Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CONTRIBUTIONS (SBTE) – PROCEEDINGS 2025

From the SBTE President and the Chair of the Scientific Committee

Dear SBTE Members

This year we will celebrate the 40th anniversary of the Brazilian Embryo Technology Society (SBTE). The history of the SBTE was built based on partnerships and the commitment of many people, including the founders, the previous boards, and all the members of our society. The legacy of these people will be celebrated at a special event: the XXXVIII annual meeting of the SBTE, by the sea on the Bahian coast at Costa do Sauípe.

We prepared a scientific program, represented here in this special issue of Animal Reproduction, that contains the full papers from twelve internationally recognized invited speakers. These world-class speakers will deliver talks that encompass the cutting-edge knowledge on basic science and the technological advances in the applied field. We thank all the speakers for joining us in the SBTE celebration and also for their commitment to preparing the scientific review articles.

The main program will be complemented by high-quality workshops independently coordinated. We are thankful to the colleagues who put their time and resources into preparing the workshops on epigenetics, nutrition and reproduction, and small ruminant reproduction. In addition, the conference will be the place for 201 abstracts. We are thankful to the authors for choosing SBTE to publicize their work. We are confident they will find in our society a vibrant atmosphere to engage in fruitful discussions during the meeting.

The SBTE annual meeting is only possible due to the dedication of several people. The members of the SBTE board worked devotedly to deliver a high-quality meeting, along with the long-lasting support of Guilherme Trevizolli, Guilherme Vanzela, and the Brix team, led by Marta Barreto. We had a lined-up team of colleagues serving the SBTE as manuscript reviewers, abstract session editors and abstract reviewers, judging committees to evaluate the students' competitions and best abstract awards, and session moderators. We are thankful to all of you who served the SBTE promptly. We also express our gratitude to the companies belonging to the 'SBTE- business condominium' for the continuous and fundamental support in maintaining our society. In addition, we were funded this year by the National Council for Scientific and Technological Development (CNPq, Brasília, Brazil), the São Paulo Research Foundation (FAPESP, São Paulo, Brazil), and the Regional Council of Veterinary Medicine of the State of Bahia (CRMV-BA, Salvador, Bahia, Brazil) to which we express our thankfulness. We are pleased to have reached an eleven-year standing partnership with the Animal Reproduction Journal and the Association of Embryo Technology in Europe (AETE) for publishing the SBTE-AETE joint proceedings.

Finally, we thank you all for attending the SBTE annual meeting.

We are looking forward to meeting you all at Costa do Sauípe – Bahia to celebrate the 40th anniversary of the SBTE!

On behalf of the entire SBTE Board,

Felipe Perecin

Chair of the SBTE Scientific Committee

Roberto Sartori

President of the SBTE

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

SBTE BOARD OF GOVERNORS (2025/2026)

President: Roberto Sartori (ESALQ/USP - Piracicaba, SP)
Vice-president: Bernardo Garziera Gasperin (UFPel – Pelotas, RS)
1st Secretary: Lindsay Unno Gimenes (FCAV/UNESP – Jaboticabal, SP)
2nd Secretary: Rafael Gianella Mondadori (UFPel – Pelotas, RS)
1st Treasurer: Cláudia Maria Bertan Membrive (FCAT/UNESP – Dracena, SP)
2nd Treasurer: Rogério Ferreira (UDESC – Chapecó, SC)
Communication director: Joanna Maria Gonçalves de Souza Fabjan (UFF – Niteroi, RJ)
Scientific director: Felipe Perecin (FZEA/USP - Pirassununga, SP)
Business director: Alexandre Barbieri Prata (Globagen Vet Science, SP)

SBTE PROCEEDINGS GENERAL EDITORS

Felipe Perecin
Roberto Sartori

SBTE WORKSHOP CHAIRS

Joanna Maria Gonçalves de Souza Fabjan
Marcella Pecora Milazzotto
Mayra Elena Ortiz D'Avila Assumpção
Pietro Sampaio Baruselli

SBTE MANUSCRIPT REVIEWERS

Alan Ealy
Beatriz Fernandes-Fuertes
Bernardo Garziera Gasperin
Claude Robert
Claudia Lima Verde Leal
Cláudia Maria Bertan Membrive
Fabiana Fernandes Bressan
Felipe Perecin
Gabriel Bó
Jo Leroy
Joanna Maria Gonçalves de Souza Fabjan
John Kastelic
José Buratini Júnior
Krishna Chaitanya Pavani
Lindsay Unno Gimenes
Marcella Pecora Milazzotto
Marcelo Marcondes Seneda
Mário Binelli
Mateus José Sudano
Rafael Gianella Mondadori
Roberto Sartori Filho
Victor Enrique Gomez Leon
Vilceu Bordignon

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

SBTE ABSTRACT SESSION CHAIRS

FTAI and AI: Leonardo de França e Melo, Luiz Francisco Machado Pfeifer, Luiz Gustavo Bruno Siqueira
FTET, ET, and superovulation: Fábio Morotti
OPU-IVF: Bernardo Garziera Gasperin
Folliculogenesis and oogenesis: Juliano Coelho da Silveira
Male physiology and semen technology: Maíra Bianchi Rodrigues Alves
Embryology, developmental biology and physiology of reproduction: Marcos Roberto Chiaratti
Cloning, transgenesis and stem cells: Ligiane de Oliveira Leme
Supporting biotechnologies: Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics": Marcella Pecora Milazzotto

SBTE ABSTRACT REVIEWERS

Alessandra Bridi
Alessandra Corallo Nicacio
Alexandre Floriani Ramos
Alexandre Hênryli de Souza
Alexsandra Fernandes Pereira
Amanda Fonseca Zangirolamo
Ana Carolina Pedrosa
Ana Rita Tavares Krause
Andrei Antonioni Guedes Fidelis
Anna Carolina Denicol
Arnaldo Diniz Vieira
Beatrice Ingrid Macente
Bernardo Garziera Gasperin
Bruna Martins Garcia
Bruno Campos de Carvalho
Bruno Gonzalez de Freitas
Camila Bortoliero Costa
Camila Bruna de Lima
Carla Fredrichsen Moya
Carlos Eduardo Cardoso Consentini
Carlos Eduardo Porciuncula Leonardi
Carlos Frederico Martins
Carolina Capobiango Romano Quintão
Cassio Cassal Brauner
Christina Ramires Ferreira
Christopher Junior Tavares Cardoso
Clara Slade Oliveira
Cláudia Lima Verde Leal
Claudia Maria Bertan Membrive
Daniela dos Santos Brum
Daniele Missio
Denis Vinicus Bonato
Eduardo Schmitt
Eliane Vianna da Costa e Silva
Elis Lorenzetti
Elizângela Mírian Moreira
Eneiva Carla Carvalho Celeghini
Ériklis Nogueira
Evelyn Rabelo Andrade Oliveira
Fabiana de Andrade Melo Sterza
Fabiana Fernandes Bressan
Fabiane Pereira de Moraes
Fábio Gallas Leivas
Fábio Lucas Zito de Moraes
Fabio Morato Monteiro
Fábio Morotti
Felipe Zandonadi Brandão
Fernando Caetano de Oliveira
Fernando Silveira Mesquita
Gildas Mbemya Tetaping
Gilson Antônio Pessoa
Guilherme Fazan Rossi
Guilherme Pugliesi
Gustavo Desire Antunes Gastal
Gustavo Guerino Macedo
Gustavo Martins Gomes dos Santos
Henderson Ayres
Hymerson Costa Azevedo
Isabella Rio Feltrin
Janaina Menegazzo Gheller
Jessica Ispada
Jessica Nora Drum
João Diego de Agostini Losano
João Henrique Moreira Viana
José de Oliveira Carvalho
José Felipe Warmling Sprícigo
Jose Nélio de Sousa Sales
José Roberto Viana Silva
Jose Victor Vieira Isola
Juliana Corrêa Borges Silva

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SBTE BOARD MEMBERS

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SPONSORS SBTE

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Additional eCG treatment at TAI increases corpus luteum size and progesterone concentration but does not affect pregnancy rates

Gustavo Martins Gomes dos Santos[1], Katia Cristina Silva Santos[1], Marcelo Marcondes Seneda[2], Jose Henrique Ayres Dias[1], Fábio Morotti[2]

