83/110 - 75.4%; D: 56/76 - 73.7%; E: 67/89 - 75.3%; F: 68/82 - 82.9%). Regarding blastocyst production, the two concentrations of stearic acid evaluated (C: 35/108 - 32.4%^{c}; D: 21/76 -27.6%^{c}) allowed blastocyst rates similar ($P > 0.05$) to the control groups (A: 30/92 - 32.6%^{bc}; B: 44/95 - 46.3%^{ab}), despite the higher numerical values observed in group C. When the medium was directly supplemented with OA, it was observed that the higher concentration (F: 42/82 - 51.2%^{a}) provided a rate superior ($P < 0.05$) to the BSA control group and did not differ from the 100 µM OA group (E: 37/89 - 41.6%^{abc}), indicating that OA, a product of the SCD1 enzyme, may have a positive effect on embryonic development in cattle, especially at higher concentrations. Financial support: Fapemig, CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Epigenetic and developmental plasticity of oocytes under heat stress

Caroline Alencar Imaeda-Carvalho[1], Marcelo Tigre Moura[1], Fabíola Freitas Paula-Lopes[1]

[1]Universidade Federal de São Paulo (*UNIFESP*)

E-mail: caroline.imaeda@unifesp.br

The decline in fertility due to heat stress (HS) has been extensively studied across different species. The oocyte is a primary target of heat stress, undergoing several cellular and molecular adaptations to withstand the challenges posed by high temperatures. This often results in a significant reduction in oocyte developmental competence and subsequent embryonic development. Previous studies in our laboratory demonstrated that the plasticity of female mice to adjust to a mild environmental temperature of 35°C involved a major variation in the epigenetic status of the oocyte with a minor effect on in vivo oocyte developmental competence. Therefore, the aim of this study was to use the mouse model to determine the effect of moderate heat stress during the first window of de novo DNA methylation reprogramming on global DNA methylation and developmental competence of murine oocytes. Therefore, Swiss mice were mated to produce offspring. Lactating females and their offspring were exposed to either HS (39°C during 12 hours of light and 21°C during 12 hours of darkness) or control group (21°C throughout the entire period) for 11 days from postnatal day 10 (P10) to 21 (P21). At P21, all animals were allocated to control environmental chamber until puberty (P35). For the first study, pubertal females (HS: n = 6; control: n = 5) were superovulated with eCG and hCG, within a 48 h interval. Animals were euthanized 15h after hCG injection for oocyte collection. Collected oocytes were denuded in 0.1% hyaluronidase, fixed in 4% paraformaldehyde, and subjected to immunofluorescence using monoclonal rabbit anti-mouse 5-methylcytosine primary antibody (1:1000) and secondary polyclonal goat anti-rabbit Alexa Fluor 488 antibody (1:500). Oocytes were counterstained with 50 µg/mL propidium iodide for DNA labeling. Oocyte analysis and image acquisition was performed under fluorescence microscope (Zeiss Axio Imager A2) and Zen Pro software. DNA methylation fluorescence intensity was quantified using Image J software. This experiment was replicated 3 times using 15 oocytes for control and 17 oocytes for HS. For the second experiment, pubertal females (HS: n = 25; control: n = 21) were superovulated and bred to fertile males. Embryo collection was performed 94 hours after breeding to determine preimplantation embryonic development and viability. Statistical analysis was conducted using SAS software (SAS User's Guide. 1989). Results indicated that HS increased (P = 0.06) oocyte global DNA methylation as compared to control. Moreover, HS reduced the percentage of embryos that reached the blastocyst (P = 0.04) and expanded blastocyst (P = 0.02) stages. Heat stress also increased the proportion of fragmented (P = 0.003) and degenerated (P = 0.04) embryos as compared to control. In conclusion, exposure of females to moderate HS during the time of oocyte growth and de novo DNA methylation caused oocyte hypermethylation, reduced embryonic development and viability.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Exposure of bovine COCs to post-ovulatory aging differentially compromised cellular function in intact COCs versus denuded oocytes

Veronica Louzada dos Reis[1], Fabíola Freitas De Paula Lopes[1]

[1]Universidade Federal de São Paulo (*UNIFESP*)

E-mail: veronica.louzada@unifesp.br

Among the major factors exerting adverse effects on female fertility, we can highlight oocyte aging. Post-ovulatory aging (POA), manifested when fertilization fails to occur within the optimal time frame causes detrimental alterations in the ovulated oocyte, impairing the functional integrity of the female gamete. It has already been demonstrated that oxidative stress is one of the main contributors of oocyte aging. However, there is a limited understanding of how exposure of cumulus-oocyte complexes (COCs) to POA affects intact COCs versus denuded oocytes. Therefore, this study used a bovine model to explore the involvement of mitochondria and reactive oxygen species (ROS) in the context of POA in bovine COCs. Slaughterhouse-derived bovine ovaries were processed for COCs collection. The post-ovulatory aging model was established with bovine COCs collected at the following stages: Immature (0h of *in vitro* maturation - IVM), Mature (COCs subjected to standard 22h IVM), and Aged (COCs subjected to prolonged 40h IVM). Oocytes from each stage (Immature, Mature and Aged) were divided into two subgroups: intact COCs and denuded oocytes, after removal of cumulus cells, to determine mitochondrial activity (Mitotracker Red CMXROS - Experiment 1) and ROS production (CellROX Green - Experiment 2). Oocyte apoptosis was evaluated in denuded oocytes by TUNEL assay (Experiment 3). Sample analysis and image acquisition was performed under fluorescence microscope (Zeiss Axio Imager A2) and Zen Pro software. Mitochondrial activity and ROS production were quantified using Image J software. Mitochondrial activity, ROS production and oocyte apoptosis experiments were replicated 3, 4 and 5 times, respectively. Non-parametric data were analyzed by Wilcoxon and parametric data were submitted to ANOVA using SAS software (SAS User's Guide. 1989). Aged COCs exhibited a notable decline in mitochondrial activity as compared to the other groups ($P < 0.0001$). Interestingly, there was no difference in mitochondrial activity between mature and aged denuded oocytes. As anticipated, immature oocytes demonstrated lower mitochondrial activity ($P < 0.0001$) relative to their counterparts from other developmental stages. ROS production was significantly elevated ($P < 0.0001$) in mature and aged COCs as compared to immature COCs. Nevertheless, examination of denuded oocytes unveiled an increase in intracellular ROS levels during post-ovulatory aging when contrasted with immature ($P < 0.0001$) and *in vitro* matured ($P = 0.0002$) oocytes. Examination of apoptosis in denuded oocytes showed no difference in the percentage of TUNEL-positive oocytes between groups. In conclusion, exposure of COCs to POA elicited a differential response in the intact COCs and denuded oocytes. Mitochondria were negatively affected by post-ovulatory aging, evidenced by increased oxidative stress and mitochondrial dysfunction.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Extracellular vesicles derived from early and late follicles significantly change cumulus and oocyte gene expression but not oocyte competence to form a blastocyst

Mirela Brochado Souza Cáceres[1], Giovana Barros Nunes[2], Cíntia Rodrigues da Silva[2], Ricardo Perecin Nociti[2], João Carlos Pinheiro Ferreira[2], Natália Marins Bastos[3], Paola Maria da Silva Rosa[3], Juliano Coelho da Silveira[3], Marcos Roberto Chiaratti[1], Gisele Zoccal Mingoti[2]

[1]Universidade Federal de São Carlos (*UFSCAR*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*UNESP*)
[3]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*

E-mail: mirela.mbs@gmail.com

The aim of this study was to investigate the impact of extracellular vesicles (EV) derived from bovine follicles at different stages of the follicular growth wave (Early and Late) on transcriptome profile of oocytes and cumulus cells. Cumulus-oocyte complexes (COCs) were selected (grades I and II) and subjected to pre-IVM (8 hours) with the addition or not of EV: Control group (no EV), Early group (EV obtained from 7-8.5 mm follicles, characterizing the pre-deviation phase), and Late group (EV obtained from ≥ 12.0 mm follicles, characterizing the post-deviation phase until the pre-ovulatory phase). After pre-IVM, a subset of five COCs per sample had oocytes and cumulus cells collected separately for RNA sequencing (RNA- Seq). Also, a subset of COCs was used for assessing the meiotic stage (e.g., GV0, GV1, GV2, GV3, MI and MII), while another subset was used for IVM and IVP to assess embryonic developmental rates and blastocyst cell number and apoptosis (TUNEL). Statistical analyses were performed on the R Studio by using ANOVA followed by Tukey's test (parametric data) or Kruskal-Wallis (non-parametric data). Groups were considered different at $P < 0.05$. For the RNA-Seq, transcripts with FDR < 0.05 and log2 fold-change ≤ -1 or ≥ +1 were considered differentially expressed genes (DEG). In cumulus cells, 750, 822 and 280 DEGs were found between Early x Control, Late x Control and Early x Late. Analysis of these DEGs by KEGG revealed an enrichment for cAMP signaling in Early and Control, but not Late. Also, Cushing syndrome was enriched in Late and Control, while MAPK signaling and Gap junction were only enriched in Control. On the other hand, in oocytes, 172, 133 and 89 DEGs were found between Early x Control, Late x Control and Early x Late. According to KEGG analysis, only p53 signaling was enriched in Early and Late. Furthermore, EV treatment had no effect on oocyte meiotic stage, embryonic rates, as well as blastocyst cell number and TUNEL rate. Finally, EV treatment, regardless of Early or Late, improved mitochondrial membrane potential in blastocysts compared to Control. In conclusion, EV treatment changed key pathways related to COC development in cumulus cell expression, however further studies are needed to better understand the implications of this effect.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Extracellular Vesicles Isolated by Size Exclusion Chromatography Increase Meiosis Progression During *in vitro* Maturation of Bovine Cumulus-Oocyte Complexes

Gislaine dos Santos[1], Angelica Camargo dos Santos[2], Yi-Kai Liu[3], Marcos Roberto Chiaratti[2], W. Andy Tao[3], Christina Ramires Ferreira[4], Flávio Vieira Meirelles[1], Felipe Perecin[1], Alessandra Bridi[1,4], Juliano Coelho da Silveira[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*
[2]Universidade Federal de São Carlos (*UFSCAR*)
[3]Purdue University
[4]Universidade do Oeste de Santa Catarina (*UNOESC*)

E-mail: gislainesantos@usp.br

Extracellular vesicles present in follicular fluid (ffEVs) play a role in cell-to-cell communication and can participate in follicular development and oocyte maturation. There is limited understanding regarding the impact of the isolation methods on ffEVs contents and their use as a supplement during *in vitro* maturation (IVM). We hypothesized that the ffEVs isolation method influences the contents of bioactive molecules impacting the cumulus cells (CC) response. We collected FF from 3-6 mm follicles from 3 pairs of bovine ovaries in stage 3 of the estrous cycle (Ireland *et al*. 1980. J Dairy Sci. 63:155–160) and pooled forming one replicate (6 replicates). For each replicate, 100 µL of FF was used to isolate ffEVs using ultracentrifugation (ffEVs-UC) or exclusion chromatography (ffEVs-SEC). The ffEVs-UC and ffEVs-SEC were used to evaluate proteins and lipids by mass spectrometry, miRNA (RT- qPCR), and mRNA (RNAseq) contents. Next, cumulus-oocyte complexes (COCs) from 3-6 mm follicles from the slaughterhouse were submitted to IVM (base medium TCM 199; 6 replicates, n = 40 COCs/treatment/replicate) with or without the ffEVs: C (Control, without the ffEVs); UC (supplemented with ffEVs-UC); and SEC (supplemented with ffEVs-SEC). COCs were matured for 9 hours to evaluate CC transcripts and the number of transzonal projections (TZPs). In addition, after 21 hours of IVM, we evaluated the maturation rate. Data was analyzed using t-test analysis or ANOVA following Tukey's test considering p-value ≤ 0.05. Based on real-time PCR, transcriptomic, lipidomic, and proteomic we determined the impact of the isolation methods. Our data demonstrated that EVs isolated by different methods were different in terms of proteins, miRNAs, transcripts, and lipids content. Proteins such as NPR2, TGFBR1, EGFR, and BMPR1B were detected in ffEVS-SEC but not in ffEVS-UC. The bioinformatic analysis of mRNA, miRNA, and proteins indicates that these ffEVs contents play a role in modulating pathways associated with the regulation of TZPs, such as the regulation of actin cytoskeleton. To investigate if the different contents can influence the CC response, we supplemented COCs during IVM with ffEVs separated by different methods and observed a lower number of TZPs (P = 0.04) and an increased maturation rate (78.9% ± 7.19) in the ffEVs-SEC group than the C group (59.34% ± 7.53; P = 0.003). The analysis of transcriptional variants in CC supplemented for 9 h revealed that *PDE5A*-203 was upregulated in ffEVs-SEC group compared to the C group. PDE5A can decrease the levels of cGMP in CC, contributing to meiosis progression and consequently TZP numbers reduction. Together, these findings suggest that EVs' isolation method can recover EVs with different contents that upon supplementation can induce changes in transcripts involved in meiosis progression and TZPs-mediated communication in COCs during IVM. Funding: FAPESP 2021/06645-0, 2021/12560-7 and 2023/00884-8; CAPES 001.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Extracellular Vesicles: Preserving Follicular Morphology and Steroidogenic Function in Ovine Ovarian Tissue Culture

Lucy Vanessa Sulca Ñaupas[1], Karina Cañón Beltrán[2], Yulia Nathaly Cajas[3], Rosane Mazzarella[4], Rensson Homero Céliz[5], Maria Encina González[6], Dimitrios Rizos[4], Ana Paula Ribeiro Rodrigues[1]

[1]Universidade Estadual do Ceará (*Laboratório de Manipulação de Oócitos e Folículos Ovarianos Pré-Antrais - LAMOFOPA/UECE*)
[2]Complutense University of Madrid (*Department of Biochemistry and Molecular Biology - UCM*)
[3]Technical University of Madrid (*Department Agrarian Production - UPM*)
[4]National Institute for Agriculture and Food Research and Technology (*Department of Animal Reproduction - INIA*)
[5]National Institute of Agrarian Innovation (*Agricultural Experimental Station - INIA*)
[6]Complutense University of Madrid (*Department of Anatomy and Embryology - UCM*)

E-mail: lucnaupas@gmail.com

In vitro culture (IVC) of ovarian tissue is a promising strategy to preserve the large population of preantral follicles. However, few viable follicles reach more advanced stages of development where they are capable of becoming fertilizable oocytes. An innovative strategy that could ensure survival and promote follicular development is the use of extracellular vesicles (EVs), as they play a crucial role in cell-cell communication, modulating cellular response and biological activity. Therefore, this study aimed to evaluate the impact of supplementation of EVs isolated from ovine and bovine follicular fluid (FF) on the *in vitro* culture of ovine preantral follicles. For this, EVs were isolated and characterized by Nanosight (NTA) and transmission electron microscopy (TEM). For *in vitro* culture, the ovarian cortex of both ovaries was fragmented and randomly divided into four groups: fresh (Control, n = 5); cultured without EVs ($IVC^{ø}$, n = 5); cultured with 1 x 10^6/ mL ovine EVs (IVC^{+O}, n = 5); and cultured with 1 x 10^6/ mL bovine EVs (IVC^{+B}, n = 5). After 8 days of culture, the morphology and follicular development were evaluated in all recovered fragments by classical histology. Additionally, estradiol (E2) and progesterone (P4) levels were measured in the culture medium on days 2, 4, 6, and 8. Statistical analysis was performed using SPSS® software, and the Kruskal-Wallis H test was applied for comparisons, considering significant differences when $P < 0.05$. The results revealed that follicular morphology remained preserved similarly ($P > 0.05$) to the control (97.3 ± 1.3%) in the groups supplemented with EVs (IVC^{+O} (85.3 ± 3.4%) and IVC^{+B} (81.3 ± 4.3%)) and significantly reduced in $IVC^{ø}$ (65.4 ± 7.4%). Additionally, there was a significant increase ($P < 0.05$) in the percentage of developing follicles in the $IVC^{ø}$ (99.4 ± 1.4%) and IVC^{+O} (99.2 ± 0.8%) compared to control (66.1 ± 12.5%) and remained similar to IVC^{+B} (89.7 ± 7.5%). Regarding E2 levels, a significant reduction ($P < 0.05$) was observed in the $IVC^{ø}$ (6.2 ± 1.6 pg/mL) and IVC^{+B} (41.0 ± 1.2 pg/mL) groups throughout the culture, while the IVC^{+O} (424.9 ± 17.6 pg/mL) group remained stable ($P > 0.05$). On the other hand, P4 levels in the $IVC^{ø}$ (2.8 ± 0.5 ng/mL) and IVC^{+B} (3.2 ± 1.2 ng/mL) groups remained stable throughout the culture days, while in the IVC^{+O} (7.6 ± 2.5 ng/mL), it showed a significant increase ($P < 0.05$) from day 8 onwards. In conclusion, the effects of EVs appear to be species-specific as the addition of ovine FF-EVs to the ovine ovarian tissue culture medium exerts positive effects on follicular morphology and development, as well as maintaining E2 and production P4 levels. These findings underscore FF-EVs' potential as a valid approach for improving follicular development *in vitro*.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

GnRH increases progesterone, extracellular vesicle concentration, and modulates miRNAs in granulosa cells from bovine preovulatory follicles after a short time

Marcela Bortoletto Cerezetti[1], Juliana Germano Ferst[1], Sergio Farias Vargas Júnior[2], Cibele Maria Prado[1], Felipe Perecin[1], Flávio Vieira Meirelles[1], Arnaldo Diniz Vieira[2], Bernardo Garziera Gasperin[2], Rafael Gianella Mondadori[2], Juliano Coelho da Silveira[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*
[2]Universidade Federal de Pelotas (UFP)

E-mail: marcela.cerezetti@usp.br

This study aimed to evaluate follicular fluid (FF) for extracellular vesicle (EV), concentration, and miRNA contents, as well as granulosa cell miRNA profile in response to an induced LH surge. To obtain the samples, cows (Bos taurus; n = 11) were subjected to a synchronization protocol. On the first day (Day -9), the intravaginal progesterone (P4) device (1 g) was inserted, estradiol benzoate (2 mg), cloprostenol (500 µg), and GnRH (gonadorelin; 250 µg) were applied intramuscularly (IM). On day -2, cloprostenol (500 µg) was applied again and the follicular diameter cows were monitored by ultrasonography until the day of follicular aspiration. After device removal (Day 0), cows with follicles ≥ 11 mm in diameter were selected and ultrasound-guided follicular aspiration was performed after the treatment to obtain granulosa cells and FF. Follicles were punctured in animals that did not receive GnRH (Control Group; n = 3), 1 (GnRH-1h; n = 4) or 2 (GnRH-2h; n = 4) hours after receiving an additional IM injection of 100 µg GnRH. For determine the serum P4 concentrations blood samples were collected before the follicular aspiration. The FF was used to measure P4 and estradiol (E2) concentrations, analyze the EVs concentration by nanoparticle tracking analysis (NTA) and evaluate the content of miRNAs present in the EVs by qRT-PCR. The total RNA isolated from granulosa cells and EVs from FF were analyzed for the relative expression levels of 382 miRNAs. Differences between treatments were analyzed by ANOVA and Tukey test. There was a gradual increase ($P < 0.05$) in mean nanoparticles concentration in EVs from FF in the GnRH stimulated groups being observed for Control ($4.58 \times 10^9 \pm 1.83 \times 10^8$ particles/mL), GnRH-1h ($8.04 \times 10^9 \pm 6.25 \times 10^8$ particles/mL) and for GnRH-2h ($1.09 \times 10^{10} \pm 6.53 \times 10^8$ particles/mL). Particles did not differ ($P > 0.05$) among the groups (Control: 166.3 ± 13.2 nm; GnRH-1h: 161.65 ± 8.6nm; GnRH-2h: 151.95 ± 4.6nm). E2 levels in FF were also similar ($P > 0.05$). The concentration of P4 increased ($P < 0.05$) in FF over time (Control: 66 ± 17 ng/mL; GnRH-1h: 120.9 ± 24.1 and GnRH-2h: 244 ± 48.2), while serum P4 did not show a significant ($P > 0.05$) increase. Relative to miRNA analysis, 254 miRNAs were common between the groups and 31 were differentially expressed in granulosa cells. Among the pathways regulated by the 31 differently expressed miRNAs are endocytosis and endocrine resistance pathways, which are linked to ovarian steroidogenesis and the regulation of EVs release. In the EVs from FF, 112 miRNAs were common among the groups. Thus, higher P4 concentrations in FF seem to increase cellular communication mediated by EVs. In conclusion, the concentration of EVs is influenced by the acute response to the LH peak, and according to miRNA analysis, the endocytosis pathway is involved in this process, indicating a role of EVs in the early stages of ovulation and luteinization processes. Support: FAPESP grants 2023/15072-9, 2021/06645-0 and CAPES - Finance Code 001.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Immunostaining of gonadotropin receptors of *Bos indicus* cows with high or low antral follicle count

Deborah Nakayama Yokomizo[1], Mariana Moreira dos Anjos[1], Ana Karolyne Alves Miguel[1], Camila Bizarro da Silva[2], Amanda Fonseca Zangirolamo[1], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Laboratório de Biotecnologia da Reprodução Animal (REPROA/UEL)
[2]Pontifícia Universidade Católica do Paraná (PUCPR)

E-mail: deborah.yokomizo@uel.br

The study aimed to evaluate the immunostaining intensity of gonadotropin receptors (FSHr and LHr) in cyclic *Bos indicus* cows (n = 20) according to the antral follicle count (AFC). The ovaries (n = 40) were obtained from a local abattoir and the AFC was classified using a caliper to measure antral follicles with a diameter ≥ 3 mm into high AFC (n = 20, ≥ 30 antral follicles) or low AFC (n = 20, ≤ 15 antral follicles) groups. Standard histological preparation was performed after 24 h of fixation in 10% formaldehyde solution. For immunostaining, a primary polyclonal antibody (rabbit IgG) against FSH receptor (dilution 1:300, ab113421, Abcam) and LH receptor (dilution 1:100, ab125214, Abcam) was used, incubated in a moist chamber at 4°C for 22 hours, followed by incubation with a secondary antibody (goat anti- rabbit IgG, dilution 1:1000, ab205718, Abcam) at room temperature for one hour. The staining was visualized using 3,3'-Diaminobenzidine (DAB) and counterstained with hematoxylin. The follicular structures assumed the positive control and the negative control was phosphate-buffered saline (PBS) instead of the primary antibody. Immunostaining intensity was measured using Image J software with the IHC profiler extension in all types of follicles, i.e. primordial, primary, secondary, and antral follicles. The immunostaining was measured in pixels from 0 (darkest) to 255 (lightest) intensity. The immunostaining intensity was evaluated by ANOVA with adjusted mixed effects, with the AFC group (high and low) and developmental stage (pre-antral and antral follicles) as fixed factors. Tukey's test was used as a post hoc mean test in case of significant effects, with significance set at $P \leq 0.05$. 1329 ovarian follicles were analyzed, and all follicular development categories were immunostained for both receptors. FSHr immunostaining intensity showed an interaction between the AFC group and the follicular development stage, where low AFC pre-antral follicles exhibited higher immunostaining intensity (120.74 ± 2.20 *vs* 139.46 ± 1.74, P = 0.026). The immunostaining intensity for FSH receptors in antral follicles of the low AFC group was higher than in the high AFC group (135.69 ± 2.88 *vs* 152.38 ± 2.27, P = 0.032). There was higher LHr immunostaining intensity in cows with low AFC compared to those with high AFC (115.54 ± 1.72 *vs* 129.38 ± 1.40, P = 0.05). In conclusion, *Bos indicus* cows with low AFC exhibited more intense immunostaining of FSH and LH receptors in their pre-antral and antral ovarian follicles.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Influence of antral follicle count on primordial follicle growth and survival, stromal cell density, collagen fibers and gene expression in bovine ovarian tissues cultured *in vitro*

Natália Martins Barbosa[1], Venância Antoia Nunes Azevedo[2], Ernando Igo Teixeira de Assis[2], Francisco das Chagas Costa[2], Felipe Ferreira da Silva[2], Nathália Alves[1], Giovanna Tavares Petrucelli[1], Anderson Weiny Barbalho Silva[2], José Roberto Viana Silva[2], José Camisão de Souza[1]

[1]Universidade Federal de Lavras (*UFLA*)
[2]Universidade Federal do Ceará (UFC)

E-mail: nataliamartinsb@hotmail.com

This study aimed to evaluate the influence of antral follicle count (AFC) on follicular development and survival, ovarian stromal cell density, distribution of collagen fibers, as well as on expression of proliferating cell nuclear antigen (PCNA), cellular communication network factor 2 (CCN2) and growth and differentiation factor 9 (GDF-9) in bovine ovarian tissue cultured *in vitro* for 6 days. Bovine ovaries obtained from abattoirs were classified as low (≤15 antral follicles; n = 20), medium (16-30 antral follicles; n = 20), and high AFC (≥31 antral follicles; n = 20). Ovarian cortical tissues (3x3x1 mm) from all ovaries in each category were cultured in an incubator at 38.5°C with 5% CO2 in air for 6 days in α-MEM. Both non- cultured and cultured tissues were fixed in 4% paraformaldehyde for histological evaluation of follicular activation, development, and survival, as well as to classify the follicles as normal or degenerated. Additionally, the density of ovarian stromal cells and the distribution of collagen fibers in the extracellular matrix were examined using the picrosirius red staining. Tissue samples were also used to perform total RNA extraction, followed by reverse transcription and real-time PCR to assess the expression of the PCNA, CCN2 e GDF-9 genes. Chi-square test was used to compare the percentage of normal follicles and those of primordial and developing follicles. Data for collagen fibers distribution, stromal cell density, were compared by ANOVA and Tukey test. Differences were statistically significant when $P < 0.05$. The results showed a higher percentage of normal follicles was observed after *in vitro* culture of tissues from ovaries of medium and high AFC (P=0.38), when compared to low AFC. *In vitro* cultured tissues had significantly increased percentage of developing follicles in all AFC categories, compared to non-cultured samples ($P < 0.0001$). A higher rate of follicular activation was observed in tissues from medium AFC ovaries when compared to high and low AFC ($P < 0.0001$). Tissues from all AFC classes had similar reduced stromal cell density after *in vitro* cultured ($P > 0.05$). There was also a reduction in the percentage of collagen fibers after culture in all AFC classes ($P < 0.0001$). However, tissues from ovaries of medium AFC had a higher percentage of collagen fibers compared to non-cultured ones ($P < 0.0001$). The AFC classes did not influence PCNA and GDF-9 gene expressions ($P > 0.05$), except for the CCN2 gene, which showed higher expression in ovaries of medium AFC category ($P = 0.03$). For tissues cultured in α-MEM, there was no difference in PCNA expression between AFC categories, but higher expression of GDF-9 and CCN2 were observed in tissues from low AFC category ($P = 0.03$). In conclusion, ovaries with medium AFC exhibit greater follicular survival, enhanced primordial follicle activation, and reduced collagen fiber loss after *in vitro* culture. However, GDF-9 and CCN2 expression is increased in ovaries of low AFC class.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Oocyte-specific mitofusin 2 knockout enhances the metabolic dysfunction of offspring born to obese female mice

Jaiane Santana da Paz[1], Victória Cristina Cardoso da Paixão Hass Gonçalves[1], Mirela Brochado Souza Cáceres[1], Angelica Camargo dos Santos[1], Lindomar de Oliveira Alves[1], Julio Cesar Valerio Roncato[1], Renan Omete Ferreira[1], Marcos Roberto Chiaratti[1]

[1]Univerdade Federal de São Carlos (*Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde - UFSCAR*)

E-mail: jaiane@estudante.ufscar.br

Growing evidence supports that maternal obesity predisposes the offspring to a metabolic syndrome, which is in part explained by mitochondrial and endoplasmic reticulum (ER) dysfunction in oocytes. Mitofusin 2 (MFN2) is a master regulator of mitochondrial metabolism and insulin signaling. Recently, we showed that oocyte-specific Mfn2 knockout (KO) results in abnormal mitochondria-ER contact sites (MERCS) as well as mitochondrial and ER dysfunction in mouse oocytes. Furthermore, offspring born to Mfn2 KO oocytes had glucose intolerance and impaired insulin signaling. Here we used a high-fat diet model to induce maternal obesity and further explore the effects of oocyte-specific Mfn2 KO on offspring. Towards this, six-week-old female mice, harboring either heterozygous (mfn2+/-) or homozygous (mfn2-/-) KO oocytes for Mfn2, were fed either a normal (NFD, 9.4% of kcal from fat)- or a high (HFD, 58.6% of kcal from fat)-fat diet for 12 weeks. Females were then mated to wild-type males and only heterozygous KO (mfn2+/-) offspring were weaned on a standard chow and considered in the analysis. Groups were compared by ANOVA followed by Tukey post-hoc test, t-Student or Wilcoxon test, depending on data distribution and number of groups. Differences with $P < 0.05$ or P-adjusted (FDR)<0.05 (transcriptomic data) were considered as significant. Diet-induced obesity of mothers was confirmed by increased weight gain ($P < 0.01$) and glucose intolerance ($P < 0.05$), regardless of maternal genotype (mfn2+/- or mfn2-/-). Ten-week-old pups born to mfn2-/- mothers on HFD had higher weight gain ($P < 0.01$), increased perigonadal fat ($P < 0.01$), hyperinsulinemia ($P < 0.05$), and glucose intolerance ($P < 0.01$) in comparison to pups born to mfn2+/- mothers on HFD, a finding that associated with insulin resistance ($P < 0.05$) and increased insulin turnover ($P < 0.05$). Interestingly, these differences between mfn2-/- and mfn2+/- groups were not observed for pups born to mothers on NFD. Remarkably, transcriptomic analysis revealed a pronounced change (FDR < 0.05) in gene expression in the heart and gastrocnemius muscle of 10-week- old pups born to mfn2-/- mothers, which was exacerbated under HFD. Gene enrichment analysis based on dysregulated genes indicated a significant alteration in pathways such as oxidative phosphorylation, insulin signaling and diabetes (FDR<0.05). Altogether, these results indicate that oocyte-specific Mfn2 KO exacerbates the metabolic dysfunction of offspring born to obese mothers, enhancing their obesity and glucose intolerance. Support: FAPESP (grants # 2020/15412-6 and 2021/04835-6).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

rbFSH on sheep embryo production

Brenda Barbosa Martins[1], Aline Matos Arrais[1], Maria Paula da Costa Plaza[1], Pedro Henrique de Mello Cotta Maia[1], Sandra Ribeiro da Silva[1], Joanna Maria Gonçalves Souza-Fabjan[1] Alejo Menchaca[2], Rodolfo Ungerfeld[2], Jeferson Ferreira da Fonseca[3,4], Felipe Zandonadi Brandão[1]

[1]Universidade Federal Fluminense (*UFF*)
[2]Universidad de la República, Montevideo (Facultad de Veterinaria - *UDELAR*)
[3]Empresa Brasileira de Pesquisa Agropecuaria
[4]Caprinos e Ovinos (*Caprinos e Ovinos*)

E-mail: medvet.brendamartins@gmail.com

The aim of this study was to compare the effectiveness of new recombinant bovine FSH (rbFSH) in superovulation of ewes subjected to non-surgical embryo recovery (nser) with that of a porcine pituitary gonodotrophin extract (pFSH). The study was performed during the reproductive transition, with 40 adult, cyclic, multiparous Santa Inês ewes (56.8 ± 2.9 kg; 3.0 ± 0.1; weight and body condition score; mean ± SD). The ewes received the same estrous synchronization protocol (Balaro *et al*., Dom Anim Endocrinol, 54:10-14, 2016), initiated by insertion of intravaginal device (ivd) containing medroxiprogesterone acetatate (60 mg; Progespon - Syntex, Buenos Aires, Argentina), before superovulation. In the group superovulated with pFSH (GpFSH), ewes (N = 20) received (333 IU i.m.; Pluset - Biogénesis Bagó, Curitiba, Brazil) in six decreasing doses (25%, 25%, 15%, 15%, 10% and 10% of the total dose) every 12 h, with the first dose given 80 h after ivd removal. In the group superovulated with rbFSH (GrbFSH), ewes received a single dose of ripafolitropina alfa bovina (100 µg i.m.; Zimbria - Ceva, Paulínea, Brazil) 80 h after ivd removal. At this time, a new ivd containing progesterone (P4; 0.36 g; Primer PR - Agener União Saúde Animal, São Paulo, Brazil) was inserted in all ewes, remaining in situ for 48 h. Twelve hours after the P4 ivd, sodium cloprostenol (0.24 mg i.m.; Estron - Agner União Saúde Animal, São Paulo, Brazil) was administered. Ewes received lecirelin (25 µg i.m.; Tec-Relin - Agener União Saúde Animal, São Paulo, Brazil), a GnRH analogue 36 h after the ivd removal. All females were single mated during estrus. The corpora lutea (CL) were counted using B- mode and color doppler ultrasonography one day before nser. The animals received a protocol for cervical dilation (Leite *et al*., Arq Bras Med Vet Zootec, 0:1671-1679, 2018) and nser was performed on Day 10 of the protocol (six days after ovulations). The recovered structures were evaluated regarding the stage of development and quality. Data were expressed as mean ± SEM and compared between groups using the Fisher test, mixed models or Mann-Whitney test, with a significance level of 5%. There were no differences on the proportion of ewes that came into estrus (100% *vs* 95%), the interval from IVD removal to estrus (66.9 ± 2.3 h *vs* 65.0 ± 2.4 h), number of CLs (8.3 ± 1.1 h *vs* 6.3 ± 1.1), number of recovered structures (3.1 ± 0.8 *vs* 1.9 ± 0.9) and number of viable embryos (1.2 ± 0.8 *vs* 1.7 ± 0.8), for GpFSH and GrbFSH, respectively. The number of anovolutary follicles was greater in GpFSH than GrbFSH (1.3 ± 0.5 *vs* 0.3 ± 0.5; P=0.04). Estrous length tended to be longer in the GpFSH than GrbFSH (44.5 ± 3.9 h *vs* 35.6 ± 3.9 h; P=0.08). In the conditions of this study, in which there was a general low embryo yield, there were no relevant differences detected with the use of pFSH and a new commercial rbFSH.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

The influence of different timings of CIDR insertion on the embryo yield in locally adapted superovulated Brazilian goats

Luana Rangel Côrtes[1], Juliana Nascimento Duarte Rodrigues[2], Alexandre Weick Uchôa Monteiro[3], Kleibe de Moraes Silva[3], Ribrio Ivan Tavares Pereira Batista[4], Joanna Maria Gonçalves Souza-Fabjan[5], Jeferson Ferreira da Fonseca[3]

[1]Fundação Universidade Federal de Viçosa (*Programa de Pós-graduação em Medicina Veterinária*)
[2]Universidade Federal Rural da Amazônia (*Campus Paragominas*)
[3]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[4]Universidade Federal Fluminense, [5]Universidade Federal Fluminense (*Faculdade de Veterinária*)

E-mail: luana.cortes@ufv.br

Financial support: CNPq (303727/2021-7), Embrapa (10.20.02.003.00.02.006) and CAPES.

This study aimed to investigate whether the variation (morning or afternoon) in the insertion of progesterone (P4) intravaginal device (IVD) affects embryo yield in locally adapted superovulated goats. A total of 16 Canindé and 22 Moxotó goats were equally assigned to two groups according to the time of insertion (G1: 06:30 to 07:30 a.m. and G2: 06:30 to 07:30 p.m.) of the IVD containing 0.3 g of P4 (Eazi-Breed CIDR®; Pfizer, São Paulo, Brazil), which remained for six days. The superovulatory protocol consisted in administration of pFSH (133 mg i.m.; Folltropin-V®; Bioniche Animal Health, Belleville, Canada) in six doses (25, 25, 15, 15, 10, and 10%) at 12 h intervals, starting 60 h before IVD removal. Three doses of cloprostenol (131.5 µg i.m.; Sincrocio®, Ouro Fino, Cravinhos, Brazil) were administered at IVD insertion, simultaneously with the fifth dose of pFSH and 8 h before non-surgical embryo recovery (NSER). At 24 h after IVD removal, goats received gonadorelin acetate (25 µg i.m.; Gestran®, Tecnopec, São Paulo, Brazil). Donors were mated every 12 hours during estrus with fertile bucks (n = 9). Three doses of flunixin meglumine (75 mg i.m.; Flumax®, J.A. Saúde Animal, São Paulo, Brazil) were administered 3, 4, and 5 days after the onset of estrus, and NSER was performed 7 d after the first mating. Ultrasound evaluations were performed before NSER for CL count. Data were subjected to ANOVA or chi-square test, and P≤0.05 was considered significant. Two goats from each group (1 Moxotó and 3 Canindé) exhibited total precocious regression of corpora lutea (PRCL) and had no structures recovered. Five goats (G1: n = 2 and G2: n = 3; 3 Moxotó and 2 Canindé) presented partial PRCL, with viable embryos successfully recovered. The interval from IVD removal to onset of estrus (20.5 ± 1.7h and 21.9 ± 1.7h), number of mating (1.8 ± 0.1 and 1.8 ± 0.1), number of CL per female (16.5 ± 1.5 and 14.8 ± 1.7), total structures recovered per female (6.9 ± 1.2 and 7.0 ± 0.8), recovery rate (42.7 ± 6.4% and 52.8 ± 5.6%), number of viable (grades 1, 2 or 3) embryos recovered per flushed female (6.2 ± 1.2 and 6.1 ± 0.9), number of morulae (2.2 ± 1.1 and 1.7 ± 0.5) and blastocysts (4.0 ± 0.9 and 4.4 ± 0.8), for G1 and G2, respectively, showed similar results (P > 0.05). Percentage of morulae (29.9 and 30.5%) and blastocysts (73.2 and 71.4%) and embryo viability (82.2 and 82.7%) were also similar (P > 0.05) between groups. As expected for NSER performed 7 d after mating, the proportions of blastocyst were superior to morulae in both groups (P < 0.01). We concluded that the difference in the timing of IVD insertion does not affect the efficiency of embryo yield in locally adapted superovulated Brazilian goats.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS AND SUPEROVULATION

Treatment of chronic cystic ovarian disease with deslorelin: preliminary results

Aline Silva Mendes Teixeira[1], Leticia Prates Martins[1], Luiz Gustavo Bruno Siqueira[2], Ricardo Alamino Figueiredo[2], João Henrique Moreira Viana[2]

[1]Universidade de Brasília (UnB)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)

E-mail: alinemendees20@gmail.com

Cows used as oocyte donors may develop chronic cystic ovarian disease (COD), characterized by the presence of multiple large follicles and refractoriness to conventional treatments (Faria *et al*. Anim Rep. 2017). Our group has previously demonstrated that such COD conditions may be treated by vaccination against GnRH. The main flaw of this approach, however, is the lack of control of the duration of immunization. We therefore hypothesized that the same effect can be obtained using a long-acting GnRH agonist. In the current study, we evaluated the effect of deslorelin implants (Suprelorin, Virbac) on ovarian activity in cows with COD. Nelore donors previously diagnosed with the disease (n = 16) were randomly allocated into four groups, which received: no treatment (control group, G0), or one (G1), two (G2), or three (G3) sc implants with 4.7 mg deslorelin. Ovaries were scanned weekly by ultrasonography and videoclips were stored for further measurement of all visible follicles and luteal tissue present. We recorded the size of the largest follicle, as well as the number of follicles above the expected size of the dominant follicle at deviation (>8mm) and at ovulation (>12mm), antral follicle count (AFC), and the presence of mucometra. Data were analyzed using the Glimmix procedure of SAS, with a statement for repeated measures. Before treatment, all cows presented a cystic follicle (21.2 ± 0.1mm), with an average 2.6 ± 0.4 follicles larger than 12 mm and 4.9 ± 0.9 larger than 8 mm. We observed the so-called "flare-up" effect after treatment, which in the current study was characterized by the luteinization of follicular cysts in the treated groups and therefore a greater ($P < 0.05$) amount of luteal tissue during weeks 1 and 2 in groups G1, G2, and G3, compared with the control group (G0). There were treatment, time, and treatment x time effects ($P < 0.02$) on all endpoints related to follicle size. In the cows receiving deslorelin implants (G1, G2, and G3), we observed a decrease ($P < 0.05$) on the size of the largest follicle and on the number of follicles larger than 12 mm from week 4 onwards, compared with G0. On group G3, no follicular cysts were observed from week 7 onwards. The number of follicles larger than 8 mm, however, only differed ($P < 0.05$) from G0 in G2 and G3, and from week 6 onwards. On the other hand, there was no effect ($P > 0.05$) of deslorelin treatment on AFC or on mucometra score. In summary, the preliminary results suggest that two sc implants (total 9.4 mg) of deslorelin can be used as a therapeutic alternative for the treatment of oocyte donors with COD.

Acknowledgements: DPG UnB, FAPDF Project 00193-00002307/2022-11 and CNPq INCT 406866/2022-8.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Aging in mice: Effects on semen, embryo and fetus

Larissa Araújo Stábile[1], Camilla Mota Mendes[1], Thais Rose dos Santos Hamilton[1], Álvaro de Miranda Alves[1], Marcelo Demarchi Goissis[1], Marcilio Nichi[1], Heriberto Barbosa-Moyano[1], Mayra Elena Ortiz D'Ávila Assumpção[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: larissa.stabile@hotmail.com

We hypothesized that aging in mice leads to alterations in sperm physiology, which negatively affects both *in vivo* and *in vitro* embryo development, and further *in vivo* conceptus growth. Experiments used C57BL/6J males aged 4 (control), 19 and 24 months (experimental). For experiments 1 (sperm) and 2 (embryo), groups were compared using One-Way ANOVA followed by posthoc test LSD. Experiment 3 (conceptus) was divided into two contemporaneous trials: groups 4 *vs*. 19 and 4 *vs*. 24; both were compared using the Student t-test. A 5% significance was considered. In experiment 1, the male reproductive potential (4, n=11; 19, n=5; 24, n=9) was studied. Serum testosterone levels, testicular weight, sperm morphology, CASA motility, integrity of plasma and acrosomal membranes- FITC/IP, oxidative stress-CELLROX green, mitochondrial membrane potential-JC1, modified chromatin susceptibility to acid-SCSA, chromatin condensation-CMA3, capacitation status- CTC, and impact on *in vivo* fertilization rate were evaluated. Aged groups showed a trend towards lower *in vivo* fertilization rate (p=0.067), and lower weight of both right and left testicles (p=0.025; p=0.011). The 24-group showed lower serum testosterone levels (p=0.018). Aged groups presented a higher percentage of individuals (p=0.0001), multi- defects (p=0.0361), and total defects (p=<.0001). The 19-group demonstrated higher tail defects (p=0.023), and the 24-group had higher midpiece defects (p=0.006). Males at 19 months showed higher intermediate mitochondrial membrane potential (p=0.032) and damaged plasma membrane with oxidative stress (p=0.003). The 24-group had more deprotaminated cells (p=0.045). Capacitated spermatozoa (p=<.0001) were reduced among aged males, with the 19-month-old showing more acrosome-reacted spermatozoa (p=0.016). In experiment 2 (4, n=5; 19, n=5; 24, n=7), *in vitro* embryo development, blastocyst stages, and immunofluorescence for CDX2 and SOX2 were analyzed. Aged groups showed lower cleavage (D1.5; p=0.0290), blastocyst (p=0.0070), and embryo development rate (D4.5; p=0.0120). The 19-group had a higher early blastocyst rate (p=0.0051), and aged groups had lower rates of expanded (p=0.0402) and hatched blastocysts (p=0.0003). In the aged groups, there was a reduction in total cells (p=0.004) and trophectoderm cells (CDX2- positive; p=0.016). The 24-group had a smaller inner cell mass (SOX2-positive; p=0.035). Aged groups exhibited reduced fluorescence intensity for CDX2 (p=<.0001), with the 24- group showing lower fluorescence intensity for SOX2 (p=<.0001). In experiment 3 (4, n = 10 *vs*. 19, n = 10; 4, n = 14 *vs*. 24, n = 9), morphometric analyses of fetuses and placentas and litter (16.5d of gestation) were conducted. The 24-group had smaller (p=0.029) and lighter fetuses (p=0.040), a lower fetal: placental weight ratio (p=0.027), and a tendency of less viable fetuses (p=0.075). In conclusion, increased age in mice impairs sperm function, affecting embryo and fetal development.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Assessment of vascular parameters of the spermatic cord in young bulls of Nellore (*Bos indicus*) and Caracu (*Bos taurus*) breeds.

Leticia Padovani da Silva[1], Marcelo Sant'Ana Borges[1], Marina de Oliveira Silva[1], Laura Fernanda Sechirolli da Silva[1], Pedro Luiz Bignardi[1], Maria Eugênia Zerlotti Mercadante[1], Fabio Morato Monteiro[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (*UNESP*)

E-mail: ltc.padovani@gmail.com

Due to anatomical differences in testicular conformation between *Bos indicus* and *Bos taurus* bulls, there are distinctions regarding vascular parameters of the spermatic cord and testicular tissue perfusion. In *Bos indicus*, the length of the testicular artery is greater, with more vessel coiling, compared to *Bos taurus* breeds. The aim of the study was to evaluate and compare blood flow assessment parameters during reproductive development in young Nellore (*Bos indicus*) and Caracu (*Bos taurus*) bulls. For the evaluation of testicular tissue perfusion and spermatic cord vascularization, the Z5 Vet ultrasound device (Mindray, Shenzhen, China) equipped with a 7.5MHz linear transducer and spectral Doppler mode was used. The analyzed variables were testicular artery diameter, peak systolic velocity (PSV), end-diastolic velocity (EDV), vascular resistance index (RI), and pulsatility index (PI). Twenty Nellore animals with a weight of 348 ± 55 kg and age of 13.82 ± 0.8 months, and fifteen Caracu animals with a weight of 364 ± 46 kg and age of 14.07 ± 0.7 months were used. Three evaluations were performed with a 120-day interval. Data were analyzed using analysis of variance and mixed models, with the assistance of the SAS® MIXED procedure. Statistical significance was set at P<0.05. In all assessments, significant differences were observed between the races. Caracu young bulls showed higher values compared to Nellore in testicular artery diameter (0.23±0.01 cm *vs*. 0.19±0.009 cm, respectively, P<0.001), PSV (11.75±0.56 cm/s *vs.* 10.58 ± 0.51 cm/s, respectively, P<0.001), and EDV (7.05±0.46 cm/s *vs.* 5.28±0.43 cm/s, respectively, P<0.001). On the other hand, Nellore young bulls presented higher values than Caracu young bulls for RI (0.47± 0.01 *vs*. 0.38±0.01, respectively, P<0.001) and PI (0.64±0.02 *vs.* 0.50±0.02, respectively, P<0.001). The RI and PI indices reflect the difficulty with which blood travels through the vessel. By observing these parameters and applying them in the comparison between taurine and zebu animals, the anatomical and hemodynamic differences between Nellore and Caracu breeds can have important implications for reproductive physiology. The greater tortuosity and smaller diameter of the testicular artery in Nellore bulls result in higher vascular resistance, reflected in higher IR and IP indices, and lower PSV and EDF compared to young Caracu bulls. In conclusion, young Caracu bulls (*Bos taurus*) present higher values of testicular artery diameter and blood velocities, while young Nellore bulls (*Bos indicus*) demonstrate higher vascular resistance indices and pulsatility indices. These findings suggest that the specific anatomical characteristics of Nellore bulls may favor more efficient thermoregulation and better adaptation to environments with high temperatures. Financial support: FAPESP (Process No. 2017/50339-5) and CAPES finance code 001.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Beyond the scrotal circumference: exploring traits associated with early sexual development in Nelore bulls

Bruna Lima Chechin Catussi[1], Romulo Germano de Rezende[2], Pedro Nacib Jorge Neto[2], Emanuele Almeida Gricio[1], Laura Nataly Garcia-Oliveros[1], Eneiva Carla Carvalho Celeghini[1], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP*
[2]IMV Technologies Brasil

E-mail: brunacatussi@usp.br

This study aimed to investigate the phenotypic, nutritional, and metabolic factors associated with early sexual development in juvenile bulls. A cohort of 128 Nelore (*Bos indicus*) bulls, aged 11.1 ± 0.14 months and weighing 340.2±3.94 kg, were selected from a commercial farm. On day 0, prepubertal bulls were assigned to a feedlot period until Day 70 for feed efficiency testing. Measurements taken on day 0 included body weight (BW), body condition score (BCS), scrotal circumference (SC), rectal temperature (RT), and testicular temperature (TT). Additionally, the testicular parenchyma was assessed using Ecotext software, capturing parameters such as black pixel (BP), white pixel (WP), and grey pixel (GP) as described in Catussi *et al.* Reprod. Fertil. Dev, 2023. Blood serum sample was collected for LC/MS metabolomics analysis. On day 70, semen samples were obtained via electroejaculation, categorizing bulls as prepubertal (PRE; n=97; fewer than 50x106 cells per ejaculate) and pubertal (PUB; n=31; produced more than 50×106 sperm cells per ejaculate with more than 10% motility). At the same time, measurements included BW, BCS, SC, RT, TT, rib eye area (REA), rump fat thickness (RFAT), subcutaneous back fat thickness (BFAT), residual feed intake (RFI), average daily gain (ADG), Ecotext, and blood sample collection. Statistical analysis was performed using the proc GLIMMIX of SAS 9.4 with age as a covariate. On day 0, BW (P=0.28), BCS (P=0.38), SC (P=0.17), RT (P= 0.09), and TT (P=0.98) were similar between PRE and PUB bulls. However, testicular parenchyma presented differences, in which PUB bulls had less BP (6.45±1.6 *vs.* 27.40±3.3; P<0.01), more GP (662.6±109.1 *vs.* 32.3±32.3; P<0.01) and more WP (111.9±2.8 *vs.* 93.8±1.5; P<0.01) than PRE bulls. Metabolomics analysis identified a total of 80 metabolites, with further analysis using Partial Least Squares Discriminant Analysis (PLS-DA) revealing eight significant metabolites. Additionally, enrichment analysis (EA) uncovered alterations in eight metabolic pathways between the groups. On Day 70, PUB bulls had higher sperm concentration (P<0.001) and motility (112.8±38 million/sptz and 54.4±2.99, respectively) then PRE bulls (3.4±1 million/sptz and 10.4±2.3, respectively). No differences between groups were observed for BW (P=0.17), BCS (P=0.85), RT (P=0.10), TT (P=0.99), REA (P=0.69), RFAT (P=0.24), RFI (P=0.15) and ADG (P=0.61). However, differences were found in SC (PUB= 32.0±0.5 *vs.* PRE=29.4±0.3; P<0.01) and BFAT (PUB= 4.16±0.1 *vs.* PRE= 3.77±0.1; P= 0.05). Additionally, any ecotext parameter differed on Day 70 (P>0.05). The metabolome analysis on Day 70 found differences in 11 metabolites and three metabolic pathways. In conclusion, on Day 0, testicular parenchyma by ecotext and metabolomics analysis presented differences between prepubertal and pubertal bulls. On Day 70, pubertal bulls presented higher scrotal circumference and subcutaneous rump fat thickness. These findings suggest that factors beyond scrotal circumference may also play a role in early sexual development in Nelore bulls.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Cryopreservation with extracellular vesicles improves post-thaw quality of epididymal cauda sperm from Nelore bulls

Laura Gabrielli Haupenthal[1], Maria Alice de Almeida[1], Amanda Nespolo Silva[1], Paola Maria da Silva Rosa[1], Gabriela Melendes Schneider[1], André Furugen Cesar de Andrade[1], Juliano Coelho da Silveira[1], Felipe Perecin[1], Maíra Bianchi Rodrigues Alves[1,2]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Faculdade de Ciências Agrárias e Veterinárias - UNESP*)

E-mail: laura.gabrielli@usp.br

Herein, we aimed to evaluate the impact on sperm post-thaw quality upon addition of extracellular vesicles (EVs) to the extender during cryopreservation of sperm from the epididymis cauda (SPCAU). For that, epididymal fluid (EF) was obtained postmortem from 21 sexual mature Nellore bulls by intraluminal perfusion by retrograde flow of the epididymal cauda. First, a pool of EF from 15 bulls was formed to obtain EVs by ultracentrifugation (119,700g/70min, 4°C). Afterward, SPCAU from EF of six bulls were isolated (600g/10min, 4ºC), analyzed for sperm concentration, and incubated with BoviFree® extender added of EVs (EVs) or not (Control; Co). Following subjective motility and vigor assessment, sperm doses were filled and cooled (-0.5°C/min) to 5°C, kept at 5°C for 2h30, and frozen (-20°C/min) using TK4000®. Sperm were evaluated post-thawing (37ºC/30s) for motility characteristics using the Sperm Class Analyzer and for plasma membrane integrity (PMI) and high mitochondrial membrane potential (HMMP) by BD Accuri flow cytometryTM C6. Rates of first cleavage (28 to 30 hpi), cleavage (D4), and blastocysts (D7) were evaluated during *in vitro* embryo production, and blastocysts were stained with Hoechst 33342 to assess the number of cells. EVs *vs.* Co data were compared by analysis of variance using SAS®. A significant difference was considered when P≤0.05. Post-thaw EVs sperm display high quality compared to Co in total (EVs:49.51±2.92%; Co:30.73±3.49%) and progressive (EVs:31.73±3.70%; Co:17.40±3.61%) motility, fast sperm (EVs:44.35±2.64%; Co:24.56±3.12%), straightness (EVs:74.95±3.67%; Co:69.00±4.02%), curvilinear velocity (EVs:105.35±5.09µm/s; Co:89.86±3.14µm/s), straight-line velocity (EVs:60.35±5.94µm/s; Co:45.00±3.7µm/s), average path velocity (EVs:79.70±4.67µm/s; Co:64.78±1.91µm/s), linearity (EVs:57.43±5.23%; Co:50.63±4.70%) and amplitude of lateral head displacement (EVs:3.25±0.18µm; Co:2.90±0.19µm). Post-thaw sperm PMI was higher in EVs (39.92±6.52%) compared to Co (28.50±6.77%), while HMMP showed no difference (P=0.39). Post-thaw sperm of EVs and Co were similar for rates of first cleavage [EVs:39.00±6.71% (78/200); Co:40.31±8.65% (77/191); P=0.79), cleavage [EVs:63.00±1.83% (126/200); Co:60.73±4.83% (116/191); P=0.64), and blastocysts [EVs:26.00±4.64% (52/200); Co:24.60±7.50% (47/191); P=0.75). The same was found for the quality of embryos: early blastocyst (EVs: 3.84±1.51%-2/52; Co: 12.77±1.51%-6/47; P=0.10), expanded blastocyst (EVs:73.08±5.82%-38/52; Co:82.98±6.42%-39/47; P=0.23), hatching blastocyst (EVs:15.38±0.78%-8/52; Co:4.26±0.86%-2/47; P=0.06) and blastocyst cells count (EVs:113.20±9.14 cells; Co:111.75±5.75 cells; P=0.92); however, blastocyst rates were higher (P=0.02) in EVs: 9.62±2.05% (5/52) vs. Co: 0±0% (0/47). Our findings show that SPCAU cryopreservation with EVs results in greater sperm post-thaw quality which may impact the blastocyst quality rates. Funding: CAPES 001; Patent application required nº BR 10 2023 020354-0.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Effect of sexual rest time on sperm parameters in bulls

Jesús Alfonso Sánchez Viafara[1], Iván Darío Rojas Gómez[1], Hennis Dorlanys Diaz Mendoza[1], Hanzel Alberto Hortua Castro[1], Raúl Sulpicio Sarmiento Cely[1], Julian Valencia[2]

[1]Centro de Desarrollo Tecnológico Del Cesar
[2]Universidad Antonio Nariño

E-mail: jesusveterinario85@hotmail.com

This study aimed to identify factors that affected the semen quality of breeding bulls in the low tropics of Colombia. Semen analysis was performed on 2000 bulls located in the department of Cesar, by electroejaculation (Electro Jac®). The sperm parameters evaluated in each andrological evaluation per bull one time were percentage of normal cells, secondary abnormalities, live and dead cells, semen volume, progressive motility, sperm concentration, turbulence and vigorous oscillatory movement. The sexual rest time of the bulls was recorded (less than 5 days (n=452), between 5 to 15 days (n=1131), 16 to 30 days (n=166), between 31 to 60 days (n=47), from 61 to 90 days (n=14) and greater than 90 days (n=190). Statistical analyzes were performed using IBM SPSS 27.0 for Windows (IBM Corp., Armonk, NY, USA). All variables were first tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). Pearson correlation analysis was performed. Also, a general linear model (inter-subject factor: race and rest period) was carried out, followed by Tukey's post hoc test for multiple comparisons. The level of significance was set at $P < 0.05$ in all cases. When bulls have a sexual rest time of less than 5 days and between 31 to 60 days, progressive mobility is less ($P < 0.05$). The percentage of normal cells is lower with a sexual rest time of less than 5 days ($P < 0.05$). An increase in secondary abnormalities is observed with a sexual rest time of less than 5 days ($P < 0.05$). The percentage of live cells increases when the bulls have a sexual rest time between 5 to 15 days ($P < 0.05$). There is a greater proportion of dead cells when bulls have a sexual rest time of less than 5 days ($P < 0.05$). In turn, it was observed that the greater the progressive mobility, the lower the percentage of secondary abnormalities and the lower the percentage of dead cells. The higher the proportion of sperm with secondary abnormalities, the lower the sperm concentration ($P < 0.05$). In conclusion, a sexual rest time should be provided to the bulls between 5 to 15 days so that their sperm characteristics such as progressive individual motility, normal and live cells increase and secondary abnormalities and the percentage of dead cells decrease. When there is a sexual rest of less than 5 days, there is not adequate sperm maturation in the epididymis and production in the testicles. Deficient sperm maturation affects membrane structure, motility and normal morphology, which reduces sperm quality.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Exploring extracellular vesicles from bovine epididymal fluid across regions: *Caput*, *corpus* and cauda

Yulizabeth Daniela Pinto Rojas[1], Maria Alice de Almeida[2], Schaienni Fontoura Saldanha[2], Fernanda Schneberger dos Santos[2], Camila Azzolin de Souza[2], Gislaine dos Santos[2], Matheus Andrade Chaves[2], Juliano Coelho da Silveira[2], Marcelo Emílio Beletti[1]

[1]Universidade Federal de Uberlândia (Instituto de Ciências Biomédicas - *UFU*)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: yulizabethpinto@outlook.com

The epididymal epithelium constitutes a communication network generating unique luminal environments exclusive to each region of the epididymis. Through apocrine secretion, extracellular vesicles (EVs) leave cells can carry important biomolecules involved in the post-testicular maturation of spermatozoa. EVs have a lipid membrane and are not equipped with transcription machinery or nuclei, so they can cross biological barriers and deliver their contents to target cells. Our aim was to compare and characterize the EVs isolated from the fluid in the caput, corpus and cauda regions of the epididymis of mature bulls. An N=22 epididymides were acquired from slaughterhouses, and epididymal fluid (EF) was collected from the three regions by dissection and flushing with 1xPBS, followed by isolation of EVs by serial centrifugation and ultracentrifugation (Alves *et al.*, Theriogenology, 161:26–40, 2021). The EVs were characterized by Transmission Electron Microscopy (TEM) (JEOL 100 CX II, BioCell) (n=2), Flow cytometry (CytoFLEX, Beckman Coulter) using EV markers CD81, Alix, Calcein and negative controls as CytC with (n=6). Nanoparticle Tracking Analysis was performed using NanoSight® NS300 (n=14). For statistical analysis and graphics, we used the program GraphPad Prism 8.0.1. (244) and p values smaller than 0.05 were considered significant. The TEM results showed the morphological structure and presence of EVs in the fluid obtained from the different regions of the epididymis. Similarly, flow cytometry demonstrated the presence of EVs through the specific markers evaluated by positive events/µL, regardless of this, no significant differences were observed in the expression of specific markers between regions (One-Way Anova). The concentration of EVs showed a significant difference in the three regions of the epididymis. The EVs in the cauda region were smaller in comparison to caput and corpus (120 nm), and the concentration of EVs was higher in the cauda (2,07 x 10^{10} particles/mL) than in the corpus (1,03 x 10^{10} particles/mL), in the caput there was no significant difference in concentration. Accordingly, the expression of specific markers in the different regions suggests the same cellular origin of the EVs. Additionally, the fact that the cauda region presents EVs with a small and increased concentration, it is important to understand the role and contents of these vesicles as well as the impact in the spermatozoa. Understanding the origin and concentration of EVs from the different regions sheds light on future biological processes that take place in sperm maturation and the epigenetic events involved in fertilization.

Acknowledgements: FAPESP#21/06645-0, #15/21829-9, 22/01505-8; CNPq 406869/2023-5; FAPEMIG (APQ-01881-22); and CNPq (306482/2022-3).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Exploring Heat Stress Effects on Epididymal Epithelial Cells: An *in vitro* Model for Studying Epididymis-Sperm Communication

Ana Beatriz Bossois Moura[1], Tiago Henrique Camara De Bem[1], Gabriela Melendes Schneider[1], Raissa Braido Rangel[1], Laura Gabrielli Haupenthal[1], Maria Alice de Almeida[1], Juliano Coelho da Silveira[1], Maíra Bianchi Rodrigues Alves[2,1], Felipe Perecin[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: ana.moura@usp.br

Funding: This study was financed in part by FAPESP 2019/23685-5; 2021/08759-2; 2022/01505-8, CNPq 308014/2021-9 and CAPES - Finance Code 001.

Sperm establishes communication with epididymal cells via extracellular vesicles (EVs). Environmental stressors can impact the epididymal microenvironment determining changes in the sperm transcript profile that may affect embryonic development and progeny health. Herein we aimed to validate an *in vitro* model of epididymal epithelial cell (EEC) homeostasis disruption in bulls. The model is based on the exposure of EEC in culture to elevated and non-physiological temperature, and further investigation of its effects on the sperm transcript profile after coincubation of sperm with EECs-derived EVs. To achieve this, three cell lines of EEC were obtained from the epididymis of mature Nelore bulls (n=3). Caudal EEC was cultured at physiological temperature (5% CO_2; 32°C) in principal cell medium and purified until the third passage. Immunohistochemistry was performed in EEC using immunomarkers for vimentin (VIM) and cytokeratin (CK) and was evaluated by epifluorescence microscopy (Axioplan 2, Carl Zeiss®, GER). Cell lines were assigned at the same quantity (5x10^4 cells/well) to two distinct groups: heat stress (HS: 5% CO_2; 38.5°C) and control (CON: 5% CO_2; 32°C), both for 48 hours. Following 48 hours, cells from each group were counted using a Neubauer chamber. Immunocytometry (CytoFLEX, Beckman Coulter, USA) was performed in EECs to investigate changes in the heat shock protein HSP70 (Santa Cruz Biotechnology, USA). We obtained EVs from the culture medium via serial centrifugation and ultracentrifugations to evaluate particle concentration and diameter by NanoSight® NS300 (Malvern Instruments, UK). Statistical analyses were performed using the R program, where statistical tests used included the Shapiro-Wilk normality test, Analysis of Variance (ANOVA), and t-test to compare means at a significance level of 5%. The culture exhibited a higher percentage of CK staining (96%) compared to VIM (28%), which implies an enrichment of epithelial cells. EECs responded to heat stress by increasing cell proliferation, with higher cell counts in HS (1.64±0.09x10^6 cells/mL; $P<0.05$) compared to CON (0.95±0.09x10^6cells/mL; $P<0.05$). Similarly, a considerable difference in HSP70 was observed in the stressed EEC group, indicated by higher fluorescence intensity (HS: 1.34±0.09; CON: 0.58±0.09; $P<0.05$), normalized by the negative control. However, no difference was found in particle concentration or diameter between the different groups ($P>0.05$). These results indicate that caudal EECs exhibited adaptive cellular responses to potential heat stress-induced injuries. This highlights the need for further understanding of their molecular response to treatment. Therefore, our results demonstrate a potential model to investigate the modulations occurring in epididymis-sperm communication in response to heat stress.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Preliminary results of post-thawing liposome-sperm interaction: a descriptive analysis of the impact on sperm morphofunctional characteristics in cattle

Maria Alice de Almeida[1], Matheus Andrade Chaves[1], Laura Gabrielli Haupenthal[1], Felipe Perecin[1], Juliano Coelho da Silveira[1], Maíra Bianchi Rodrigues Alves[2]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Departamento de Patologia, Reprodução e Saúde Única - UNESP*)

E-mail: alicealmeida_02@hotmail.com

Funding: FAPESP.

Cryopreservation triggers damage to the sperm plasma membrane (SPM), resulting in cryoinjuries, impairing sperm quality and fertility potential. Although advances were performed to minimize damage to sperm quality during cryopreservation, post-thaw sperm still display poor quality. Recognizing that liposomes, composed of a lipid bilayered carrier that can incorporate various beneficial compounds, may positively influence SPM structure, the addition of liposomes to post-thaw sperm holds promise for SPM reorganization and restoration of sperm quality. In this study, our aim was to evaluate the interaction between post-thawing sperm and nanoliposomes and its impact on the post-thaw morphofunctional features (motility and abnormalities). For this, commercial doses of cryopreserved semen from one bull were thawed (37°C/30s), sperm were selected by Percoll® density gradient (45 and 90%) The sperm were then divided into three groups: Control (without liposome), Lip1x (in a 1:1,000 sperm/liposome ratio) and Lip10x (in a 1:10,000 sperm/liposome ratio). Liposomes, d between 100-200 nm and composed of phospholipids and cholesterol at an 80:20 ratio, were labeled with the fluorophore rhodamine (Avanti Polar Lipids) at 1% molar, and coincubated with sperm for 3 h at 38.5°C, 5% CO2 in air, and high humidity. The interaction sperm-liposome was evaluated after staining sperm with Hoechst 33342 (ThermoFisher Scientific) and evaluating images of 180 sperm cells obtained using a Thunder 3D Imager microscope (Leica Microsystems) with a magnification of 63x. Total and progressive motility were assessed using iSperm computer-assisted sperm analysis (Aidmics Biotechnology Co, LTD), and four videos were obtained for each group. Sperm abnormalities were assessed by fixing sperm in pre-heated 4% paraformaldehyde in PBS and classifying 200 cells as normal and defects (major, minor, and total) using phase contrast microscopy (Zeiss®) at 100x magnification. The GraphPad Prism 8.0 program was used for descriptive data analysis. The interaction sperm-liposome in the Lip1x was 66% and in the Lip10x was 95%, while in Control there was no interaction. Liposomes were identified in the acrosome and post-acrosomal regions of the sperm head, in the midpiece, and in the flagellum for both groups. Total and progressive motility were, respectively 13.63±4.59% and 8.87±3.28% for Control; 23.93±2.41% and 20.61±2.08% for Lip1x; and 24.62±2.45% and 18.20±2.76% for Lip10x. Regarding abnormalities, major, minor, and total defects were, respectively, 5.65%, 7.53%, and 13.18% for Control, 4.00%, 10.00%, and 14.00% for Lip1x and 0.49%, 6.43%, and 6.92% for Lip10x. Our results indicate that post-thaw sperm interact with liposomes, potentially influencing post-thaw motility without significant alterations in sperm abnormalities. However, further analysis involving a larger sample are underway to validate these preliminary results and verify their implications for post-thaw fertility.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Seminal parameters in relation to testicular biometrics in Nelore bulls

Ketlyn kaylane Xavier[1], Alexandra Albertina Oliveira[2], Ludmila Carvalho[1], Laís Reis Carvalho[1], Nathália Alves[1], Giuliana Sales[1], José Camisão de Souza[1], Miller Pereira Palhão[1]

[1]Universidade Federal de Lavras
[2]Universidade José do Rosário Vellano

E-mail: ketlyn.xavier@estudante.ufla.br

The objective was to investigate the correlation between scrotal circumference (SC) and seminal parameters in sexually mature Nelore bulls (*Bos indicus)*. Twenty bulls of the Nellore breed, aged between 5 and 10 years, underwent an andrological examination were evaluated for scrotal circumference and seminal parameters at of the breeding season, following the protocols established by the Brazilian College of Animal Reproduction (2013). The SC was initially measured using a measuring tape placed around the area with the largest diameter of the scrotal sac. Based on these measurements two classes were created: SC ≤ 38cm (n=12) and SC > 38cm (n=8). In addition, the dorso-ventral length of the testicles was assessed using a caliper, while the testicular consistency was determined by palpation, using a scale from 1 to 5. Semen was collected by electroejaculation and analyzed immediately using light microscopy techniques to evaluate physical and morphological characteristics, such as color, volume, sperm concentration, progressive sperm motility, vigor and presence of morphological abnormalities. Sperm cell count was performed in a Neubauer chamber after dilution of semen in formalin-saline solution to a ratio of 1:200. Sperm morphology was evaluated in 200 cells by direct staining with Rose Bengal. The variables relating to testicular biometry (scrotal circumference, testicular length and consistency) and the seminal variables (ejaculate volume, motility, vigor, concentration and total number of pathologies found) were checked for distribution and homoscedasticity. The correlation between seminal parameters was investigated using Pearson's correlation analysis, with statistical analyses performed using SAS® on demand software. A positive correlation (0.73, p<0.001) was observed between sperm motility and vigor. Furthermore, sperm motility and vigor were positively correlated with sperm concentration (0.55 and 0.52, p= 0.02 and 0.03, respectively). Testicular length was greater in bulls with SC > 38cm (P=0.03), while testicular consistency showed no significant difference between SC classes (P=0.3). As for the seminal parameters, the ejaculate volume (SC ≤ 38 = 4.8 ± 2.2 and SC > 38 = 3.7 ± 1.29 mL; P=0.2), sperm motility (SC ≤ 38 = 78.3 ± 10.0 and SC > 38 = 78.8 ± 13.5%; P=0.5), vigor (score 0 to 5; SC ≤ 38 = 3.6 ± 0.6 and SC > 38 = 3.8 ± 0.5%; p=0.3) and total pathologies (SC ≤ 38 = 10.6 ± 3.3 and SC > 38 = 10.0 ± 6.2%; p=0.4) were similar in bulls with SC ≤ 38 and > 38. The absence of significant differences in ejaculate parameters and scrotal circumference between groups can be explained by the fact that the bulls evaluated in this research were of similar ages. Thus, it is concluded that scrotal circumference had no influence on the seminal parameters in sexually mature Nelore bulls.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Spermatozoa binding to bovine oviduct cells: The influence of endocrine milieu on the effectiveness of the binding test *in vitro*

Paula Renata Cortat[1], Camila Azzolin de Souza[2], Alessandra Bridi[3], Mirela Balistrieri Dias[1], Lucas Oliveira e Silva[1], Fernanda Schneberger dos Santos[2], Eneiva Carla Carvalho Celeghini[4], Juliano Coelho da Silveira[2], José de Oliveira Carvalho[5], Roberto Sartori[1]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[3]Universidade do Oeste de Santa Catarina (*UNOESC*)
[4]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[5]Universidade Federal do Espírito Santo (UFES).