[1]Sheep Embryo Reprodução Animal, [2]Universidade Estadual de Londrina

This study evaluated the impact of eCG administration during artificial insemination on CL quality and pregnancy/TAI. In Study I, multiparous cows (n=52; 45 days postpartum; 3.46±0.13 body condition scores - BCS) were submitted to a conventional TAI protocol, beginning on a random day of the estrous cycle (D0) with progesterone (P4) device insertion (1.2g, Fertilcare®, MSD, Brazil), an injection of estradiol benzoate (2mg, i.m., Fertilcare Sincronização®, MSD), and cloprostenol (0.530mg, Ciosin®, MSD). On D8, the P4 device was removed, and cows received i.m. 0.530mg cloprostenol, 1mg estradiol cypionate (Fertilcare Ovulação®, MSD), and 300IU eCG (Folligon®, MSD). On D10, the estrus intensity (no, low or high) was monitored according to the painting at the base of the tail, and the diameter of the largest follicle (LF) was measured. Then, the cows were randomized into Control (no treatment) or eCG400 (received i.m. 400IU of eCG) groups. On D17, CL was evaluated by ultrasound (E2V Sonoscape®, Domed, Valinhos, Brazil) for diameter (cm), area (cm2) and using color Doppler for luteal blood flow score (Low/vascularization <40%; Medium/vascularization >45% and <50%; and High/vascularization >50%). Blood was also collected for serum P4 measurement (ng/mL) by chemiluminescence. In Study II, 603 multiparous Nelore cows (45 days postpartum), with BCS 2.99±0.38, from a single farm, received the same TAI protocol and design (Control, n=349 vs. eCG400, n=254) to evaluate pregnancy/TAI. All inseminations were performed by one technician using frozen semen from a single proven bull. Pregnancy diagnosis was performed 30 days later by ultrasound. In Study I, data were analyzed by ANOVA with GLM, including treatment, estrus intensity, flow score, BCS, LF diameter, and their interactions. Associations were tested using Chi-square or Fisher's exact test. Pregnancy/TAI was analyzed using logistic regression including treatment effects, estrus intensity, BCS, and interactions ($P<0.05$). In Study I, among groups, BCS, LF diameter, ovulation rate on D10, and estrus intensity were similar ($P>0.1$). Use of eCG on D10 increased CL diameter ($P=0.02$; 2.26±0.07 vs. 2.03±0.07) and luteal area ($P=0.02$; 4.10±0.26 vs. 3.35±0.21) compared to the Control. Estrus intensity also influenced CL diameter and area ($P<0.05$), with greater values in cows showing high estrus expression. No interactions were found ($P>0.1$). The eCG400 resulted in a higher P4 concentration ($P=0.002$; 10.91±0.96) compared to the Control (7.51±0.54) and no effects were observed for other variables or interactions ($P>0.1$). The pregnancy/TAI was influenced by estrus expression ($P<0.0001$; High 66.6% vs. No-low 45.1%, but no effect was observed for the eCG treatment ($P=0.70$; Control 58.2% (203) vs. eCG400 54.7% (139). Interactions or BCS did not influence conception results. In conclusion, additional gonadotropic support with eCG at the time of TAI improves CL quality and P4 levels but does not improve pregnancy/TAI.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

A novel intravaginal device developed for heifers (Repro one Novilhas): progesterone profile, animal welfare measurements and fertility in timed AI protocols

Carlos E. C. Consentini[1], Moacir Marchiori Filho[2], Alexandre Barbieri Prata[1], Rodrigo Brüner[3], Frederico Jardim[4], Leuton Luis Alves Barbosa[5], João Fábio Duarte[6], Eduardo Rossignolo[1], Pedro Henrique Déo[7], Mauro Meneghetti[1]

[1]GlobalGen Vet Science, [2]UCBVET Saúde Animal, [3]Tulipa Agropecuária, [4]Genex Brasil, [5]Agrobeef Consultoria Agropecuária, [6]Nelore Simioni, [7]Fazenda Nova Piratininga

Optimizing pregnancy per AI (P/AI) in beef heifers is key for profitability but remains challenging, either in yearling (~12 mo) or older heifers (~24 mo). In response to a demand from technicians and producers, a novel intravaginal progesterone (P4) device (IVD) — Repro One Novilhas (**OneN**) — was developed, maintaining the P4 concentration (0.5 g) and surface area as the conventional Repro One (**One**), but with a smaller anatomical design to improve welfare and usability. The study compared OneN and One in terms of hormonal profile, animal welfare measurements, usability, and P/AI. Initially, 40 Nelore heifers (18 mo) were used to assess: 1) P4 levels from d0 (insertion) to d10 (removal), 2) circulating cortisol at 0 (H0), 1 (H1) and 2 (H2) hours post-insertion, 3) degree of discomfort based on tail raising, back arching, and animal agitation over a period of time (scored as 1 = mild discomfort; 2 = moderate discomfort of short duration; and 3 = greater discomfort of longer duration), and 4) presence of inflammatory cells in the vaginal cytology at IVD removal. Additionally, 3,888 heifers (7 herds, age: 9 to 28 mo) were used to evaluate: 1) body weight (BW) change during the TAI protocol (n=708), 2) IVD loss during the protocol, 3) presence of mucus at IVD removal, and 4) P/AI on d30. The heifers were randomly assigned to OneN and One groups, differing only in the IVD. The remaining treatments of the protocol were: d0: 1.5 mg of E2 benzoate and insertion of a new 0.5 g P4 IVD (OneN or One); d7: IVD removal, 0.5 mg of E2 cypionate, 0.530 mg of PGF, and 200 UI of eCG; d9: TAI. All hormones were provided by GlobalGen. Analyses were performed using the Proc Glimmix of SAS 9.4. The P4 concentration did not differ within any given day of IVD insertion (mean P4 from d0 to d10: 1.90 vs. 1.91 ng/mL for OneN and One). Cortisol levels were lower in the OneN group at H1 (1.86 vs. 2.70 ng/mL) and H2 (2.00 vs. 2.89 ng/mL). The OneN heifers exhibited a higher proportion of discomfort degree 1 (63 vs. 11%) and fewer cases of degree 3 (13 vs. 67%), resulting in a lower mean discomfort degree (1.5 vs. 2.6). Fewer inflammatory cells were observed at IVD removal in the OneN (31.0 vs. 70.7%). The average daily gain of BW from d0 to d7 was greater in OneN group (0.590 vs. 0.180 g/d). The IVD loss during the protocol was 0% for both groups. The proportion of IVD with mucus present at removal was lower in OneN (12 vs. 58%). There was no difference on P/AI between OneN and One (39.2 vs. 41.1%). In summary, Repro one Novilhas provided similar P4 release and fertility outcomes compared to conventional Repro one, while offering improvements in animal welfare indicators, including lower circulating cortisol, reduced discomfort and vaginal inflammation, and greater BW gain. Its anatomically adapted and smaller design offers a secure, practical, and effective targeted solution for beef heifers, enhancing well-being, physiological and behavioral responses during TAI protocols.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

A novel pre-synchronization strategy enhances ovarian dynamics and fertility in high-producing dairy cows

Caroline Cervi Vandresen[1], Lucas O. e Silva[1], Mirela Balistrieri[1], Sebastian Albavi[1], Paula R. Cortat[1], Flávia Aguiar Carrieri[1], Guilherme Silva de Oliveira[1], Matheus de Oliveira Dini Nascimento[1], Carlos E. C. Consentini[2], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil., [2]GlobalGen Vet Science

This study evaluated a pre-synchronization strategy using GnRH instead of estradiol esters to induce ovulation, that reduces expression of estrus between the end of pre-synchronization and onset of timed-AI (TAI) protocols and allows for initiation of the TAI protocol on day 6 of the estrous cycle. A total of 219 Holstein cows (110 primiparous and 109 multiparous; 46.1±0.1kg/d of milk; body condition score of 3.2±0.2; 37±0.3 days in milk) were randomly assigned to two groups: pre-synchronization (PS; n=108) or control (CON; n=111). On D-11 (D0=onset of the TAI protocol), cows in the PS group received an intravaginal progesterone device (IVP4; 0.5g) and 0.53mg cloprostenol sodium (PGF, IM). On D-6, the IVP4 was removed and 16.8µg buserelin acetate (GnRH) was given IM. Both groups underwent the same TAI protocol for the 1st postpartum service, that started on D0 with a 2g IVP4 and 16.8µg GnRH IM. On D7, 0.53mg PGF was administered IM, followed by a second dose of PGF on D8, when the IVP4 was removed and 1mg estradiol cypionate was given IM. On D10, TAI was performed. Ultrasound evaluations were done on D-11, D-6 (PS group only), D0, and D7 (all groups), to evaluate presence of CL, diameter of the largest follicle (LF), presence of a follicle with ovulatory capacity (≥10 mm), and ovulation rate (determined as the disappearance of a Fol≥10 mm followed by formation of a new CL), and on D42 to assess pregnancy per AI (P/AI). Statistical analyses were performed by PROC GLIMMIX (SAS 9.4; P≤0.05). Presence of CL on D-11 was similar between groups (CON: 67.6 [75/111] vs PS: 68.5% [74/108]). Ovulation to the GnRH on D-6 in PS group was 77.8% (84/108), resulting in a greater proportion of cows with CL on D0 than in CON group (92.6 [100/108] vs 78.4% [87/111]). The proportion of cows with Fol≥10 mm on D0 was similar between groups (CON: 93.7 [104/111] vs PS: 93.5% [101/108]). However, the diameter of the LF on D0 was greater in CON (14.0±0.4 vs 13.0±0.4 mm). Ovulation to the GnRH on D0 was greater in PS (74.1 [80/108] vs 54.1% [60/111]). On D7, although the LF diameter was similar between CON and PS (13.4±0.1 vs 12.9±0.1 mm), the proportion of cows with CL was greater in PS cows (98.2 [106/108] vs 91.0% [101/111]), and there was a greater proportion of cows with ≥2 CL in PS than in CON (74.1 [80/108] vs 33.3% [37/111]). The P/AI was greater in PS than in CON group (41.6 [45/108] vs 28.8% [32/111]). In conclusion, this pre-synchronization strategy effectively improved ovarian dynamics during the TAI protocol, as evidenced by the greater proportion of cows with CL and greater ovulatory response to the GnRH at the onset of the protocol, as well as greater proportion of cows with ≥2 CL at the first PGF, resulting in a 44% increase in P/AI for the 1st postpartum service. This pre-synchronization protocol represents a practical and efficient strategy for enhancing reproductive performance in high-producing dairy cows.

Acknowledgments: São Jorge Farm, CAPES, FAPESP, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Are there benefits on early eCG administration in postpartum anestrous *Bos taurus* cows?

Helena Leites Freitas[1], Fabricio Gularte[1], Gabriel Maggi[1], Larissa Lacerda dos Santos[2], Mariana Gehres Rodrigues[2], Rafael Gianella Mondadori[1], Sergio Farias Vargas Júnior[1], Fernando Caetano de Oliveira[2], Gustavo Desire Antunes Gastal[1], Bernardo Garziera Gasperin[2,1]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Universidade Federal do Rio Grande do Sul

This study aimed to evaluate the best timing for eCG administration, either one day before or on the day of progesterone (P4) device removal and to assess whether early eCG application influences follicular growth and pregnancy rate. On Day 0 (D0), the cows received a P4 intravaginal device (IVD, Primer 1 g; Agener União, São Paulo, Brazil) and 2 mg of estradiol benzoate (RicBe; Agener União, São Paulo, Brazil). The cows were allocated into three groups: a control group (n=94), which did not receive eCG; the eCG D7 group (n=84), which received 400 IU of eCG (SincroeCG; Ouro Fino Saúde Animal, São Paulo, Brazil) on Day 7; and the eCG D8 group (n=92), which received the same dose on Day 8. On D8, IVD was removed, and all animals received 520 µg of sodium cloprostenol (Estron; Agener União, São Paulo, Brazil) and 1 mg of estradiol cypionate (SincroCP – Ouro Fino Saúde Animal). Fixed-time artificial insemination (FTAI) was performed 48 hours after device removal by a single technician. A subgroup of cows with dominant follicle diameters between 5 and 8 mm on D7 (control, n=18; eCG D7, n=15; and eCG D8, n=16) was selected for measurement of dominant follicle diameter on D7 and D10, these values were compared using paired data analysis, and pre-ovulatory follicle diameter, daily and cumulative growth rates were compared using ANOVA, followed by Tukey's test, with $P \leq 0.05$ being considered significant (JMP18 software). Pregnancy rate was compared using logistic regression. Regarding follicular diameter, paired data analysis performed on a subgroup of animals revealed significant effects of day ($P \leq 0.0001$) and group ($P=0.02$), but without day x group interaction, indicating that follicular diameter increased from D7 to D10 in all the groups, but was more pronounced in cows from eCG D7. No significant difference was observed in pre-ovulatory follicle diameter at the time of FTAI. Regarding pregnancy rates, the analysis showed a significant effect of replicate ($P=0.02$) and treatment ($P=0.02$), but without interaction. Pregnancy rate was lower in the control group (43.6%) compared to the eCG D7 group (63.1%), while the eCG D8 group (54.3%) did not differ significantly from the others. Preliminary results suggest a potential benefit in anticipating eCG administration, however, this benefit may depend on factors such as the degree of postpartum anestrus, body condition score (BCS), and energy balance, which should be further investigated in future studies.

Acknowledgment: The authors thank FAPERGS, CNPq and CAPES for their financial support.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Assessment of progesterone device duration and recombinant eCG dose on reproductive performance of primiparous and multiparous Nelore cows submitted to fixed-time AI

Alexandre Hênryli de Souza[1], Laís Ângelo de Abreu[2], Federico Randi[1], Rafael Moreira[1], Luciano Penteado da Silva[3], André Mancini Carreira[4], Marcos Wilson Vargas[5], Sofía Albertini[2,6,7], Yngrid Cristina[5], Pietro Sampaio Baruselli[2,6,7]

[1]Ceva Saúde Animal, [2]Universidade de São Paulo, [3]Firmasa Tecnologia Para Pecuaria Ltda., [4]Bos Serviços Veterinários, [5]Marcos Vargas Consultoria, [6]University of São Paulo, [7]Animal Reproduction Department, University of São Paulo

This study evaluated the influence of the progesterone device exposure period and the dosage of recombinant equine chorionic gonadotropin (reCG) on the reproductive performance of primiparous (PRI) and multiparous (MUL) Nelore cows submitted to fixed-time AI (TAI). A total of 1,124 suckled cows (PRI= 373 and MUL= 751) with an average age of 56 ± 1.1 months and body condition score (BCS; 1 to 5 scale) of 2.6 ± 0.02 from two commercial farms located Brazil (states of Minas Gerais and Tocantins) were used. Two experiments were developed with the same arrangement according to the category: Experiment 1) PRI and Experiment 2) MUL. At the onset of protocol (day 0 or 1), cows received an intravaginal P4 device (P4D; Prociclar®) and were aleatory randomized according to either one of the treatments: 8D-eCG) P4D and 2 mg of estradiol benzoate (EB; Benzoato HC®; i.m) on day 0 (D0; AM), and 300 UI of conventional eCG (Novormon®; i.m) on D8 (PRI= 94; MUL= 192); 7D-reCG) P4D and 2mg of EB on D1 (AM), and reCG adjusted according to the category [Foli-Rec®; 1) PRI received 105 UI (1.5 mL; n= 96) and 2) MUL received 84 UI (1.2 mL; n= 190), i.m] on D8; 7.5D-1.2reCG) P4D and 2 mg of EB on D1 (PM), and 84 UI of reCG on D8.5 (PRI= 94; MUL= 188) and 7.5D-1.5reCG) P4D and 2 mg of EB on D1 (PM), and 105 UI reCG on D8.5 (PRI= 89; MUL= 181). All animals also received 0.530 mg of PGF (Luteglan®, i.m) and 1mg of estradiol cypionate (Cipionato HC®; i.m) concomitant with P4D removal. The groups 7D-reCG, 7.5D-1.2reCG and 7.5D-1.5reCG additionally received 100 µg of gonadorelin (GnRH; Cevarelin®; im) at the day of fixed-time AI. The TAI of all groups was performed at D10 (AM). A subset of animals (n= 52) was evaluated by US (Mindray®, DP-10) on day of P4d withdrawal and at TAI. The following variables were evaluated: daily growth rate follicle between the day of P4D withdraw (DGRF; mm/day), early ovulation (EO; %) and pregnancy rate (P/AI). Statistical analyses were performed using PROC GLIMMIX of SAS (9.4). No interaction (P= 0.20) between PRI*MUL was observed and data was presented by main effects (P4D exposure period and dosage of reCG). No effect of treatment was observed for DGRF [8D-eCG) 0.91; 7D-reCG) 1.9; 7.5D-1.2reCG) 1.2; 7.5D-1.5reCG) 1.8 mm; P= 0.34], EO [8D-eCG) 32.1% (92/286); 7D-reCG) 34.6% (99/286); 7.5D-1.2reCG) 28.0% (79/282); 7.5D-1.5reCG) 34.0% (92/270); P= 0.88], and P/IA [8D-eCG) 51.4% (147/139); 7D-reCG) 57.3% (164/122); 7.5D-1.2reCG) 51.4% (145/137); 7.5D-1.5reCG) 52.2% (141/129); P= 0.67]. In conclusion, no differences were observed on reproductive efficiency between the conventional eCG and the reCG groups.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Association of a GnRH analogue with estradiol benzoate in early resynchronization of *Bos taurus* heifers