E-mail: paulacortat@usp.br

The aim was to evaluate the effect of endocrine environments on the ability of sperm to bind to bovine oviduct epithelial cells (BOEC) cultured *in vitro*. In experiment 1, 12 reproductive tracts of cows were collected in a slaughterhouse and transported to the laboratory at 35°C. The tracts were classified according to ovarian structures: with CL or without CL with a dominant follicle (DF) ≥ 10mm. In experiment 2, 7 *Bos indicus* cows were synchronized to have a 14d old CL (n=3) or a DF≥10 mm, in absence of CL (n=4), at the time of slaughter, when blood samples and reproductive tracts were collected. In both experiments, the oviduct isthmus region was dissected and categorized according to the location of the ovarian structures in relation to the oviduct (contra [C] or ipsilateral [I]): CLC, CLI, DFC and DFI. Follicular fluid (FF) from all follicles >5mm of each ovary was collected to analyze P4 and E2 concentrations. BOEC from each group were cultured to form explants, then were co-incubated with $1x10^5$ motile sperm/mL, using a pool of frozen-thawed sperm from 3 fertility bulls. The number of sperm-bound/mm of explant was evaluated at 0.5, 12, and 24h. Circulating and FF concentrations of E2 and P4 were analyzed by chemiluminescence. Statistical analyses were done by GLIMMIX of SAS (P≤0.05). In experiment 1, P4 concentration in FF was greater in CLI than in other 3 groups (CLI: 193.0±26.2^a, DFI: 62.7±19.9^b, DFC: 39.5±13.7^b and CLC: <20.0^b ng/mL), while E2 concentrations did not differ. At 0.5h of co-incubation, DFC had more sperm-bound/mm (DFC: 63.8±3.7^a [172 explants], DFI: 51.1±2.6^b [242], CLC: 51.4±4.4^{bc} [72], CLI: 44.3±3.3^c [134]). At 12 and 24h,CLI had less sperm-bound/mm (12h: DFC: 48.7±2.8^a [212], DFI: 46.9±2.9^{ab} [221], CLC:40.6±2.8^b [91], CLI: 27.1±2.2^c [111]; 24h: DFC: 35.2±2.4^a [248], DFI: 32.3±1.9^a [223], CLC:30.2±2.6^a [71], CLI: 20.9±1.5^b [131]). In experiment 2, cows with CL had greater circulating P4 than cows from DF group (5.4±1.1 *vs* 2.0±0.3 ng/mL). P4 concentrations in FF was greater in CLI than DFC, but did not differ from the other 2 groups (CLI: 112.7±32.9^a, DFI: 81.2±21.9^{ab}, CLC: 34.4±8.6^{ab}, DFC: 24.5±2.6^b ng/mL). E2 concentration in FF was greater in DFI than other 3 groups (DFI: >200.0^a, CLC: 75.5±62.4^b, CLI: 19.3±8.0^b, DFC:13.7±1.3^b ng/mL). At 0.5 and 12h, the DF groups had more sperm-bound/mm (0.5h: DFC: 102.3±5.3^a [126], DFI: 97.2±4.4^a [162], CLI: 87.3±5.0^b [122], CLC: 72.2±3.9^b [129]; 12h: DFI: 83.1±4.8^a [126], DFC: 74.3±4.2^a [131], CLC: 55.2±4.3^b [124], CLI: 42.3±3.1^b [137]). However, at 24h, the CLC did not differ from DF groups (CLC: 45.5±3.7^{ab} [120], DFC: 48.9±2.8^a [177], DFI: 56.7±3.7^a [148], CLI: 36.2±3.0^b [129]). In conclusion, the endocrine milieu affected the capacity of sperm to bind to BOEC. Therefore, the best *in vitro* model to evaluate sperm binding as a strategy for fertility assessment is using the oviduct from tracts with DF and without CL. Ack: FAPESP#2018/03798-7 and #2022/01433-7, CAPES, ST Repro.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Treatment with magnetic nanoparticles improves the sperm quality of bovine semen

Daniel Castilla Balcazar[1], Rolando Mamani Chullo[1], Juan Ramirez Paredes[1], Silvia Leon Trinidad[1], Carlos Quilcate Pairazaman[1], Jean Feugang[2], Deysi Dipaz Berrocal[1]

[1]Instituto Nacional de Innovacion Agraria (*Dirección de Desarrollo Tecnológico Agrario*)
[2]Mississippi State University (*Department of Animal and Dairy Sciences*)

E-mail: danielcastillabalcazar@gmail.com

Incredible advances in nanotechnology tools have opened a new world of possibilities in post-collection semen manipulation and important innovation for reproductive biotechnologies such as AI and IVF. The last application involves the use of these tools to detect and eliminate damaged sperm through the synthesis of magnetic nanoparticles conjugated with proteins, among which lectin stands out for its ability to bind to sperm with acrosomal reaction to through externalized glycosidic residues on its surface membrane (Feugang *et al.* 2015). In this study, we evaluated the impact of conjugated magnetic nanoparticles on sperm parameters in bull ejaculates. We used magnetic iron nanoparticles that were conjugated with lectins from Arachis hypogaea (PNA), along with a combination of PNA with 20%w/w PHA (Phaseolus vulgaris), to nanopurify ejaculates that had less than 50% motility. The semen samples were collected three times from three bulls housed at the Central Genetic Nucleus of the INIA – Peru, using an artificial vagina. Each ejaculate sample was diluted (Optixcell) until obtaining a concentration of 80 x 106 spermatozoa/mL and then was divided into a T0-control (without nanoparticles) and T1 (PNA 4 mg/mL); T2 (4mg/mL PNA+PHA); T3 (8 mg/mL PNA) and T4 (8 mg/mL PNA+PHA). After 15 minutes, motility (iSperm®), acrosomal reaction and sperm membrane viability were analyzed (Nikon eclipse Ts2R fluorescence microscope). To evaluate the acrosome was used FITC-PNA-PI staining (A. intact acrosome-bright green or B. reacted-no fluorescence signal) and H33342-PI for sperm viability (A. Intact-no signal fluorescence or B. Damaged-Fluorescence red). For both cases, one hundred sperm were evaluated per repetition from each treatment. The Sigma Plot 12.0 software was used to analyze the data obtained from the study. A general linear model was employed to identify differences between control and nanopurified semen samples, both within and between them. The Shapiro–Wilk Test was used to analyze the distribution of data, which revealed that all parameters followed a normal distribution except for the Acrosomal reaction parameters. The results of the study showed that nanopurification using lectin-coated magnetic nanoparticles significantly improved the viability and acrosome reaction of bull ejaculate samples. T2 exhibited higher viability compared to the control ($p=0.006$), while T1 ($p=0.01$) and T3 ($p=0.03$) showed a significant increase in the number of live sperm with an intact acrosome compared to the control. Interestingly, T3 also showed a trend ($p=0.053$) of having more live sperm with acrosomal reaction after nanopurification. However, there were no statistical differences found in total and progressive motility in any of the treatments. In summary, the study concludes that nanopurification using lectin-coated magnetic nanoparticles is an effective method for improving the quality of bull ejaculate samples, resulting in improved viability and acrosomal reaction.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Vascular parameters in relation to testicular biometry in Nelore bulls

Ludmila Carvalho[1], Alexandra Albertina Oliveira[2], Laís Reis Carvalho[1], Nathália Alves[1], Ketlyn Kaylane Xavier[1], Giuliana Sales[1], Karine Rabelo de Oliveira[1], José Camisão de Souza1, Miller Pereira Palhão[1]

[1]Universidade Federal de Lavras (UFLA)
[2]Universidade José do Rosário Vellano (UNIFENAS)

E-mail: ludmila.andrade1@estudante.ufla.br

The objective was to investigate the relationship between scrotal circumference (SC) and vascular parameters of the testicles in sexually mature Nelore bulls (Bos indicus). Twenty Nelore bulls, aged between 5 and 10 years, had an ultrasound examination in Doppler mode to evaluate the vascular parameters of the testicular artery along the spermatic cords and in the testicles. Initially, SC was measured with a measuring tape in the region with the largest diameter of the scrotum, dividing the males into two groups [SC ≤ 38cm (n=12) and SC > 38cm (n=8)]. During the examination, both testicles were evaluated at two different points: in the spermatic cord and in its initial third. The parameters measured included the diameter of the testicular arteries at the sampling points and the resistivity indices (RI) and pulsatility (PI). In addition, a blood sample was collected to measure serum testosterone concentrations. Pearson correlations were performed between vascular and biometric parameters and serum testosterone profile. Statistical analyzes were carried out using the SAS on demand software. Pearson's correlation analysis revealed that there was no significant difference in the vascular parameters (RI and PI) of the testicular artery between the two SC groups, neither in the spermatic cord nor in the initial third of the testis. However, regardless of the categorization into testicular biometric groups, a negative correlation was observed between testicular length and the indices of RI (-0.56 in the spermatic cord and -0.51 in the initial third of the testis) and PI (-0 .55 in the spermatic cord and -0.52 in the initial third of the testis). Furthermore, the RI and PI values of the testicular artery in the spermatic cord correlated positively with the same parameters measured in the initial third of the testis (high correlation for RI/RI: 0.80, $p<0.0001$; and moderate correlation for PI/PI: 0.58, $p<0.005$). Finally, PI measured in the testicular artery in the initial third of the testis showed a negative correlation with testosterone concentrations (-0.53, $p<0.02$). In short, while testicular biometry did not demonstrate any influence on vascular parameters, the RI and PI indices were lower in the testicular artery in the initial third of the testis. Furthermore, a negative correlation was observed between testosterone levels and PI in the testicular artery, suggesting that these indicators may be useful in evaluating the reproductive health of Nelore bulls.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Biostimulation with the ram effect increases the embryo quality in superovulated ewes

Ana Paula Pereira Schmidt[1], Aline Matos Arrais[1], Felipe Zandonadi Brandão[2], Ribrio Ivan Tavares Pereira Batista[3], Maria Emilia Franco Oliveira[3], Joanna Maria Gonçalves Souza- Fabjan[1], Jeferson Ferreira da Fonseca[4], Rodolfo Ungerfeld[5]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - UFF*)
[2] Universidade Federal Fluminense (*Departamento de Reprodução Animal - UFF*)
[3]Universidade Federal Fluminense (*UFF*)
[4]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)
[5]Universidad de la República (*Facultad de Veterinaria - UDELAR*)

E-mail: paula_schmidt@id.uff.br

The pulsatile release of luteinizing hormone (LH) induced by the male effect may play a beneficial role in embryonic viability during superovulation. The objective of the present study was to determine if biostimulation with males for 72 h enhances the ewes' superovulatory response, including the embryo quality. Twenty ewes were used in a latin square design, so all ewes underwent both experimental treatments (CG = control group and REG = ram effect group), with an interval of 119 days between both periods. All ewes were isolated from rams 60 days before the start of the study (without visual, olfactory and auditory contact). While CG ewes remained isolated from rams throughout the study, REG ewes were joined and stimulated with rams. Estrous synchronization and superovulation were performed according to protocols previously described (Balaro *et al.*, Domest Anim Endocrinol, 54:10-14, 2016; Taira *et al*., Animal Reproduction Science, 181: 140-146, 2022). The rams were joined with the ewes simultaneously with the administration of the third dose of pFSH and had abdominal protection to prevent mating. Each ram remained with up to 7 ewes, being replaced by a new one every 12 hours, up to 36 hours after the last dose of pFSH, totaling 72 hours of biostimulation. Artificial insemination (AI) was performed at 24, 36, and 48 h after the administration of the fifth pFSH dose. The animals underwent an ultrasound exam to determine the total number of ovarian cysts and corpora lutea (CL) before the non-surgical embryo collection. Subsequently, all collected structures were examined and classified based on developmental stage and morphological characteristics indicative of quality, as described by the IETS. Only class 1 (grades I, II, and III) embryos were considered viable. Thirty grades I and II blastocysts (n=15 per group) were dry-frozen in pools of five embryos (n=3 pools per group) for analysis of differential expression of genes related to cellular stress (PRDX.1 and HSP70), embryonic quality (AQP3, CDH1, CDX2, and SIRT2) and apoptosis (BAX and BCL2), using RT-qPCR and 2−ΔΔCt method (endogenous control; ACTB and GAPDH). The data were analyzed using mixed models with Bioestat statistical software, including the treatments, time, and their interactions as the main effects. Data are presented as LS mean ± SEM. Differences were considered significant when $P \leq 0.05$. No difference was observed in the means of ovarian cysts (0.2±0.1 *vs.* 0.9±0.5), CLs (9±1 *vs.* 8±1), recovered structures (6±1 *vs.* 5±1), viable structures (4±1 *vs*. 3±1 in CG and REG treatments. However, bioestimulation increased the abundance of transcripts associated with energy metabolism (SIRT2) and cell adhesion (CDH1) and reduced the expression of those related to stress and apoptosis (PRDX.1 and BCL2). In conclusion, biostimulation promoted healthier embryo development according to gene expression, impacting positively therefore the quality of the embryos recovered.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effect of epidermal growth factor on biometric aspects and nuclear maturation of oocytes from Spix's yellow-toothed cavy (Galea spixii Wagler, 1831)

Alexsandra Fernandes Pereira[1], Leonardo Vitorino Costa de Aquino[1], Samara Lima Olindo[1], Yara Letícia Frutuoso e Silva[1], Vinicius Dantas da Silva[1], Lhara Ricarliany Medeiros de Oliveira[1], Moacir Franco de Oliveira[1]

[1]Universidade Federal Rural do Semi-Árido (UFERSA)

E-mail: alexsandra.pereira@ufersa.edu.br

The oocyte meiotic competence depends on the intricate bidirectional communication between oocytes and *cumulus* cells, which can be modulated by epidermal growth factor (EGF). In rodents, during the *in vitro* maturation (IVM), 10 and 50 ng/mL EGF appear to improve the oocyte maturation. For Spix's yellow-toothed cavy, a wild rodent native to the Caatinga biome, this knowledge represents a milestone for obtaining protocols related to *in vitro* embryo production (IVEP). Therefore, we evaluated 10 and 50 ng/mL EGF on the extrusion of the first polar body (1PB) and biometric aspects of oocytes matured *in vitro.* The study was approved by the Animal Ethics and Use Committee (no. 23091.010566/2017-20) and Chico Mendes Institute for Biodiversity Conservation (no. 60428-1). Ovaries were collected from seven anesthetized and euthanized individuals and transported to the laboratory in saline solution with 0.05 mg/mL penicillin (30 °C). In the laboratory, oocytes were recovered by slicing in medium composed of TCM199 with 0.2 mM sodium pyruvate, 10% fetal bovine serum and 1% antibiotic-antimycotic (OCM medium). Subsequently, oocytes were selected and matured in OCM medium with 0.1 mM cysteamine, 10 µg/mL FSH/LH, in the presence of 10 ng/mL EGF (EGF10) or 50 ng/mL EGF (EGF50). After 24 h IVM (38.5 °C; 6.5% CO2), oocytes were denuded and classified according to the presence of 1PB under a stereomicroscope. Posteriorly, photomicrographs of oocytes containing 1PB were obtained for biometric evaluation using Image J software. All the structures were analyzed for total oocyte diameter (OD), oocyte internal diameter (OID), zona pellucida thickness (ZPT), ooplasm diameter (OPD), perivitelline space diameter (PSD), internal oocyte area (IOA), ooplasm area (OA), perivitelline space area (PSA). All results were expressed as mean ± standard error and 1PB rates were compared using Fisher exact test, while biometric data were compared using ANOVA and Tukey test. After three repetitions, 106 viable structures were recovered (7.6 oocytes/ovary), which were divided between EGF10 (no. oocytes: 54) and EGF50 (no. oocytes: 52). Regarding 1PB extrusion, no difference ($P > 0.05$) was observed between EGF10 (64.8% ± 2.4; 35/54) and EGF50 (55.8% ± 29/52). Nevertheless, after biometric analyses, oocytes derived from EGF10 had a higher OD (78.8 ± 5.2 µm and 74.9 ± 2.6 µm), ZPT (9.4 ± 0.8 µm and 8.5 ± 0.9 µm), OPD (69.6 ± 4.2 µm and 64.8 ± 2.8 µm), IOA (15502.1 ± 2466.5 µm² and 13877.3 ± 957.6 µm²) and OA (12058.6 ± 1630.6 µm² and 10412.9 ± 901.7 µm²) when compared to EGF50 ($P < 0.05$). These results are positive, as oocytes that present a higher OD and OPD indicate better quality. Although EGF did not influence nuclear maturation, EGF10 guaranteed a better biometric quality of oocytes after IVM. This is the first step towards the development of protocols related to IVEP in Spix's yellow-toothed cavy, and it is now necessary to evaluate the efficiency of EGF10 on embryonic production.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effect of residual feed intake on reproductive characteristics in Nelore (*Bos indicus*) heifers: Preliminary results

Fabio Morato Monteiro[1], Marina de Oliveira Silva[2], Marcelo Sant'Ana Borges[2], Leticia Padovani da Silva[1], Laura Fernanda Sechirolli da Silva[2], Pedro Luiz Bignardi[2], Maria Eugênia Zerlotti Mercadante[1]

[1]Instituto de Zootecnia (IZ - APTA)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*UNESP*).

E-mail: fabiomoratomonteiro@yahoo.com.br

Residual Feed Intake (RFI) is an important method for assessing feed efficiency in cattle. However, the influence of RFI on reproduction-related parameters remains controversial. Studies indicate that females with negative RFI (more efficient) have lower body fat compared to females with positive RFI (less efficient). The reduction of adipose tissue can lead to a decrease in the blood concentration of leptin and IGF-I. Consequently, females with negative RFI may present a reduction in reproductive performance, since the decrease in leptin and IGF-I levels results in a decrease in GnRH pulses, causing delays in both puberty and sexual maturity. Therefore, the objective of the present study was to evaluate the reproductive characteristics of females classified as negative and positive for RFI. In this study, 199 Nellore heifers (Bos indicus) genotyped, with an average age of 17 months and 250 kg, were used, with 94 negative RFI heifers and 105 positive RFI heifers. Heifers' RFI was estimated using the BLUPF90 family programs, based on the combination of genomic and phenotypic information from their ancestors and collateral relatives. Seven ultrasound evaluations were performed at 28-day intervals, assessing the following phenotypes: vulvar biometry (rima, vulva and anogenital), antral follicle count (AFC), ovarian diameter, and uterine pixel intensity. The data were analyzed using the Statistical Analysis System (SAS) software with the MIXED procedure, with a P-value of 0.05. The values of rima and anogenital measurements were higher for negative RFI (75.9±0,4 mm and 105.6±0,5 mm, respectively) compared to positive RFI (73.6±0,4 mm and 103.5±0,5 mm, respectively). Vulva measurement values were similar between divergent RFI groups (P=0.10). No differences were observed between negative and positive RFI heifers, both for AFC (P=0.74) and follicular diameter (P=0.66). Negative RFI heifers showed larger ovarian diameter (19.3±0,1 mm) than positive RFI heifers (18.9±0,1 mm). From the preliminary results, it is suggested that negative RFI heifers exhibit higher vulvar biometry and ovarian diameter measurements than positive RFI heifers.

Acknowledgments: The Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted. FAPESP (Process nº. 2022/08182-0).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effect of Sphingosine-1-phosphate on trophectoderm differentiation in bovine embryos

Francieli Perroni Berling[1], Camilla Mota Mendes[1], Marcelo Demarchi Goissis[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: francieliberling@gmail.com

The early development of the embryo is marked by the first event of cell differentiation that defines the inner cell mass (ICM) and the trophectoderm (TE). There is evidence in mouse embryos and in cultured cells that sphingosine 1-phosphate (S1P) can regulate pathways involved in this event. We sought to study the role of S1P stimulation in TE differentiation by adding S1P or the S1P receptor inhibitor JTE-013 during embryonic culture. Our hypothesis was that S1P induces TE differentiation while JTE-013 would impair TE development. *In vitro*-produced embryos were cultured in KSOM medium without serum and without glucose in 4-well plates without mineral oil. Cleaved embryos were randomly allocated to treatments at 90 hours post fertilization (hpf) in two different experiments. The first experiment consisted of a control group, vehicle (NaOH 30uM) and 200 nM S1P, while the second consisted of a control group, vehicle (DMSO 0,0125% (V/V) and 1mM JTE-013 The development rates (blastocysts/cleaved) and blastocysts were obtained at 186 hpf in 10 IVP replicates for the S1P treatment and 5 replicates for the JTE-013 treatment. The blastocysts had their zona pellucida removed and fixed with paraformaldehyde 3.8% for subsequent immunofluorescence targeting GATA3 (n = 10 embryos). The images were obtained using a laser scanning confocal microscope. Total cell count, TE (GATA3-positive) and ICM (GATA3-negative) were obtained. The embryos were also collected for qRT-PCR in four replications to analyze gene expression of *GATA3*, *YAP1*, *SOX2*, *CDX2*, *TFAP2C* and *OCT4*. *YWHAZ* and *H2A* were used as reference genes. Rates and cell counts were analyzed by ANOVA using and qRT-PCR was analyzed using a linear mixed model with pre-planned comparisons in SAS 9.4 software. A significance level of 5% or less was considered statistically significant. There was an increase on the development rate in the S1P group compared to control and NaOH (31.70 ± 3.27 *vs.* 19.52 ± 3.73, 19.83 ± 3.56 respectively; p=0.02), although there was no difference in the JTE-013 experiment. There was an increase in the S1P group compared to control and NaOH considering total cells (160.70 ± 8.78 *vs.* 119.12 ± 6.94, 122.08 ± 8.01 respectively; p = 0.002) and ICM cells (58.00 ± 4.24 *vs.* 35.25 ± 3.35, 38.83 ± 3.87 respectively; p = 0.0005). No effect was observed in TE cell number or TE/TOTAL ratio. Cell count data were not different in the JTE-013 experiment. The expression of *CDX2* (p=0.0249) was increased in the S1P and NaOH groups, while *TFAP2C* (0.0352) decreased in the S1P group compared to the control. *GATA3*, *SOX2*, *YAP1* and *OCT4* were not different. The addition of JTE-013 did not interfere with the expression of any gene. In conclusion, TE differentiation was not positively modulated by S1P as expected, despite increases in *CDX2*. However it was observed an increase in the total cell number and ICM cell number, suggesting a different action of S1P in bovine embryos. FAPESP 17/09576-3, 22/02748-1.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of α-pinene on follicular morphology, stromal cell density and expression of genes related to antioxidant defense in bovine ovarian tissues cultured *in vitro*

Venância Antoia Nunes Azevedo[1], Ernando Igo Teixeira de Assis[1], Francisco das Chagas Costa[1], Anderson Weiny Barbalho Silva[1], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará (UFC)

E-mail: venancianunes@gmail.com

The objectives of this study were to evaluate the effect of α-pinene on primordial follicle activation, development and survival, as well as on ovarian stromal cells density and mRNA expression of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX1), peroxiredoxin 6 (PRDX6) and nuclear factor erythroid 2-related factor 2 (NRF2) in bovine ovarian tissues cultured *in vitro* for 6 days. To this end, ovarian cortical tissues (3x3x1mm) were cultured for 6 days in α-MEM alone or supplemented with α-pinene (1.25, 2.5, 5.0, 10.0 or 20.0 µg/ml). Non-cultured and cultured fragments were fixed and processed histologically to evaluate follicular activation, development and survival. These follicles were classified as morphologically normal or degenerated. Ovarian stromal cells density was also evaluated. Ovarian tissues cultured in control medium (α-MEM) alone or supplemented with 10.0 µg/mL α-pinene were selected to investigate the levels of mRNA at the end of 6 days of cultures by real-time PCR technique. The primers used specifically amplified the RNAs for SOD, CAT, GPX1, PRDX6 and NRF2. The housekeeping gene glyceraldehyde3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The delta-delta-CT method was used to demonstrate the relative expression of the mRNAs studied. The Chi-square test was used to compare the percentage of normal follicles and the percentages of primordial and developing follicles (GraphPad Prism 9.0). Data of stromal cell density was analyzed by analysis of variance (ANOVA) and Tukey test. The association between stromal cell density and percentage of normal preantral follicles was evaluated by linear regression analysis. Unpaired Student's t-test was used to compare the levels of mRNA and the differences were statistically significant when $P < 0.05$. The results showed that 2.5, 5.0 or 10.0 µg/mL α- pinene increased the percentages of normal follicles when compared to those cultured in α- MEM alone ($P < 0.05$), but did not have an effect on follicular growth ($P > 0.05$). Different from tissues cultured in control medium, tissues cultured with 10.0 µg/ml α-pinene maintained a well-preserved ovarian structure with the density of stromal cells similar to those uncultured ovarian tissue ($P<0,05$). Furthermore, it showed a positive correlation between ovarian stromal cell density and the percentage of morphologically normal follicles. The presence of 10.0 µg/ml α-pinene in culture medium significantly increased mRNA levels for NRF2 and PRDX6 when compared to those cultured with α-MEM alone ($P< 0.05$). However, α-pinene did not influence the expression of mRNA for CAT, SOD and GPX1 ($P>0.05$). In conclusion, 10.0 µg/mL α-pinene improves the follicular survival, preserves the stromal cells density and increases the expression of NRF2 and PRDX6 mRNA after *in vitro* culture of bovine ovarian tissue.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of circulating progesterone concentrations on ovarian dynamics and hepatic transcripts expression of genes coding for enzymes associated with progesterone metabolism in prepubertal Nelore (*Bos indicus*) heifers

Rodrigo L. O. R. Alves[1], Aurea M. O. Canavessi[1], Mirela Balistrieri[1], Giselly B. Kolling[1], Paula R. Cortat[1], Patrícia R. Cavalcanti[1], Livia B. Lanza[1], Caroline Vandresen[1], Lucas O. e Silva[1], Roberto Sartori[1]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)

E-mail: rodrigolemos@usp.br

The aim was to study ovarian dynamics and gene expression of hepatic enzymes associated with progesterone (P4) metabolism in prepubertal Nelore (*Bos indicus*) heifers exposed to P4. 1st period (P1): on d-1, heifers (322.7±5.2kg of body weight; 20.5±0.2mo old) underwent follicle ablation (FA) and, on d0, they were assigned to 3 P4 treatments: Control (CON; n=11): 2 sham (no P4) intravaginal devices (IVD); lower P4 (LP4; n=11): one 0.5g IVDP4 and one sham IVD; or higher P4 (HP4; n=10): 2 IVDP4 (0.5 and 2g). The IVD were kept until d18 when FA was performed again. 2nd period (P2): on d23, all heifers underwent FA and, on d24, they received a 1g IVDP4, which was kept until d42. Ultrasound was performed daily from d0 to 18, and from d24 to 43, to evaluate follicular waves (FW) and uterine diameter (UD). Blood samples were collected daily from d0 to 19, and from d24 to 43, for circulating P4. Liver biopsies were performed on d-2, 17 and 22, to evaluate gene expression of the enzymes CYP3A4, CYP2C19, AKR1C4, and AKR1D1, quantified by RT-qPCR. The relative mRNA abundance was analyzed using ΔCt (cycle threshold) values, and results are presented as $2^{(-\Delta Ct)}$. Statistical analyses were done by GLIMMIX of SAS 9.4 ($^{a-c}P\leq0.05$; $^{A,B}0.05>P\leq0.10$). All results are presented in this order: CON, LP4 and HP4. Mean circulating P4 over P1 was different among treatments (0.2±0.1^{c}, 1.0±0.1^{b}, 4.0±0.3^{a} ng/mL). P4 treatment affected: number of FW (2.6±0.2^{a}, 2.0±0.2^{b}, 2.7±0.2^{a}), FW duration (FWd; 7.8±0.4^{ab}, 9.2±0.9^{a}, 6.4±0.4^{b} d), follicular growth rate (FGR; 1.3±0.1^{a}, 1.2±0.1^{ab}, 1.1±0.1^{b} mm/d), maximum diameter of dominant follicle (DFm; 13.7±0.4^{b}, 16.9±0.7^{a}, 12.5±0.6^{b} mm) and UD on d18 (16.1±0.4^{ab}, 17.8±0.7^{a}, 14.4±0.4^{b} mm). During the 1st FW, P4 treatment affected FGR (1.2±0.1^{a}, 1.0±0.1^{b}, 1.1±0.1^{b} mm/d), DFm (12.9±0.4^{b}, 15.3±1.0^{a}, 10.1±0.6^{c}mm) and FWd (7.9±0.5^{ab}, 9.1±1.0^{a}, 6.0±0.7^{b} d). During the 2nd FW, P4 treatment affected the time of follicle deviation (3.3±0.3^{a}, 2.6±0.3^{ab}, 2.3±0.3^{b} d) and DFm (13.5±0.4 ^{b}, 15.6±0.7^{a}, 12.2±0.8^{c}). Different P4 exposure during P1 affected mean P4 concentrations during P2 (2.3±0.2^{a}, 1.7±0.1^{b}, 2.0±0.1^{ab} ng/mL). There was no difference in the gene expression of the evaluated enzymes on d-2. However, on d17, there was an effect of P4 treatment on the expression of CYP2C19 (1.76±0.29^{AB}, 1.23±0.22^{B}, 1.94±0.19^{A}), AKR1C4 (5.55±0.37^{ab}, 4.79±0.38^{b}, 6.30±0.43^{a}) and AKR1D1 (0.24±0.02^{ab}, 0.17±0.04^{b}, 0.29±0.03^{a}). On d22, P4 treatment affected only the expression of AKR1C4 (6.16±0.84^{a}, 3.96±0.86^{b}, 4.11±0.90^{b}). In conclusion, exposing prepubertal *Bos indicus* heifers to LP4 resulted in a reduced number of FW, greater DFm and UD, and longer FWd. Heifers exposed to HP4 had greater expression of genes of enzymes associated with P4 metabolism. Additionally, heifers that were not previously exposed to P4 seem to have lower P4 metabolization. Ack: FAPESP #2018/03798-7, #2021/09904-6, CAPES, CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of estradiol on enzymes involved in endometrial PGF2α synthesis during early pregnancy in beef heifers

Isabella Rio Feltrin[1], Igor Garcia Motta[1,2], Amanda Guimarães da Silva[1], Samuel Volpe Souza[1], Ana Clara Degan Mattos[1], Karine Galhego Morelli[1], Thiago Kan Nishimura[1], Guilherme Pugliesi[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Ourofino Saúde Animal

E-mail: isabella.feltrin@unesp.br

We aimed to evaluate the effects of estradiol benzoate (EB) on the components of the endometrial cascade of PGF2α in beef heifers 13 days after ovulation. Nelore heifers were submitted to an estrous synchronization protocol, but ovulation was not induced. The protocol consisted of the insertion of an intravaginal P4-device (Progestar, Biogénesis Bagó) along with i.m. administration of 2mg of EB (Bioestrogen, Biogénesis Bagó) and 0.150mg of sodium D-Cloprostenol (Croniben, Biogénesis Bagó). After 6 days, 0.150mg of sodium D-Cloprostenol was given. After 48 hours (h), the P4 devices were removed and a new dose of 0.150mg of sodium D-cloprostenol was given. Estrous detection patches were applied to determine the occurrence of mounting behavior associated with estrus. Heifers that demonstrated estrus were randomly subdivided into non-inseminated (N=21) or inseminated (N=39) groups. Daily ultrasonography exams were performed to determine ovulation (D0). On D13, heifers were randomly assigned to two experimental groups to receive 0 or 1mg of EB (Sincrodiol, Ourofino Saúde Animal) (EB-0:N=10 non-inseminated and 18 inseminated; and EB-1:N=11 non-inseminated and 21 inseminated). The treatment time was considered as hour 0 (H0). On H3, the heifers were subjected to endometrial cytology, and the samples were subsequently analyzed by qPCR. Doppler ultrasonography was performed daily from D13-D19. A pregnancy check was done on D28. Data were analyzed as a 2x2 factorial design (factor 1: non-inseminated and inseminated; and factor 2: EB dose) by ANOVA in SAS. The day of structural luteolysis did not differ (P>0.1) between non- inseminated and inseminated non-pregnant heifers; however, it was anticipated (P=0.007) in the EB-1 than in the EB-0 group (16.6 ± 0.2 *vs.* 17.1 ± 1.1 days). The proportion of pregnant heifers did not differ (P>0.1) between EB groups (BE-0:36.8% *vs* BE-1:38.1%). For CL blood perfusion (BP), there was a significant interaction (P=0.05) between the treatment group and time. The CLBP was lower between D16 to D19 in the EB-1 group compared to the EB-0 group. In non-inseminated heifers, CLBP decreased from D15-D16 and continued to decrease until D18; while in inseminated pregnant heifers, CLBP decreased from D16- D17 and continued to decrease until D18 (P=0.09). The abundance of transcripts for PGR and OXTR was, respectively, 2.3 and 7 times greater in the EB-1 group than in the EB-0 group, regardless of pregnancy status. For the gene expression of ESR2, the expression was 9.5-fold greater (P=0.04) in non-inseminated heifers compared to pregnant heifers. For IL1β (pro-inflammatory cytokine), the transcript abundance was 2.9-fold greater (P=0.005) in the EB-0 group than in the EB-1 group, regardless of pregnancy status. In conclusion, estrogen treatment 13 days post-ovulation promoted uterine modulation, since PGR, OXTR, and IL1β were affected by the systemic administration of 1mg EB, regardless of pregnancy status, in Nelore heifers. Ack:FAPESP,CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of increased progesterone concentration induced by hCG on initial conceptus size

Manuela Brauner Freitas[1], Eduardo Pradebon da Silva[1], Suzana Rossato Feltrin[1], Alfredo Quites Antoniazzi[1], Ana Paula Silva, Manuela Wolker Manta[1], Valério Marques Portela, Murilo Faciochi[1], Carlos Miguel Staudt[1]

[1]Universidade Federal de Santa Maria (*UFSM*)

E-mail: manuelabraunerf@gmail.com

Progesterone (P4) is crucial for maintaining pregnancy in various species. In cattle, elevated concentrations of P4 during the initial pregnancy period are associated with improved embryonic development and increased pregnancy rates. An alternative to increase endogenous P4 concentrations during the initial embryonic development period is the use of human Chorionic Gonadotropin (hCG). The objective of this study was to evaluate the effect of hCG administration as an inducer of accessory corpus luteum (aCL) 5 days after Fixed- Time Artificial Insemination (FTAI) on bovine embryo development. For this purpose, clinically healthy cows, aged 3 to 6 years, crossbred between Brangus and Braford (n=29) were subjected to the same estrous cycle synchronization protocol, receiving intramuscular (IM) administration of 5.5 mg of 17beta-estradiol and 50 mg of P4 (Betaproginn®, Boehringer Ingelheim), along with the insertion of an intravaginal (IV) device containing 0.96g of P4 (Progestar®, Boehringer Ingelheim) on day 0. On day 7, 1 mg of estradiol cypionate (SincroCP®) and 0.5 mg of sodium cloprostenol (SincroCio®; both Ourofino Animal Health) were administered IM, and the IV device was removed; on day 9, FTAI was performed with semen from the same bull. Two groups of cows were randomly separated: the control group (CG; n=13) and the treated group (hCG; n=16). After 5 days of FTAI, cows in the hCG group received a dose of 2500 IU of hCG (Chorulon®, MSD Animal Health, IM). The cows in the hCG group had a 62.5% aCL formation rate, and none in the CG developed aCL. Seven conceptuses were recovered by the CG and 6 from the hCG. After 14 days of AI, US was performed to evaluate the aCLs, and day 27 the cows were slaughtered, the reproductive tract was collected and transported to the laboratory in refrigerated thermal boxes at 4°C. Following, ovaries were removed and the uterus was dissected to remove connective tissue and ligaments. Subsequently, uterine flushing was performed with 20 mL of Dulbecco's Phosphate Buffered Saline Solution (DPBS, pH 7,2). The uterine horns were massaged simultaneously, and the conceptus was recovered through the opening of the cervix, they were evaluated under a stereomicroscope and immediately stored in liquid nitrogen. For serum P4 measurement, blood samples were collected from all cows on days 5 and 14 after FTAI, determined by chemiluminescent assay kit (ADVIA Centaur, Siemens). Data analysis was performed using JMP14 software (SAS Institute Inc., Cary, NC, USA), P4 concentration and conceptus by ANOVA, followed by Student's t-test. Results were considered different when $P < 0.05$. Cows in the hCG group showed increased serum P4 compared to animals in the CG group. Conceptuses were not different between the groups. These results indicate that the elevation in serum P4 concentration triggered by hCG does not influence conceptus on day 18 in cattle.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of IVF sperm concentration and IVF incubation period on bovine *in vitro* production

Amanda Nespolo Silva[1], Thiago Fernandes Amaral[1], Paula Rodriguez-Villamil[1], Rebeca Dias[1], Yasmin Pereira Ribeiro[1], Nelyane José Lopes[1], Juliana Hayashi Tannura[1], Rebecca Krisher[1]

[1]ABS Pecplan

E-mail: amanda.silva@genusplc.com

Maximizing the *in vitro* fertilization (IVF) procedure success is commercially important, where the sperm concentration and sperm:oocyte coincubation period contribute to this success. The sperm concentration used in the bovine IVF procedure contributes to the fertilization rates and good quality *in vitro* produced (IVP) embryos (Grade I and II embryos). Here it was hypothesized that a higher sperm concentration and IVF period increase the polyspermy rate of bovine IVP embryos. The objective was to evaluate two different sperm:oocyte concentrations and two different IVF incubation periods. *In vitro* matured viable oocytes (n=3075) were inseminated using a purified sperm pellet from a single Girolando sire. The inseminated dose was adjusted to reach 1800 or 600 sperm:oocyte. The IVF incubation period was 22h *vs* 8h. This experiment was replicated 4 times. Putative zygotes (n=2059) were *in vitro* cultured, for 7 days, following a commercial lab protocol, under 38.5ºC and 5% O^2 and 5% CO^2. A total of 694 presumptive zygotes were harvested 18h after the IVF, and were fixed and stained with 5µg/mL of Hoeschst 33342 for nuclear labeling for 15 minutes. The 18h period was used for both incubation groups to increase the success to observe pronuclei. Zygotes harvested from the 8h group were placed in IVF media drops lacking sperm cells until the zygotes from the 22h group were harvested, at 18h after IVF procedure; when remaining zygotes were placed in culture after 22h of IVF. Embryos were rinsed in DPBS/BSA and placed on a slide containing an 8µL drop of SlowFade Gold Antifade reagent covered with a coverslip and observed with a 40x objective using an epifluorescence microscope and a DAPI filter. Polyspermy rate, cleavage rate (72hpi), total produced blastocyst, and grade I and grade II embryos at 144hpi were evaluated. Data was analyzed using the GLM procedure from the SAS 9.4 software. The model included sperm concentration, IVF period, and replicate as fixed effects. Results are presented as least-square means. Results showed a higher polysperm rate in the 1800 sperm:oocyte group with a 22h IVF (38.13%) compared to the 600 sperm:oocyte group with 8h IVF period (18.92%; p=0.0190). There was no difference in the total blastocyst production regarding the treatment. However, the rate of good quality produced embryos (GI/GII) was higher in the 600 sperm:oocyte within an 8h IVF period group (26.63%;p=0.0289). Here it was shown that the polysperm embryo is a consequence from the higher sperm concentration used in the IVF dose; also the rate of GI/GII embryo production depends on the semen concentration and IVF incubation period. Besides the sperm ratio and incubation period, attention is required regarding the oocyte quality as an important risk factor in the percentage of polyspermic zygotes. Commercial IVF labs need to optimize IVP protocols to mitigate the risks of developing polyspermic embryos, reflecting better quality produced embryos.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of long-acting dexamethasone on luteolysis in *Bos taurus* cows

Gabriel Longo Rodrigues[1], Fabiane Pereira de Moraes[1], Jéssica Lazzari[1], Sergio Farias Vargas Júnior[1], Arnaldo Diniz Vieira[1], Ligia Cantarelli Pegoraro[2], Giovani Batista Pastre[3,4], Rogério Ferreira[5], Bernardo Garziera Gasperin[1], Rafael Gianella Mondadori[1]

[1]Universidade Federal de Pelotas (*UFPel*)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*
[3]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[4]Virbac saúde animal
[5]Universidade do Estado de Santa Catarina (*UDESC*)

E-mail: glongorodrigues@gmail.com

Glucocorticoids, such as cortisol and its synthetic analogues (e.g., dexamethasone), have been shown to modulate prostaglandins and cytokines in reproduction, inhibiting prostaglandin F2alpha (PGF) production and aiding in maintaining the corpus luteum (CL). Physiologically, maternal recognition of pregnancy in cattle occurs through the release of interferon-tau (IFNT) by the embryonic trophoblast. Disturbances in this process induce luteolysis and pregnancy loss, and the cow resumes cyclicity. IFNT inhibits luteolysis by reducing PGF pulses, thus maintaining progesterone (P4) levels to sustain pregnancy. This study aimed to evaluate the effect of a recently launched long-acting dexamethasone injection (Dexaforce, Virbac, São Paulo – SP) on luteolysis in non-pregnant, non-lactating, Jersey, *Bos taurus* cows (n=20). To induce ovulation, all animals had their estrous cycle synchronized with an eight-day protocol. Briefly, at day -10 (D-10), all animals received a 1 g P4 intravaginal device (IVD) and 3 mg of estradiol benzoate; at day -2 (D-2), with the IVD removal, the cows received PGF analogs. Ovulation was induced with a GnRH analogue at day 0 (D0). On day 13, to confirm ovulation, the ovaries were scanned by transvaginal ultrasound to measure the CL (diameter and volume) and the blood perfusion assessed by Doppler (0 (no perfusion) to 3 (high perfusion) scale). Immediately after the analysis, the animals were evenly distributed into the following groups: Dexamethasone (Dex; 0.06 mg/kg BW IM; n=10) and control (Cont; n=10). To determine the moment of luteolysis, daily ultrasonography and blood sample collection for P4 measurement were performed from days 14 to 23, with some animals evaluated up to day 30. Luteolysis was determined by P4 concentration ≤1.0 ng/mL or CL perfusion ≤1, whichever came first. The moment of luteolysis was compared between the groups by survival analysis (JMP Statistical Discovery LLC), with $P \leq 0.05$ being considered significant and $P < 0.1$ considered a tendency. At Day 30 (D30), there was a trend (P=0.07) towards a higher proportion of animals in the Dex group not undergoing luteolysis. It is noteworthy that, on day 22, all the animals from the Cont group had undergone luteolysis, while 40% of the animals from the Dex group had viable CL 30 days after ovulation. The inhibition of cyclooxygenases (mainly COX-2) may be the main factor responsible for extending luteal phase duration. Further studies are needed to confirm these findings and elucidate the potential role of glucocorticoids in modulating luteolysis and pregnancy maintenance in cattle. The authors thank FAPERGS, CNPq, CAPES and Virbac for their financial support.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Electroporation preceding *in vitro* fertilization of *in vitro* matured oocytes: Implications for bovine embryo development