Laura Rohde Brondani[1], Larissa Thaísa Weide[1], Yasmim Rosa de Oliveira[1], Ingrid Leticia Trindade Vieira[1], Camila Rohde Brondani[2], Ana Paula Martini[2], Gilson Antônio Pessoa[2], Daniela dos Santos Brum[1], Fábio Gallas Leivas[1]

[1]Fundação Universidade Federal do Pampa, [2]Universidade Federal de Santa Maria

Early resynchronization is a widely used tool in cows, as it allows two services within a 32-day interval. The use of estradiol benzoate (EB) is well established for D22 resynchronization, however, its use at a 1 mg dose in taurine heifers does not appear to uniformly synchronize the follicular wave. GnRH analogues are also used to synchronize the follicular wave, as they induce ovulation. Thus, the aim of this study was to evaluate the efficacy of Buserelin Acetate associated with EB in D22 resynchronization of the follicular wave in Bos taurus heifers. A total of 463 nulliparous heifers, pubertal with a CL, 14 months old, with an average body condition score of 3.99 ± 0.2 were enrolled in the study. Heifers were resynchronized 22 days after the first FTAI by inserting reused (1x) 1.0 g intravaginal P4-releasing device (Sincrogest®, Ourofino Saúde Animal, São Paulo, Brazil) and randomly assigned to two groups: group 1 (G1; n=231; 1 mg EB IM, Sincrodiol®, Ourofino Saúde Animal, São Paulo, Brazil) and group 2 (G2; n=232; 1 mg EB plus 10 µg Buserelin Acetate IM (Sincroforte®, Ourofino Saúde Animal, São Paulo, Brazil). On Day 30, the P4 device was removed and pregnancy diagnosis was performed by transrectal ultrasonography (US) on all females. Heifers diagnosed as pregnant (n=270; 58.3%) returned to pasture. Non-pregnant heifers (G1=91/193 and G2=102/193) continued in the protocol, receiving 200 IU of eCG (Sincro eCG®, Ourofino Saúde Animal, São Paulo, Brazil), 0.5 mg of Sodium Cloprostenol (Sincrocio®, Ourofino Saúde Animal, São Paulo, Brazil) 1mg of E2 Cypionate IM (SincroCP®, Ourofino Saúde Animal, São Paulo, Brazil) and they had tail-chalk applied on the base of their tailhead for estrus detection. FTAI was performed 48 hours later (D32) using semen with previously known fertility bulls. The largest follicle (LF) diameter was measured by US on D22 (n=463), D30 (n=193) and D32 (n=165). Heifers showing a LF on D30 and disappearance of that in the same ovary on D32 were considered with early ovulation. Pregnancy diagnosis was performed 30 days after FTAI. Data were analyzed using ANOVA with a General Linear Model and Tukey's test for comparisons (significance level 5%). Similar follicular diameter on D22 was found (G1=11.6 mm ± 2.2 vs G2=11.9 mm ± 2.2). On D30 and D32, the follicular diameter was larger in G1 than G2 (G1=13.7 mm ± 2.7 vs G2=12.1 mm ± 2.03; G1=15.1 mm ± 2.6 vs G2=13.4 mm ± 2.1, respectively; p=0.0001). Early ovulation was higher in G1 than G2 (18.8% vs 8.8%; p=0.038). Estrus occurrence was similar between groups (G1=73.2% vs G2=68%; p=0.422). G2 heifers achieved a higher conception rate in the R22 (G2=55.7% vs G1=34.8%; p=0.004). Although follicular diameter were higher when using only EB, this study demonstrated that the use of 10 µg Buserelin Acetate associated with 1 mg EB to initiate the resynchronization protocol (22 days after the previous TAI) improved conception rates in 14 months old Bos taurus heifers.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Bovine maternal appeasing substance (MBAS) mitigates the effects of excitable temperament in timed artificial insemination of nellore cows - preliminary results

Nicolas Moreira Piedras Monnerat Caparelli[1], Samuel Rodrigues Bonamichi do Couto[2], Júlia Medeiros Cruz de Lima Martins[2], Thiago de Souza Vieira[1], Ana Clara Souza Resende de Aguiar[1], Isabella Barbosa Oliveira de Macedo[1], Marco Roberto Bourg Mello[1], Felipe Zandonadi Brandão[2]

[1]Universidade Federal Rural do Rio de Janeiro, [2]Universidade Federal Fluminense

The objective of this study was to evaluate the effects of administering a synthetic analogue of the bovine maternal appeasing substance (mBAS – FerAppease®) on follicular dynamics, conception rate (CR), and pregnancy loss (PL) in Nellore cows submitted to timed artificial insemination (TAI), considering the influence of animal temperament (calm or excitable). Two experiments were conducted in two different farms using a hormonal protocol for ovulation synchronization with three management steps over a 10-day period with a 0.5g intravaginal P4 device (D0) and 300UI of ECG (D8). In Experiment I, 87 cows were allocated to control (CON; n=40) and mBAS (n=47) groups, allocated according to body weight, BCS and days postpartum. The mBAS group received 10 mL of the product on days 0 (start of the protocol) and 10 (day of insemination). Ultrasound B-mode and Doppler imaging were used between D8 – D10 to assess the volume and pixel intensity of the pre-ovulatory follicle (POF), as well as ovulation rate (OR) (D17). Temperament was classified on D0 as calm (CALM; score ≤ 2) or excitable (EXC; score > 2) based on behavioral assessments conducted during animal restraint in the chute and at the time of release, according to the methodology described by Couto et al. (Theriogenology, 192:14–21, 2022). In Experiment II, 597 cows underwent the same treatments (CON, n=292; mBAS, n=305) to evaluate temperament, CR and PL 30 and 60 days after AI. Statistical analyses were performed using generalized linear mixed models (SPSS Statistics, IBM Corp.). The mBAS treatment did not affect POF volume (CON: 1.02 ± 0.4 cm³; mBAS: 1.02 ± 0.5 cm³; p=0.96), but mBAS tended to increase pixel intensity of the POF (1.67 ± 0.9 vs. 1.41 ± 0.7; P=0.07). The OR was greater in mBAS compared to CON (80.8% vs. 62.5%; P=0.03). No differences in follicular volume at the time of TAI or pixel intensity were found between temperament groups (p>0.05). In CALM cows, a trend was observed in OR (mBAS = 74.3% - 26/35 vs. CON = 58.6% - 17/29; p = 0.09), while among EXC cows, OR was higher in the mBAS group (100% -12/12 vs. 72.7% - 8/11; p = 0.02). Overall CR did not differ between treatments (mBAS: 66.9% - 204/305; CON: 63.0% - 184/292; p = 0.16). Among CALM cows, CR was similar (mBAS: 66.5% - 155/233; CON: 66.2% - 147/222; p = 0.47), while in EXC cows, the mBAS group had a higher CR (68.1% - 49/72 vs. 52.8% - 37/70; p = 0.03). In the CON group, EXC cows had lower CR than CALM cows (52.8%; 37/70 vs. 66.2%; 147/222; p = 0.02), a difference not observed in the mBAS group (CALM 66.5% - 155/233 vs. EXC 68.1% - 49/72; p = 0.4). PL was lower in the mBAS group compared to CON (1.47% - 3/204 vs. 4.35% - 8/184; p = 0.04). When considering temperament, PL was similar among CALM cows, but in EXC cows, PL was reduced in the mBAS-treated group (0% vs. 5.26%; p = 0.02). It is concluded that the use of mBAS in Nellore cows submitted to TAI improves reproductive outcomes in excitable females.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of estradiol cypionate dose in the cyclicity induction protocol and the use of prostaglandin F2α on D0 of the FTAI protocol in 14-month-old Nelore heifers

Augusto Rodrigues Felisbino Neto[1], Thiago Alves de Lima[2], Juliana Kátia de Souza[2], Murilo Fregnani Moraes[2], Paulo Vicente Ferreira Junior[3], Emanuele Almeida Gricio[1], Sofía Albertini[1], Rogério Fonseca Guimarães Peres[4], Jose Nélio de Sousa Sales[5], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo, [2]Fazenda Santa Cruz da Serra, [3]Ourofino Saúde Animal, [4]Trilha pecuária inteligente e gestão, [5]Universidade Federal de Lavras