Diana Rangel de Lemos[1], Eliza Diniz de Souza[2], Carolina David Vieira[3], Wasim Al Shebli[1], Naiara Zoccal Saraiva[2], Luiz Gustavo Bruno Siqueira[2], Clara Slade Oliveira[2], Carolina Capobiango Romano Quintão[2], Luiz Sérgio de Almeida Camargo[2]

[1]Universidade Federal de Viçosa (UFV)
[2]Empresa Brasileira de Pesquisa Agropecuaria (*Embrapa Gado de Leite - Laboratório de Reprodução Animal*)
[3]Universidade Federal de Juiz de Fora (UFJF)

E-mail: dianalemosvet@gmail.com

Electroporation (EP) offers promise for delivering exogenous molecules into cells and embryos for gene editing purposes. However, mosaicism poses a common challenge in genetic modification. It arises from incomplete genetic editing in embryo cells, influenced by the timing of gene editing component delivery into the zygote cytoplasm, coinciding with DNA replication onset at the one cell stage. We have assessed EP before *in vitro* fertilization (IVF) to potentially reduce mosaicism. However, voltage strengths and embryo development post-EP have remained unreported. To enlighten this topic, *in vitro* matured *cumulus*-oocyte complexes were randomly assembled in the following groups: A) Control A (n=149): IVF of oocytes surrounded by *cumulus* cells; B) Control B (n=171): IVF of denuded oocytes; C) EP plus IVF (EP of denuded oocytes followed by IVF; n=358); D) EP minus IVF (EP of denuded oocytes without IVF; n=339). The oocytes of groups C and D were randomly distributed into the following voltages: 3, 5, 10, 15, and 20 V/mm. EP was performed using the Nepa21 electroporator system (Nepagene, Japan) and the presumptive zygotes were cultured for seven days, with at least three replicates. Data was analyzed using the mixed linear model of the SAS Software, and presented as mean±SEM. Voltages of 3 and 5 V in Group C (EP with IVF) resulted in cleavage (74.7±5.2% and 69.4±3.7%, respectively) and blastocyst (33.3±5% and 35.1±5.9%, respectively) rates similar (P>0.05) to control groups A (81.5±3.6% and 43.5±8.3) and B (71.4±5.1% and 37.1±3.8%). There was no difference (P>0,05) between groups A and B. However, voltages of 10, 15 and 20 V resulted in cleavage (52.8±13.2%, 37.8±4% and 22.1±3.9%, respectively) and blastocyst (7.33±3.7%, 3.3±2.3% and 2.8±1.5%, respectively) rates lower (P<0.05) than voltages of 3 and 5 V and than control groups A and B. When IVF after EP was omitted (group D), all tested voltages resulted in cleavage (20.8±6.2%, 40.0±22.7%, 44.6±19.9%, 28.3±10.9% and 10.8±5.5% for 3, 5, 10, 15 and 20 V, respectively) and blastocyst (3.4±1.7%, 0%, 2.3±2.3%, 3.4±1.7% and 0% for 3, 5, 10, 15 and 20 V, respectively) rates lower (P<0.05) than control groups A and B. In conclusion, EP preceding IVF impairs the production of blastocysts when voltages of 10 V/mm and above are applied. Also, EP alone can induce the cleavage of in-vitro matured oocytes that eventually can develop up to blastocyst stage at very low rates. Voltages of 3 and 5 V/mm hold promise in delivering gene editing components before fertilization to reduce mosaicism, but further investigation is warranted to evaluate their efficacy and potential implications for gene editing. This study provides valuable insights into optimizing parameters for EP preceding IVF, demonstrating embryo potential to reach the blastocyst stage.
Financial support: FAPEMIG, CNPq, CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Evaluation of the antioxidant potential of Astaxanthin in the vitrification of bovine embryos - Preliminary results

Amanda Bianchini Rosa[1], Thalis de Melo Oliveira[1], Yumi Camila Tsukamoto Taguti[1], Nelise Kasue Samecima Prosperute[1], Mayara Paula Paglione[1], Maíra Bianchi Rodrigues Alves[2,1], Lindsay Unno Gimenes[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (*Departamento de Patologia, Reprodução e Saúde Única, Faculdade de Ciências Agrárias e Veterinárias - UNESP*)
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: amandabianchinirosa@gmail.com

Vitrification is extensively used commercially, being a technique of fast freezing, however, the extreme temperatures to which embryos are subjected promote oxidative stress, which is related to the imbalance in the production and elimination capacity of reactive oxygen species (ROS), altering the viability and efficiency of the procedure. In order to optimize the results of the technique, astaxanthin (AST), a carotenoid with antioxidant characteristics, was employed with the objective of inhibiting or minimizing oxidative damage. The aim of this study was to evaluate the antioxidant potential of AST as a supplementation in the vitrification medium of bovine *in vitro* produced embryos. Bovine ovaries from a slaughterhouse were aspirated to obtain grade I to III oocytes, which were destined for the *in vitro* embryo production. On the seventh day of *in vitro* culture, embryos at the blastocyst (BL, n=51) and expanded blastocyst stage (BX, n=44) were vitrified using commercial medium (Vitrogen, Cravinhos, São Paulo, Brazil), in 4 experimental replicates. Each embryo category was assigned to one of the four groups with different doses of AST added to the vitrification medium (V1 and V2): 0nM (Control Group), 1nM, 500nM, and 1000nM. The embryos remained vitrified in cryogenic containers for at least 24 h and then were warmed (medium D1 to D3), following the manufacturer's instructions. After 24 h in culture medium (SOF), they were fixed on slides with the fluorescent probes, Cell Rox and Hoechst, from Thermo Fisher Scientific (Massachusetts, United States), according to the methodology described by Rocha-Frigoni *et al.* (Reproduction, Fertility, and Development, 26:797-805, 2014). Images were obtained by fluorescence microscopy through the Axion Vision program and analyzed with ImageJ software, which allowed the estimation of fluorescence intensity. Statistical analyses were performed by non-parametric analyses (Kruskal-Wallis; RStudio software), considering significant values of $P < 0.05$ and tendency, when $0.05 < P < 0.1$. For BL embryos, there was no difference between treatments (mean ± SEM; AST 1nM: $63.40^{A} \pm 4,60$; AST 500nM: $68.83^{A} \pm 4,29$; AST 1000Nm: $66.04^{A} \pm 3,70$; CON: $71.53^{A} \pm 5,39$; $P = 0.60$). However, in the BX category, treatment with any dose of AST promoted higher fluorescence intensity, i.e., indicating a higher amount of ROS, compared to the control group (mean ± SEM; CON: $46.74^{A} \pm 3,17$; AST 1nM: $59.49^{B} \pm 4.98$; AST 500nM: $63.97^{B} \pm 4,92$; AST 1000: $60.80^{B} \pm 3,88$; $P = 0.09$). In conclusion, at the BL stage, AST did not promote any protective effect and, paradoxically, at the BX stage, it increased oxidative stress.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Exploring the role of MTFAS pathway in mitochondrial metabolism regulation of bovine oviductal epithelial cells

Ana Beatriz dos Reis Bartoli[1], Thays Antunes[1], João Vitor Alcantara da Silva[1], Aldcejam Martins da Fonseca Junior[1], Ana Beatriz Florencio[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*Centro de Ciências Naturais e Humanas - CCNH/UFABC)*

E-mail: bartoli.ana@aluno.ufabc.edu.br

Recently, it was discovered that octanoic acid, through the Mitochondrial Fatty Acid Synthesis pathway (MTFAS), potentially regulates the Tricarboxylic Acid (TCA) cycle and the formation of electron transport chain complexes, which can impact mitochondrial function. However, this pathway, as well as its metabolic impacts have been poorly characterized in most cellular models. With this in mind, the aim of this study was to evaluate the role of the MTFAS pathway in regulating mitochondrial metabolism of bovine oviductal epithelial cells (BOECs). Gene silencing of OXSM, a mediator of the MTFAS pathway, was carried out using siRNA during different incubation periods. BOECs were cultured until reaching 60% confluency. At this point (t = 0), cells in the treated group received lipofectamine 0.3% [v/v] + OXSM siRNA (0.25 pmol), while the control group received lipofectamine 0.3% [v/v] + Scramble siRNA. Subsequently, cells were evaluated by microscopy at three timepoints (t = 24h, t = 48h, and t = 72h) for mitochondrial membrane potential (MMP) using Mitotracker® Red (Invitrogen) (≈ 15 cells/group, 3 replicates), and the level of target gene (OXSM) silencing by RT-qPCR (≈ 600 cells/group, 3 replicates). Fluorescence analyses were performed by quantification using ImageJ software, and MMP and RT-qPCR data were subjected to Student's T-test using GraphPad Prism, considering P <0.05. Results show that the siRNA treatment decreased OXSM transcript levels in treated cells compared to the control group at 24h ($p < 0.0001$) and at 72h ($p = 0.001$), indicating that the siRNA treatment can efficiently modulate the MTFAS pathway. Surprisingly, an increase in MMP levels was observed only at 48h in the treated group compared to the control group ($p = 0.0424$), suggesting a potential mechanism for regulating mitochondrial metabolism during BOECs cultivation. Taken together, these preliminary data suggest that MTFAS pathway plays a role for BOECs cellular activity, but its low activity can be compensated during cell cultivation. This work emphasizes the need for further investigations into the role of mitochondrial metabolism in BOECs to improve oviductal cell culture systems. Acknowledgments to FAPESP (2023/15734-1).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Extracellular vesicles and uterine cells from the uterotubal junction of Nelore cows are affected by the body's energy reserve

Schaienni Fontoura saldanha[1], Natalia Marins Bastos[1], Juliana Germano Ferst[1], Ricardo Perecin Nociti[1], Rodrigo Silva Goulart[1], Flávio Vieira Meirelles[1], Felipe Perecin[1], Juliano C Da Silveira[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: schaiennifontouras@usp.br

Body conditions changes can affect the concentration of hormones and metabolites which impacts the bovine female's reproduction. Animals with a high body energy reserve (BER) exhibit reduced embryonic recovery on day 4th of the embryonic development compared to those with a moderate BER. Extracellular vesicles (EVs) have been identified in uterine fluid and recognized as a novel mediator of maternal-embryonic communication, delivering bioactive materials such as mRNAs, miRNAs and proteins. However, the influence of increased BER on the uterine receptivity of the uterotubal junction (UTJ), where the embryo establishes its initial contact with the maternal endometrium, remains poorly understood. This study aimed to evaluate the effects of increased BER on the miRNA and mRNA profiles of EVs and endometrial cells from the UTJ of cows, respectively. For this, Nelore cows from the same herd underwent two different nutritional plans during a 70-day feedlot period to either maintain (MBER) or increase (HBER) their BER. At the end of the feedlot period, animals underwent estrous synchronization, artificial insemination, and were slaughtered approximately 120 h after ovulation induction. The reproductive tracts were collected, and the UTJ ipsilateral to the corpus luteum were flushed with 2 mL of PBS. After this, UTJ cells were collected by ex-vivo endometrial biopsies. Only UTJ samples from animals containing an 8-cell embryo in the isthmic portion of the oviduct were used in this study (MBER group, n=3; HBER group, n=3). EVs were isolated from UTJ fluid, and their miRNA content was assessed using a panel of 382 miRNAs, with 9 showing differential expression (DE) between groups, all upregulated in the MBER group ($P<0.05$). Additionally, RNA content was extracted from UTJ cells and subjected to sequencing analysis, revealing 435 DE genes between the groups, with 249 upregulated and 186 downregulated in the MBER group ($P<0.1$) compared to HBER. To evaluate whether transcripts in UTJ cells are related to miRNAs in UTJ EVs, we examined if downregulated genes from RNAseq were predicted targets of the upregulated miRNAs. Interaction analysis revealed that 80 of the 186 downregulated genes are targets of the 9 upregulated miRNAs in the same group. To delve deeper into the correlated genes, we initially focused on DKK3 and ITGA5. Both were predicted to be regulated by 4 of the 9 upregulated miRNAs and already described with a significant role in early embryonic development in cell signaling, migration and differentiation processes. Based on these previous results, we can infer that the impact of BER of cows on the UTJ cell transcriptome seems to be mediated by EVs through their miRNA content, modulating the dynamic of the UTJ environment during the early embryonic development. Funding: FAPESP 2023/02746-1 and CAPES001.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Fatty acids supplementation alters the synthesis of prostaglandins $F_{2\alpha}$ and E_2 in bovine embryos produced *in vitro*

Ana Karolyne Alves Miguel[1], Laura Chuba Machado Rolniche[2], Lucas de Oliveira Bezerra[2], Adriano Felipe Mendes[2], Maria Eduarda Rocha Pinto[2], Amanda Guimarães da Silva[1], Isabella Rio Feltrin[1], Alan Ealy[3], Guilherme Pugliesi[1], Claudia Maria Bertan Membrive[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Faculdade de Ciências Agrárias e Tecnológicas - UNESP*)
[3]Virginia Polytechnic Institute and State University

E-mail: anakarolyne.miguel1234@gmail.com

PGE2 and PGF2α are synthesized by the maternal endometrium and the embryo trophoblastic cells. PGE2 exerts luteotrophic effects favoring maternal pregnancy recognition (MRP), while PGF2α exerts luteolytic actions. Specific fatty acids (FAs) alter prostaglandins biosynthesis. The promotion of increased PGE2 synthesis, decreased PGF2α synthesis, and/or increased PGE2/PGF2α ratio, may benefit the embryo implantation during MRP. This study aimed to evaluate the effects of supplementing specific FAs in the *in vitro* culture medium (IVC) of bovine embryos on the synthesis of PGE2, PGF2α, PGE2/PGF2α ratio and embryo production. Ovarian follicles measuring 2 to 10 mm in diameter were aspirated from slaughtered Nelore females using a 10 mL syringe attached to an 18G needle. Oocytes with homogeneous cytoplasm and multiple layers of *cumulus* cells were submitted to *in vitro* maturation (IVM) in 100 µL drops containing IVM medium with 5% fetal bovine serum (FBS) under mineral oil, and incubated at 38.5°C in an atmosphere humidified at 5% CO2 for 24 hours. After IVM, the oocytes were submitted to *in vitro* fertilization (IVF) with semen from a single Nelore bull, for 18 hours under the same conditions as IVM (D0=day of IVF). After IVF, presumptive zygotes (n=1117) were allocated into 4-well cell culture dishes with 700 µL of culture medium/well with 1.25% FBS. The following treatments were added: 100 µM conjugated linoleic acid (CLA Group; n=223 oocytes); 100 µM linoleic acid (LA Group; n=227); 100 µM oleic acid (OA Group; n=225) or 50 µM eicosapentaenoic acid - EPA + 50 µM docosahexaenoic acid - DHA (EPA + DHA Group; n=219); in addition to medium without FAs (Control Group; n=223); in 10 replicates. PGE2 and PGF 2α concentrations were determined by ELISA. Embryo production was evaluated on D7 and D9. Statistical analysis was performed using the SAS PROC MIXED software. PGE2 synthesis in the LA and OA groups did not differ from the Control Group; however, synthesis was lower in the CLA and EPA + DHA groups compared to the Control (0.2676 ± 0.0552 and 0.0920 ± 0.0082 *vs.* 1.1376 ± 0.2528 ng/mL respectively; P=0.0001). PGF2α synthesis was higher in the LA and OA groups compared to the Control (0.7026 ± 0.1644 and 0.4110 ± 0.0391 *vs.* 0.2658 ± 0.0378 respectively; P=0.0001), and lower in the CLA and EPA + DHA groups compared to the Control (0.7078 ± 0.0077 and 0.0570 ± 0.0046 *vs.* 0.2658 ± 0.0378 respectively; P=0.0001). The PGE2/PGF2α ratio did not differ between the LA, OA, CLA, and Control groups. The EPA + DHA group compared to the Control showed a lower PGE2/ PGF2α ratio (1.6340 ± 0.1360 *vs.* 3.5760 ± 0.9255 respectively; P=0.0001), lower blastocyst rate on D7 (3.98 ± 1.43 *vs.* 14.94 ± 3.13% respectively; P=0.0378) and D9 (2.34 ± 1.29 *vs.* 12.86 ± 3.10% respectively; P=0.0036). In conclusion, embryos treated with EPA + DHA showed lower synthesis of PGE2, PGF2α, PGE2/PGF2α ratio, as well as a lower blastocyst rate on D7 and D9. Acknowledgments: FAPESP and CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Fatty acid supplementation alters prostaglandin synthesis in bovine trophoblastic cells cultured *in vitro*

Gabrielle Ceragioli Damaceno[1], Lucas de Oliveira Bezerra[2], Laura Chuba Machado Rolniche[2], Catarina Lazarevicius Prestes[2], Adriano Felipe Mendes[2], Amanda Guimarães da Silva[1], Isabella Rio Feltrin[1], Alan Ealy[3], Guilherme Pugliesi[1], Claudia Maria Bertan Membrive[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Faculdade de Ciências Agrárias e Tecnológicas - UNESP*)
[3]Virginia Polytechnic Institute and State University

E-mail: gabrielle.ceragioli@unesp.br

PGE2 and PGF2α are synthesized by the maternal endometrium and embryo trophoblastic cells. PGE2 exerts luteotrophic effects favoring maternal pregnancy recognition (MPR) and PGF2α exerts luteolytic actions. Specific fatty acids (FA) alter prostaglandins biosynthesis. This study aimed to evaluate the effects of specific FAs supplementation in the culture medium of bovine trophoblastic cells (TC-1) on the synthesis of PGE2, PGF2α, and the PGE2/PGF2α ratio, what could benefit the embryo in MPR. TC-1 was cultivated in culture bottles at 38.5°C in a humidified atmosphere with 5% CO2. Afterward, they were transferred to 6-well cell culture dishes (10 x 10^6 cells per 9.6 cm^2 well) containing medium with 10% fetal bovine serum (FBS) and cultured for 5 days. TC-1 received an FBS-free culture medium for 24 hours. Then, the wells received FBS-free medium containing 100 µM conjugated linoleic acid (CLA), 100 µM linoleic acid (LA), 100 µM oleic acid (OA), or 50 µM eicosapentaenoic acid (EPA) + 50 µM docosahexaenoic acid (DHA) for 5 minutes, 24, 48 and 72 hours; in a total of 5 replicates. The Control contained the same concentration of ethanol, used to dilute FA, as the experimental groups. PGE2 and PGF 2α concentrations were determined by ELISA. Statistical analysis was performed by SAS PROC MIXED, considering the well as the experimental unit. There was no difference in the PGE2 and PGF2α synthesis within 5 minutes between the groups. LA group, compared to the Control, had a greater (P<0.001) synthesis of PGE2 after 24 (301.19 ± 61.83 *vs*. 45.59 ± 4.00 ng/mL respectively), 48 (272.42 ± 56.82 *vs*. 48.15 ± 6.59 ng/mL respectively) and 72 hours (310.58 ± 81.54 *vs*. 97.40 ± 22.40 ng/mL respectively). PGF2α synthesis was higher (P<0.001) in the AL and OA groups compared to the Control at 24 (151.67 ± 17.01 and 105.71 ± 30.86 *vs*. 45.89 ± 9.86 ng/mL respectively), 48 (112.94 ± 40.95 and 159.41 ± 43.67 *vs*. 80.10 ± 19.10 ng/mL respectively) and 72 hours (317.67 ± 55.52 and 200.81 ± 20.39 *vs*. 98.77 ± 21.81 ng/mL respectively). PGF2α synthesis was lower (P<0.001) in the EPA+DHA group compared to the Control group at 48 (7.28 ± 0.54 *vs*. 45.89 ± 9.86 ng/mL respectively) and 72 hours (11.07 ± 0.71 *vs*. 98.77 ± 21.81 ng/mL respectively). The PGE2/PGF2α ratio was higher (P = 0.007) in the CLA, LA, EPA+DHA groups compared to the Control for 24 (2.23 ± 0.31, 2.04 ± 0.40 and 2.32 ± 0.31 *vs*. 1.11 ± 0.15 respectively), 48 (3.16 ± 0.68, 2.42 ± 0.29 and 2.77 ± 0.50 *vs*. 0.74 ± 0.15 respectively), and was maintained for 72 hours in the EPA+DHA group compared to the Control (4.19 ± 1.44 *vs*. 1.09 ± 0.18 respectively). In conclusion, LA increases the synthesis of PGE2 and PGF2α at 24, 48, and 72 hours of culture, however, the PGE2/PGF2α ratio was increased at 24 and 48 hours of treatment. EPA+DHA decreased PGF2α synthesis and increased PGE 2/PGF2α ratio at 48 and 72 hours of treatment. The increase of PGE2/PGF2α ratio suggests that both treatments can be beneficial to increase MPR in cows.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Fertility after three-days progesterone treatment in ewes during the transition period to the breeding season

Julia Bopp de Andrade[1], Julia Nobre Blank Camozzato[2], Cesar Andrei Marques da Silveira[1], Gabriel Maggi[2], Monique Tomazele Rovani[1], Fernando Caetano de Oliveira[1], Bernardo Garziera Gasperin[2]

[1]Universidade Federal do Rio Grande do Sul (*UFRS*)
[2]Universidade Federal de Pelotas (*Veterinary graduate program - PPGV UFPel*).

E-mail: juliabop@hotmail.com

The objective of this study was to evaluate the influence of the duration of exposure to progesterone intravaginal device (IVD) in ewes during the transition period to the breeding season on estrus (rate and timing of estrus onset), pregnancy and conception rates. One hundred and thirty-five crossbred ewes from three farms (replicates) were subjected to a hormonal protocol and natural mating (NM). The control group (8DP4; n=67) received an intravaginal device (IVD) containing 360 mg of progesterone (P4; Primer-PR, Agener União, São Paulo, Brazil) on day -8, while the treatment group (3DP4; n=68) started the protocol on day -3. On day 0, all ewes had their IVD removed and received 125 µg of sodium cloprostenol (Estron, Agener União) IM and 300 IU of eCG (Novormon, Zoetis, Campinas, Brazil) IM. Ewes underwent NM with rams at a 10% ratio, which were marked on the chest with a crayon. Data were analyzed using ANOVA, logistic regression, and survival analyses for estrus timing (JMP Statistical Discovery LLC), with replicates and treatments included in the model. P≤0.05 considered as significant. The 3DP4 treatment decreased estrus expression (3DP4: 83.8%, 57/68; 8DP4: 94.0%, 63/67; P=0.05) and pregnancy rate (3DP4: 27.8%, 19/68; 8DP4: 43.4%, 29/67; P=0.03) and tended to decrease conception rate (3DP4: 33.3%, 19/57; 8DP4: 46.0%, 29/63; P=0.07) compared to 8DP4. An interaction between replicates and treatment on pregnancy rate, was observed (P=0.04), suggesting a possible effect of seasonal variations across farms and animals. The timing of estrus onset did not differ between groups (3DP4: 46.5±3h; 8DP4: 48.6±3h; P=0.51). In conclusion, the duration of P4 exposure significantly influenced estrus expression and pregnancy rate in ewes bred by NM, suggesting that short P4 exposure may not be feasible for timed artificial insemination protocols during the transition into the breeding season. Acknowledgements: FAPERGS, CAPES and CNPq. The authors thank FAPERGS, CNPq and CAPES for their financial support.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

First detection of *Leptospira* spp. in *cumulus*-oocyte complexes from naturally infected cows

Paulo Victor dos Santos Pereira[1], Ana Paula da Silva Cupello[1], Lucas Francisco Leodido Correia[1], Walter Lilenbaum[2], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - UFF*)
[2]Universidade Federal Fluminense (*Laboratório de Bacteriologia Veterinária - Instituto Biomédico*)

E-mail: victor_paulo@id.uff.br

Despite a few reports showing the presence of *Leptospira* spp. in COCs from experimentally infected cows, it is still unclear if the bacterium can penetrate the COCs in naturally infected cows. This study aimed to identify the presence of *Leptospira* spp. in COCs from naturally infected cows. Ovaries and uterine body fragments were collected from 40 clinically healthy cows immediately after slaughter. The follicular fluid (FF) and COCs were recovered by follicle aspiration. The COCs were fixed for 24 h in a 4% formalin solution and stored in PBS + 1% BSA, to carry out the direct immunofluorescence technique. Then, FF and uterine fragments were analyzed by PCR to detect the *Lip*L32 gene, which is only present in pathogenic *Leptospira* spp. The COCs were permeabilized with PBS + 1% PVA + 0.5 X Triton-100 for 40 min and blocked with PBS + 1% PVA incubation for 40 min. Afterward, COCs were incubated overnight at 4 °C in a solution of PBS + 0.1% PVA with 2 µg/mL of the primary monoclonal mouse IgG2a *Lip*L41 antibody (Thermo Fisher Scientific®), washed three times, and then incubated for 1 h at 4 °C in a solution of PBS + 0.1% PVA with 1 µg/mL secondary antibody - goat anti-mouse IgG2a Alexa 488 (Thermo Fisher Scientific®). Finally, COCs were again washed three times, and cells were counter-stained with 0.5 µg/mL propidium iodide in PBS + 0.1% PVA for 5 min. The COCs were then analyzed to identify the *Leptospira* spp. by fluorescent labeling under epifluorescence microscope (Eclipse E200 - Nikon®), and cows were then classified as positive (POS) or negative (NEG) regarding the bacterium presence. Data are presented as Mean ± SEM. In total, 67.5% (27/40) of the cows were positive for bovine genital leptospirosis (BGL) in the PCR test. Regardless of the infection status, an average of 8.7 ± 6.3 COCs/cow (range: 1 - 41 COCs/cow) was obtained. It was possible to observe spirochetes in 90% of the females (36/40) and in 62% of the overall COCs (181/293). Considering those POS cows, the bacterium was visualized in 75.3 ± 4.1% of the COCs (ranging from 20 to 100%). In fact, 13 cows had 100% of their COCs infected by *Leptospira* spp. Curiously, despite negative results in the PCR samples, 11 cows had the bacterium present in 61.3% (54/88) of their COCs, showing that the presence of *Leptospira* spp. may not have been detected in the PCR, possibly due to the low bacterial load, obtaining little DNA in the samples. Of note, it was possible to identify the presence of the pathogen adhered to the zona pellucida and *cumulus* cells of COCs. In conclusion, this is the first study to demonstrate the presence of *Leptospira* in contact with COCs from naturally infected cows, showing that the detection of this pathogen in the FF signals not only a migration through the ovary but also an interaction with the COC, which could be one of the many causes of the reproductive problems triggered by BGL and a potential risk for the IVP. Acknowledgments: Capes (code 001), CNPq, and FAPERJ.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Gene targeting of *GATA6* alters gene expression of bovine embryos

Marcelo Demarchi Goissis[1], Leticia Escobar Carreiro[1], Francieli Perroni Berling[1], Camilla Mota Mendes[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*

E-mail: mdgoissis@usp.br

The second event of cell differentiation during mammalian development is the segregation between the epiblast (EPI) and the primitive endoderm (PE). This occurs within the inner cell mass (ICM) of blastocysts. It is known in mice that *GATA6* drives PE differentiation by repressing *NANOG*, which in turn drives EPI formation repressing *GATA6*. In the bovine embryo, recent data show that *NANOG* gene targeting does not interfere with GATA6 expression. Thus, we hypothesize that *GATA6* is not required for the formation of the PE in bovine embryos. The specific objective of this study was to assess expression of genes related to this second cell differentiation event after gene editing of *GATA6* using CRISPR/Cas9. The experimental set comprised *in vitro* fertilization (IVP)-derived embryos subjected to microinjection 16 hours post-fertilization with 100 ng/µl of two distinct guide RNAs (gRNA) targeting the DNA binding domain of *GATA6* and 100 ng/µl of TrueCut™ Cas9 Protein v2 (ThermoFisher). Conversely, the control group underwent microinjection without Cas9 protein. Embryos were then cultured *in vitro* for 186 hours post fertilization (hpf) until blastocyst stage and harvested individually for gene expression analysis of *GATA6, NANOG* and *SOX17* by absolute q-RT-PCR, using 10 gRNA+Cas9 embryos and 4 control embryos. Cleavage rates at 90 hpf, blastocyst formation (number of blastocysts/number of zygotes), and development rates (blastocysts/cleaved embryos) were evaluated across six replicates. The data was subjected to ANOVA analysis, followed by Tukey's mean comparison, with significance set at 5% or lower. We did not observe significant difference between *GATA6*- Cas9 or control groups on cleavage rate (85.68 ± 3.78% and 75.93 ± 3.78%, p=0.21), blastocyst rate (20.29 ± 2.66% and 11.75 ± 2.66%, p=0.12) and development rate (22.38 ± 2.47%, and 15.51 ± 2.47%, p=0.24). Expression of *GATA6, NANOG* and *SOX17* was verified in all collected embryos. Four out of the 10 embryos injected with gRNA+Cas9 did not present reduction in *GATA6* and were excluded from the other analyses. Considering only the embryos that presented reduced *GATA6*, we observed a significant reduction in gene expression of both *NANOG* (p=0.02) and *SOX17* (p=0.03). This latter result hints at the rejection of our hypothesis, as *SOX17* is a marker for PE cells. Ongoing studies on protein localization will provide further data to support this conclusion. Financial support by FAPESP 17/09576-3.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Identification of biomarkers in the vaginal microbiota of high-producing dairy cows

Ana Vitória Querido de Oliveira Ramalho[1], Anne Kemmer Souza[1,2], Amanda Zangirolamo Fonseca[1,2], Ricardo Guella Droher[1], Francieli Gesleine Capote Bonato[1], Amauri Alcindo Alfieri[1,2], Marcio Carvalho da Costa[3], Marcelo Marcondes Seneda[1,2]

[1]Universidade Estadual de Londrina (*Laboratório de Biotecnologia da Reprodução Animal - REPROA/UEL*)
[2]Instituto Nacional de Ciência e Tecnologia da Cadeia Leiteira (INCT-LEITE)
[3]Département de Biomédecine Vétérinaire, University of Montreal, Montreal, Canada

E-mail: anavitoriaramalho72@gmail.com

This study aimed to prospect significant biomarkers in the vaginal microbiota of high- producing dairy cows that became pregnant or not after artificial insemination to establish an association between bacterial communities and reproductive performance. HPB cows, multiparous and high production (n = 10), were used. On the day of estrus, the cows were inseminated, and after 30 days, the pregnancy diagnosis was made. The cows were classified as pregnant (PG) or non-pregnant (NP). The vaginal swab of each female was obtained immediately before artificial insemination. The samples were submitted to DNA extraction and sequencing performed by the PacBio platform. To select and filter the samples within the range of 1,500 bp, the DADA2 software was used, while the SBanalyzer 2.4 software was used to assign taxonomic identification to all readings. Of the 10 cows inseminated, seven became pregnant. For the characterization of the vaginal microbiota of both groups, 366,509 bacterial readings were used by the PacBio SNG, grouped into 22 phyla and 476 species. The LEfSe method was performed to identify bacterial genera that are most likely to explain the differences between the PG and NP cow group, aiming to predict significant biomarkers. Four genera were associated with the PG group and two with the NP group. Regarding the genus, Clostridiales_unclassified, Fermicutes_unclassified Helicobacter and Intestinimonas were associated with the PG group both by distribution and abundance. In the NP group, the genera Pseudomonas and Jeotgalicoccus were detected by LEfSe with a high LDA score, reflecting not only the marked abundance in non-pregnant cows but also the similarity between the animals in this category. The results highlight the differences in bacterial composition between two reproductive profiles, pregnant and non- pregnant. These findings represent a starting point to improve the understanding of the dynamics of the vaginal microbiota of dairy cows, allowing the tracking of beneficial bacterial populations to enhance reproduction.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

IGF1 release after administration of 325 mg and 500 mg of recombinant bovine somatotropin in crossbred beef heifers

Lígia Mattos Rebeis[1], Angélica Simone Cravo Pereira[1], Sofía Albertini[1], Jose Alfonso Echavarria Martínez[2], Ester Costa Fabricio[1], Bruna Lima Chechin Catussi[1], Samuel Volpe Souza[1], Lucas Cárnio de Siqueira Branco[1], Marco Rogério Dalalio[3], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidad de los Llanos
[3]Agener União Química

E-mail: ligia.rebeis@gmail.com

Bovine Somatotropin (bST) and Insulin-like Growth Factor 1 (IGF1) play pivotal roles in cattle reproduction. bST, a protein hormone produced by cattle, enhances milk production and influences reproductive processes. IGF1, stimulated by bST, is crucial for follicular development and embryo survival, directly impacting fertility rates. The interaction between these hormones suggests that bST boosts milk yield and potentially improves reproductive efficiency through IGF1. Recombinant bovine somatotropin (rBST) is an analog of growth hormone naturally produced by cows. Therefore, this study aims to measure the IGF1 release (hormonal curve) after administration of 500 mg or 325 mg of rBST in crossbred beef heifers. A total of 29 crossbred beef heifers located in Pirassununga-SP (Beef Cattle Research Laboratory-USP, Brasil) were used. At the beginning of the synchronization protocol (D-9), the animals received an intravaginal P4 device (Primer Monodoses®, Agener União), 2mg of estradiol benzoate (EB; RIC-BE®, Agener União), and 0.530 mg of sodic cloprostenol (Estron®; Agener União). After 9 days (D0), the P4 device was removed, and heifers received 0.530 mg of sodic cloprostenol (PGF; Estron®, Agener União) and 0.5 mg of EC (Cipiotec®; Agener União). At this moment, the animals were divided into three groups: G1 (n=10; without rBST administration), G2 [n=10; administration of 325 mg of rBST (Posilac®, Agener União)], G3 [n=9; administration of 500 mg of rBST (Lactotropin®, Agener União)]. From D0 on, blood was collected from the animals every 48 hours for 14 days (D14) for subsequent hormonal analysis of IGF1. Statistical analyses were performed using GLIMMIX in SAS 9.4. There was treatment*time interaction (P<0.0001). On D0 and on D2, there was no difference between the groups in the concentration of IGF: D0:G = 190.4 ± 15.1 *vs.* G2 = 190.1 ± 14.4 *vs.* G3 = 179.7 ± 15.1 ng/mL, P =0.84 and D2: G1 = 195.4 ± 18.9 *vs*. G2 = 264.0 ± 18.9 vs. G3= 260.8 ± 19.9, P=0.053. On D4, IGF1 was higher in animals in the G2 group (G1 = 185.0 ± 15.9^{B} vs. G2 = 247.4 ± 15.5^{A} *vs*. G3 = 237.0 ± 16.3^{AB}, P=0.03). On D6, IGF1 increased in both G2 and G3 groups (G1 = 191.7 ± 23.7^{B} *vs*. G2 = 278.0 ± 22.5^{A} *vs*. G3 = 308.0 ± 23.7^{A}, P=0.005) and this increase remained on D8 (G1 = 189.9 ± 20.3^{B} *vs.* G2 = 328.8 ± 19.2 ^{A} *vs.* G3 = 361.7 ± 21.5 ^{A}, P<0.0001), D10 (G1 = 177.0 ± 21.1^{B} *vs*. G2 = 314.6 ± 20.1^{A} vs. G3=339.5 ± 21.1^{A}, P<0.0001), D12 (G1 = 180.4 ± 20.2^{B} *vs.* G2 = 316.3 ± 19.2^{A} *vs.* G3 = 343.2 ± 20.2^{A}, P<0.0001) and D14 (G1 = 172.7 ± 16.7^{B} *vs.* G2 = 309.4 ± 15.8^{A} *vs.* G3 = 331.1 ± 16.7^{A}, P<0.0001). In conclusion, after the fourth day, administration of 325 mg or 500 mg of recombinant bovine somatotropin increases IGF1 hormone levels in crossbred beef heifers.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Impact of centrifugation on oocyte developmental potential and mitochondrial DNA quantification

Marilin Ivask[1], Marcos Roberto Chiaratti[2], Flávio Vieira Meirelles[1], Lawrence Charles Smith[3,1]

[1] Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]Universidade Federal de São Carlos (*Departamento de Genética e Evolução - UFSCAR*)
[3]Université de Montreal (*Faculty of Veterinary Medicine*)

E-mail: marilin.ivask@usp.br

The role of mitochondrial methylation in oogenesis is poorly understood. Ooplasmic biopsy can be used to correlate methylation with oocyte competence, but the low amount of mitochondrial DNA (mtDNA) in biopsy complicates single cell studies. Centrifuging oocytes before biopsy concentrates mitochondria on one side of the oocyte, enabling getting more mtDNA for methylation analysis. This study aimed to assess the impact of centrifugation, biopsy and individual culture on oocyte development, also to compare mtDNA content between biopsies from centrifuged *vs* non-centrifuged oocytes. Bovine oocytes were matured in TCM199 medium with 10% of FCS, 5 U/mL hCG, 0.5 µg/mL FSH, 0.2 mM pyruvate and 50 µg/mL gentamicin at 38.5°C and 5% CO2 for 18 h. Oocytes were thereafter divided into two main groups: 1) centrifuged (C) at 10000 g for 15 min after incubation with 7.5 µg/mL cytochalasin B; 2) incubated just with 10 µg/mL Hoechst 33342 (nC). The two main groups were further divided into three subgroups: biopsied and individually cultured (B+), non-biopsied individually cultured (B-) and non-biopsied group cultured (G). Approximately 1% of the oocyte volume was biopsied and frozen. Biopsied oocytes were frozen or parthenogenetically activated for developmental assessment. All biopsied oocytes were individually activated and cultured, while non-biopsied ones were activated in groups, then randomly divided into individual or group culture. Cleavage (CL%) and blastocyst (BL%) rates were evaluated at day 3 and 8, respectively. MtDNA copy number in biopsies and corresponding oocytes were assessed via qPCR as described in detail elsewhere (Chiaratti *et al*., Biology of Reproduction, 82:76-8, 2010). Statistical analysis was performed with SAS OnDemand for Academics using one-way ANOVA with Tukey's test and Spearman's correlation test. P<0.05 was considered significant. The CL% (mean±SD) were 48.6±17.6% for CB+ (n=140), 62.7±18.5% for nCB+ (n=110), 44.6±16.4% for CB- (n=110), 60±19.5% for nCB- (n=110), 47±22.4% for CG (n=357) and 60.6±14.6% for nCG (n=362). The BL% were 14.5±9.3% for CB+, 25.5±15.7% for nCB+, 13.6±12.9% for CB-, 26.4±19.1% for nCB-, 20.1±11.7% for CG and 29±9.5% for nCG. Centrifugation had a significant effect on CL% (P=0.002) and BL% (P=0.002), but not biopsy or culture type. MtDNA copy number in biopsies notably differed (P=0.004) between C (13777±12024; n=11) and nC (1888±1520; n=11) groups. MtDNA copy number was significantly associated between biopsies and oocytes in the C (R=0.75; P=0.007), but not in the nC (R=0.08; P=0.81), group. Centrifugation resulted in ~8x more mtDNA compared to regular cytoplasm biopsy, 8.6% *vs* 1.1% of the total oocyte mtDNA, enabling faithful prediction of mtDNA content in oocytes. These preliminary results show the potential for obtaining more mtDNA from biopsies for downstream applications, though centrifugation seems to negatively impact oocyte developmental capability. Funding: FAPESP 2021/09886-8, 2023/08037-2

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Influence of lipid metabolism related miRNAs in follicular fluid extracellular vesicles during *in vitro* maturation of bovine *cumulus*-oocyte complexes, preliminary results

Fernanda Schneberger dos Santos, Alessandra Bridi[1], José Roberto Quirino de Oliveira[1], Ludimila Cardoso Zoccal Janini[1], Lorena Calixto Munhoz[1], Juliano Coelho Da Silveira[1], Cláudia Lima Verde Leal[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: fschneberger@usp.br

The interaction between the oocyte (OO) and its *cumulus* cells (CC) within the follicular environment is essential for maturation. The follicular fluid (FF) surrounding the *cumulus*-oocyte complex (COC) contains small extracellular vesicles (fEV) that participate in intrafollicular communication. Recent studies indicate that IVM and EV-mediated communication may affect lipid metabolism, which if altered, can compromise oocyte quality. As fEV carry miRNAs that can regulate lipid metabolism, this study aimed to investigate the effect of fEV during IVM on oocyte maturation and lipid content; lipid metabolism-related miRNA in fEVs and in cultured cells were assessed as well. FF and COC were obtained from slaughterhouse bovine ovaries by aspiration of 3-6mm follicles. fEV were isolated from FF by exclusion chromatography followed by ultracentrifugation (100,000 x g; 70 min; 4°C). FCS was depleted of its EV (dFCS) by ultracentrifugation (100,000 x g; 18 h; 4°C). COC were placed in maturation medium [TCM199 with 0.4 mM glutamine, 0.2 mM pyruvate, gentamicin (50 mg/mL), EGF (20 ng/ml), FSH (0.5 µg/ml)] supplemented with 10% dFCS (control) or 10% dFCS with fEV (fEV group), for 22h at 38.5°C and 5% CO2 in air. After IVM, CC and OO were separated, and OO evaluated for maturation rates (MII: 1st polar body extrusion). Part of matured OO was fixed and stained (Bodipy 493/503) for lipid droplet (LD) content analysis (% LD area relative to cytoplasm area) by confocal microscopy. The other part of OO and respective CC were stored separately at -80°C for analysis of lipid metabolism related miRNAs by RT-qPCR; fEV were analyzed for the same miRNAs. Data were analyzed by Mann-Whitney U test (MII rates and LD contents) or Tukey test (miRNA expression, ΔCt values); significance was 5%. Maturation rates (4 replicates) were not affected by fEV (80% MII for ~100 OO/group, P>0.05). LD contents (3 replicates) were also similar between control (dFCS) and fEV (13%, ~30 OO/group, P>0.05). Studied miRNA were detected in fEV (27), OO (11) and CC (41). All miRNAs in fEV were also in CC, 8 were common to all samples, and 3 were only common to OO and CC. miRNA levels in OO and CC did not change with fEV during IVM (3 replicates, P>0.05). Considering present results, it may be concluded that fEV do not affect maturation of oocyte or its lipid contents, and although lipid metabolism-related miRNA were detected in fEV, they were probably not transferred to cells as miRNA expression in OO or CC did not vary. However, since fEV also carries mRNAs and other miRNAs, other effects on lipid metabolism or other functions in COC cannot be disregarded. Financial support: FAPESP (SPEC Grant # 2021/09886-8; AR Grant #2021/06760-3); FS - DS Scholarship (Capes 88887.694635/2022-00); AB - PD Scholarship (FAPESP 2023/01524-5); JRQO - ScI Scholarship (FAPESP 2023/12424-1); LCZJ DS Scholarship (Capes 88887.836321/2023-00); LCM - ScI Scholarship (PUB-USP 2023/83-1).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Influence of Nitric Oxide on oxidative stress and expression of mitochondrial aconitase in bovine oviductal epithelial cells

Ana Beatriz Florencio[1], Heloise Cale da Rocha[1], Patricia Kubo Fontes[1], Joana Claudio Pieretti[1], Amedea Barozzi Seabra[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*UFABC*)

E-mail: anaflorencio2015@gmail.com

Reproductive processes in the bovine oviduct involve synchronized cell signaling events and the nitric oxide (NO) plays an important role in coordinating these events. Nevertheless, in other cell types the presence of high NO levels negatively affects mitochondrial metabolism and gene expression, inhibiting aconitase activity, reducing energy production, and increasing oxidative stress, leading to enzyme damage and reactive oxygen species production. Therefore, we aimed to identify if this event occurs in the bovine oviduct. We hypothesized that bovine oviductal epithelial cells (BOECs) exposed to pathological NO levels would present higher ROS levels and lower *ACO2* abundance. For that, BOECs were collected from an abattoir at two phases of the estrous cycle, the Luteal Phase (LP) and Follicular Phase (FP), by ovarian morphology evaluation (n=6 animals/phase, 3 replicates). The cells were cultured (DMEM + 10% FBS, 38.5°C, 5% CO□) until 70% confluence when they were treated with S-Nitrosoglutathione (GSNO), a NO donor (at pathological levels, 500 µM, GSNO group). At four- and 48-hours incubation with GSNO or not (control), BOEC were submitted to ROS levels analysis (CellROX® Green reagent, with pixels number measured in each area by ImageJ-FIJI software) and *ACO2* abundance measurement (by RT- qPCR, using SybrGreen system with bovine specific primers, beta-actin as a reference gene, and normalized as Delta-Delta-Ct). Data were analyzed by ANOVA and Tukey's test using GraphPad Prism software, with a significance level of 5%. As a result, no difference in ROS levels was observed in BOEC from FP when comparing either different times or treatments (C_4h 42.7 ± 11.2, C_48h 31.1 ± 7.9, GSNO_4h 39.8 ± 14.5, GSNO_48h 42.9 ± 8.8), but in BOEC from LP the ROS levels decreased as the culture time progressed (4 h > 48 h) in both groups (GSNO: 65.7 ± 7.7 *vs.* 38.07 ± 15.5, p<0.0001 and control: 62.1 ± 14.7 *vs.* 22.3 ± 7.0, p<0.0001, respectively), moreover, there was an effect of treatment in these cells, which higher levels of ROS were present in the GSNO group than the control, but only at 48 h (p=0.032), with no difference at 4 h (p>0.05). Interestingly, we also observed an effect of the estrous cycle on the ROS levels, which were higher in cells from LP than FP either in control (p=0.0028) and GSNO groups (p<0.0001), but only at 4 h, with no difference at 48h. Regarding the mRNA abundance of *ACO2*, no statistical differences were observed between any experimental groups (FP: C_4h 0.5 ± 0.6, C_48h 0.5 ± 0.04, GSNO_4h 1.3 ± 0.9, GSNO_48h 1.0 ± 0.1 and LP: C_4h 0.4 ± 0.2, C_48h 1.0 ± 0.8, GSNO_4h 0.8 ± 0.5, GSNO_48h 1.2 ± 1.3). In conclusion, we demonstrated that BOECs exposed to pathological levels of NO exhibited higher ROS levels (only in cells from LP). However, this increase is not influenced by *ACO2* levels in this specific cell type. Supported by FAPESP (20/09051-0, 2024/04153-0, 2022/00321-0 and 2020/03646-2).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Influence of the lunar cycle on serum progesterone and estradiol concentrations, and follicular count in guinea pigs

Fernando P. Perea[1], Luz Isabel Camas Carangui[1], Natalia Soledad Chabla Morquecho[1], Maria Silvana Mendez Alvarez[1], Alejandro A. Aguirre[2], Hugo Hernandez Fonseca[3]

[1]Universidad de Cuenca (*Facultad de Ciencias Agropecuarias*)
[2]Fort Bend Animal Hospital
[3]Saint George's University (*Anatomy, Physiology & Pharmacology*)

E-mail: ferromi9@gmail.com

Evidence has accumulated that the lunar cycle influences diverse aspects of reproductive activity in farm animals (Aguirre *et al.*, Journal of Biological Rhythms, 36:160-168, 2021; Chinchilla-Vargas *et al.*, Animal Reproduction Science, 193:117-125, 2018). A retrospective study demonstrated an effect of the moon cycle on reproductive and productive aspects in guinea pigs (Perea *et al.*, Chronobiology International, 41:127-136, 2024). Studies on the subject have analyzed the effect of the moon on reproductive performance, but there is a lack of evidence on aspects supporting the biological basis of this phenomenon. Since guinea pigs are an excellent animal model to study in more depth the relationship between the moon and reproduction, this study aimed to assess the influence of the lunar cycle (LC) on serum progesterone and estradiol concentrations, and follicular count in this species. Eighty nulliparous guinea pigs, aged 3-3.5 months and weighing 1061±90 g were randomly assigned to four time points (n=20 each; 5 animals per phase for 4 consecutive LCs) of the LC: new moon (NM); crescent quarter (CQ); full moon (FM); waning quarter (WQ). Animals were fasted for 12 hours and anesthetized with sevoflurane. In an unconscious state, blood samples were taken by cardiac puncture and the animals were immediately euthanized by cervical dislocation. Ovaries were removed and weighed, and one of them was histologically processed. Different categories of follicles in the whole ovary were counted by duplicate (two slices separated by 250 µm) under a stereoscope. Progesterone and estradiol concentrations were quantified by chemiluminescence. Data were analyzed by the general linear model and the least square means test of SAS. In the statistical model, the phase and number of the lunar cycle were included as independent variables, and body weight and stage of the estrous cycle (determined by vaginal cytology) as covariates. Ovarian weight and gonadosomatic index were not influenced by moon phase. LC influenced serum progesterone ($P=0.0489$) and estradiol ($P<0.0001$) concentrations. Progesterone was greater ($P<0.05$) in NM (5 ± 0.7 ng/ml) than in FM (2.6 ± 0.7 ng/ml) and WQ (3.1 ± 0.7 ng/ml). Estradiol was greater ($P<0.0001$) in NM (86.8 ± 3 pg/ml) than in CQ (71.7 ± 2.9 pg/ml), FM (77.1 ± 2.9 pg/ml) and WQ (70.3 ± 3 pg/ml). LC affected the number ($P=0.0005$) and percentage ($P=0.0014$) of large antral follicles (LAF). Number of LAF was greater in NM (3.0 ± 0.49) than in CQ (2.0 ± 0.48; $P>0.05$), FM (0.6 ± 0.46) and WQ (0.8 ± 0.49) ($P<0.01$). The number ($P=0.0019$) and percentage ($P=0.0013$) of primordial follicles (PF) were influenced by moon phase, with lower values in the number of PF in WQ (13.3 ± 1.7) than in NM (18.8 ± 1.6), CQ (20.3 ± 1.6) and FM (22.5 ± 1.5) ($P<0.01$). The number of corpora lutea and other follicular categories were similar among lunar phases. In conclusion, LC influenced circulating concentrations of ovarian steroids and proportions of large antral and primordial follicles in guinea pigs.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Intensive reproductive management in cyclic Morada Nova sheep by alternated use of double cloprostenol estrus synchronization protocols

Andreia Souza Lopes[1], Alice Lima Martins[2], Luana Rangel Côrtes[2], Lucas Oliveira Pinheiro[1], Matheus Modesto do Amaral[3], Sérgio Novita Esteves[4], Rui Machado[4], Jeferson Ferreira da Fonseca[4]

[1]Universidade Federal do Recôncavo da Bahia (*UFRB*)
[2]Universidade Federal de Viçosa (*UFV*)
[3]Centro Universitário Presidente Antônio Carlos Juiz de Fora
[4]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*

E-mail: deealopes@gmail.com

The objective of this study was to test the effectiveness of combining two synchronization protocols for integrated reproductive management using cloprostenol in sheep. This study was approved by the Ethics and Animal Care Committee of Embrapa Dairy Cattle Research (7945200723). A total of 35 nulliparous Morada Nova ewes and two fertile rams were used. Ewes were synchronized with two doses of 131.5 µg of cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, Brazil) i.m. with intervals of 7.5 days (T7.5, n=18, 28.0± 0.5 kg and 1.4±0.0 years) and 11.5 days (T11.5, n=17, 27.0±0.5 kg and 1.4±0.0 years) (P >0.05). The animals were kept in two groups (17 and 18 sheep), with half belonging to each group in each pen. The first dose was administered to all sheep on day 0 (D0) in the morning, and the second doses on days 7.5 and 11.5 in the afternoon, with a difference of 4 days between the groups. After the second dose of the T7.5 group, a ram equipped with a colored marker was introduced into each pen and remained until D15. Estrus was recorded early in the morning and late in the afternoon (marked ewes) and B-mode transrectal ultrasound and Doppler were used for early diagnosis of pregnancy on day 21, where the embryonic vesicle and active corpus luteum could be visualized, as well as at 30 days post-estrus. Data were analyzed using Bio Estat 5.0 software (Belém, Brazil) and submitted to ANOVA (parametric, mean ± SEM) or Fisher exact test and chi-square test (non-parametric, %) and differences were considered significant when P≤0.05. Five animals showed estrus 43.2±9.7 h before the second dose of cloprostenol T11.5 and were mated and considered group T11.5B. Thus, 100.0 (18/18) and 91.7% (11/12) of the ewes came into heat in groups T7.5 and T11.5, respectively. The interval until estrus was similar (P>0.05) between the T-7.5 and T11.5 groups (47.3±6.9 and 53.5±3.7 hours, respectively). The pregnancy rate at 21 days was 100% for T7.5 (18/18), 83.3% for T11.5 (10/12) and 100% for T11.5B (5/5). Pregnancy rates at 21 and 30 days and embryonic loss were similar (P>0.05) to T7.5 (100.0% or 18/18; 94.4% or 17/18; 6.6%), T11.5 (83.3% or 10/12; 66.7% or 8/12; 16.6%) and T11.5B (100% or 5/5; 60.0% or 3/5; 40.0%), respectively. A total of 34 ewes came into heat and were mated within a 15-day interval, resulting in an overall pregnancy rate of 80.0% (28/35). The results indicate that the association of two estrus synchronization protocols was effective in inducing estrus and pregnancy in Morada Nova ewes, making it suitable for planning reproductive management. Financial support: EMBRAPA (20.22.03.004.00.02.013) and FAPEMIG (BPD-00308-22).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Interaction of embryos and glandular spheroids under progesterone and estrogen stimulation: A new *in vitro* insight to mimic the uterine environment.

Thais Sayuri Imura Oshiro[1], Anna Eloísa Serafim Viana[2], Amanda Nespolo Silva[3], Eduardo Ribes Martínez[4], Cibele Maria Prado[2], Marcia de Almeida Monteiro Melo Ferraz[4], Juliano Coelho Da Silveira[2], Tiago Henrique Camara De Bem[2], Flávio Vieira Meirelles[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Cirurgia - FMVZ/USP)*
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]ABS Pecplan
[4]Ludwig Maximilians Universität München (*Faculty of Veterinary Medicine / Clinic of Ruminants*)

E-mail: thais.imura@usp.br

Mimicking the uterine environment *in vitro* may contribute to understand the mechanisms involved in early pregnancy. Our aim was to establish spheroids from endometrial glands to study maternal-embryonic interaction. Our hypothesis is that *in vitro*-cultured endometrial spheroids are responsive to hormonal treatments and the presence of the embryo. Endometrial glands (EG) and epithelial (Epi) and stromal (Stro) cells were isolated from non-pregnant cows' uterus. Intercaruncular biopsies were kept in collagenase (100mg/mL) for 50 min at 38.5°C. EG were collected using 100 and 40 µm cell strainers. After isolation, the EG were frozen and stored in liquid nitrogen until use. Epithelial and stromal cells were seeded in the flasks, cultured and purified until passage II. Two different groups: G1 only EG (0.06v/v), and G2 EG (0.06v/v) + Epi (3,000 cells/10uL) + Stro (80,000cells/mL), were submitted (G1+ and G2+) or not (G1- and G2-) to hormonal stimulation. The co-culture was carried out in SOF medium +10% FBS +1% PSF in Matrigel (2.5mg/mL). Hormonal stimulation was performed by adding 200pg Estrogen (E2) + 4.5ng Progesterone (P4) on D2; 3pg E2 + 4.5ng P4 on D3; 3pg E2 + 7.5ng P4 on D4 and D5; 3pg E2 + 15ng P4 on D6 and followed by 3pg E2 + 30ng P4 until D9. On D8, blastocysts (D7) were added to G1+ (G1+E) and G2+ (G2+E) and co-cultured for 24 h. On D9, the embryos were removed, and cells were collected to analyze the profile of 383 microRNAs (miRNAs) from the 6 groups in triplicates. Relative expression levels were compared based on ANOVA followed by Tukey. Total RNA was extracted according to Trizol protocol (Thermo Fisher), reverse transcription was performed with MystiCq microRNA kit and RT-PCR with SYBR Green (Thermo Fisher). RT-PCR data were normalized by the geometric mean of Hm/Ms/Rt U1 snRNA and bta-miR- 99b. In the G1 246 miRNAs were detected, while the exclusive miRNAs for G1+E (6), G1+ (4), and G1- (60). In the G2 group, 278 miRNAs were detected showing exclusive miRNAs for G2+E (11), G2+ (5) and G2- (10). Enriched pathways modulated by these miRNAs were determined by bioinformatics analysis using DAVID. Metabolic Pathway, MAPK, PI3K-AKT, Hippo and WNT, were common pathways found in all groups from both G1 and G2. However, when we enriched the pathways individually for each group, the Regulatory Pluripotency Signaling Pathway (80 genes), were more modulated in the G1+E group. In the G2+E group, the most enriched pathway was the Cancer Pathway, followed by Focal adhesion, ECM-receptor interaction, and Cell cycle. Based on the results, we argue that the embryo presence, different cell types and hormones, modified the profile of miRNA expression in the maternal tissues. These modifications are indicative of a crosstalk with the maternal environment created *in vitro*. Furthermore, the bovine endometrial environment *in vitro* model becomes a powerful tool to study the mechanisms and physiology of maternal- embryonic communication. FAPESP 2021/13948-9.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Melatonin supplementation in pre-maturation media does not affect the bovine IVP outcome

Laryssa Ketelyn Lima Pimenta[1], Nayara Ribeiro Kussano[2], Ana Caroline Chaves Vall Nicolás[3], José Eduardo Vieira Chaves[1], Pedro Henrique Déo[1], Venâncio Augusto Oliveira Silva[1], Marcos Coura Carneiro[1], Alice Caroline Souza e Castro[1], Margot Alves Nunes Dode[2], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás (*Escola de Veterinária e Zootecnia - UFG*)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*
[3]Universidade de Brasília (*UnB*)

E-mail: lketelyn@discente.ufg.br

The use of meiotic resumption inhibitors such as natriuretic peptide type C (NPPC) during the pre-maturation period (pre-IVM) provides the oocyte additional time to undergo cytoplasmic changes which can improve oocyte quality. Melatonin (MTn), in turn, has been evaluated during IVM for its ability to reduce levels of oxidative stress due to its antioxidant action. To minimize the deleterious effects of oxidative stress and provide better conditions for the acquisition of oocyte competence, we evaluated the effect of MTn during pre-IVM. Grades I and II COCs (n=1752) were recovered from slaughterhouse ovaries (n=219) and, were randomly distributed into three groups: 1) Control IVM: COCs were IVM for 24h; 2) pre-IVM control: COCs were cultured for 6 h in the presence of 100 nM of NPPC, followed by 24 h of IVM and 3) pre-IVM + MTn: COCs were cultured for 6 h in the presence of 100 nM of NPPC and MTn [10-9 M], followed by 24 h of IVM. After maturation, the COCs were co-incubated with sperm cells for 18 h. Zygotes were then transferred to IVC medium, where they remained for seven days. Cleavage rate was assessed on day 2 after fertilization (D2) and the blastocyst rate on D6 and D7. For IVM, IVF and IVC the atmosphere was set at 5% of O2 and 5% CO2, at 38.5 °C. Data were analyzed by chi-square test (p≤ 0.05). The cleavage rate was higher (P≤ 0.05) in the groups pre-IVM control (n= 442/557; 79.4%) and pre-IVM + MTn (n= 451/601; 75%) in compared to IVM Control (n= 392/594; 66.0%). On D6, pre-IVM + MTn (n= 85/601; 14.1%) showed a lower rate of blastocysts (p≤ 0.05) compared to IVM Control (n= 110/594; 18.5%) and pre-IVM control (n= 113/557; 20.3%). However, on D7, the blastocyst rates of both pre-MIV groups, pre-IVM control (n= 200/557;35.9%) and pre-IVM + MTn (n= 185/601;30.8%) were higher than observed in the control IVM group (n= 153/594; 25.8%). In conclusion, a treatment of pre-maturation supplemented with MTn for 6 h has insufficient effect to increase the production of IVP bovine embryos. Research in our lab is in progress to assess the synergistic impact of MTn supplementation during pre-IVM and during IVM.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Mini-oviducts: spheroids as a model to *in vitro* study the oviductal functionality and physiology

Patricia Kubo Fontes[1], Ana Beatriz Florencio[1], Heloise Cale da Rocha[1], Thays Antunes[1], Jessica Ispada[1], João Vitor Alcantara da Silva[1], Aldcejam Martins da Fonseca Junior[1], Ana Beatriz dos Reis Bartoli[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*UFABC*)

E-mail: pkfontes@gmail.com

In the magnetic bioprinting system, cells tagged with magnetic nanoparticles can rapidly be printed into specific three-dimensional patterns using external magnetic force. Hence, the oviductal magnetic spheroid (OMS) culture was standardized by our research group, resulting in a stable spheroid structure, that can be customized in terms of cell size (up to 10,000 cells), cell proportion (epithelial:stromal cells), culture period (up to 21 days), cellular self-organization capability (internal stromal cells location with external epithelial cells layer) with the presence of oviductal glycoprotein 1 (OVGP1) and acetylated-alpha tubulin (acTUB, cilia marker). Next, in this study, we aimed to evaluate the OMS competency as a model to study the bovine oviduct hormone responsiveness to estradiol (E2) and progesterone (P4). Therefore, the OMS was submitted to protocols to mimic the estrous cycle phases, specifically the luteal, follicular, and post-ovulatory phases respectively as high P4 (100 ng/mL, for 7 days), followed by high E2 (300 pg/mL, for 3 days), and no hormone (only DMEM, for 4 days), totaling 14 days of culture. Thereafter, the OMS was submitted to immunoassay analysis for OVGP1 and acTUB (raw intensity density was normalized by the spheroid area using ImageJ) and relative mRNA quantification by RT- qPCR using Taqman™ system with bovine-specific primers and normalized by Delta-Delta-Ct (*ACTB* was used as reference gene). Data were analyzed by ANOVA and Tukey's test using GraphPad Prism software, with a significance level of 5%. As a result, we observed no difference in acTUB level ($p>0.05$), but the OVGP1 protein level was progressively increased among the groups (P4<E2<DMEM, $p<0.05$, n=3 spheroids/group). When evaluating the mRNA abundance (n=3 spheroids/group), the levels of *OVGP1*, *FOXJ1* (cilia marker), and hormone receptors (*ESR1, ESR2,* and *PGR*) were similar between groups ($p>0.05$). Therefore, we cultured the OMS again for 7, 10, and 14 days (n=3 spheroids/group) but in the absence of hormones (DMEM only, during the entire period). As before, no difference was observed in acTUB levels ($p>0.05$), while the OVGP1 protein level increased by the culture time (D7<D10<D14, $p<0.05$), indicating that the OVGP1 levels might increase as a result of culture time, but not by the hormonal activity. In conclusion, the OMS has the potential to be used as a model to study the oviduct tissue. With some further evaluations of different hormonal designs, we might expand the capacity of modulating the OMS hormone responsiveness. Nevertheless, the malleability of this model regarding cell size, cell proportion, and culture duration associated with the maintenance of the tissue functionality enhances the OMS as an approach that might allow applicability beyond mere oviductal cell culture model, but also a co-culture system for embryo development. Supported by FAPESP (21/11747-6, 20/02500-4, 22/12169-9).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Modulation of the WNT/β-CATENIN pathway during *in vitro* maturation of swine oocytes using DKK1 and RSPO2

Thais Naomi Gonçalves Nesiyama[1], Juliano Rodrigues Sangalli[1], Edna de Fátima Elias Macule[2], Lawrence Charles Smith[3], Vilceu Bordignon[4], Flávio Vieira Meirelles[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Faculté de Médecine Vétérinaire, Université de Montreal
[4]Faculty of Agricultural and Environmental Science, McGill University

E-mail: thais_ngn@usp.br

Among the *in vitro* embryo production (IVP) steps, *in vitro* maturation (IVM) is crucial for producing good-quality swine embryos. Incomplete formulation of the IVM medium affects the capacity of oocytes to acquire competence. Improving the medium composition modulating development pathways during the IVM can benefit the swine IVP. The WNT (Wingless) pathway modulates and is involved in gametogenesis, oocyte maturation, and embryonic development. Targeting proteins from the canonic pathway of WNT (Wnt/β- catenin) with Dickkopf-related protein 1 (DKK1) and R-Spondin (RSPO2) improved nuclear and cytoplasmatic maturation in other species. However, it is not clear how proteins with antagonistic activities can contribute to increasing gamete competence. Thus, we interfered in the Wnt/β-catenin in this study by adding the recombinant proteins DKK1 or RSPO2 (PeproTech®), in the IVM medium. To this end, *cumulus*-oocyte complexes (COCs) obtained from gilt ovaries were matured in two steps (IVM I and II). First, IVM I (0-22h) consisted of TCM-199 medium supplemented with 20% (v/v) porcine follicular fluid (PFF), previously collected from sows and stored in 1 mL aliquots, 0.91 mM sodium pyruvate, 3.05 mM D- glucose, 0.57 mM L-cysteine, 10 ng/mL EGF, 5 IU/mL hCG, 10 µg/mL eCG, 1 mM cAMP and 20 µg/mL gentamicin. Second, IVM II (22-44h) consisted of IVM I medium in the absence of hCG, eCG, and cAMP, maintained at 38.5°C, in 5% CO^2, and humidified air. To test the modulation of the Wnt/β-catenin pathway, we used 4 groups: 1- Control group as described above; 2- adding 200 ng/mL DKK1; 3- adding 100 ng/mL RSPO2; 4- Negative control group without PFF. After 44 hours, all four groups (CTL, DKK1, RSPO2, CTL-) were denuded with TrypLE™ Express (Gibco), and the IVM rates were evaluated by observing the 1st polar body extrusion. The maturation rate in the CTL- was the lowest (41,5 ± 2,98%; 98/236; $P<0,005$) among the groups. However, the other groups had similar maturation rates (67,21 ± 2,84%; 203/302) CTL (66,02 ± 2,23%; 204/309) DKK1, and (67,71 ± 2,08%; 184/280) RSPO2 ($p>0,05$). To test the competence, matured oocytes were parthenogenetically activated using 15 µM Ionomycin for 5 min, followed by exposure with 200 µM TPEN (Milipore®) for 15 min, and incubation with 7.5 µg/mL Cytochalasin B for 4h in PZM-3. The parthenotes were *in vitro* cultured (IVC) in PZM-3 media at 38ºC, in 5% CO^2, 5% O^2, and 90% N^2 humidified atmosphere for 7 days. The group CTL- produced no blastocysts (0/19), while the other showed similar results, 48,46% (16/33) CTL, 48,57% (17/35) DKK1, and 50% (12/24) RSPO2. We conclude that PFF is essential for oocyte maturation, and modulators of the Wnt/β-catenin pathway did not improve IVM in gilts. Future studies are developing to better understand the DKK1/RSPO2 effects on oocyte maturation. This study was financed by the CAPES-001 and FAPESP grant#: 21/09886-8 SPEC, 23/12977-0, and 13/08135-2 CEPID. Partnership with the Santa Rosa Meatpacking Company.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Molecular diagnosis of the presence of *Leptospira spp*. in different anatomical regions of the genital system of naturally infected cows – Preliminary results

Ana Paula da Silva Cupello[1], Paulo Victor dos Santos Pereira[1], Lucas Francisco Leodido Correia[1], Gicele Santos Apolinario[1], Walter Lilenbaum[1], Joanna Maria Gonçalves Souza- Fabjan[1]

[1]Universidade Federal Fluminense (*UFF*)

E-mail: anacupello@id.uff.br

Leptospira spp. has previously been detected in the ovaries, oviducts, uterus, vaginal secretion, follicular fluid, and semen of cattle. In this context, among the clinical samples used to diagnose bovine genital leptospirosis (BGL), cervical-vaginal mucus collection and *in vivo* uterine biopsy are viable alternatives compatible with animal welfare. However, it is not yet known whether this bacterium is evenly distributed in all regions of the bovine uterus and, therefore, whether biopsy collection could result in false-negative results, keeping the pathogen circulating silently in the herd. Given the above, this study aims to evaluate the diagnostic potential of uterine samples in the context of BGL through the relationship between the presence of *Leptospira* spp. in different uterine regions, oviducts, and ovaries of naturally infected cows. Samples from 19 clinically healthy cows destined for slaughter were used. The follicular fluid (FF) from the ovaries of each animal was collected, as well as uterine samples from the uterine body (UB), base of the uterine horns (BUH), apex of the uterine horns (AUH), and oviducts (OVID), for analysis of the presence of *Leptospira* spp. DNA, totaling five samples from each animal. At the slaughter line, the reproductive tract was aseptically removed, and the samples were collected individually for each animal and packed separately in conical tubes, avoiding cross-contamination. The collected samples were refrigerated at 4 ºC until they arrived at the laboratory. Upon arrival at the laboratory, the ovaries had their follicles punctured to obtain FF. All the uterine samples had their endometrium and myometrium dissected and frozen until they were processed for the PCR technique, while the whole OVID was dissected and frozen. The PCR technique aimed to detect the *Lip*L32 gene, which is only present in pathogenic *Leptospira* spp. species. Animals with genital samples showing the *Lip*L32 gene were considered positive for BGL. In total, 89.5% (17/19) of the cows had at least one sample with the presence of *Leptospira* spp. DNA, indicating that they had BGL. Among the FF samples collected, 84.2% (16/19) were positive (POS-FF). Concerning UB, 52.6% (10/19) of the samples had the presence of this bacterium (POS-UB). In addition, it was also possible to associate the results obtained in FF and UB, in which 47.4% (9/19) of the cows were positive in both. About the uterine horns and oviducts, 10.5% (2/19) of the AUH samples were positive, while all 19 BUH and OVID samples were negative. These results may indicate that there is no connection to the presence of *Leptospira* spp. in different regions of the genital tract of naturally infected bovine females, leading to the belief that this agent is not uniformly distributed throughout the genital tract during BGL infection. Acknowledgements: Capes (code 001), CNPq and FAPERJ.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Natriuretic peptide precursor C during pre-IVM sustained transzonal projection integrity, altered chromatin and epigenetic features and accelerated meiotic progression in bovine COCs – A study based on a refined GV classification system

Helena Fabiana Reis de Almeida Saraiva[1], Juliano Rodrigues Sangalli[1,2], Juliano Coelho Da Silveira[1,2], Lawrence Charles Smith[1,3], Flávio Vieira Meirelles[1,2], Felipe Perecin[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]University of Montreal (*Département de Biomédecine Vétérinaire*)

E-mail: helenasaraiva@alumni.usp.br

Oocyte competence is affected by its developmental stage. We proposed a refined classification of immature oocytes based on differences in chromatin compaction and nuclear lamina features, subdividing GV1-3 into .1, .2, and .3. We employed this system to test the effects of a pre-IVM with natriuretic peptide precursor C (NPPC) on meiotic parameters and *cumulus*-oocyte communication at the time points 0h (end of pre-IVM period), 6h, 9h, and 19h of IVM. Abattoir COCs were pre-matured for 6h in TCM199 bicarbonate plus 50 µg/mL gentamicin, 0.2 mM sodium pyruvate, 0.4% BSA (base medium) supplemented with 100 nM NPPC, 10-4 IU/mL rhFSH, 500 ng/mL oestradiol, 50 ng/mL progesterone, 50 ng/mL androstenedione (PM group) or the same pre-IVM added 5 µM AG1478 (inhibitor of EGF receptors; PM-Ei group). IM group: immature COCs as 0h-control, and those directly *in vitro* matured as IVM-control. At 0h, we analyzed the GV distributions, GV breakdown (GVBD) rates, oocyte H3K9 acetylation, and density of intact transzonal projections (TZP) after immunostaining assays. H3K9ac content (arbitrary units) and TZP densities (n° TZP/10 µm oocyte perimeter) were quantified with Fiji software. Thereafter, immature and pre-matured COCs were *in vitro* matured for 19h in base medium added 100 ng/mL AREG, 10-2 IU/mL rhFSH, 10 ng/mL IGF1, 150 ng progesterone and 50 ng oestradiol. We evaluated the meiotic progression at 6h IVM, and MII rates at 9h and 19h IVM. Data were submitted to ANOVA followed by Tukey-Kramer or Kruskal-Wallys test, with 5% significance. Distinct responses of the immature oocytes at early (1.1-2.2) and advanced (2.3-3.3) GV stages to both pre-IVM protocols promoted increases in GV1.1 and 1.2 categories while decreased 2.1 and 2.2 in pre-matured oocytes (0h). Immature GV3 oocytes were less susceptible to PM arrest, since GV3.1 decreased and 3.3 increased in pre-matured groups, with 1.85% and 5.28% of GVBD rates at 0h in PM and PM-Ei, respectively. Both pre- IVM sustained TZP integrity similar to immature COCs and independently of GV stage. PM oocytes at GV1 (58.5; p=0.0004) and GV2 (82.3; p=0.0009) showed lower H3K9ac compared to IM (GV1: 107.8; GV2: 82.3) and PM-Ei (GV1: 91.9; GV2: 76.2), whereas GV3 H3K9ac differed (p<0.0001) between all groups (IM: 83.3a; PM: 44.0c; PM-Ei: 64.8b). Although the increased GV1 category at 0h, pre-matured oocytes progressed faster in meiosis up to 6h IVM, with most of them at GVBD (PM: 44.4%; PM-Ei: 21.7%) and MI stages (PM: 36.1%; PM-Ei: 39.2%) whereas IM oocytes were mainly at GV3.2 (26.3%), GV3.3 (22.4%), and GVBD (34.2%) stages. At 9h IVM, 9.4% of PM and 2.2% of PM-Ei oocytes were at MII stage, however, similar MII rates were observed at 19h IVM (IM: 82.10%; PM: 76.09%; PM-Ei: 89.23%). The refined GV classification proved to be a useful tool in delineating differences among GV stages, which influenced COC responses to pre-IVM strategies, potentially impacting oocyte competence *in vitro*. Funding: Fapesp (2024/03347-6).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Ozone uterine insufflation for treating mares with *Escherichia coli* lipopolysaccharide-induced endometritis

Nathália Alves[1], Marcelo Siqueira El Azzi[2], Luciana França Maciel[1], Daniel Wouters[1], Geraldo Márcio da Costa[1], Ludmila Carvalho[1], Natáli Martins Barbosa[1], Rafaela Negreiros Alvarenga Diniz[1], Miller Pereira Palhão[1], José Camisão de Souza[1]

[1]Universidade Federal de Lavras (UFLA)
[2]University of Wisconsin Madison

E-mail: nathalia.alves3@estudante.ufla.br

The objective was to evaluate the effectiveness of ozone (O3) uterine insufflation in mares with induced endometritis using Escherichia coli (E. coli) lipopolysaccharide (LPS) in reducing polymorphonuclear (PMN) counts. Twenty mares underwent LPS infusion on d1. Following LPS infusion, mares were randomly assigned to one of three treatments: Ozone treatment 1, (OT1), n=7: a single O3 application directly into the uterus on d2, via insufflation for 6 minutes at 35 mg/mL with a flow of 0.25 L/min in 1.5 liters of gas (Gerador Z-Vet, Ozonebras, Brazil); Ozone treatment 2 (OT2), n=7, identical to OT1 but administered on d2 and 3; and Negative Control (NCT), n=6, no O3 insufflation. Uterine Cytobrush samples were collected on d0 (pre-treatment), 1, 2 (6 hours post-LPS infusion), 3, and 4. D5 samples were collected only from animals receiving 2 ozone applications. The slides were stained with a rapid panoptic kit, two hundred cells were counted and those with a proportion ≥0.5% were considered positive. The uterine tissue was fixed with a buffered 10% formaldehyde solution and submitted to alcohol, xylol, and paraffin processing. The samples for bacterial culture were collected by a low-volume flush and inoculated on 5% ovine agar blood. The agar Sabouraud was used to isolate fungi. Bacterial culture was done by additional inoculation on MacConkey agar and methylene eosin-blue agar. Bacteria and fungi were identified based on the colony morphologic and culture characteristics. The fixed effects of treatment, day, and interaction on uterine inflammation were tested as repeated measurements using a mixed model and mean compared by paired Student's t-test (JMP Pro-12, SAS), and mare within treatment as the random error. The proportion of PMN was compared by chi-square. Polymorphonuclear numbers were similar among treatments across the first four cytology evaluations. However, PMNs were higher on d4 in OT2 ($p<0.01$; 10.6±2.9) compared to OT1 (0.21±2.7). PMNs were lower ($p<0.01$) in the OT1 group, both before LPS infusion (d0, 0.77±2.5) and on d4 (0.21±2.5). In OT2, PMN levels also decreased ($P<0.01$) on the day of O3 treatment and the final day of cytology compared to the other days, with an even greater reduction observed in comparison to OT1 ($P<0.001$). The NCT group exhibited consistently higher PMN values throughout the study than the ozone-treated groups. O3 treatment had no effect on histopathological aspects. Regarding bacterial culture, 12.5% of animals in OT1 and 28.6% in OT2, initially positive for bacteria on d0, tested negative after treatment. In summary, the results suggest that a single application of O3 effectively reduced PMN counts, but two applications demonstrated greater potential to reduce the number of microorganisms in the culture.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Prevalence of cytological endometritis and milk yield of postpartum Holstein and Girolando cows housed in compost barn systems