This study evaluated the effects of estradiol cypionate (EC) doses (0.5 vs. 1.0mg) in a cyclicity induction protocol and prostaglandin F2α (PGF) administration on D0 of a FTAI protocol in 842 14-month-old Nelore heifers (mean age=13.9±0.02 months; BCS=2.81±0.01; weight=243.7±0.80 kg; 0.8% CL on D-24) at a farm in Barra do Garças, MT, Brazil. Heifers were assigned to three groups: Control (P4 only, n=283), P4EC0.5 (P4 + 0.5mg EC, n=281), or P4EC1.0 (P4 + 1.0mg EC, n=278). On D-24, all heifers received 150mg P4 (IM; Sincrogest, Ourofino). On D-12, P4EC0.5 and P4EC1.0 groups received 0.5 or 1.0mg of EC (IM; Fertilcare Ovulação, MSD), respectively; the Control group received none. On D0, heifers were randomized to receive (n=425) or not (n=417) 530µg cloprostenol sodium IM (PGF; Estron, Agener), followed by 2mg of EB IM (Fertilcare Sincronização, MSD), and P4 device (Fertilcare 600, MSD). On D7, heifers underwent device removal and received 530µg PGF (IM; Estron), 0.5mg EC (IM; Fertilcare Ovulação), and 200IU eCG (IM; Folligon, MSD). FTAI occurred 48 hours post-device removal. Ultrasound exams assessed the presence of CL (D-24 and D0), pregnancy diagnosis (30 days post-FTAI), and pregnancy loss (final pregnancy check, 170 days). Data were analyzed using PROC GLIMMIX in SAS 9.4. EC-treated groups had higher CL presence on D0 [P4CE0.5=47.7%a (134/281); P4EC1.0=55.4%a (154/278); Control=28.3%b (80/283); P<0.0001] and P/AI [P4EC0.5=48.0%a (135/281); P4EC1.0=46.8%a (130/278); Control=36.0%b (102/283); P=0.01]. Pregnancy loss was similar across groups [P4EC0.5=15.4% (21/136), P4EC1.0=15.4% (20/131), Control=17.6% (18/102); P=0.85]. No treatment*PGF interaction was observed for P/IA (P=0,62). There was treatment*weight interaction for CL presence on D0 (P=0.04). The use of 1.0mg CE improved CL presence in lighter (≤243 kg) heifers [P4EC0.5=38.4%b (56/146), P4EC1.0=54.9%a (79/144), Control=21.0%c (30/143)], while both CE doses were equally effective in heavier (≥244 kg) heifers [P4EC0.5=58.4%a (80/137), P4CE1.0=56.7%a (77/136), Control=37.3%b (53/142)]. Heavier heifers had higher P/AI [47.2% (196/415) vs. 39.9% (173/433); P=0.02]. No treatment*weight interaction was observed for P/AI (P=0.93). PGF on D0 did not affect P/AI [PGF=43.5% (185/425), noPGF=43.6% (182/417); P=0.89], regardless of CL presence [CL: noPGF=50.5% (95/188), PGF=47.6% (89/187); noCL: noPGF=37.7% (88/233), PGF=40.4% (97/240); P=0.44]. Heifers with CL on D0 had higher P/AI [49.1% (184/375) vs. 39.1% (185/473); P=0.02]. EC treatment (0.5 and 1.0mg) enhances cyclicity and P/AI without affecting pregnancy loss. Treatment with 1.0 mg of CE improved CL presence in lighter heifers. These results indicate that EC treatment offers a practical strategy to improve reproductive efficiency in prepubertal Nelore heifers. PGF on D0 provided no additional benefit.

Acknowledgments: Farm Santa Cruz da Serra.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of injectable progesterone at the time of pregnancy diagnosis on pregnancy loss in early Bos indicus heifers submitted to TAI

Laís Reis Carvalho[1], Matheus Pedroso Vicente[1], Rafael Resende Rabelo Silva[1], Lucas Araújo Lemos[1], Thiago Alves de Lima[2], Juliana Kátia de Sousa[2], Roger de Medeiros Maluf Wutke[3], Igor Garcia Motta[4], Bruno Gonzalez de Freitas[4], Jose Nélio de Sousa Sales[1]

[1]Universidade Federal de Lavras, [2]Fazenda Santa Cruz da Serra, [3]Sereno Consultoria, [4]Ourofino Saúde Animal

The objective was to evaluate the use of injectable progesterone (P4i) at the time of pregnancy diagnosis (day 40) on pregnancy loss in early Bos indicus heifers submitted to the ovulation synchronization protocol. In the study, 1042 pregnant Nelore heifers aged 14 months and body condition score of 3.18 ± 0.01 (scale of 1 - 5) were used. All heifers were submitted to the ovulation synchronization protocol based on P4 and E2. Thirty days after TAI (D40), pregnancy diagnosis (PD) was performed and the females considered pregnant were divided into two experimental groups (Control and P4i groups). At the same time, heifers in the P4i group (n = 524) received 300 mg of P4i (Sincrogest Injectable, Ouro Fino, Brazil) and heifers in the Control group (n = 518) did not receive additional treatment. After 30 days, a new PD was performed to evaluate pregnancy loss between 30 and 60 days of pregnancy. Furthermore, body weight was measured at the beginning of the protocol (D0) and at the time of PD (D40). Statistical analyses were performed by GLIMMIX procedure of SAS by logistic regression. The heifers in the control and P4i groups started the protocol with 259.1 kg and 259.0 kg, respectively. At the time of PD (D40), the weight was 296.5 kg (Control) and 296.2 kg (P4i). The pregnancy loss rate was similar between the experimental groups [Control = 11.1% (66/524) and P4i = 10.4 (54/518); P=0.73]. There was no interaction between weight at the beginning of the protocol and treatment at the time of PD (P=0.32). However, in early heifers weighing less than 250 kg there was a tendency towards greater pregnancy loss in the control group (Control = 11.6% and P4i = 6.7%; P=0.10). In heifers weighing more than 250 kg, no difference in pregnancy loss was observed between the experimental groups (Control = 10.9% and P4i = 12.1%; P = 0.62). It is concluded that the use of P4i at the time of pregnancy diagnosis did not reduce pregnancy loss in early Bos indicus heifers submitted to the ovulation synchronization protocol.

Support: OuroFino, Sereno Consultoria, Santa Cruz da Serra and Cifrão farms.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of Injectable Trace Mineral Supplementation on the Fertility of 12-Month-Old Nelore Heifers Undergoing Cyclicity Induction

José Luiz Severo da Silva[1], Guilherme Felipe dos Santos, Marcello Guadagnini[2], Edjalma Rodrigues da Silva Junior[1], David Giraldo Arana[3], Guilherme de Paula Nogueira[1]

[1]Universidade Estadual Paulista, [2]Axiota Animal Health, [3]Fazenda Água Limpa

This study aimed to evaluate the effect of injectable trace mineral supplementation (Zn, Cu, Mn, and Se; Multimin® 90) on the fertility of prepubertal Nellore heifers subjected to estrous cyclicity induction and fixed-time artificial insemination (FTAI). A total of 224 Nellore heifers, 12 months old with an average body weight of 267 ± 22.1 kg, were maintained on Brachiaria brizantha pasture and supplemented with a protein-energy diet (8 kg/animal/day; 25% CP and 64% TDN), along with a commercial mineral premix (Núcleo Campo BI; Premix®). Twelve days before FTAI (D–12), animals were randomly assigned to two groups: MI+ (n = 113), receiving 1 mL/50 kg BW of Multimin® 90 subcutaneously, and MI– (n = 111), without supplementation. Both groups received 150 mg of injectable progesterone (P4i) and doramectin-based endectocide on D–12. On D0, a progesterone intravaginal device (0.5 g) was inserted, and 1 mg of estradiol benzoate (EB) was administered. Body weight, rump height, CPM scores (conformation, precocity, muscling), and blood samples (5 mL) for progesterone analysis were collected, and all animals received a reproductive vaccine (CattleMaster®). On D7, the device was removed and 1 mg of estradiol cypionate (EC), 0.52 mg of PGF2α, and 100 IU of equine chorionic gonadotropin (eCG) were administered. FTAI was performed on D9 using semen from 10 randomly assigned bulls. Statistical analysis was conducted using Jamovi® (v2.6.0). Shapiro-Wilk test assessed normality. Parametric data were analyzed by Student's t-test, nonparametric by Mann-Whitney U, and categorical logistic regression. A multiple logistic regression model with fixed effects for body weight, rump height, and bull was used to estimate pregnancy odds. Heifers in MI+ had 2.09 times greater odds of conception than those in MI– (OR = 2.09; 95% CI: 1.08–4.07), even after adjustment. On D42, conception rate was significantly higher in MI+ (31.9%; 36/113) than in MI– (19.8%; 22/111; p = 0.04). Progesterone levels on D0 did not differ between groups (p > 0.05) but were lower in pregnant (0.95 ± 0.59 ng/mL) vs. non-pregnant (1.48 ± 1.40 ng/mL; p = 0.01) heifers. Rump height was also associated with fertility: pregnant heifers averaged 130 ± 3.8 cm vs. 132 ± 2.9 cm in non-pregnant (p < 0.05). In conclusion, supplementation with injectable trace minerals prior to cyclicity induction improved conception in prepubertal Nellore heifers and may benefit reproductive performance in young beef females.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of PGF2α administration (D0) and protocol length (7 vs 8 days) in 13-15 months Bos Indicus Nelore Heifers