Milena Carvalho Coelho, Marcilene Daniel Damasceno[1], Pedro Felipe Rodrigues e Oliveira[1], Elaine Maria Seles Dorneles[1], Andrea Micke Moreno[2], Alessandro de Sá Guimarães[3], Maria de Fátima Ávila Pires[3], Bruno Campos de Carvalho[3]

[1]Universidade Federal de Lavras (Departamento de Medicina Veterinária - UFLA)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]EMBRAPA Gado de Leite

E-mail: milenacarvalhocoelho111@gmail.com

Compost barn systems are spreading in Brazil very rapidly, for housing Holstein or Girolando herds. Farmers frequently house the prepartum cows in the compost barn, aiming for the care of the transition period. This housing provides comfort and avoids the heat stress effects on the upcoming lactation and calf health. However, with calvings occurring on the composting bed it is still necessary to determine the negative effects of the composting bed on uterine health. The objective was to evaluate the prevalence of cytological endometritis in Girolando and Holstein cows calving in compost barns and its impact on milk yield. Fifteen dairy farms participated in the study. One visit was realized in each farm and all cows between 21 and 42 days postpartum were enrolled. A total of 181 multiparous and primiparous cows (88 Holstein and 93 Girolando) were submitted to endometrial cytology by the cytobrush technique according to Kasimanickan *et al.*, Theriogenology, 62:9-23, 2004. The smears were Panoptic fast (Laborclin, Pinhais, Brazil) stained and at least 300 epithelial and leukocyte cells were counted under optical microscopy (1000X) (Leica, Wetzlar, Germany). Cows with 6% or more polymorphonuclear leukocytes counts were considered as positive for cytological endometritis. Cow milk yield also was recorded. The cows were categorized according to days of postpartum in weeks (week 3 – 21-27 days postpartum, week 4 – 28-34 days postpartum and week 5 – 35-42 days postpartum). The prevalence of cytological endometritis (CE) was analyzed by chi-square test according to breed, postpartum week and parity. Milk production was analyzed by proc GLM considering the effects of CE status, breed, week, parity and breed x CE status. The data was analyzed in SAS v. 9.4. The overall CE prevalence was 26.49% (49/181). The prevalence of CE in Holstein cows (22.73%, 20/88) was lower (p=0.0593) than in Girolando cows (31.18%, 29/93). There was no parity or parity x breed effect on CE (P>0.05). Primiparous cows presented 31.03% (18/58) of CE while 22.09% (19/86) of multiparous cows presented CE. The prevalence of cytological endometritis was higher (P=0.0008) on week 3 (48.48%, 16/33) than on weeks 4 (25.00, 9/36) and 5 (15.56%, 14/90), regardless of the breed. Girolando cows yielded less milk (28.32±1.16 kg/day) than Holstein cows (38.23±1.60 kg/day) (P=0.0018), with no parity effect (P>0.05). Cows with cytological endometritis produced less kg/milk/day than healthy cows (P<0.05; 28.26±2.00 *vs* 34.31±1.21, respectively). As expected the prevalence of CE was higher on cows evaluated on week 3 (48.48%). However the 15.56% of CE cows evaluated on week 5 postpartum demonstrates that uterine inflammation was not completely resolved by this time. Environmental factors, especially composting bed quality can play a major role in uterine inflammation and this association must be cleared. Support: Fapemig APQ-00665-22 and RED-00132-22.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Progesterone does not exert negative feedback on the GnRH-induced LH surge in ovariectomized *Bos taurus*, *Bos indicus* and crossbred beef heifers

Lucas Oliveira e Silva[1,2], Sarah West[2], Viviana Garza[2], Jessica Sustaita-Monroe[2], Leslie King[2], Sarah Bynum[2], Emily Ecord[2], George A. Perry[3], Roberto Sartori[1], Rodolfo C. Cardoso[2]

[1]Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (*Departamento de Ciência Animal - ESALQ/USP*)
[2]Department of Animal Science, Texas A&M University
[3]Texas A&M AgriLife Research and Extension Center

E-mail: l.silva@usp.br

The aim was to evaluate the effect of progesterone (P4) in modulating the GnRH-induced LH surge, using heifers from distinct genetic groups (Gen): *Bos taurus*, *Bos indicus* and crossbred. Initially, 21 pubertal heifers were ovariectomized (OVX) and received 2 subcutaneous estradiol (E2) implants, providing basal concentrations (3.1±0.6 pg/mL). Overall, 6 Hereford (HF); 8 Brahman (BR) and 7 Braford (F1) OVX heifers were enrolled, over 9 replicates, and each heifer was submitted to all P4 treatments. Group High P4 (HP4) received 2 intravaginal P4 devices (1.34 g) on d -5, kept over 5 d. Group Decreasing P4 (DP4) received the same treatment, however, the 1st device was removed on d -1.5, and the 2nd was removed ~18 h later (d -1). Group No P4 (NoP4) did not receive P4 device. On d -1, jugular catheters were placed, and heifers were allocated in individual pens. On d0, heifers received 100 µg of gonadorelin acetate im at 0 min (GnRH challenge), and blood samples were collected every 15 min over 2.5 h (-30 to 120 min), and every 30 min over 4 h (120 to 360 min) to evaluate the LH surge. Circulating LH concentrations were measured by RIA. Sensitivity, intra and inter-assay CV were 0.2 ng/mL, 8.1% and 11.3%. Statistical analyses were done by GLIMMIX of SAS ($P \leq 0.05$). Circulating P4 concentrations overtime were different among P4 groups, but there was no effect of Gen or interaction P4*Gen. At the GnRH challenge (d0), circulating P4 was greater in HP4 than in DP4 and NoP4 (3.0±0.2, 0.1±0.03, and 0.2±0.1 ng/mL). No interaction P4*Gen was observed on LH outcomes. Regarding the P4 effect, although HP4 group had a lower LH baseline (average -30, -15, and 0 min concentration) than DP4 and NoP4 (0.3±0.1, 0.7±0.1, and 0.8±0.1 ng/mL), there was no effect of P4 on mean LH concentration, LH peak concentration (11.7±1.7, 11.3±1.7, and 11.0±2.0 ng/mL), nor LH surge AUC (1,420±214.9, 1,127.3±171.6, and 1,200.7±226.6 ng/mL*min). However, in the HP4 group, the interval between GnRH and the LH peak was delayed compared to DP4 and NoP4 (60.7±7.5, 40.0±7.5, and 44.3±6.3 min). Regarding the Gen effect, F1 heifers had a greater baseline than BR and HF (0.9±0.1, 0.5±0.1, and 0.6±0.1 ng/mL). However, HF heifers had markedly greater circulating LH overtime, greater LH peak concentration and AUC, and a delayed time to peak, compared to BR and F1 (16.6±2.5, 8.2±1.2, and 10.3±1.3 ng/mL; 2012.3±304.5, 882.2±135.7, and 1164.2±152.3 ng/mL*min; 66.7±8.0, 44.4±5.6, and 37.1±5.4 min, respectively). In conclusion, distinct P4 concentrations were not able to modulate the GnRH-induced LH surge in OVX heifers, regardless of the Gen. These results indicate that the well-known regulatory effect of P4 on pituitary is not independent, but possibly mediated and dependent on E2 concentrations. BR and F1 heifers had similar LH outcomes and were less sensitive to the GnRH stimulus than HF heifers, regardless of the P4 environment. Ack: FAPESP 2021/09924-7, 2022/12348-0, USDA-NIFA2018-67015-27595.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Semi-solid formulation of micro-encapsulated recombinant bovine interferon tau with anti-luteolytic activity demonstrated in cows

Emilio Lamazares Arcia[1], Aleikar Vazquez[1], Florence I. Hugues[2], Felipe Sandoval[1], Ignacio Cabezas[2], Karel Mena-Ulecia[3], Frank Camacho[4], Miguel Gutierrez[5], Jorge Roberto Toledo Alonso[1]

[1]Pathophysiology Department, Universidad de Concepción
[2]Universidad de Concepción
[3]Science Biolgical department, Universidad Católica de Temuco
[4]Pharmacology Department, Universidad de Concepción
[5]Pathophysiology Department Universidad Técnica de Cotopaxi

E-mail: elamazares@udec.cl

This work focuses on the embryonic losses in cattle related to artificial insemination methods. This fact affects cattle breeders worldwide, where Brazil, Argentina, and Uruguay represent a high percentage in the South American cone. This impacts the number of seasonal births and the costs of maintaining breeders with empty bellies. The percentages of gestation using assisted reproduction methods are between 55 and 65% of effective gestation, which means up to 45% of open cows, representing a significant impact on the costs of cattle farms. The general objective is to increase gestation percentages using an antiluteolytic prototype containing an encapsulated and prolonged-release formulation. For that, the production process of interferon tau (IFN-trb) at 1L volume scale and the purification conditions using ion exchange have to be adjusted as well as the Standardization of the IFN-trb encapsulation process and its incorporation into a semi-solid matrix (hydrogel). Also, we aim to demonstrate the antiviral activity of IFN-trb in higher cells and cows. Our research is at the forefront of innovation, studying the *in vitro* release kinetics of IFN-trb encapsulated and incorporated into a hydrogel. The production of IFN-trb was achieved from a pichia pastoris culture using a Winpact bioreactor and controlled conditions. Purification was performed using Giga Cap S 650s Cation Exchange Matrix. The electrospraying technique generated IFN-trb encapsulation using low-weight chitosan as a polymer. The encapsulated IFN-trb was incorporated into an 8% starch hydrogel. Solid structures of precise dimensions were achieved using precise molds to be incorporated into the intrauterine administration device. The *in vitro* antiviral activity was determined using Mengo virus in Hep-2 and MDBK cell lines by using the cytopathic effect inhibition method. The formulation's antiluteolytic effect was determined in 10 cows previously synchronized and administered with the formulation by measuring progesterone levels in blood for 25 days post-administration. IFN- trb was obtained, purified, encapsulated, and incorporated into a stable matrix. The release of IFN-trb was achieved under agitation at 150 rpm and a temperature of 37 °C for up to 18 days. Protection was evidenced in all samples from day 8 to day 23, confirming the maintenance of the antiviral activity of IFN-trb. Blood progesterone levels were maintained in elevated ranges (8-13 ng/mL, 20-25 days) in treated animals compared to control animals (15-19 days). This represents up to 5 days more than the normal cycle, evidencing the antiluteolytic effect. In conclusion, we have developed an innovative and safe formulation containing IFN-trb, with a slow and prolonged release, exerting an antiluteolytic effect evidenced by the extension of the estrous cycle up to 5 days concerning the control animals. We are testing in Chile and intend to evaluate it in Brazil (Fortgen company). We are also testing the formulation in embryo implantation in Ecuador.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Small extracellular vesicles from fetal bovine serum modify lipid metabolism-associated miRNA expression in *cumulus* cells and alter the bovine oocyte lipid accumulation

Alessandra Bridi[1,2], Fernanda Schneberger dos Santos[2], José Roberto Quirino de Oliveira[2], Lorena Calixto Munhoz[2], Ludimila Cardoso Zoccal Janini[2], Juliano C Da Silveira[2], Cláudia Lima Verde Leal[2]

[1]Universidade do Oeste de Santa Catarina (UNOESC)
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP*)

E-mail: alessandra.brid@gmail.com

IVM medium contains fetal bovine serum (FBS), an important factor influencing oocyte (OO) lipid accumulation. Small extracellular vesicles (EVs) are components of FBS (EVsfbs) and transport bioactive molecules, including microRNAs (miRNAs). These miRNAs are small non-coding RNA molecules involved in post-transcriptional regulation and may influence COC modulation during IVM. Therefore, we investigated the effect of EVsfbs on OO lipid accumulation and the expression of miRNAs associated with lipid metabolism in EVsfbs and *cumulus* cells (CCs). For this, EVsfbs were isolated from 1 mL of FBS using exclusion chromatography (SEC, Izon), followed by ultracentrifugation. The pellets were resuspended in 1 mL of TCM199 and stored at -20°C. Bovine COCs obtained from follicles (3-6 mm) of slaughterhouse ovaries were matured *in vitro* for 22 hours at 38.5°C, 5% CO2 in air, in IVM medium supplemented with: (1) 10% FBS (control); (2) 10% FBS depleted of its EVs (FBSd); and (3) 10% FBSd plus EVs isolated from FBS (EVsfbs). After IVM, the COCs were denuded, oocytes were fixed for lipid droplet (LD) analysis, and CCs samples were snap-frozen for transcript analysis. For LD analysis, we followed a staining protocol using BODIPY 493/503 according to Garcia *et al*., Animal Reproduction, 20:2, 2023. CCs samples were pooled for miRNA analysis (20 COCs/pool; 3 biological replicates). Relative miRNA expression levels were assessed using RT-qPCR. The ratio between the LD area and OO area and miRNA expression were subjected to ANOVA, followed by Tukey's test with a 5% significance level. The lipid content was lower in the FBSd and EVsfbs groups than in the control group (p=0.0089). Twenty-five and forty-one bovine miRNAs associated with lipid metabolism were detected in EVsfbs and CCs, respectively. Only five miRNAs (miR-193a, miR-122, miR-23b-3p, miR-188, and miR-192) exhibited differential expression among the treatments in CCs. MiR-193a, miR-122, miR-188, and miR-192 had increased expression in CCs derived from COC treated with EVsfbs compared to the control group (P<0.05). Of these, miR-122 and miR-192 were also expressed in EVsfbs, which may have contributed to the increased expression of these miRNAs in the EVsfbs CCs group. Moreover, miR-23b-3p exhibited increased expression in CCs from the FBSd-treated group compared to the control group (P<0.05). Taken together, the differential expression of lipid metabolism miRNAs in CCs and the difference in OO lipid accumulation suggest that FBS bioactive molecules, but not EVs, are responsible for increasing oocyte lipid accumulation during IVM. In conclusion, this study reveals that FBS supplementation during IVM alters COC lipid metabolism at the molecular level by modifying the expression of related miRNAs in CCs, as well as morphologically for increasing lipid content in OO. Funding: FAPESP 2021/06760-3; 2023/01524-5; 2023/12424-1; Capes 88887.694635/2022-00; 88887.836321/2023-00 and PUB-USP 2023/83-1.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

The effect of fullerol addition to bovine oocyte *in vitro* maturation media

Victor Mutti Drummond Ribeiro Prata[1], Philipe Pimenta Nunes[1], Marina S ladeira[1], Ana Carolina Leite Albeny[1], Eliane Beatriz Magalhães Silva[1], Luciano Rezende Carvalheira[1], Teresa Rodrigues Passos[1], Ângela Maria Quintao Lana[1], Pedro Henrique Silva Ribeiro[1], Alan Maia Borges[1]

[1]Universidade Federal de Minas Gerais (UFMG)

E-mail: Muttidrummond@gmail.com

Oxidative stress is one of the most important causes of decreased efficiency in oocyte maturation and *in vitro* embryonic development due to an imbalance between reactive oxygen species (ROS) and antioxidants. *In vitro* culture conditions are related to elevated oxygen tension and are affected by factors like sperm presence and maternal antioxidant deficiency, leading to higher ROS generation compared to *in vivo* conditions. Fullerol is a spherical carbon nanostructure and potent antioxidant, known for its high electronegativity and stability, which allows it to effectively target specific sites within cells. This study hypothesizes that adding fullerol to the *in vitro* maturation (IVM) culture media of bovine oocytes will mitigate oxidative stress, thereby improving oocyte maturation and embryo quality. Different concentrations of fullerol, diluted in phosphate-buffered saline (PBS), were added to the IVM culture media, composed of TCM-199 Bicarbonate (Gibco®, USA) supplemented with fetal bovine serum (100µL/mL), amikacin (5µL/mL), pyruvate (2µL/mL), LH (10µL/mL), FSH (1µL/mL), and estradiol (1µL/mL). Four different IVM culture media were used: Control Group 1 (CT; n=461 oocytes) - TCM 199 bicarbonate medium; Group 2 (F1; n=461 oocytes) - TCM 199 bicarbonate medium with 1 nM fullerol; Group 3 (F10; n=439 oocytes) - TCM 199 bicarbonate medium with 10 nM fullerol; Group 4 (F50; n=451 oocytes) - TCM 199 bicarbonate medium with 50 nM fullerol. The doses were determined based on the method used by Ladeira *et al.* (2013). Oocytes were matured for 24 hours at 38.5°C, 5% CO2, and 95% humidity, fertilized, and cultured under the same *in vitro* conditions. Embryos were fixed in 4% paraformaldehyde for apoptosis assessment using the TUNEL method, and oocytes were evaluated for nuclear maturation using the Hoechst stain. Matured oocyte rate (Metaphase II) did not differ (P>0.05) among treatments (CT=53.3%; F1=48.2%; F10=48.0%; F50=33.3%). Cleavage rate and blastocyst production rate per cleaved embryo and per the total number of mature oocytes were assessed on days 2 and 7, respectively. No statistical differences (P>0.05) were found in cleavage rates among experimental groups CT, F1, F10, and F50 (73.96%, 73.53%, 73.12%, 76.94%, respectively). Lower blastocyst production (P>0.05) was found in treatments CT (30.15%), F1 (27.11%), and F50 (31.04%). Total cells per embryo (CT=130.84; F1=129.78; F10=110.72; F50=134.05) and apoptotic cells (CT=8.52; F1=7.68; F10=7.54; F50=5.85) did not differ (P>0.05) among treatments. Apoptotic rate was higher (P>0.05). Addition of different concentrations of fullerol did not affect (P>0.05) apoptotic rate of embryonic cells. In conclusion, the addition of fullerol to IVM culture media did not affect oocyte maturation and cleavage rates but reduced blastocyst production at a 10 nM concentration. At 50 nM, cleavage and blastocyst production were similar to the control group, with reduced apoptotic rates.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

The role of prostaglandins E2 and F2α in ovulation and the establishment of an ovulation blockade model using flunixin meglumine in cattle

Fabiane Pereira de Moraes[1], Jéssica Lazzari[1], Gabriel Maggi[1], Sergio Farias Vargas Júnior[1], Arnaldo Diniz Vieira[1], Ligia Cantarelli Pegoraro[2], Rafael Gianella Mondadori[1], Rogério Ferreira[3], Monique Tomazele Rovani[4], Bernardo Garziera Gasperin[1]

[1]Universidade Federal de Pelotas (UFPel)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA)*
[3]Universidade do Estado de Santa Catarina (Departamento de Zootecnia - UDESC)
[4]Universidade Federal do Rio Grande do Sul (Setor de Grandes Ruminantes - UFRS)

E-mail: fabypmoraes@gmail.com

This study investigated the role of prostaglandins E2 (PGE) and F2α (PGF) in ovulation through two experiments. The objectives were to: 1) evaluate the effect of intrafollicular injection (IFI) of PGE or PGE+PGF on ovulation and luteal function; and 2) establish na ovulation blockade model using the intraovarian injection (IOV) or IFI of the antiinflammatory flunixin meglumine (FM). In experiment 1, cyclic cows (n=31) were synchronized using an estrogen and progesterone (P4) protocol. On day 9, the P4 intravaginal device (IVD) was removed and the cows sacro-caudal region was marked for estrus detection. IFIs were performed 12 h after removal of IVDs in follicles ≥ 10 mm, in cows with no estrous manifestation, according to each group: Control (PBS IFI; n=4), PGE (500 ng/mL PGE IFI; n=6), and PGE+PGF (500 ng/mL PGE and 100 ng/mL PGF IFI; n=4). PGE and PGF were obtained from Cayman Chemical, Ann Arbor, Michigan, USA. Ovulation was monitored by transrectal ultrasound (US) every 12 h until 84 h after IFI. Luteal function was assessed by serum P4 concentrations 7 and 14 days post-IFI, which were analyzed by ANOVA, followed by Tukey's test. In experiment 2, 21 cows were synchronized as previously described and received 25 µg of lecirelin (TEC-Relin, Agener União, São Paulo, Brazil) IM, 20 h after P4 IVD removal. After 16 h, animals with follicles ≥ 10 mm were allocated to three groups: Control (0.2 mL PBS IFI; n=6), FM IFI (0.2 mL containing 338 µM FM; Vernunft *et al*., J. Reprod. Dev., 68: 246–253, 2022; n=6), and FM IOV (0.2 mL containing 10 mg FM; Banamine, MSD Saúde Animal, São Paulo, Brazil; n=6). Ovulation was monitored by US every 12 h. Ovulation rate was analyzed by logistic regression and the contrast was used for comparisons among groups. For both experiments, $p \leq 0.05$ is considered significant, and $P < 0.1$ is considered a trend. In experiment 1, while ovulation timing did not differ between groups (Control: 42 ± 25.46; PGE: 56 ± 12.81; PGE+PGF: 54 ± 22.45 h; $P > 0,05$), P4 concentrations (7 days after IFI, Control: 2.34 ± 0.8; PGE: 4.29 ± 0.65; PGE+PGF: 2.23 ± 0.8 ng/mL; 14 days after IFI, Control: 7.67 ± 0.8; PGE: 12.21 ± 0.65 and PGE+PGF: 8.67 ± 0.8 ng/mL) tended to be higher in the PGE group ($P = 0.09$). In the second experiment, all animals in the Control and FM IOV groups ovulated within 48 h, while only one animal (1/6) ovulated in the FM IFI group ($P < 0.05$), with the others developing anovulatory structures. In conclusion, IFI of PGE or PGE+PGF did not alter ovulation timing, but PGE tended to increase P4 concentrations. Furthermore, FM administered IOV at the given concentration did not block ovulation in cattle. The authors thank FAPERGS, CNPq and CAPES for their financial support.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

The role of the S-adenosylmethionine sensor (SAMTOR) in mitochondrial DNA methylation dynamics of pre-implantation bovine embryos

Thays Antunes[1], Ana Beatriz dos Reis Bartoli[1], João Vitor Alcantara da Silva[1], Camila Bruna de Lima[2], Marc-André Sirard[2], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*UFABC*)
[2]Université Laval

E-mail: thaysantunes1@hotmail.com

One of the main events in the early stages of embryonic development is the DNA methylation reprogramming that occurs during the pre-implantation stages. During this phase, the embryo undergoes a process of demethylation and de novo methylation when it comes to nuclear DNA. On the other hand, the opposite profile is observed in mitochondrial DNA (mtDNA) with lower methylation levels in the beginning of development, peaking at 16 cell stage, and subsequent demethylation. Both nuclear and mtDNA events are important for proper embryonic development and rely on various controlling mechanisms, one of which is SAMTOR, acting as a sensor of S-adenosylmethionine (SAM), the primary donor of the methyl group for DNA methylation. Additionally, SAMTOR influences the mammalian target of rapamycin complex 1 (mTORC1) pathway, which regulates the transcription of DNA methyltransferase (DNMT1). With this knowledge, the objective of this study was to elucidate the role of SAMTOR in the reprogramming of mtDNA methylation at the earliest stages of the pre-implantation embryonic development. For this purpose, embryos were subjected to conventional IVM and IVF protocols. After that, all zygotes had their zona pellucida removed and were transferred to 20µl drops. Lipofection was performed with complementary siRNA treatment (0.25 pmol) targeting the SAMTOR transcript, or a control group (Scramble), and maintained until day 2 (D2) of culture (peak of SAMTOR transcription levels). After 48 hours, embryos (pools of ≈ 10 embryos/group, 3 replicates) were evaluated for SAMTOR levels by qPCR, using beta-actin gene as a normalizer. Furthermore, total DNA libraries were prepared using NEBNext® Enzymatic Methyl-seq Kit, and the average mtDNA methylation levels were assessed for cytosines in all contexts. Data were analyzed by Student T-test, considering $P<0.05$ and tendency of P up to 0.07. Embryos treated with target siRNA showed a decrease in SAMTOR transcription levels compared to the scramble group ($p<0.0074$). Regarding the mtDNA methylation, SAMTOR group on D2 showed a tendency ($p=0.06$) towards higher average methylation levels compared to the Scramble control group for CG context, whereas on day 4 (D4), the SAMTOR group showed a tendency ($p=0.06$) towards lower methylation patterns compared to the Scramble group for CGH context. Regarding the day of development, the Scramble group showed the same pattern as previously reported, with an increase in methylation profile from D2 to D4, but in contrast, SAMTOR group showed a decrease in methylation levels from D2 to D4 ($p=0.01$). In conclusion, SAMTOR plays an important role in modulating the epigenetic profile, leading to a disturbance in the methylation dynamics of mtDNA in pre-implantation embryos. Further studies are needed to elucidate the pathways involved in this process and its role in the dynamics of nuclear DNA methylation and impacts on embryonic quality. Acknowledge: FAPESP 2023/15734-1 and CAPES.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Uterine diameter of pre-pubertal Nelore heifers (*Bos indicus*) submitted to pre-synchronization 10 days before the start of the TAI protocol

Augusto felisbino neto[1], Denis Fernando Cirino de Souza[2], Adecir Cardoso Silva Junior[2], Vitor de Almeida Souza[2], Victor Arantes Ferreira[2], Italo Ferreira de Paula[2], Jose Nélio de Sousa Sales[3], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Ferty+ Animal Reproduction
[3]Universidade Federal de Juiz de Fora (*UFJF*)

E-mail: augusto.felisbino@usp.br

The greatest difficulties that negatively affect reproductive efficiency are late puberty, failure to detect estrus, postpartum anestrus, and the interval between calving (Buss, 2017). The objective of this study was to evaluate the effect of injectable P4 administration 10 days before FTAI protocol on the uterine development in prepubertal Nelore heifers. One hundred thirty two heifers (18 months; 327,1±28,3 kg), from one farm in the state of MT were used. The heifers underwent a US evaluation before the experiment and were then distributed into groups. Only those without CL participated in the experiment. Furthermore, follicular dynamics were performed by US on D0 (beginning of the TAI protocol) and uterine diameter on D0 and D9 (TAI). Heifers were divided into 3 groups: 1. Control (n=43), did not receive any treatment. 2. P4-D24, treatment with long action P4 (150mg of injectable P4 (1.0ml; Sincrogest® injectable, Ourofino Saúde Animal, Cravinhos-SP, Brazil) on D-24 (n=42) and 0.5mg IM of EC (SincroCp®, Ourofino) after 12 days (D-12). And 3. P4-D10, treatment with the same dose of long action P4 (n=47) 10 days before on D0. On D0, 2mg IM of BE (Sincrodiol®, Ourofino), 0.530mg IM of cloprostenol sodium (PGF2α; Sincrocio®, Ourofino) and a third-use intravaginal P4 device (Sincrogest®) were administered. After seven days, the P4 device was removed, and 0.5 mg IM of EC (SincroCP®), 0.53 mg IM of sodium cloprostenol (PGF2α; Sincrocio®) and 200 IU of eCG (Sincroecg®) administered. TAI was performed 48 hours after removing the P4 device. Statistical analyses were performed using BIOSTATIC® (v. 5.3) and SAS® (v. 9.4) with a P-value of 0.05. Differences in uterine diameter between groups were only observed on D0 (Control=13.3±0.3mm^{b}; P4-D24=14.2±0.3mm^{ab}; P4-10=14.6±0.3mm^{a}; P=0.02). Group P4-10 presented a larger uterine diameter than Control group. However, no differences were observed related to P4-24 group. At TAI (D9), the groups presented similar uterine diameter (Control=15.1±0.5mm; P4- D24=15.4±0.8mm; P4-10=15.7±0.7mm; P=0.83). Also, the presence of CL on D0 was higher on the P4-24 group [Control=0%^{b} (0/32); P4-D24=42,9%^{a} (18/42); P4-10=0%^{b} (0/42); P<0.05]. Furthermore, the diameter of the largest follicle at the beginning of the protocol (D0) differs between groups (Control=9.12±0.3mmb; P4-D24=7.19±0.4mm^{c}; P4-10=10.69±0.5mm^{a}; P<0.05). P4-10 group presented larger follicle diameter than Control and P4-24 groups. It is concluded that heifers pre-synchronized 10 days before D0 with long-acting P4 have a larger uterine and follicle diameter at the beginning of the protocol (D0). However, there is a lower presence of CL on D0 than P4-24 group. The P4-10 could be a great alternative for pre-synchronization in prepubertal heifers over a shorter period (10 days).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Uterine fluid extracellular vesicles collected during estrus from high fertility and infertile cows are similar in diameter and concentration

Raissa Braido Rangel[1], Laura Gabrielli Haupenthal[1], Maria Alice de Almeida[1], Juliana Germano Ferst[1], Juliano Coelho da Silveira[1], Guilherme Pugliesi[2], Mateus José Sudano[3], Rui Machado[4], Felipe Perecin[1], Maíra Bianchi Rodrigues Alves[1,5]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (FZEA/USP)
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (Departamento de Reprodução Animal - FMVZ/USP)
[3]Universidade Federal de São Carlos (Departamento de Genética e Evolução - UFSCAR)
[4]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[5]Universidade Estadual Paulista Júlio de Mesquita Filho (Departamento de Patologia, Reprodução e Saúde Única, Faculdade de Ciências Agrárias e Veterinárias - UNESP)

E-mail: raissabraido@usp.br

Extracellular vesicles (EVs) are nanoparticles able to carry molecules (e.g. proteins, RNAs) that play a key role in cell communication. While present in the uterine fluid (UF), EVs features remain unknown in females of different fertile potentials during estrus. The aim was to investigate characteristics of EVs isolated of UF collected from high-fertility (HF) and infertile (IF) cows during estrus. For that, Canchim nulliparous cows with 65.94±0.23 months of age, 625.72±10.60 kg and 7.67±0.11 of body condition score, classified in a previous study as HF (n=11) or IF (n=7) based on 3 successive cycles of embryo transfer and pregnancy diagnosis were used; HF confirmed pregnancy in all 3 cycles while IF were diagnosed as non-pregnant in all cycles. HF and IF cows were submitted to estrous cycle synchronization using 2 mg estradiol benzoate intramuscular (IM), 0.5 mg prostaglandin (PGF; IM), and a single-dose progesterone intravaginal release device (0.5 g) at first day. After 8 days, intravaginal device was removed and 0.5 mg PGF (IM), 1 mg estradiol cypionate (IM), and 300 IU eCG (IM) were applied. Following 48 hours, UF was obtained using a Foley catheter transcervically (flushing with 50 mL of DMPBS) and the estrogenic response was assessed by measuring the diameter of preovulatory follicle (PF) and the thickness of endometrium (END) by transrectal ultrasound (ExaPad/IMV Technologies), assessment of uterine consistency by transrectal palpation, presence of vaginal mucus (MUC) by inspection and heat expression (HEAT) by teaser bull marks. EVs-UF were isolated by centrifugation and ultracentrifugation protocols, then characterized by diameter and concentration in nanoparticle tracking analysis (NanoSight®NS300), morphology in transmission electron microscopy (FEI200kV Tecnai20 LAB6) and specific markers in flow cytometry (Cytoflex®Beckman Coulter). Next, EVs from HF and IF cows that showed higher PF and END were compared. For statistical analysis, data were analyzed using Statistical Analysis System by Analysis of Variance after testing normality by Shapiro-Wilk. Significance level was considered when $P<0.05$. Regarding estrogenic response, HF and IF were similar. Isolation of EVs was validated by their cup-shape morphology, concentration of 1.54x109±0.22x109 particles/mL, diameter of 165.15±2.64 nm and detection of EV markers (ALIX; CD81; Calcein). After, 10 cows were selected to HF (n=5) and IF (n=5) based on PF and END. They were similar to PF (HF:13.51±0.64 mm; IF:12.93±1.32 mm), END (HF:11.18±0.83 mm; IF:9.64±0.66 mm), HEAT (HF:1.00±0.63; IF:0.80±0.50) and MUC (HF:0.80±0.20; IF:0.20±0.20). Regarding EVs, similar concentrations (HF:1.35x109 ±0.34x109 ; IF:1.32x109 ±0.32x109 particles/mL) and diameters (HF:162.48±4.21; IF:168.38±4.66 nm) were found. Thus, high-fertile and infertile females display similarities in estrogenic response and EVs-UF features. Further studies will be performed to explore the molecular cargo and role of these EVs. CAPES001

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS, AND STEM CELLS

Embryo development after chemical or electrical activation and IVF of cat oocytes (*Felis catus*)

Irving Mitchell Laines Arcce[1], Federico Andrés Allegroni[2], Minerva Yauri Felipe[2], Maiana Silva Chaves[1], Luciana Magalhaes Melo[1], Vicente José de Figueiredo Freitas[1], Daniel Felipe, Salamone[2]

[1]Laboratory of physiology and control of reproduction, School of Veterinary Medicine, State University of Ceará
[2]Animal Biotechnology Laboratory, School of Agriculture, University of Buenos Aires

E-mail: irving.mitchell@aluno.uece.br

Currently, wild felids are among the most threatened species in the world. Conservation initiatives aimed at safeguarding endangered felid species benefit from cutting-edge advancements in Assisted Reproductive Technologies (ARTs), such as *in vitro* fertilization (IVF) and somatic cell nuclear transfer (SCNT). Given the great availability of biological material from domestic cats (*Felis catus*), these are often used as a research model to improve ARTs in wild felids. Consequently, the aim of the present study was to evaluate the chemical and electrical activation of cat oocytes matured *in vitro* that is important for SCNT compared to a standard *in vitro* fertilization (IVF) protocol. In summary, 43 domestic cat ovaries from ovariohysterectomies were transported to the animal biotechnology laboratory at the Faculty of Agronomy, University of Buenos Aires (Argentina), and processed within 2 hours post-castration. Cumulus-oocyte complexes (COCs) were released from the follicles using the slicing technique and matured in TCM-199 supplemented with 50 ng/mL EGF, 0.3 mM sodium pyruvate, 100 µM cysteamine, 5 µg/mL myo-inositol, 1 µg/mL ITS, 1% antibiotic, 10 µg/mL FSH, 10% porcine follicular fluid for 24 hours in a CO_2 incubator. The matured oocytes were subjected to IVF or chemical and electrical activation. For *in vitro* fertilization (IVF), the *in vitro* matured oocytes were co-incubated for 18 hours with 1.5 x 10^6 sperm/mL, which were recovered from the epididymis and selected using the Swim-up technique. For both electrical and chemical parthenogenetic activation, cumulus cells from matured oocytes were removed using 0.2% hyaluronidase. The electrical activation was performed using a unidirectional pulse "Direct pulse" with a voltage of 0.063 kV/cm. Each pulse lasted for 0.032 µs. Chemical activation was induced by exposure to 5 µM ionomycin in TALP-H for 4 minutes followed by immediate incubation in 2 mM 6-DMAP and SOF for 3 hours. The presumptive zygotes (both from IVF and parthenogenetic) were cultured *in vitro* in SOF medium in an incubator with a gas mixture containing 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. Cleavage and blastocyst rates were assessed on days 2 and 7 of culture, respectively. The data were statistically analyzed using one-way analysis of variance (ANOVA), and the results, expressed as percentages, were compared using the chi-square test, employing R software version 3.4.3. There were no differences ($P > 0.05$) in the cleavage rates for IVF (47.87%; 45/94), electrical activation (59.01%; 36/61) and chemical activation (55.78%; 53/95). In contrast, blastocyst development was significantly higher ($P < 0.05$) in IVF (27.65%; 26/94) and chemical activation (32.63%; 31/95) compared to electrical activation (14.75%; 9/61). In conclusion, the use of calcium ionophores as oocyte activators appears to be an alternative for the production of blastocysts in cats. However, more studies are needed to strengthen its potential use in SCNT.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS, AND STEM CELLS

Homology-directed gene editing using electroporation of bovine zygotes

Diana Rangel de Lemos[1], Eliza Diniz de Souza[2], Carolina David Vieira[3], Naiara Zoccal Saraiva[2], Luiz Gustavo Bruno Siqueira[2], Hyago Passe Pereira[2], Clara Slade Oliveira[2], Carolina Capobiango Romano Quintão[2], Luiz Sérgio de Almeida Camargo[2]

[1]Universidade Federal de Viçosa
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA – Gado de Leite)
[3]Universidade Federal de Juiz de Fora

E-mail: dianalemosvet@gmail.com

Financial support: Fapemig, CNPq, CAPES.

Gene editing can occur via two pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). While NHEJ is rapid but prone to errors, HDR employs a homologous DNA template for precise outcomes, albeit being a rare event. Electroporation (EP) offers a straightforward method for delivering gene editing components into the cytoplasm of bovine embryos; although, its efficacy in facilitating knock-ins by HDR remains uncertain. Here we used EP to introduce a stop codon in the prolactin receptor gene, testing two HDR enhancers: SCR7, a NHEJ inhibitor, and RS1, a HDR stimulant. SgRNA, Cas9 and single strand oligodeoxynucleotide (ssODN) containing a stop codon were delivered by EP (Nepa21 electroporator, Nepagene, Chiba, Japan) to *in vitro*-fertilized oocytes, which were randomly allocated to the following groups: A) Control: no EP (n = 77); B) ControlHDR: EP of sgRNA, Cas9, and ssODN performed at 8h post-fertilization (n = 74); C) RS1: as in B followed by 24h incubation with RS1 at 7.5 µM (n = 70); D) SCR7: as in B followed by 24h incubation with SCR7 at 1 µM (n = 74); E) RS1+SCR7: as in B followed by 24h incubation with RS1 at 7.5 µM and SCR7 at 1 µM (n = 72). Embryos were cultured for seven days in three replicates. Single blastocysts underwent DNA extraction. PCR-generated DNA fragments from the target site were sequenced and analyzed by SeqScreener Gene Edit Confirmation (Thermo Fisher, Waltham, USA). Presence of indels (insertions/deletions) and HDR (stop codon insertion) was considered relevant when their rates per embryo exceeded 5%. Data were compared by Fisher's exact test (proportion of embryos with indels or HDR) or ANOVA and shown as mean ± SEM when appropriated. Significance was set at $P < 0.05$. No difference ($P = 0.95$) in blastocyst rates among Control (25.7 ± 9.5%), ControlHDR (19.8 ± 5.6%), RS1 (20.1 ± 2.7%), SCR7 (19.7 ± 7%) and RS1+SCR7 (25.1 ± 8.3%) groups was found. The proportion of embryos with indels (91.6% [11/12], 62.5% [5/8], 85,7% [6/7] and 87,5% [7/8] for ControlHDR, RS1, SCR7 and RS1+SCR7, respectively; $P = 0.25$) and the indel mean rate per embryo (71 ± 8.3%, 51.2 ± 11.5%, 42.2 ± 8.4% and 65.3 ± 14.4% for ControlHDR, RS1, SCR7 and RS1+SCR7, respectively; $P = 0.24$) were similar. Likewise, no difference ($P = 0.12$) in the proportion of embryos with HDR among ControlHDR (41.6% [5/12]), RS1 (12.5% [1/8]), SCR7 (57.1% [4/7]) and RS1+SCR7 (25% [2/8]) groups was found. HDR mean rates per embryo ranged from 5.4% to 70.1%, with no significant difference ($P = 0.21$) among ControlHDR (31.8 ± 10.6%), SCR7 (9.2 ± 2.4%), and RS1+SCR7 (18.5 ± 8.9%) groups. The RS1 group was excluded because only one embryo had HDR. No indels or HDR were found in the Control group. Despite the limited number of embryos, this study demonstrates EP's ability to deliver gene editing components for HDR in bovine embryos. However, the tested molecules did not enhance HDR efficiency post-EP. Therefore, EP offers a potential method for knock- in insertion in bovine embryo genome, yet further research is needed to enhance HDR rates.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS, AND STEM CELLS

SCNT using bone marrow mesenchymal stem cells (BM-MSC) results in normal telomere length in horses

Mariana Suvá[1], Matias Gabrielli[1], Diego Mengual Gomez[2], Belen Pose Ortiz de Rozas[1], Elisabet Wiedenmann[1], Roberto Jordan[1], Gabriel Vichera[1]

[1]Kheiron Biotech S.A.
[2]Laboratorio de Oncología Molecular, UNQUI

E-mail: marianasuva@gmail.com

After the birth of Dolly, one of the first concerns regarding somatic cell nuclear transfer (SCNT) was that the cloned animals would be born with shorter telomeres and decreased cell replication capacity, resulting in a reduced lifespan. Since then, telomere length of clones of different species was analyzed, reaching highly variable conclusions. Equine cloning has been performed over the last 20 years and became a successful industry; however, to the best of our knowledge there is no information regarding telomere length after horse SCNT yet. In this study we compared the relative telomere length of young cloned horses derived from aged donors with age-matched controls, by real-time quantitative PCR. To achieve this, zone free SCNT was performed using BM-MSCs obtained from different donors with ages from 10 to 30 years. Only cell cultures with low passage numbers (< 5) were used. After that, we isolated genomic DNA from whole blood samples of 36 cloned horses of 1-3 years old. As controls, genomic DNA was isolated from newborn (n = 3), 1-3 years old (n = 8), 7-8 years old (n = 2), and 21-22 years old (n = 2) naturally bred horses. For all the samples, relative telomere length was measured in triplicate, and data were analyzed with the Livak's Delta-delta Ct method. Statistical differences were determined using Student's t-test on log-transformed data. When comparing telomere length, no differences were observed between the cloned horses and the aged-matched controls, regardless of the age of the donor horses. However, the telomere length of natural breeding horses significantly decreased with age, with no differences observed between animals of the same age (Spearman r = -0.945). Therefore, any possible difference in telomere length between samples of donor animals of different ages would not affect the telomere length of the cloned born foals. In conclusion, these results suggest that telomere length is restored after SCNT in horses cloned using BM-MSC as nuclear donors. Whether telomeric reconstitution depends on the donor cell type remains to be evaluated. A higher number of samples from animals with different ages are being analyzed in order to reinforce our conclusion.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Absolute concentration of circulating metabolites after use of the injectable multimineral based on organic and inorganic phosphorus, selenium, copper, magnesium and potassium in primiparous beef cows

Giovani Batista Pastre[1,2], Edson Guimarães Lo Turco[3], Bruna Lima Chechin Catussi[1], Guilherme Felipe dos Santos[1,2], Arnaldo Gonçalves Soares Neto[2], Guilherme Felipe dos Santos, Luc Durel[2], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Virbac saúde animal
[3]Universidade Federal de São Paulo (*UNIFESP*).

E-mail: giovani.pastre@virbac.com.br

The aim of this study was to analyse the absolute concentrations of circulating metabolites and identify significant changes in metabolic pathways in response to treatment with an injectable multimineral supplement containing organic and inorganic phosphorus, selenium, copper, magnesium and potassium in primiparous beef cows. The experiment was carried out on a farm in Água Clara, MS, Brazil. A total of 50 Nelore (*Bos indicus*) primiparous cows (3.05 ± 0.34 of BCS) were randomly assigned to one of two groups on D0 of fixed timed artificial insemination (FTAI) protocol: 1) Control (no treatment; n = 25) or 2) Group 15 mL (treated with 15 mL of Fosfosal® on D0; n = 25). The cows received (40 days after calving) FTAI protocol. Blood samples were collected for metabolomics analysis on D0 (beginning of FTAI protocol), D10 (FTAI), D21, and D39. Metabolites were evaluated using targeted LC- MS/MS analysis in tandem with a triple quadrupole mass spectrometer. Multivariate analysis as partial least squares discriminant analysis (PLS-DA) was performed by MetaboAnalyst 5.0. The top significant features were selected for Over Representation Analysis. An absolute concentration (micromol) of 45 metabolites was used to identify altered metabolic pathways following the multimineral treatment. On D0, no differences were observed between groups. However, on D10, metabolic pathways were increased by treatment with 15 mL of Fosfosal, which were tryptophan metabolism, and spermine and spermidine biosynthesis (p=0.05/Q2=0). Tryptophan is a precursor for several pivotal molecules and is involved in physiological functions. Spermine and spermidine are polyamines involved in cell metabolism, cellular dynamics for stress adaptation. No difference on D21 and D39 for those metabolites with absolute concentration were found between groups. The observed deviations underscore the extensive influence of the multimineral treatment on essential amino acid pathways. Tryptophan, a precursor for pivotal molecules like serotonin and melatonin, modulates cattle behavior and physiological functions. Also influences niacin (Vit B3) synthesis, immune function, and inflammatory functions and improves animal welfare. Our results also reveal the impact of the mineral treatment on polyamine metabolism, which govern essential roles in cellular processes (cell growth, proliferation, and differentiation). Also, presents antioxidant properties (stress adaptation responses), modulates sperm function and embryonic development, and improves animal welfare. In conclusion, the treatment with injectable minerals impact the metabolism pathways on day 10, reflecting alterations in amino acid metabolism, energy production, and lipid utilization. The presence of spermidine and spermine biosynthesis underscores their role in modulating cellular growth and stress response, expanding our comprehension of Fosfosal's metabolic footprint.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Abundance of target-transcripts in vitrified-warmed IVP bovine embryos cultured with CNP

Camila Bortoliero Costa[1], Elisa Mariano Pioltine[2], Thaisy Tino Dellaqua[2], Amauri Alcindo Alfieri[1], Marcelo Marcondes Seneda[1], Marcelo Fábio Gouveia Nogueira[2]

[1]Universidade Estadual de Londrina (*Centro de Ciências Agrárias - UEL*)
[2]Universidade Estadual Paulista Júlio de Mesquita Filho (*Instituto de Biociência - UNESP)*

E-mail: cbortoliero@gmail.com

The use of C-type natriuretic peptide (CNP) is already well established in maturation (or pre-maturation). However, there are scarce reports of its use in *in vitro* culture and potential related effect on embryo cryotolerability. We aimed, with this study, to screen the effect of *in vitro* culture with 400 nM of CNP on the transcript's abundance of cryopreserved-warmed bovine embryos. On D7 and D8, only expanded and grade I (IETS) *in vitro* produced blastocysts were vitrificated using the Open Pulled Straw technique. After warming and culture (24 - 48 h), hatched blastocysts were collected [n = 3/group in 4 replicates from control (no CNP), and CNP (400 nM CNP) groups]. The hatching rate was evaluated at the following times: 24h [39.1 ± 0.7 and, 32.0 ± 0.9] and, 48 hours [28.5 ± 0.7 and, 40.0 ± 1.1] in control groups and CNP, respectively. The Biomark HD platform was used to relatively quantify the mRNA of interest and gene expression analyzes were performed using probes TaqMan in specific assays for *Bos taurus*. To the statistical analysis, it was calculated the ΔCq values relatively to the geometric mean of the reference genes - i.e., GAPDH, and ACTB - among the chosen 96 genes set. Fold-change (FC) was calculated using the 2−ΔCq method. All analyzes were performed using SigmaStat 4.0 and MetaboAnalyst 5.0. The evaluation of the transcripts data was initially performed with the univariate statistical method and, secondly, with a multivariate method. Significance was considered when $P < 0.05$ and FC >1.5. Transcript abundance was upregulated in the CNP group in the following genes: ATF4 (P = 0.0004), BMP15 (P = 0.0043), GFPT2 (P = 0.0043), PNPLA2 (P = 0.0322) and SOX (P = 0.0302). Those transcripts are related to the endoplasmic reticulum stress, cholesterol biosynthesis, metabolism of insulin, and degradation of triacyl glycerides pathways. The results are suggestive that the use of CNP (400 nM during *in vitro* culture) can modulate embryo's gene expression after vitrification and warming. However, more studies are needed to prove the functionality (i.e., *in vivo* study) of the observed alteration. Financial support: CAPES (Finance Code 001), CNPq (311899/2022-6) and FAPESP (2019/10732-5).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Analysis of the expression of long non-coding RNAs in cytoplast and karyoplast samples from bovine oocytes and embryos

Ricardo Perecin Nociti[1,2], Marcos Roberto Chiaratti[3], Tiago Henrique Camara De Bem[2], Juliano Rodrigues Sangalli[2], Luis Aguila Paredes[4], Alessandra Bridi[2,5], Lindomar de Oliveira Alves[3], Dewison Ricardo Ambrizi[2], Lawrence Charles Smith[1,2], Flávio Vieira Meirelles[2]

[1]Université de Montreal (*Faculty of Veterinary Medicine - UdeM)*
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*
[3]Universidade Federal de São Carlos (*Departamento de Genética e Evolução - UFSC*)
[4]Universidad de la Frontera (*Centre of Reproductive Biotechnology (CEBIOR-BIOREN), Faculty of Agriculture and Forestry - UFRO*)
[5]Universidade do Oeste de Santa Catarina (UNOESC - Campus Xanxerê).

E-mail: rnociti@gmail.com

Evidence accumulated over the last decade shows that long non-coding RNAs (lncRNAs) are widely expressed and have important roles in gene regulation. Depending on the location and specific interactions with DNA, RNAs and proteins, lncRNAs can modulate chromatin function, regulate the assembly and function of membrane-less nuclear bodies. Also, they alter the stability and translation of cytoplasmic mRNAs and interfere with signaling pathways. Characterization in murine oocytes and embryos demonstrated that maternal and zygotic lncRNAs form two distinct populations, indicating a large accumulation during oogenesis and a possible participation in the acquisition of totipotency. In cattle, our previous work identified 1,083 differentially expressed lncRNAs from a total of 6,752 differentially expressed genes in oocytes and zygotes. Since the location of lncRNAs in the cell is a good indicator of their functions, we aimed in this work to analyze the expression of lncRNAs in the cytoplast and karyoplast of bovine oocytes and embryos. Cytoplast (Cyto) and karyoplast (Karyo) biopsies from bovine oocytes in germinal vesicle (GV) and metaphase II (MII) and from zygotes (15 h after ICSI) were isolated and processed by RNA-Seq. Using bioinformatics, we identified a total of 21,889 expressed genes, 5,903 differentially expressed genes, of which 499 were differentially expressed lncRNAs. Genes with >two-fold difference in expression and adjusted P < 0.05 were considered differentially expressed. Results indicated exclusive expressed genes and lncRNAs in our groups Cyto-GV (844; 105), Karyo-GV (214; 13), Cyto-MII (273; 32), Karyo-MII (69; 2), Cyto-zygote (88;6) and Karyo- zygote (106; 3), respectively. The non-location specific lncRNAs KANTR and TUNAR were more expressed in Cyto-MII, Karyo-MII, while TUG1 was in Cyto-zygote and Karyo-zygote. Moreover, we detected that NONBTAG000722.2, NONBTAG016769.2, and LOC112447501 where Cyto-zygote specific and NONBTAG016950.2, LOC783163, and LOC101902809 Karyo-zygote specific. In conclusion, our findings indicate that it is possible to explore separately stage and cell-location specific transcriptomic differences between bovine oocytes and zygotes. Also, many lncRNAs are present exclusively in specific stages of development, with greater accumulation in the cytoplast, suggesting cytoplasmic functions in translation and/or posttranscriptional gene regulation. Future experiments will characterize the dynamics of expression of lncRNAs throughout early embryonic development in the two cellular fractions studied. Support: FAPESP: 2019/04738-0, 2021/11912-7, 2021/09886-8; NSERC: CRDPJ536636-18, RGPIN-2020-05278; MITACS: IT15216; ANID-FONDECYT: 11230091 (LA).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Assessing nuclear integrity and embryo developmental potential of matured bovine oocytes subjected to high hydrostatic pressure before vitrification

Alice Caroline Souza e Castro[1], Marcos Coura Carneiro[1], José Eduardo Vieira Chaves[1], Venâncio Augusto Oliveira Silva[1], Laryssa Ketelyn Lima Pimenta[1], Bruna Alves Abud Ribeiro[1], Ana Cecília Fernandes Vidigal[1], Danyela Lima da Costa Pena[1], Leonardo de França e Melo[1], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás (*Escola de Veterinária e Zootecnia - UFG*)

E-mail: alicecaroline@discente.ufg.br

High Hydrostatic Pressure (HHP) induces sublethal stress in cells, thereby influencing molecular pathways and potentially enhancing cellular resilience. The primary objective of this study was to assess two key parameters: chromatin configuration (Experiment 1; n = 376) and embryonic development (Experiment 2; n = 237) of matured COCs following exposure to HHP before vitrification. Grade I and II COCs, obtained from abattoir ovaries, were selected and subjected to IVM for 21 h. Subsequently, they were randomly assigned to 1 of 4 treatment groups: 1) Control (CON; n = 161): COCs maintained in holding media at a constant temperature of 36°C; 2) Vitrification (VIT; n = 150): COCs subjected to vitrification and subsequent warming (Cryotop®); 3) HHP (n = 141): COCs exposed to HHP; and 4) HHP+VIT (n = 161): COCs subjected to HHP before undergoing vitrification and warming. For the HHP treatment, COCs were loaded into a straw containing 0.25 mL of holding media and placed in a pressure chamber (Cryo-Innovation®), where they were exposed to 200 bar pressure at a temperature of 36°C for 30 min, the setting was determined in a previous study (not published). Following the treatment, all groups underwent an additional 30 min of incubation in holding media before being returned to *in vitro* maturation to reach the final period of 24 h. In Experiment 1, oocytes were denuded, fixed in a solution consisting of ethanol and acetic acid in a 3:1 ratio for 24 h, and subsequently stained with Lacmoid dye. The percentage of oocytes reaching the metaphase II stage of meiosis was then assessed under an optical microscope (Olympus®). In Experiment 2, oocytes were subjected to IVF and subsequent IVC, following which the percentage of cleavage on Day 2 and blastocyst development on Days 7 and 8 were evaluated. Statistical analysis of the data was performed using the Chi-square test (P≤0.05). The results obtained from Experiment 1 indicated that both the CON and HHP groups exhibited similar percentages of oocytes reaching metaphase II of meiosis [78.5 (73/93) and 81.6% (80/98), respectively], as well as VIT and HHP+VIT groups [21.9 (18/82) and 24.2% (25/103), respectively), although the fresh groups showed greater chromatin preservation than the VIT groups. In Experiment 2, the CON group had higher cleavage rate compared to other experimental groups [CON = 60.2 (97/161)a; VIT=35.3 (24/68)b; HHP = 39.5 (17/43)b and HHP+VIT = 22.4% (13/58)b]. Considering embryonic development on Day 7, the CON group had a higher blastocyst rate than HHP and VIT groups [CON = 22 (15/68)a; VIT = 7.3 (5/68) b; HHP = 11.6 (5/43)b and HHP+VIT = 3.4% (2/58)b]. Similar rates of embryo development were observed on Day 8. In summary, exposing COCs to 200 bar didn't improve the percentage of oocytes reaching MII after vitrification and IVM. Furthermore, HHP exposure itself compromised embryo development. Although pressure is described as a cell protection tool before cryopreservation, the tested protocol was ineffective.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Beta-hydroxybutyrate modulates epigenetic marks and nucleolar size in bovine fibroblasts.

Edna de Fátima Elias Macule[1], Helena Fabiana Reis de Almeida Saraiva[2], Flávio Vieira Meirelles[2], Juliano Rodrigues Sangalli[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*

E-mail: ednaelias2022@usp.br

Financial support: FAPESP 2022/06581-4 and CAPES.

The Dairy industry has several problems affecting the health of the cows, being the ketosis one of the most important, causing reduced milk production and other sanitary and reproductive problems. The beta-hydroxybutyrate (BHB) is a metabolite that accumulates in the blood of cows suffering from ketosis. Besides its canonical functions (i.e. energy provider during states of nutrient deprivation), BHB was discovered to be a potent epigenetic modifier and affect longevity. Curiously, smaller nucleoli were found in long-lived animals and correlated with longevity. Thus, we decided to investigate the effects of BHB on several epigenetic marks (H3K4me3, H3K27me3, H3K9me3) and on the nucleolar (using the nucleolar marker protein fibrillarin) in cells. To this end, 5 x 10^4 bovine adult fibroblast cells were plated in 35-mm petri-dishes, cultured for 48 hours in alpha-MEM + 10% SFB, and then treated for 24h with 6 mM BHB (BHB group) or not (Control). Since BHB is promptly water-soluble, it was directly diluted in the culture medium, and vehicle control was not necessary. After treatment, we fixed the cells in PFA 4%, and carried out immunofluorescence to detect the global levels of the epigenetic marks and fibrillarin. Images for a given experiment were taken in the same laser intensity between the groups, thereby allowing quantification of the signal intensity. At least 7 images were taken per group in random fields in each glass-slide, and >140 cells per treatment were analyzed. Each nuclei or nucleoli were manually selected and the fluorescence levels measured using the software Image J. As a result, the treatment did not affect the repressive mark H3K27me3 ($p = 0.6954$, Mann-Whitney test). However, it increased the other repressive mark H3K9me3 by ~1.12 fold in BHB-treated cells compared with the control ($p<0.0001$, Mann- Whitney test). Additionally, the levels of H3K4me3, a dietary responsive and associated transcription mark, was also increased ~1.08 fold in relation to control ($p<0.0001$, Mann- Whitney). Regarding the fibrillarin, while the expression did not differ between the groups ($p=0.318$), it was observed a reduction of ~34% in the nucleoli area ($p< 0.0001$, Mann- Whitney) as well as number of nucleoli per cells (6 Control x 5 BHB cells, $p<0.0001$, Mann- Whitney). These data show that BHB is capable of modulating epigenetics marks associated with transcription and repression in cells, and causes a reduction in the nucleolar area in cells, a phenotype associated with increased longevity. However, the genes and pathways repressed or activated by those epigenetic marks will be investigated in the future to fully understand the mechanisms by which BHB regulates several biological processes.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Can the time and conditions of incubation of *in vivo*- derived goat embryos compromise embryonic cryosurvival?

Ribrio Ivan Tavares Pereira Batista[1], Gicele Santos Apolinario[1], Viviane Lopes Brair[2], Luana Rangel Côrtes[3], Paulo Sérgio Cerqueira Rangel[1], Mariana Pedrosa de Paula Guimarães[1], Jeferson Ferreira da Fonseca[4], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*UFF*)
[2]Universidade de Vassouras (*USS*)
[3]Universidade Federal de Viçosa (*UFV*)
[4]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)

E-mail: ribrio@yahoo.com.br

Evidence suggests that the conditions after collection may compromise the survival of *in vivo*-derived (IVD) embryos after cryopreservation. To test this hypothesis, 150 IVD embryos (grade I and II) from 25 Alpine goats were divided into three groups after recovery on day 6 (D6; D0 = AI): P3 (n=50) – embryos maintained in a 35 mm cell culture dish with 2 mL of holding medium (HM; 301 mOsm/kg) at 37 °C for 3 h on a hotplate; P6 (n = 50) – embryos kept under the same conditions as the P3 group for 6-8 h; and T6 (n = 50) embryos maintained in cryovials containing 1 mL of HM at 37°C, for 6-8 h, in a transporter. After incubation, the embryos were reclassified and subjected to slow freezing and the media from each group was frozen for osmolarity analysis. After thawing, 45 blastocysts (n = 15 per group) were dry-frozen in pools of five embryos for analysis of differential expression of genes related to cellular stress (PRDX.1 and HSP70), embryonic quality (CDX2, NANOG, TGFB1 and NRF1) and apoptosis (BAX and BCL2), using RT-qPCR and $2^{-\Delta\Delta Ct}$ method, after normalization with ACTB and GAPDH genes. After thawing and before IVC, 10 blastocysts from each group were analyzed for mitochondrial activity (MitoTrackerRed), glutathione (GSH; Cell Tracker Blue) and reactive oxygen species (ROS; H2DCHFDA) levels. Results are presented as percentage (%) or mean ± SDM. Differences were considered significant when P≤0.05 in ANOVA followed by Tukey's test (parametric data), Kruskal-Wallis followed by Dunn's test or chi-square test (non-parametric). Increase in medium osmolarity was observed in all groups after the incubation period: 15% (347/301) in P3, 49% (301/447) in P6 and 1% (301/307) in T6. Regarding the stage of development after incubation, frequencies were assessed by Kappa test revealing a significative increase ($p<0.05$) of blastocysts and expanded blastocysts in the T6 group (27,5% and 42,5%, respectively), meanwhile P6 group had no difference ($p>0.05$) among these stages (14,1 and 34,62%, respectively). Despite this, no difference ($P>0.05$) was observed in the survival rate after cryopreservation (61, 70 and 78%). Levels of GSH ($44 \pm 4^{a, b}$, 48 ± 6^{a} and 39 ± 2^{b}), ROS (10 ± 3^{a}, 10 ± 1^{a} and 18 ± 2^{b}) and mitochondrial activity (38 ± 20^{a}, 32 ± 3^{a} and 81 ± 46^{b}) differed between groups. Higher expression ($P<0.05$) of genes associated with cellular stress (HSP70 and PRDX1) and proapoptotic (BAX) were observed in the P6 group, compared to the T6 group. However, both groups were similar ($P>0.05$) to the P3 group. The abundance of other transcripts did not differ ($P>0.05$) between the groups. Despite previous incubation on the transporter did not modify the survival rate after cryopreservation, when compared to incubation on the hot plate, the analysis performed revealed that the experimental condition of the T6 group is able to better maintain molecular aspects of IVD goat embryos after thawing, such as apoptotic incidence and ATP production.

Acknowledgments: CAPES, FAPEMIG, FAPERJ, and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Cumulus-Oocyte Complexes Matured in Microfluidics System Present Decrease Lipid Accumulation and Favorable Oxidative Profile

Franciele Flores Vit[1], Juliano Rodrigues Sangalli[1], Mariani Farias Fiorenza[1], Helena Fabiana Reis de Almeida Saraiva[1], Juliana Germano Ferst[1], Felipe Perecin[1], Flávio Vieira Meirelles[1], Lucimara Gaziola de la Torre[2], Juliano Coelho Da Silveira[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade Estadual de Campinas (*Faculdade de Engenharia Química - UNICAMP*)

E-mail: ffvit@usp.br

The *in vitro* maturation of oocytes (IVM) is pivotal to allow *in vitro* fertilization of oocytes retrieved from non-stimulated follicles. The *in vitro* production can significantly impact embryo rates. Based on this premise, novel techniques applied to *in vitro* maturation may help increase *in vitro* production rates. In this regard, the objective of this study was to construct a reversible and reusable microdevice enabling dynamic culture of *cumulus*-oocyte complexes (COCs) with constant medium exchange. COCs were cultured in the microdevice and plates using TCM119 B medium supplemented with 10 µg/ml sodium pyruvate, 25 µg/ml gentamicin, 0.4% BSA, 10-2 IU/ml recombinant human FSH, and 50 ng/ml recombinant human luteinizing hormone (LH). COCs were cultured in plates containing drops with 30 µL of medium and 15 COCs per drop, while oocytes were cultured in microfluidics with 4 µL of medium and each cultivation chamber containing 2 COCs. The flow rate used throughout the experiment was 1 µL/min for 22 hours. We performed 5 routines with a total pool of 60 COCs per routine (30 COCs in control plates and 30 COCs in microfluidic device). After 21 hours of maturation, the COCs were removed, and only matured COCs (evaluated by extrusion of the first polar body) were stained with BODIPY to assess the lipid profile and the lipid droplets in COCs from both groups. Additionally, oxidative stress (ROS) was measured using CellROX Green, and the production of antioxidants was evaluated by reduced glutathione (GSH) using the Celltracker Blue CMF2HC marker. We also activated parthenogenetic 10 COCs per routine cultured in plates and microdevice to verify whether microfluidics affects the blastocyst rate. Data were expressed as means ± SEM (T-test), and $p < 0.05$ was considered statistically significant. We did not observe statistical differences between the groups' maturation rates and blastocysts. The lipid droplets in COCs matured in microdevices were two times smaller than those in COCs matured in plates ($p<0.0004$). Regarding ROS and GSH levels were higher for COCs matured in microdevices ($p=0.0001$ and $p=0.0003$, respectively), closely resembling those of *in vivo* matured COCs as reported in the literature (Del Collado *et al*., 2017). The results suggest that the microdevice does not interfere with COCs maturation process. Importantly, it can change lipid accumulation reducing droplets. However, the oxidative stress and antioxidants increased in the COCs matured in microdevice. In conclusion, *in vitro* maturation using the microdevice can improve oocyte characteristics resembling COCs matured *in vivo*, which supports it as a promising alternative to improve *in vitro* embryo production.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

DNA detection of *Neospora caninum* in follicular fluid from oocyte donor cows

Ligia Cantarelli Pegoraro[1], Katielen Mota da Silva[1], Bernardo Garziera Gasperin[2], Sergio Farias Vargas Júnior[2], Ísis Soares da Cunha[2], Rafael Gianella Mondadori[2], Fabiane Pereira de Moraes[2], Fabricio Dias Torres[3], Caroline Pinto de Andrade[3], Monique Tomazele Rovani[4]

[1]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[2]Universidade Federal de Pelotas (*UFP*)
[3]Laboratório Axys Diagnóstico Veterinário
[4]Universidade Federal do Rio Grande do Sul (*Setor de Grandes Ruminantes - UFRG*)

E-mail: ligia.pegoraro@embrapa.br

Neospora caninum is a major cause of spontaneous abortion and bovine carriers can eliminate the protozoa intermittently in their germ cells, if subjected to stressful and immunosuppressive processes. Therefore, its transmission by gametes and its implications in early embryonic development in cattle should be further explored. The present study tested the hypothesis that follicular fluid and endometrial tissue of naturally infected cows contain *N. caninum* DNA. Five Jersey cows previously diagnosed as positive in a serological test to *N. caninum* (EIA kit IDEXX® Neospora X2, IDEXX Laboratories, Inc., USA) were included in the study. Follicular fluid (FF) and endometrial samples were collected 30, 60, 90, 120 and 160 days after normal birth or abortion. Briefly, cows underwent low epidural anesthesia, and follicular aspiration was performed with the aid of an ultrasound device equipped with a 7.5 MHz probe and a needle system coupled to a syringe. Endometrial samples were collected using cytobrush. *N. caninum* DNA detection was performed with Sybr Green with the following rtPCR protocol: 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 seconds; 60°C for 30 seconds, and 73°C for 30 seconds (quantStudio, ThermoFisher Scientific). Molecular analyzes via rtPCR demonstrated *N. caninum* DNA in the follicular fluid of 2 out of 5 cows. In one cow, *N. caninum* DNA was detected in FF at 90 days after abortion, whereas in another cow DNA was detected in FF at 90, 120 and 160 days after parturition. Meanwhile, endometrial samples collected at all timepoints were negative for *N. caninum* DNA. These preliminary results indicate that the agent circulates intermittently in the reproductive tract of cows with positive serology for *N. caninum*. Also, additional studies are necessary to elucidate a possible transmission via gametes and the use of assisted reproductive technologies in *N. caninum* positive donors. The authors thank FAPERGS, CNPq and CAPES for their financial support.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Embryonic cryoresistance is nuclear size dependent.

Luan Henrique da Silva[1], Júlia Aparecida da Silva[1], Mateus José Sudano[1]

[1]Universidade Federal de São Carlos (*Departamento de Genética e Evolução - DGE/UFSCAR*)

E-mail: luanhenrique@estudante.ufscar.br

The application of cryopreservation on *in vitro* embryo production (PIVE) has been widely disseminated in recent years, being an excellent alternative for the preservation and subsequent use of these embryos. However, this technique still presents limitations, such as cellular and nuclear damage caused to the embryo, often making it impossible for its development. Thus, nuclear morphometry emerges as a useful and effective tool in investigating damage caused by cryopreservation to embryos. Therefore, we sought to verify the effects that cryopreservation causes in relation to the different nuclear characteristics found in cryoresistant (Crio) and non-cryoresistant (NC) embryos through nuclear morphometry. For this purpose, morphometric data were collected from *in vitro* produced bovine blastocysts classified as Crio and NC (Valente *et al*., Theriogenology, 158:290-296, 2020). For the analysis, intervals were defined for the classification of nucleus with the aid of ImageJ 1.54d software. Small nuclei were considered those in the area range of 5.045μm²< X ≤ 17.0003μm², medium nuclei ranged from 17.0003μm² < X ≤ 30.751μm², large nuclei ranged from 30.751μm² < X ≤ 51.171μm², and very large nuclei ranged from 51.171μm² < X ≤ 122.042μm². Next, the counting of nuclei in each embryo was performed to define the percentage of nuclear content in relation to the sizes of the nuclei present in the blastocyst. Finally, the data were subjected to unpaired t-test analysis adopting a significance level of 5% (P<0.05). The analysis compared cryoresistant embryos hatched at 24h (Crio24) (n = 15) versus only re-expanded non-cryoresistant embryos at 12h (NC) (n = 21) and Crio24 embryos versus cryoresistant embryos hatched at 48h (Crio48) (n = 15). Crio24 embryos showed a higher (P<0.05) percentage of large nuclei (24.9%) and very large nuclei (32.1%) compared to NC embryos (16.5% and 15.5%, respectively). In contrast, NC embryos showed a higher (P<0.05) percentage of small nuclei (41.0%) compared to Crio24 embryos (19.4%). Additionally, Crio24 and Crio48 embryos showed no difference (P<0.05) in the size of their nuclei. Thus, we can infer that nuclear morphometry made it possible to identify a higher prevalence of large and very large nuclei in Crio embryos, as well as a higher presence of small nuclei in NC embryos, indicating a clear disparity in the nuclear characteristics of the two groups, reflecting their post-cryopreservation behavior.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Enrichment of H3K27ac mediated by changes in pyruvate metabolism in bovine embryos produced *in vitro*

Aldcejam Martins da Fonseca Junior[1], Jessica Ispada[2], Pablo Juan Ross[3], Haja N. Kadarmideen[4], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC (*Centro de Ciências Naturais e Humanas - CCNH/UFACB)*
[2]AgResearch
[3]STgenetics
[4]Aarhus University (AU)

E-mail: aldcejamjunior@gmail.com

Chromatin state and the gene expression profile of cells are delicately mediated by complex changes in metabolic conditions that dictates the developmental prospects of the pre-implantation bovine embryo. This work explores the metabolomics of bovine blastocysts cultured under influence of modulators - DCA (dichloroacetate, increasing the pyruvate to acetyl-CoA conversion) and IA (iodoacetate, leading to glycolysis inhibition), from day 5 of culture; and examines their spatial epigenomics profile regarding H3K27ac, to determine the relationships between the metabolic modulations and the presence of this permissive histone mark. Embryos were cultured in 2 experimental groups [(SOFaa) + 4% BSA]: DCA (addition of 2mM of DCA) or IA (addition of 2µl of IA). Blastocysts were collected on Day 7 and their inner cell masses were submitted to Raman Spectroscopy and H3K27ac Cut&Tag (Cleavage Under Targets and Tagmentation) analysis (3 ICM per group per replicate; 3 replicates). Spectroscopic data (10 spectra/sample, 532 nm excitation on a 5-mW spot, 20s exposition) were plotted and pre-processed using the Origin 8.0 software (OriginLab, USA). Data were further analyzed using the Spectrograpy 1.2.15 software. Cut&Tag results underwent quality control with FastQC, genome alignment (Bos_taurus_UMD_3.1.1, NCBI) was done by Bowtie2, peak-calling with MACS2 and the genomic annotation by ChipSeeker. Normalization and differential expression measurement of peaks was performed by Deseq2. Results from normalized peaks found consonance with the metabolomics and helps to understand the changes induced by our experimental design. Several membrane transporters were found to be differentially accessible within peaks in DCA and IA groups. DCA group presented around 6% increase of peaks related to membrane transporters when compared to IA, such as GLUT2 and SLC2A8, glucose transporters, as well as the glycolysis regulators PFKFB3, GFPT1 and GFPT2. This suggests a turn to hexosamine biosynthesis pathway in IA group, corroborating with the decrease of fructose-6-P, even though the consumption of glucose and pyruvate were similar between groups. Peaks binding to ATP synthesis showed an increment of 15,4% in the IA group, correlated with diminished levels of ACL in this group, together with higher levels of fatty acids, indicating oxidative phosphorylation as the main ATP yield for IA. Acetyl-CoA presented decreased consumption levels on the ICMs of both groups, nevertheless, IA presented a 10% supplement of their associated peaks, giving a hint about the preferred mechanism used in these cells, with ACAA2 and ACACAB, for instance, up-represented in IA, connecting the lower levels of acetyl-CoA and the fatty acid metabolism. These findings together with our previous molecular and cell differentiation results pinnacles the complex adaptations that embryos undergo as an attempt to regulate their epigenetic reprogramming and establish an equilibrated development. FAPESP-2019220251.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Extracellular vesicles from bovine follicular fluid can delivery synthetically incorporated bta-miR20a to bovine cumulus cells

Camila Azzolin de Souza[1], Gislaine dos Santos[1], Matheus Andrade Chaves[1], Tiago Henrique Camara De Bem[1], Mariani Farias Fiorenza[1], Alessandra Bridi[1], Cibele Maria Prado[1], Flávio Vieira Meirelles[1], Felipe Perecin[1], Juliano Coelho Da Silveira[1]

[1]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP).*

E-mail: camilaazzolin@usp.br

The acquisition of oocyte competence involves cross-talk between the gamete and the follicular somatic cells. Within the follicular environment, one vital mode of communication involves extracellular vesicles (EVs), which transport and transfer various bioactive molecules, including miRNAs. MicroRNAs have the capacity to modulate essential physiological pathways in target cells, regulating key events in female reproductive biology. With this in mind, our hypothesis is that EVs isolated from follicular fluid (FF) can be enriched with synthetic miRNAs and delivered to *cumulus* cells *in vitro*. To investigate this hypothesis, ovarian follicles with a diameter between 3 to 6 mm were aspirated and the follicular fluid was used to isolate EVs using exclusion chromatography and then loaded with either a miRNA scramble or bta-miR-20a, both conjugated with a fluorophore (Alexa Fluor 488, Qiagen, Germany), using the Exo-fectTM Exosome Transfection Kit (System Bioscience, USA). Incorporation validation was performed using flow cytometry (n = 3), transmission electron microscopy (TEM), and nanoparticle tracking analysis (n = 3) (NTA). Subsequently, cumulus cell cultures were divided into the following experimental groups: (1) negative control (cumulus cells only), (2) cells treated with EVs isolated from FF alone, (3) cells treated with EVs loaded with a miRNA scramble, and (4) cells treated with EVs loaded with bta-miR-20a. Supplementation occurred for 2 h, and endocytosis was evaluated using fluorescence microscopy (n = 4) and RT-PCR (n = 6). Statistical analysis was performed one-way analysis of variance (ANOVA) followed by Tukey's test ($P \leq 0.05$). Results from the incorporation validation demonstrated successful loading of exogenous bioactive molecules into EVs without altering their and morphology of the vesicles. Fluorescence microscopy revealed significantly higher fluorescence intensity ($p<0.05$) in the group supplemented with EVs loaded with miRNA compared to negative controls. RT-PCR results indicated an increase in the relative expression of miR-20a ($p<0.05$) in cells supplemented with EVs loaded with miR-20a compared to all control groups. These findings highlight the feasibility of incorporating synthetic miRNAs into bovine FF EVs using the Exo-fectTM method without affecting EVs endocytosis by *cumulus* cells cultured *in vitro*. Thus, miRNA delivery via EVs emerges as a promising strategy for modulating physiological pathways, such as PI3K/AKT, in *in vitro* matured *cumulus*-oocyte complexes to enhance oocyte quality. Funding: FAPESP 21/06645-0, 22/01433-7, 22/01505-8 and 2022/09461-0 and by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" – Brasil (CAPES) – Financing Code 001.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Feline pre-antral follicle viability through AFP I supplementation in ovarian tissue cryopreservation

Lucas Francisco Leodido Correia[1], Nicole Oliveira Rocha[1], Gabrielle Pereira Lessa Fontes[1], Rachel Ferreira Braga[1], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Faculdade de Medicina Veterinária - UFF*)