Igor Garcia Motta[1], Emanuele Almeida Gricio[2], Bruno Silva do Espirito Santo[3], Andressa Rodrigues[3], Helio Costa Silva[3], Bruno Gonzalez de Freitas[1], Pietro Sampaio Baruselli[2]

[1]Ourofino Saúde Animal, [2]Animal Reproduction Department, University of São Paulo, [3]Agropecuária Jacarezinho

The experiment assessed PGF2α (PGF) administration on Day 0 (D0) of a fixed-time artificial insemination (TAI) protocol, as well as the impact of intravaginal progesterone device (P4D) permanence for 7 or 8 days. A total of 602 Nelore (Bos indicus) heifers, with 13 to 15 months, BW of 312.6±1.22 kg, BCS 3.4±0.02 on D-24, and average daily gain (ADG) 1.01±0.04 kg/day (D-24 to D0), from a commercial farm in Bahia, Brazil (Agropecuária Jacarezinho), were enrolled. On D-24, all heifers received a third-use intravaginal P4D (Sincrogest, Ourofino). On D-12, 0.5mg of estradiol cypionate (EC; SincroCP, Ourofino) was administered. On D0, heifers were allocated based on cyclicity to one of four experimental groups in a 2×2 factorial design: 1)PGF-8D (n=156), 2)Cont-8D (n=153), 3)PGF-7D (n=147), and 4)Cont-7D (n=150). To ensure synchronization of TAI (D10), heifers in the 8D groups received treatment on D0, while those in the 7D groups on D1. Heifers assigned to the Control groups received a new intravaginal P4D (1g; Sincrogest, Ourofino) and 2mg of estradiol benzoate (Sincrodiol, Ourofino), those in the PGF groups additionally received 0.53mg of sodium cloprostenol (PGF; Sincrocio, Ourofino). On D8, all heifers received 0,5mg of EC, 0.53mg of PGF, and 200IU of eCG (SincroeCG, Ourofino), concomitant with P4D removal. TAI was performed 48h later (D10) in all groups. Ultrasound examinations were performed to assess the presence of CL on D0, pregnancy rate (P/IA) on D40, D70, and pregnancy loss (PL). In a subset (n=213), the largest follicle (LF) diameter was measured at P4 removal and at D10. Premature ovulation (PO) was defined as the absence of the previously identified LF. Statistical analyses were performed using LOGISTIC procedure for logistic regression to model probability and GLIMMIX SAS 9.4. LF diameter at P4 removal was greater in the PGF group [PGF=10.6±0.1 vs. Cont=9.8±0.1mm; P=0.001] and larger in the 8D than the 7D groups [7D=9.9±0.1 vs. 8D=10.6±0.1mm; P=0.009], with no PGF*Length interaction. A linear effect (P=0.01) was observed between LF diameter and PO probability, with a more pronounced curve for PGF heifers, consistent with the higher PO incidence in this group [PGF=29.6% (32/108); Cont=13.3% (14/105); P=0.005]. Moreover, a significant ADG*PO linear effect was observed (P=0.03). In PGF heifers, higher ADG significantly increased the risk of PO, whereas in Control heifers, it was associated with a lower probability. P/AI was negatively affected by PGF treatment [PGF=33.9% (103/301); Cont=42.2% (127/301); P=0.03], but not by protocol length [7D=38.4 % (113/294); 8D=37.7% (116/308); P=0.91]. No effects were observed on P/IA on D70 (P=0.16) or PL (P=0.30). In conclusion, PGF administration on D0 increased the risk of PO and decreased P/IA in confined Nelore heifers with an ADG of 1 kg/day. A positive correlation was observed between LF diameter and PO probability across all groups, and higher ADG enhanced PO risk only in PGF-treated heifers.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of pre-synchronization with injectable progesterone on ovarian morphofunctional parameters in Nelore females undergoing FTAI

Diane Duarte Dantas[1], Wendel Denian Silva dos Santos[2], Maria Antonia Moreira Maciel[2], Raquel Gomes do Amaral[2], Artur Azevedo Menezes[1], Mariana Fernandes Souza[1], Rafaella Sanches de Jesus[1], Tiago Oliveira Brandão[2], Marcos Chalhoub Coelho Lima[1], Alexandra Soares Rodrigues[2]

[1]Universidade Federal da Bahia, [2]Universidade Federal do Oeste da Bahia

The purpose of this study was to evaluate the effect of pre-synchronization with injectable progesterone (P4) on the morphofunctional follicular and luteal parameters of acyclic and lactating Nelore females submitted to an FTAI program. For this, 42 females were randomly distributed into two groups on day -10 (D-10): Group P4i (n=23), which received 150 mg of long-acting P4 (Sincrogest®2, Ourofino, São Paulo, Brazil), and control group (n=19), treated with 1 mL of saline solution, both IM. On D0, all animals received an intravaginal device containing 1.0 g of P4 (Sincrogest®, Ourofino, São Paulo, Brazil), associated with the application of 2.0 mg of E2 benzoate IM (Sincrodiol®, Ourofino, São Paulo, Brazil). On D8, the device was removed and 500 µg of sodium cloprostenol (Sincrocio®, Ourofino, São Paulo, Brazil), 300 IU of equine chorionic gonadotropin (Sincro eCG®, Ourofino, São Paulo, Brazil) and 1 mg of E2 cypionate were administered, all IM. Assessments of the diameter of the pre-ovulatory follicle (DFOL), the total area of the pre-ovulatory follicle wall (AFOL), the area of vascularization of the pre-ovulatory follicle wall (VFOL) and the percentage of vascularization in the area of the pre-ovulatory follicle wall (%VFOL) were performed on D10, by B-mode ultrasonography and color Doppler (Sonoscape S2VET®, SonoScape, Goiânia, Brazil), before of artificial insemination itself. On D23, luteal structures were evaluated, including the diameter of the corpus luteum (DCL), the total area of the corpus luteum (ACL), the area of vascularization of the corpus luteum (VCL) and the percentage of vascularization of the corpus luteum of the corpus luteum (%VCL). Statistical analysis was conducted using SPSS software (version 19), considering a significance level of 5%. The results showed no significant differences between groups for any of the evaluated parameters ($p > 0.05$). The mean values for follicular parameters were: DFOL (CON = 1.08 ± 0.20 cm; P4i = 1.05 ± 0.20 cm), AFOL (CON = 0.26 ± 0.08 cm²; P4i = 0.24 ± 0.09 cm²), VFOL (CON = 0.10 ± 0.04 cm²; P4i = 0.09 ± 0.04 cm²) and %VFOL (CON = 40.21 ± 19.66%; P4i = 44.54 ± 21.87%). Regarding luteal parameters, the mean values were: DCL (CON = 1.68 ± 0.29 cm; P4i = 1.64 ± 0.26 cm), ACL (CON = 1.77 ± 0.60 cm²; P4i = 1.96 ± 0.68 cm²), VCL (CON = 0.34 ± 0.14 cm²; P4i = 0.34 ± 0.15 cm²) and %VCL (CON = 20.16 ± 6.83%; P4i = 18.83 ± 8.44%). These findings reinforce that the follicular and luteal morphofunctional parameters evaluated in this study were not influenced by pre-synchronization with injectable P4 prior to the beginning of the FTAI protocol.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of pre-synchronization with long-acting injectable progesterone on Day -7 of E2-P4 TAI protocol in Holstein cows

Pietro Sampaio Baruselli[1], Emanuele Almeida Gricio[1], Tiago O Carneiro[2], Gabriel Matos do Couto[2], Bruna Lima Chechin Catussi[3], Igor Garcia Motta[4], Bruno Gonzalez de Freitas[4]