E-mail: lucascorreia@id.uff.br

Due to the vast follicular reserve, ovarian cryopreservation is becoming an important fertility preservation for humans, aiming to maintain the viability of oocytes from preantral follicles. In this sense, aiming for biodiversity conservation, domestic cats could be a great biological model for ovary preservation in endangered wild felids. However, cryopreservation can lead to cell damage, impairing viability and follicle development. Supplementing cryopreservation solution with antifreeze proteins (AFP) could mitigate this damage, minimizing ice crystal formation. Thus, this study evaluated two concentrations of antifreeze protein type I (AFP I) on the feline follicles viability after ovarian cryopreservation. Ovaries were collected from five queens after routine ovariectomy, transported to the laboratory at 4 ºC in 0.9% saline solution, sectioned into 5 mm × 3 mm × 3 mm fragments, and kept in TCM-199 medium with 20% fetal bovine serum (base medium, BM) for 15 min. Then, fragments from each ovary were randomly assigned to the following groups: fresh control (FC); vitrification control (VC); vitrification with 10 µg/mL (V10) or 50 µg/mL (V50) of AFP I in vitrification solution, and a two-step protocol was performed. Firstly, fragments were equilibrated in a petri dish containing BM with 7.5% ethylene glycol (EG) and 7.5% dimethylsulfoxide (DMSO) for 10 min at 4 ºC. Afterward, fragments were transferred to vitrification solution (BM with 15% EG and 15% DMSO, containing or not AFP I according to each experimental group), for 10 min at 4 ºC. Then, placed into cryogenic tubes and stored in liquid nitrogen until warming/analysis. Warming was performed in a 4-step protocol at 37 ºC containing: (1) 1 M sucrose in BM for 5 min; (2) 0.5 M sucrose in BM for 5 min; (3) 0.25 M sucrose for 5 min; (4) BM for 5 min. Pre-antral follicle viability assay was performed by fragments incubation in Neutral Red solution at 50 µg/mL in phosphate buffer saline (PBS) for 30 min, followed by three washes in PBS for 10 min. Evaluation was conducted in a stereomicroscope and viable and non-viable follicles were counted. Follicles were considered viable when cells surrounding the oocyte as well as the oocyte itself were both stained red. Data were submitted to Shapiro-Wilk normality test and Levene homoscedasticity test, and compared by ANOVA followed by Tukey post-hoc test. A $P<0.05$ was considered significant and data are presented as Mean ± standard error of mean (SEM). A total of 2,223 follicles were evaluated (69.5 ± 2.7 per fragment). The percentage of viable pre-antral follicles in the VC group (57.9 ± 2.6%) and V10 group (64.4 ± 3.1%) were lower compared to the FC group (79.3 ± 3.0%). Interestingly, no difference was observed in the V50 group (72.2 ± 1.8%) compared to FC or V10, but it differs from the VC group. In conclusion, addition of 50 µg/mL of AFP I could maintain feline pre-antral follicle viability after ovarian cryopreservation. Acknowledgments: Capes (code 001), FAPERJ, and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Follicular development of allotransplanted ovarian fragments in two implantation sites of goats

Antonio Renilson Sousa Vieira[1,2], Fabiana Aparecida Santili Brandão[1], Celso Henrique Souza Costa Barros[2], Francisco Carlos de Sousa[3], Dárcio Ítalo Alves Teixeira[1]

[1]Universidade Estadual do Ceará (UFC)
[2]Centro Universitário INTA (UNINTA)
[3]Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE)

E-mail: renilsonvieirasq@gmail.com

This study aimed to investigate the effects of intra-auricular (IA) subcutaneous site compared to intramuscular (IM) in the cervical portion (cervical splenius muscle) of the neck on follicular activation and morphology, in experiments of goat ovarian transplantation. Eight pairs of goat ovaries were obtained from mixed-breed goats by bilateral ovariectomy. After washing in 70% alcohol and 0.9% saline solution, each pair of ovaries was transferred and maintained in Minimal Essential Medium until the puncture was realized with a 2 mm punch to obtain the cortical fragments. These fragments were distributed to the recipient goats and separated into the different treatments: non transplanted fresh control (FC); intra-auricular allotransplantation for 7 (IA-7) and 15 days (IA-15); intramuscular allotransplantation for 7 (IM-7) and 15 days (IM-15). The fragments were inserted into the neck and ear on both sides (left and right grafts were removed after 7 and 15 days, respectively). An average of four fragments were transplanted per site, and two fragments/animal considered as the FC group, which served as reference for comparison. After classical histology procedures, the PAS-stained sections (7 µm) were evaluated histologically using light microscopy at 400x. Preantral follicles were classified according to the stage as primordial or growing (transitional, primary or secondary), as previously reported (Silva *et al.,* Theriogenology, 61:1691-1704, 2004) and considered morphologically normal or degenerated, according to Silva *et al.* (Small Ruminant Research, 43:203-209, 2002). Follicular activation was considered to have occurred when the primordial follicles left the follicular reserve and developed into the transitional and primary categories. Analysis of variance followed by Fisher's LSD post hoc test was used to compare means for the follicular activation and morphology. Data are presented as absolute number or percentage, with statistical significance defined as $P<0.05$. A total of 5,642 histological sections were evaluated, and 451 preantral follicles were counted (167 normal follicles and 284 degenerated follicles). The percentage of normal primordial follicles showed a significant difference ($P<0.05$) between the treatments, with significantly higher values for the IA-7 (39.40 ± 12.49ab), IM-7 (18.00 ± 12.00bc) and FC group (24.30 ± 9.44a). On the other hand, significantly ($P<0.05$) lower values were observed for the IA-15 (3.33 ± 3.33c) and IM-15 (5.11 ± 2.57c) treatments. The percentage of developing follicles differed ($P<0.05$) between the groups: IA-7 (61.01 ± 17.17ab) and IM-7 (79.17 ± 12.50ab) were similar to FC (85.94 ± 6.05a), with a significant reduction in IM-15 (33.33 ± 33.33b) and no follicular activation in IA-15. In conclusion, the IA-7 and IM-7 site showed more satisfactory values in terms of morphology and follicle activation. In view of the results obtained, further studies are necessary to investigate the viability of the sites studied.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Handmade biopsy for genotyping of *in vitro*-produced bovine embryos

Clara Slade Oliveira[1], Carolina Capobiango Romano Quintão[1], Júlia Medeiros Cruz de Lima Martins[1], Marcos Vinícius Gualberto Barbosa da Silva[1], Agostinho Jorge dos Reis Camargo[2], Luiz Fernando Feres[3], Marco Antônio Machado[1], Celio Freitas[1], Naiara Zoccal Saraiva[1], Luiz Sérgio de Almeida Camargo[1]

[1]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA Gado de Leite*)
[2]Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro (*Pesagro-RIO*)
[3]Universidade José do Rosário Vellano (*UNIFENAS*).

E-mail: clara.oliveira@embrapa.br

Financial support: Fapemig, CNPq.

Genomic selection has been used to accelerate the genetic gain in livestock in several countries. In cattle, based on genomic estimated breeding value (GEBV) young bulls and cows can be selected. In order to shorten the generation interval, embryos can also be selected based on their GEVB, enhancing the genetic gain. However, embryo biopsy usually requires equipment for embryo manipulation in a well-established laboratory. This study aimed to evaluate an inexpensive and simple procedure of biopsy performed by hands (herein called handmade embryo biopsy) on *in vitro*-produced embryos using a splitting microblade and a stereoscope. Results were statistically analyzed in SAS 9.1 software, adopting a 95% confidence level. Rates were compared by chi-square and means were compared by analysis of variance among groups. In the first experiment, crossbred *Bos taurus x Bos indicus* blastocysts at day 6 or 7 after *in vitro* fertilization (IVF) were biopsied and cultured *in vitro* for 48 h (n = 223, 28-79 per group, 4 replicates). Biopsy reduced ($P<0.01$) embryo development as noted by the lower blastocoel formation/re-expansion rates on both day 6 (34.2% *vs.* 71.4%) and day 7 (66.6% *vs.* 89.8%) blastocysts, when compared to non-biopsied control embryos, respectively. Biopsy on day 7 after IVF decreased ($P<0.01$) total cell number (112.8 ± 6.3 *vs.* 149.9 ± 5.6) and increased ($P<0.01$) apoptotic index (14.9 ± 1.4 *vs.* 6.2 ± 1.2). In the second experiment crossbred *Bos taurus x B.os indicus* blastocysts on day 7 post IVF were produced and biopsied in the farm and then cultured for 3 h in buffered medium on a warm plate (n = 96, 46-50 per group, 3 replicates). Blastocysts with re-expanded blastocoel were then transferred to synchronized recipients. Blastocoel re- expansion rate after 3h in culture was 78.3 . Pregnancy rate (58.7% *vs.* 62%), birth rate (52.1% *vs.* 56%) and birthweight (29.6 ± 2.0kg *vs.* 30.07 ± 1.2kg) were similar ($P>0.05$) between handmade biopsy and non-biopsied control embryos, respectively. The whole genome amplification of DNA obtained from biopsies samples resulted in 89.6 ± 46.6 ng/µL DNA, with fragment length ranging between 2.3 ± 0.6 and 5.6 ± 0.6 kb. In conclusion, handmade biopsy can be performed in *in vitro*-produced blastocyst without compromising pregnancy, calf delivery and birth weight. Cell samples can be suitable for downstream application as genotyping. This procedure is suitable for small laboratories and at farm level without requiring expensive equipment, which contributes to reducing costs and making embryo genomic selection more affordable for farmers.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Influence of maternal category on the Nelore heifer's metabotype

Ana Laura dos Santos Munhoz Gôngora[1], Larissa Christina Montezano[2], Thiago Kan Nishimura[2], Eduardo Solano Pina dos Santos[2], Fernanda Maria Marins Ocampos[2], Paulo Roberto Leme[2], Arlindo Saran Netto[2], Guilherme Pugliesi[2], Nara Regina Brandão Consolo[2]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos (*FZEA/USP)*.

E-mail: analauragongora@usp.br

In the context of cattle animal production, heifers perform an important role in the production process, due to their performance, both in productive and reproductive terms, which are essential to ensure efficient and profitable production. This performance correlates with their mother. Therefore, the care and selection of breeding females have a significant impact on the quality and efficiency of the herd. The study aimed to evaluate the serum metabotype of Nelore heifers according to the maternal category. We hypothesized that the maternal category influences the metabolomic profile of the offspring. To this end, blood samples of 53 Nelore heifers (15 ± 1 month of age and 290 ± 10 kg of body weight), being 28 daughters of multiparous cows (MUL) and 25 of secundiparous cows (SEC), were taken by puncture of the jugular vein. The samples collected in vacuum tubes with coagulum activator were centrifuged at 2500xg for 15 minutes. The serum was pipetted out and stored in conical tubes at -80°C. The metabolomic analysis was carried out using nuclear magnetic resonance spectroscopy (^{1}H-NMR) using Bruker equipment (Bruker Corporation, Ettlingen, Germany) at 600 MHz for hydrogen frequency, on the premises of the EMBRAPA Instrumentação, São Carlos, SP. The spectrum images were processed using Chenomx NMR Suite 7.5 software (Chenomx, Edmonton, Alberta, Canada). The metabolites were identified and quantified using the Profiler and Library Manager in the software. Statistical analysis was carried out using univariate and multivariate tools in the R software and on the MetaboAnalyst website. The partial least squares discriminant-analysis (PLSDA) demonstrated a slight difference between the groups, suggesting that the metabolic profile of the heifers differs depending on the maternal category (multiparous or secundiparous). To better understand these differences, a Volcano Plot analysis was carried out. The metabolites phenylalanine (P = 0.01; 0.02557 *vs.* 0.03284 mM for SEC and MUL, respectively), creatine phosphate (P = 0.03; 0.02248 *vs.* 0.02771 mM for SEC and MUL, respectively), and glutamine (P = 0.05; 0.06163 *vs*. 0.08870 mM for SEC and MUL, respectively) were found in higher concentrations in heifers from multiparous cows compared to secundiparous cows. This result indicated enriched metabolic pathways of phenylalanine and tyrosine metabolism, pyrimidine metabolism, nicotinate and nicotinamide metabolism, and arginine and proline metabolism. In conclusion, the category of parity order of cows changed the metabolic profile of heifers with enriched metabolic pathways mainly correlated with protein and energy metabolism.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Influence of Ovarian Structures on oviductal explants formation and Gene Expression

Isabelle Cristina Pereira[1], Juliana Horta Diniz[1], Ana Carolina Bahia Teixeira[1], Raphael Rocha Wenceslau[1], Julia Meireles Nogueira[1], Jade Carceroni[1], Erika Cristina[1], Gabriela Ponath Peruzzo[1], José de Oliveira Carvalho[2], Leticia Zoccolaro Oliveira[1]

[1]Universidade Federal de Minas Gerais (UFMG)
[2]Universidade Federal do Espírito Santo (UFES)

E-mail: isabellecristina792@gmail.com

Oviductal cells are dynamic structures that change in quantity and depending on the estrous cycle and gene expression ESR1, ESR2 e PGR. The objective of this study was to evaluate oviductal explant formation from bovine reproductive tracts with distinct ovarian characteristics, as well as the expression of the genes ESR1, ESR2 and PGR in the isthmus. As hypothesized, oviductal isthmus ipsilateral to different ovarian structures present differences in the expression of the genes that influence the ability to form cell aggregates. Reproductive tracts of slaughtered cows (n = 30) were categorized in groups: ovaries with ≤5 mm follicles (small-follicles group; SF; n = 10); ovaries with follicles between 8-10 mm (large-follicles group; LF; n=10); and ovaries containing corpus luteum (CL Group; n = 10). Isthmus cells from the three groups were cultivated to form cellular aggregates (oviductal explants) during 24h and the number of aggregates in each group was evaluated. Then, qPCR assessment of ESR1, ESR2, and PGR expression was performed in oviducts from the experimental groups. Statistical analysis of the oviducal cell culture was performed using a Kruskal-Wallis test followed by Dunn's test in R3.6.1 at 5% of significance. Differential gene expression was analyzed between the LF, SF and CL groups using the REST 2009 software. The number of cellular aggregates differed ($P=0.004$), with LF presenting more aggregates (364.0 ± 139.1) than SF group (197.6 ± 127.7) and both the LF and SF groups presenting more aggregates than the CL group (8.0 ± 11.0). Gene expression analysis showed reduced PGR expression in the SF than in the CL group and greater PGR expression in the LF than in the SF group ($P<0.001$). ESR1 had a 3x higher level of expression in the SF than in the LF group ($P<0.01$). No significant differences in ESR2 expression were found between groups ($P>0.05$). ESR1 is considered a gene with mitogenic actions on cells. This may justify, at least in part, the greater number of explants formed from tubal cells under greater estrogenic influence (LF and SF). On the other hand, progesterone is a steroid responsible for acting on intercellular connections, weakening them. In this sense, it is speculated that, in the explants of the CL group, the connection between the isthmus cells during the cultivation period was made unfeasible, a step of utmost importance for the formation of the explants. Furthermore, the PGR gene, abundantly expressed in this group, has anti- proliferative actions, contributing to less explant formation in this experimental group. It was concluded that the steroidogenic structures present in the ovary influence the oviductal explants formation capacity from bovine isthmus cells, with oviductal cells. Moreover, the expression of the ESR1 and PGR in the oviduct varied in accordance with the ovarian structures present. However, more studies are needed to evaluate functionally and structurally characterize these explants.

Acknowledgments: FAPEMIG.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Intrafollicular immature oocyte transfer (IFIOT) for *in vivo* maturation of vitrified bovine oocytes

José Eduardo Vieira Chaves[1], Nayara Ribeiro Kussano[2], Laryssa Ketelyn Lima Pimenta[1], Venâncio Augusto Oliveira Silva[1], Bruna Alves Abud Ribeiro[1], Alice Caroline Souza e Castro[1], Ana Cecília Fernandes Vidigal[1], Leonardo de França e Melo[1], Margot Alves Nunes Dode[2], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás (*Escola de veterinária e zootecnia - EVZ/UFG*)
[2]Empresa Brasileira de Pesquisa Agropecuária (*EMBRAPA*)

E-mail: josechaves@discente.ufg.br

The cryopreservation of bovine oocytes has great potential for the expansion and use of animal reproduction biotechniques, as well as for genetic conservation. The aim of this study was to increase the embryo development and quality of fresh and cryopreserved COCs following *in vivo* maturation through IFIOT. *Cumulus*-oocyte complexes (COCs), obtained from abattoir ovaries, were randomly assigned to 4 treatment groups: 1) fresh COCs matured *in vitro* (MF; n = 210); 2) fresh COCs matured *in vivo* by IFIOT (TF; n = 236); 3) vitrified/warmed immature COCs matured *in vitro* (MV; n = 216); and 4) vitrified/warmed immature COCs matured *in vivo* by IFIOT (TV; n = 215). The vitrification and heating protocols followed the Cryotop® methodology, the IFIOT was carried out following the methodology described by IFIOT-Embrapa®. For IFIOT, 26 Nelore females were subjected to a synchronization protocol based on P4/E2 to ensure the presence of a pre-ovulatory follicle (≥10mm) on the day of the injection. After IFIOT, 50 mg IM of lecirelin (GnRH, Tec- Relin®, Agener União) was administered to induce the LH peak. For IVM, the oocytes remained in maturation medium for 20-22 h, just like the *in vivo* groups in the preovulatory follicle, until the matured COCs were retrieved by OPU. After maturation, the COCs from all groups were subjected to IVF and IVC. Cleavage rate was evaluated on D2 and blastocyst rates on D6 and D7. Moreover, the kinetics development was evaluated on D6 and D7. Finally, on D7, Bx embryos were evaluated for diameter (µm), total cell number, and percentage of apoptotic cells (TUNEL®). Statistical analyzes were performed using the Chi- square test, ANOVA and Tukey test (SAS; $P \leq 0.05$). Cleavage rate was lower for the vitrified groups (MV = 22.6 and TV = 21.8%) compared to the fresh groups (MF = 90.0 and TF = 86.0%). Blastocyst rate on D6 differed among all groups (MF = 22.3^{b}; TF = 31.3^{a}; MV = 0.9^{d}; and TV = 4.6%^{c}). However, on D7 differences in blastocyst rates were only observed between fresh and vitrified (MF = 44.2^{a}; TF = 47.0^{a}; MV = 2.3^{b}; and TV = 4.6%^{b}). Regarding blastocyst quality evaluations, both cryopreserved groups (MV and TV) did not achieve enough blastocysts for statistical analysis. On D6, TF embryos developed faster than MF embryos, since 24.3% (18/74) of the embryos reached the Bx stage compared to 8.5% (4/47), respectively. However, no difference in developmental stages were observed on D7 [TF =54 (60/111) and MF = 44.0% (41/93)]. Finally, TF blastocysts presented greater diameter [192 ± 16.4 (n =48) *vs.* 179 ± 16.3 µm (n = 26)], number of cells [165 ± 11.4 (n = 38) *vs.* 156 ± 11.3 (n = 24)] and fewer proportion of apoptotic cells [2.8 ± 0.01 (n = 38) *vs.* 4.2% ± 0.01 (n = 24)], compared to MF blastocysts. In summary, *in vivo* maturation using the IFIOT technique for immature vitrified COCs did not improve embryo development or quality. However, for fresh oocytes, IFIOT accelerated embryo development on D6 and increased the blastocyst quality on D7.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Replacement of bovine fetal serum in oocyte maturation media aiming to improve cryopreservation of IVP bovine embryos

Luiz Felipe Fachin Pazianoto[1,2], Marcella Pecora Milazzotto[3], Marcelo Demarchi Goissis[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]CPEX Embriões
[3]Universidade Federal do ABC (*UFACB)*

E-mail: felipe.fachin@usp.br

The freezing of bovine IVP embryos using the direct transfer method (DT) is important for the dissemination of high-value genetics. The inconsistent results may be influenced by the addition of fetal bovine serum (FBS) during oocyte maturation, as this can influence the lipid composition of blastocysts generated in IVP. Therefore, the objective of the present study was to evaluate and compare the benefits of replacing FBS with another source of lipids during IVM. The working hypothesis was that the addition of apolar or polar phase of FBS replacing full FBS during *in vitro* maturation would improve post-cryopreservation embryonic survival. The experiment had 3 experimental groups, with 4 replicates containing approximately 600 oocytes per group. Oocytes were *in vitro*-matured using 10% FBS supplementation as a control group, 10% supplementation with the nonpolar fraction or 10% of the polar fraction from FBS, separated by centrifugation. Mature oocytes were *in vitro*-fertilized and zygotes were cultivated in medium without FBS for seven days. The cleavage and blastocyst rates were evaluated after 96 and 168 hours of cultivation. The blastocysts were then subjected to slow freezing with ethylene glycol and stored until thawing for subsequent analyses. A cell membrane integrity test was performed using differential staining with Hoechst 33342 and propidium iodide. A permeability assay of the trophectoderm (TE) epithelial membrane was performed with Dextran-FITC 4000 KDa. We assessed apoptosis occurrence in thawed embryos using a caspase detection kit and fluorescence intensity analysis. Data were analyzed with ANOVA using SAS PROC GLM or PROC NPAR1WAY, except for the TE permeability test, in which we used PROC GLIMMIX considering a binomial distribution. Significance was set at 5% or lower. It was observed that there was no statistical difference among the 3 groups about both cleavage and blastocyst rates. After thawing and culturing for 24h, there was no significant difference in embryonic survival assessed by blastocoel re-expansion. There was also no difference in the total number of intact cells, number of injured cells or in the ratio of injured and intact cells. The permeability assay indicated that there was no difference among the groups considering TE epithelial integrity. The Apolar group presented a decrease in active caspase compared to the other two groups. In conclusion, even without a significant difference in the number of injured cells compared to intact ones, the caspase assay indicates that supplementation with the apolar phase could present an advantage in embryo survival after transfer.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Resveratrol and antifreeze protein type I during cryopreservation increased mitochondrial activity in *in vivo*-derived sheep embryos

Mariana Pedrosa de Paula Guimarães[1], Thais de Almeida de Oliveira[1], Gabriela Ramos Leal[1], Gicele Santos Apolinario[1], Ribrio Ivan Tavares Pereira Batista[1], Felipe Zandonadi Brandão[1], Lucas Francisco Leodido Correia[1], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*Faculdade de Medicina Veterinária - UFF*)

E-mail: marianappguimaraes@gmail.com

The cytoprotective effect of resveratrol (RESV) and antifreeze protein (AFP) in mitigating the harmful outcomes of cryopreservation has been reported. We hypothesized that a possible synergistic effect of both could be a strategy to minimize the cryoinjuries. This study evaluated the effect of exposure of *in vivo*-derived ovine embryos to RESV and AFP I before and during slow-freezing, respectively. For that, 157 GI and GII embryos were randomly distributed in three experimental groups: i) G-RESV (n = 49) – embryos were incubated for 6 h at 38.5°C and 5% CO2 in Holding medium (PBS+20% FBS) supplemented with 1 µM RESV before cryopreservation; ii) G-AFP (n = 58) – embryos were frozen in a solution supplemented with 0.1 µg/mL AFP; and iii) G-RESV+AFP (n = 49) – embryos exposed to RESV and cryopreserved with AFP. After thawing, a subset of 45 embryos (15 per group) were dry frozen in pools of five embryos for gene expression analysis (*HSP70*, *CDX2*, *CDH1*, *PRDX1*, *BAX*, *BLC2*, *AQP3*, *SIRT2*) by RT-qPCR. All the other embryos were stained immediately after thawing, with fluorescence dyes for glutathione (GSH; Cell Tracker Blue), reactive oxygen species (ROS) levels (H2DCHFDA), and mitochondrial activity (MitoTracker Red) in a single solution, or to apoptotic index (Annexin V Alexa Fluor 488, Propidium Iodide, Hoechst 33342). After that, the embryos were *in vitro* cultured for 24 h in a SOF medium at 38.5 °C and 5% O2. The results are presented as mean ± SEM, and differences were considered significant when $P<0.05$ in the ANOVA followed by Tukey's test (parametric data), Kruskal-Wallis followed by Dunn's test, or the $\chi 2$ test (non-parametric). The G-RESV, G-AFP, and G-RESV+AFP embryos, respectively, had similar results in arbitrary units (AU) for ROS (1.5 ± 0.2; 1.6 ± 0.2 and 1.8 ± 0.4) and GSH (1.5 ± 0.8; 1.6 ± 1.1 and 1.8 ± 1.4) levels, although the ROS/GSH ratio (14.0 ± 13.2^{b}, 0.1 ± 0.1^{a}, and 2.9 ± 0.9^{b}) differed. Interestingly, the G-RESV + AFP presented the highest levels of mitochondrial activity (28.2 ± 2.5^{ab}, 21.4 ± 1.4^{a}, and 31.3 ± 2.9^{b}). Lastly, the apoptotic index (27.9 ± 4.5%, 50.8 ± 7.8%, and 45.7 ± 7.9%), did not differ among groups, resulting in a similar survival rate after culture (47.4%, 32.3%, and 44.0%) for G-RESV, G-AFP, and G-RESV + AFP, respectively. Gene expression showed no difference in RESV *vs* AFP+RESV, however, AFP embryos were downregulated for *PRDX1* and *AQP3* compared to RESV. Altogether, the results point to a beneficial effect of AFP I on redox balance; moreover, the combination of both molecules ameliorates mitochondrial activity after embryo thawing, being a possible tool to mitigate cryodamage, preserving important molecular aspects of frozen-thawed *in vivo*-derived ovine embryos. In conclusion, exposure of sheep embryos to RESV and AFP I, before and during cryopreservation, respectively, does not improve *in vitro* cryosurvival but increases mitochondrial activity. Acknowledgments: Capes (001), FAPERJ, and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Role of bovine seminal plasma in modulating uterine: a metabolomic analysis

Guilherme Felipe dos Santos[1,2], Manoel Francisco Sá Filho[3,2], Edson Guimarães Lo Turco[4], Pietro Sampaio Baruselli[2]

[1]Axiota Animal Health
[2]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[3]Alta Genetics
[4]Universidade Federal de São Paulo (*UNIFESP*).

E-mail: guilhermefs@usp.br

It is believed that a portion of gestational losses may be related to a uterine environment with low receptivity. Some metabolites present in seminal plasma (SP) can modulate uterine metabolism and promote a better maternal-embryonic tolerance state. In the present study, SP was obtained by centrifugation of semen from 8 Nellore sires previously evaluated by the Concept Plus® program as high fertility (HF; 56.2 ± 1.85% P/AI) or low fertility (LF; 47.0 ± 1.98% P/AI). The seminal plasma was packaged in 0.25 straws and stored at -20°C for later treatment. Seventy Nellore cows were synchronized and treated with SP at the scheduled time of AI. Treatments were performed according to sire fertility (HF or LF). A group of cows was used as control (no SP treatment; n = 14). Seven days after treatment with seminal plasma, a biopsy of the uterine endometrium (UB) was performed and stored by snap freezing for late metabolomics analysis. One hundred and thirty-eight metabolites were analyzed in UB fragments by mass spectrometry using the target method. Multivariate statistical analysis, such as PLS-DA, to identify the most important metabolites (VIP score) in the model construction and group separation was performed using MetaboAnalyst 5.0. Metabolic pathway (MP) analysis was performed by integrating enrichment analysis and pathway topology using the KEGG database. The most relevant MP (P≤0.05) in cows treated with SP from HF sires compared to those treated with LF sires and control group were: glycerophospholipid metabolism (FDR = 0.15) and linoleic acid metabolism (FDR = 0.75), which directly participate in lipid and cholesterol metabolism; citrate cycle (FDR = 0.35), responsible for carbohydrate and fatty acid oxidation and also related to the metabolism of important amino acids such as arginine, alanine, aspartate, and glutamate, which in turn participate in the regulation of ornithine and polyamines. In conclusion, there is evidence that the deposition of SP from HF sires in both the fornix and uterine body at the time of AI may alter the local metabolic profile, activate important pathways that control the uterine environment to support early embryonic development.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Spatiotemporal gene expression of antioxidant enzymes in the bovine oviduct epithelium

Rachel Ferreira Braga[1,2], Paulo Victor dos Santos Pereira[2,3], Ana Lucia Rosa e Silva Maia[1,2], Pascal Mermillod[2], Joanna Maria Gonçalves Souza-Fabjan[1], Marie-Véronique Dattei[2], Marie Saint-Dizier[2]

[1]Universidade Federal Fluminense (*UFF - Faculdade de Veterinária*)
[2]Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (*INRAE*)
[3]Universidade Federal Fluminense (*Departamento de Patologia e Clínica Veterinária - Faculdade de Veterinária*).

E-mail: rachelfb@id.uff.br

The migration and capacitation of spermatozoa, oocyte capture, fertilization, and early embryonic development are events that take place in a time- and space-specific manner during the periovulatory period in the oviduct. Unraveling the antioxidant activity of this organ can be the key to improve IVP as gametes and embryos are subjected to oxidative stress during manipulations and culture. This study aimed to compare the gene expression of antioxidant enzymes in bovine oviduct epithelial cells (bOECs) from both regions of the oviduct (ampulla *vs.* isthmus) at different estrous cycle stages. Pairs of oviducts and ovaries of *Bos taurus* cyclic cows (n = 18) were transported on ice from a local slaughterhouse. After classification into pre-ovulatory (PRE), recent post-ovulatory (POST) and luteal (LUT) cycle stages according to ovarian morphology, oviducts ipsilateral to ovulation side were dissected to eliminate vessels, fat, and infundibulum. Then, 3-cm sections of the isthmus and ampulla at the uterine and ovarian extremity, respectively, were collected (6 PRE, 6 POST, and 6 LUT in each region). Bovine oviduct epithelial cells (bOEC) were collected by squeezing and stored at – 20 °C. Total RNA was extracted using a column-based method and analyzed by qRT-PCR for expression of [Cu-Zn] and [Mn] superoxide dismutase (SOD 1 and 2), catalase (CAT), glutathione peroxidase (GPX) 1-3 and peroxiredoxin (PRDX) 1-6 using three housekeeping genes (*YWAZH*, *GAPDH*, and *PPIA*). Normalized gene expression data were calculated using the geNorm software and delta-delta CT method. Data were analyzed by two-way ANOVA followed by Tukey's test, with $P < 0.05$ as significant. The expression of *SOD1*, *GPX1*, *PRDX1*, *PRDX2*, *PRDX3*, and *PRDX5* did not change between oviduct regions or among stages. Differences between regions were observed for GPX2, which had higher expression in the ampulla than in isthmus at PRE ($P < 0.01$), and for *GPX3* and *PRDX6*, with higher expression in the isthmus than in ampulla at all stages and at LUT, respectively ($P < 0.01$). Furthermore, in the ampulla, *CAT* and *SOD2* mRNAs were more abundant at LUT compared to PRE stages ($P < 0.05$) whereas *PRDX4* mRNAs were more abundant at PRE compared to LUT ($P < 0.01$) and POST ($P < 0.05$). In the isthmus, *PRDX6* mRNAs were more abundant at LUT compared to PRE and POST stages ($P < 0.01$). In conclusion, a region and time-specific expression of enzymes neutralizing reactive oxygen species, especially H2O2 (GPX and PRDX), was evidenced in the oviduct epithelium across the estrous cycle, calling for further investigation on the regulatory role of ovarian steroid hormones.

Acknowledgements: CAPES-COFECUB, CAPES-PrInt, FAPERJ and ANR ORGALOG (Grant number ANR-21-CE20-0042).

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

The redox state of *in vivo*-derived ovine embryos is not altered by the cryopreservation process regardless of their developmental stage - Preliminary results

Thais de Almeida de Oliveira[1], Mariana Pedrosa de Paula Guimarães[1], Gicele Santos Apolinario[1], Ana Paula da Silva Cupello[1], Lucas Francisco Leodido Correia[1], Felipe Zandonadi Brandão[1], Ribrio Ivan Tavares Pereira Batista[1], Joanna Maria Gonçalves Souza-Fabjan[1]

[1]Universidade Federal Fluminense (*UFF*)

E-mail: almeidathais@id.uff.br

During cryopreservation, an imbalance between oxidants and antioxidants occurs, increasing the chance of developing oxidative stress and its negative impact on embryonic development. On the other hand, the beneficial effect of antifreeze protein type I (AFP I) on cryopreservation has been widely reported. Therefore, the present study aimed to evaluate the impact of the slow freezing process on both oxidative stress and mitochondrial activity within the same embryo and if AFP I can alleviate the cryodamage. To reach that, Grade I and II embryos (n = 28) were non-surgically recovered and classified according to their stage of development into two groups: MO (Morulas + Compact Morulas) or BL (Blastocysts + Expanded Blastocysts). The MO (n = 12) and BL (n = 16) groups were subdivided into two groups according to the supplementation (AFP+; n=14) or not (AFP-; n = 14) of 0.1 µg/mL AFP I to the freezing extender (1.5 M of ethylene glycol in PBS). The embryos were kept in a holding medium (PBS + 20% SFB) in an incubator at 38.5 °C in 5% CO2 after collection and before freezing. The embryos were individually stained and analyzed for mitochondrial activity (MitoTracker Red, Invitrogen™), reactive oxygen species [ROS (H2DCHFDA, Invitrogen™)] and glutathione [GSH (CMF2HC, Cell Tracker Blue, Invitrogen™)] levels, immediately before freezing and after thawing. Pictures of each embryo were taken, and the fluorescence intensity was measured using the ZEN 3.5 Blue Edition software (Carl Zeiss Microscopy) and normalized to the background average intensity. Results were presented as relative fluorescence intensity levels (Mean ± SEM of arbitrary units, AU). Data were submitted to Shapiro-Wilk normality test and Levene homoscedasticity test. Normal data were compared by ANOVA, and non-normal data by the Mann-Whitney test. There was neither difference (P>0.05) between the MO and BL embryos in ROS (6.748 *vs.* 8.647 AU), GSH (3.881 *vs.* 4.596 AU), and mitochondrial activity (8.991 *vs.* 9.635 AU). Regarding the AFP addition or not to the freezing medium, there was no difference (P>0.05) in ROS, GSH, and mitochondrial activity (7.285 *vs.* 8.111; 4.303 *vs.* 4.174; 8.946 *vs.* 9.681 AU, respectively) among groups. In conclusion, our preliminary results provided that the cryopreservation process did not alter per se the redox status, regardless of the embryo development stage and the AFP supplementation. Acknowledgments: Capes (001), FAPERJ, and CNPq.

Animal Reproduction

THEMATIC SECTION: 37TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Using repeated transcervical cytobrush sampling before and after embryo transfer to probe the uterine environment in cattle: Preliminary results

Samuel Volpe Souza[1], Isabella Rio Feltrin[1], Thiago Neder Lisboa[1], Gabrielle Ceragioli Damaceno[1], Ana Karolyne Alves Miguel[2], Amanda Guimarães da Silva[1], Andressa Minozzo Oliveira[3], Nelyane José Lopes[4], Mateus José Sudano[3], Guilherme Pugliesi[1]

[1]Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia (*Departamento de Reprodução Animal - FMVZ/USP)*
[2]Laboratório de Biotecnologia da Reprodução Animal (REPROA/UEL)
[3]Universidade Federal de São Carlos (UFSCAR), [4]ABS Pecplan

E-mail: samuelsouza@usp.br

We aimed to assess the feasibility of probing the uterine environment by using repeated transcervical cytobrush sampling before and during early pregnancy in beef heifers after embryo transfer (ET). In Exp. 1, Canchim heifers (n = 23) were submitted to a P4/E2-based estrous synchronization protocol for fixed-time ET. Estrus (D0) was determined by observation twice a day with the assistance of a teaser bull. On D7, a single *in vitro*- produced (IVP) grade I blastocyst (*Bos indicus* oocyte's donors fertilized with a single Nelore sire) was transferred into the ipsilateral uterine horn containing the corpus luteum (CL). On D4, 16, and 20 the heifers were submitted to an epithelial cell collection using the cytobrush technique (Cardoso *et al*., 2017). In Exp. 2, Nelore heifers were submitted to a P4/E2-based estrous synchronization protocol, but ovulation was not induced. Estrous detection patches were used to determine the occurrence of estrous behavior. Daily ultrasonography exams were performed to confirm ovulation. Heifers that demonstrated estrus (D0) and the ovulation was confirmed were randomly subdivided into ET (n = 28) or Sham-ET (n = 9) groups, and respectively, to receive or not, an IVP grade I blastocyst (*Bos indicus* oocyte's donors fertilized with a single Brangus sire) on D7, as performed in Exp 1. On D6, 10, 15, and 20, heifers were subjected to epithelial cell collection as in Exp 1. Cytological samples from the Sham-ET group (n = 6) and from recipients of the ET group that had an active CL (>25% of CL blood perfusion) on D15 but with luteolysis detection on D20 (CL-D15 subgroup; n = 7) and with an active CL until D25 (CL-D25 subgroup; n = 7) were analyzed by qPCR to determine gene expression of interferon-tau (IFN-τ) and WNT5A (IFN-τ stimulated gene). Pregnancy was confirmed by visualization of an embryo with heartbeats on D30. The gene expression data were analyzed by PROC MIXED of SAS. For Exp. 1, the pregnancy rate on D30 was 60.1% (14/23). For Exp. 2, the pregnancy rate in the ET group was 3.6% (1/28). For gene expression, only an effect of time on IFN-τ expression (P = 0.07) and WNT5A (P<0.0001) was observed. Regardless of the experimental group (Sham-ET, CL- D15, and CL-25 subgroups), a greater expression of IFN-τ and WNT5A was observed on D6. In conclusion, this first report using repeated transcervical cytobrush sampling of the uterus during early pregnancy indicates that the success of pregnancy establishment after this method may diverge, requiring further studies to validate the technique at the proposed time without harming the pregnancy. The absence of an increase in IFN-τ and WNT5A expression in the cytological samples suggests that the failure in the pregnancy establishment is not related to injuries to the conceptus by the cytobrush; but factors related to breed, number or moment of sampling, and pregnancy loss related to the sire used for IVP could be associated with the success of this method in beef heifers.

Acknowledgements: ABS.