[1]Animal Reproduction Department, University of São Paulo, [2]Fazenda São José, [3]Universidade de São Paulo, [4]Ourofino Saúde Animal

This study evaluated the effects of pre-synchronization using 300mg of long-acting injectable progesterone (iP4) seven days before the TAI protocol (D-7) on reproductive parameters in lactating Holstein cows. A total of 822 cows (416 multiparous and 406 primiparous) producing 40.1±0.3 L of milk and 61.6±0.1 days in milk were used. On D-7, cows were randomly assigned to one of two groups: iP4 (n=416), receiving 300mg of iP4 (Sincrogest Injetável, Ourofino), or Control (n=406), receiving no treatment. On D0, all cows received an intravaginal P4 device (Sincrogest, Ourofino), 2mg EB (Sincrodiol, Ourofino), and 10µg of buserelin (Sincroforte, Ourofino). On D7 and D8, cows received 0.53mg cloprostenol (PGF; Sincrocio, Ourofino). On D8, 1mg of EC (SincroCP, Ourofino) was administered with P4 device removal, followed by TAI 48 hours later (D10). Ultrasound examinations were performed on all animals to assess the presence of CL on D-7 and D0, ovulation rate (OR) at the end of the protocol on D17 (presence of CL), pregnancy rate on D42, and on D64. Additionally, in a subset of cows (n=233), the largest follicle (LF) diameter on D0 and OR in response to GnRH administered on D0 (presence of a new CL on D7) were evaluated. Statistical analyses were conducted using PROC GLIMMIX of SAS 9.4. The data is presented as main effects when no iP4*Category interaction was found. An interaction iP4*CL D-7 was observed for LF diameter on D0. In the iP4 group, cows without CL on D-7 had a larger LF compared to those with CL. In the control group, no differences were found according to CL status [iP4-NoCL=15.6±1.0mma; iP4-CL=13.5±0.4mmb; Cont-NoCL=13.7±0.8mmab; Cont-CL=14.5±0.3mmab; P=0.02]. A similar interaction was detected for OR after GnRH treatment on D0. Cows without CL on D-7 from the iP4 group exhibited a greater OR compared to those with CL, no differences were observed in the control group [iP4-NoCL=77.3%a (17/22); IP4-CL=44.3%b (35/79); Cont-NoCL=55.6%ab (10/18); Cont-CL=57.1%ab (48/84); P=0.04]. The OR on D17 was similar between groups [iP4-NoCL=83.6% (51/61); iP4-CL=85.3% (266/612); Cont-NoCL=76.3% (45/59); Cont-CL=86.8% (262/302); P=0.25]. Moreover, a tendency for interaction iP4*CL D-7 for P/AI at 30 days was observed [iP4-NoCL=41.6% (25/60); iP4-CL=30.0% (93/310); Cont-NoCL=28.1% (16/57); Cont-CL=30.9% (93/301); P=0.06]. Additionally, there was a significant interaction iP4*CL D-7 for P/IA on D64 [iP4-NoCL=40.0% (24/60); iP4-CL=27.5% (85/309); Cont-NoCL=24.6% (14/57); Cont-CL=28.7% (86/300); P=0.04]. Pregnancy loss did not differ between treatments (P=0.31). In conclusion, cows without CL receiving iP4 on D-7 had a larger LF compared to cows with CL treated with iP4. The administration of iP4 on D-7 increased OR in response to GnRH in Holstein cows without CL compared to cows with CL treated with iP4. The iP4 treatment tended to improve P/IA at 30 days and significantly increased the P/IA at 60 days in cows without CL.

Acknowledgments: Fazenda Bela Vista.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of proestrus length on pregnancy rate in 15-month-old beef heifers subjected to FTAI

Adrian Vater[1], Matias Malinarich[1], Lucio Corbera[1], Marcelo Rodriguez[2]

[1]IA TOTAL GROUP, [2]Universidad Nacional del Centro de la Provincia de Buenos Aires

The objective of this study was to evaluate two different synchronization protocols with different proestrus length and the use of ECP in 15 months-old beef heifers. A total of 1152 Aberdeen Angus (Bos taurus) females from a commercial farm located in B. Juarez, Buenos Aires, Argentine were used. Ovarian structure (OS) was evaluated using US determining that 68% of the heifers had a CL. On Day 0 (D0), all heifers received an intravaginal device containing 0.6 g of progesterone (P4) and 2 mg of estradiol benzoate administered intramuscularly (im). On Day 6, animals were randomly assigned to one of two treatment groups. In the JSYNCH group (n = 576), the device was removed on D6, and heifers received 0.15 mg of cloprostenol sodium and 300 IU of eCG im. At 60 hours after device removal, estrus detection was performed: heifers showing estrus were inseminated at that time, while those not showing estrus received 10 µg of GnRH and were inseminated 12 hours later. In the CONV group (n = 576), the device was removed on D7, followed by administration of 0.15 mg of cloprostenol sodium, 0.5 mg of estradiol cypionate, and 300 IU of eCG, all via im injection. Forty-eight hours after device removal, non-estrous heifers received 10 µg of GnRH, and all animals were inseminated at 54 hours, regardless of estrous expression. Estrous was detected in 78% (447/576) and 71% (411/576) of the heifers in CONV and JSYNCH groups, respectively. A logistic regression model was fitted to compare the Pregnancy Rate (PR) between treatment (CONV vs. JSYNCH), between heifers showing oestrous or not after device removal. Pregnancy diagnostic was made by US on day 32 post TAI. The PR was 52% (297/576) for the CONV group and 65% (372/576) for JSYNCH group (P<0,005). Comparing heifers with and without estrous, the PR was 52% (231/447) and 51% (66/129) for the CONV group, and 67% (274/411) and 59% (98/165) for the JSYNCH group respectively. The JSYNCH group with ESTROUS (67%) showed a significant difference in PR compared to all other groups (p<0,005), while no significant difference was found among the other groups. The JSYNCH protocol increase the PR in TAI in 15 months-old beef heifers, which aligns with our expectation of better results in heifers exhibiting estrous at the time of TAI compared to those not showing oestrus and those in the CONV protocol.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effect of timed-AI progesterone device length (6d vs 7d) and body condition change (from AI to pregnancy diagnosis) on fertility of Nelore cows

Rafaela Schoma Cardoso[1], Federico Randi[2], Elikismei Denilson Araujo Kuiava[3], Rafael Moreira[2], Alexandre Hênryli de Souza[2], Fábio Morotti[1,4], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina, [2]Ceva Saúde Animal, [3]GestPec - Consultoria e Gestão Pecuária, [4]Universidade Pitágoras Unopar Anhanguera

This study evaluated the impact of intravaginal progesterone device duration (6 vs. 7 days) on pregnancy per artificial insemination (P/AI) in Nelore (Bos indicus) cows subjected to timed artificial insemination (TAI). Additionally, the influence of body condition score (BCS, scale 1–5) at TAI and pregnancy diagnosis, as well as BCS changes between these time points, on fertility was assessed. A total of 547 (primiparous = 157, multiparous = 390) postpartum cows from two commercial herds in Mato Grosso-Brazil were randomly allocated to one of two groups: seven-day progesterone device (7P9AI, n = 272) or six-day progesterone device (6D8AI, n = 275). All animals received a synchronization protocol consisting of an intravaginal progesterone device (Prociclar 750mg, CEVA) and 2mg estradiol benzoate (Benzoato-HC, CEVA) on D0, followed by device removal and administration of PGF2α (Luteglan, CEVA), estradiol cypionate (Cipionato-HC, CEVA), and eCG (Folirec 84 IU multiparous/105 IU primiparous, CEVA) either on D6 or D7, with GnRH (Cevarelin, CEVA) and TAI performed respectively on D8 (6D8AI) or D9 (7D9AI). Estrus expression was assessed with the use of a tail-adhesive patch (Estrus Alert) placed at device removal, and classified at (E1 = no scratch, E2 = near 50% scratch or E3 = greater than 75% scratched devices). Pregnancy diagnosis was performed by ultrasound 30 days after insemination. Body condition score were recorded at the time of device insert (BCS-AI) and again at pregnancy diagnosis (BCS-Preg). Based on these two BCS measurements, cows were then classified as losing (BCS-L, n = 105 - 47 primiparous and 58 multiparous), maintaining (BCS-M, n = 268 - 82 primiparous and 186 multiparous) or gaining (BCS-G, n = 174 - 28 primiparous and 146 multiparous) BCS from AI to pregnancy diagnosis. Overall, there was no significant impact of intravaginal progesterone device length on estrus expression (7D9AI = 68.0% vs 6D9AI = 65.8%; P = 0.82) and P/AI (7D9AI = 42.1% vs 6D9AI = 42.0%; P = 0.98). In addition, cows showing estrus had greater P/AI (E1 = 33.0%, E2 = 40.7%, E3 = 53.1%, P < 0.01); but there were no significant impacts of sire used, AI technician or even interactions between P4 treatment length and category (multiparous vs primiparous) and herd in terms of P/AI results. In contrast, BCS-AI, BCS-Preg and BCS change between AI and Pregnancy diagnosis all had significant effects on fertility, but again no interactions with P4 length of the timed-AI protocol. As expected, body condition losing had a major impact on P/AI (BCS-L = 33.1%, BCS-M = 45.2%, BCS-G = 48.4%, P = 0.03). In conclusion, duration of intravaginal progesterone device (6 vs. 7 days) had minimal impact on fertility in Nelore cows undergoing TAI, allowing flexibility in protocol design. However, BCS and its losses from TAI to pregnancy diagnosis significantly influenced P/AI, emphasizing the importance of monitoring and mitigating BCS loss to maximize reproductive success in beef cattle.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effects of a Novel Cloprostenol–Lecirelin Combination (Promov) on Ovulation and Pregnancy in Beef Cows

Luiz Francisco Machado Pfeifer[1], Cintia Batista Hurtado[2], Vanessa Lemos de Souza[2], Samira Alves de Souza Silva[3], Lucas Silva Gomes[1], Renata Reis da Silva[1], Monique Tomazele Rovani[4], Ingrid Pedraça Barbosa[2], Bernardo Gasperin[3]

[1]Empresa Brasileira de Pesquisa Agropecuária - Rondônia, [2]Universidade Federal do Pará, [3]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [4]Universidade Federal do Rio Grande do Sul

A series of 5 experiments were performed to validate a novel hormonal combination used to induce ovulation in cattle. Exp. 1: Nelore cows (n = 25) had their ovulation synchronized by a TAI protocol based on estradiol benzoate (EB), progesterone (P4), cloprostenol, estradiol cypionate (EC), and GnRH. Six days after ovulation detection, 500 µg of cloprostenol sodium (CS) was administered to all cows. Thirty-four hours later, the cows were allocated into two groups, in a cross-over design, to receive: 25 µg of Lecirelin (GnRH, n = 25) or 25 µg of Lecirelin + 500 µg of CS (Promov, n = 25). Ultrasound was performed every 6 hours to detect ovulation. There were no differences (P > 0.05) on the time of ovulation and ovulation rate between the groups. Exp. 2: Nelore cows (n = 19) received the same treatment as in Exp 1. GnRH group (n = 9) and Promov group (n = 10). Twenty hours after treatment, follicular aspiration was performed in all cows for analysis of intrafollicular (IF) concentrations of E2, P4 and PGFM and analysis of expression of genes involved in steroidogenesis and ovulation in granulosa cells. Cows treated with Promov had an increase (P = 0.05) in PTGS gene expression. The relative mRNA abundance of CYP19A1, HSD3B1, PGR, and STAR did not differ (P > 0.05) between the groups. Cows treated with GnRH had higher (P = 0.04) IF E2 concentration than cows treated with Promov. Exp. 3: Nelore cows (n = 17) received a TAI protocol based on E2, P4, and PGF. Thirty-four hours after the removal of the intravaginal P4 device (IPD), the cows received: GnRH (n = 17) or Promov (n = 17). The experiment was performed in a cross-over design. There were no differences (P > 0.05) between the groups on the time of ovulation, and ovulation rate. Exp. 4: Postpartum Nelore cows (n = 263) were treated as described in E3 and distributed into 2 groups to receive: GnRH (n = 134) or Promov (n = 129), 34 hours after IPD removal. Timed AI was performed 48 h after the IPD removal. Cows that received Promov tended (P = 0.1) to have greater P/IA (60.5%) than cows treated with GnRH (50.7%). Exp. 5: Postpartum Nelore cows (n = 695) were treated as in E3 except that at the time of IPD removal, all cows received 1 mg of EC. At TAI (48 h after IPD), cows that were detected in estrus were considered as the control group (CTL+, n = 337). Cows that did not express estrus received: GnRH (n = 173) or Promov (n = 185). The P/IA of cows that showed estrus (61.1%) was higher (P < 0.05) than cows treated with GnRH (45.1%). However, the P/IA of cows in the CTL+ group and the Promov group (54.0%) did not differ (P > 0.05). Cows treated with Promov tended (P = 0.07) to have higher P/IA than cows treated with GnRH. In conclusion, Promov affected intrafollicular gene expression (PTGS) and steroid concentrations (E2), suggesting it may affect the ovulation process. Moreover, cows subjected to TAI protocols treated with Promovtend to have greater P/AI than cows treated with GnRH.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effects of Ovulation Induction Protocols and Protected Fat Supplementation on Reproductive Performance in Yearling Nelore Heifers

Thiago Neder Lisboa[1], Gabryel Henrique Cavelani Moura[2], Pedro Henrique Déo[2], Pedro Vinícius Souza Alves[2], Osvaldo Alex de Sousa[3], Bruno Pietsch Cunha Mendonça[3], Igor Garcia Motta[4], Bruno Gonzalez de Freitas[4], Isabella Rio Feltrin[1], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]Fazenda Nova Piratininga, [3]Nutricorp, [4]Ourofino Saúde Animal

This study evaluated the effects of hormonal induction protocols and omega-6-rich protected fat supplementation on the reproductive performance of yearling Nelore heifers undergoing timed artificial insemination (TAI). A total of 1,600 heifers (13–18 mo; 287.5 ± 46 kg) from a commercial farm (Nova Piratininga, São Miguel do Araguaia, GO, Brazil) were maintained in a feedlot system. A 2×2×2 factorial design was used with three factors: (1) number of iP4 inductions (1 vs. 2), (2) estradiol cypionate (EC; with vs. without), and (3) supplementation with protected fat (Nutri Gordura Reprodução®, Nutricorp, Brazil [NG]; with vs. without). Day 0 (D0) marked the start of the TAI synchronization protocol. On D−36 and D0, animals were weighed (BW), evaluated for body condition score (BCS; 1–5), and reproductive tract scores (uterus and ovaries; 1–5) by palpation and ultrasonography. On D−36, heifers in the two-iP4 group (n=800) received 150 mg intramuscular (IM) iP4 (Sincorgest injetável®, Ourofino). On D−24, all animals received 150 mg iP4. On D−12, EC-treated heifers (n=800) received 1 mg EC IM (SincroCP®, Ourofino). NG-treated animals (n=800) received 100 g/head/day from D−35 to D70. At FTAI (D10), a subset (n=560) was assessed for pre-ovulatory follicle . Pregnancy diagnosis occurred on D38 and D70. Data were analyzed using PROC MIXED and GLIMMIX of SAS software. NG supplementation increased (P<0.01) BW (344±0.8 vs. 333±0.8 kg), BCS (3.33±0.008 vs. 3.29±0.008), and ADG (1.6±0.01 vs. 1.3±0.01 kg/d). The EC treatment increased (P<0.01) the proportion of heifers with a corpus luteum (CL) on D0 and the percentage of pubertal heifers (presence of CL or dominant follicle plus uterus score ≥4), with a trend (P=0.08) indicating this effect was more pronounced in heifers receiving a single iP4 dose. A trend (P=0.06) suggested that NG enhanced proportion of CL on D0 only in animals receiving two iP4 doses. Treatments with two iP4 doses, EC, and NG also improved uterine development (P<0.05). Pre-ovulatory follicle (11.9mm) was unaffected (P>0.1) by treatments. A significant iP4 × EC interaction (P=0.02) was observed for conception rates on D28 and D70: EC improved conception in heifers with one iP4 (**EC:** 62% [246/398] and 57% [227/398] vs. **No EC:** 54% [215/396] and 48% [192/396]), but not in those receiving two (**EC:** 58% [231/398] and 53% [211/398] vs. **No EC:** 61% [246/400] and 54% [216/400]). The pregnancy loss was only affected by EC treatment (EC: 7.3% [35/473] vs. No EC: 11.4% [53/461]; P=0.03). Conclusion: NG supplementation improved BW, BCS, and uterine development but not conception rates. Uterine development prior to FTAI was enhanced by both two iP4 administrations and EC treatment, while EC also improved ovulatory response. However, conception rates were maximized either with two iP4 treatments or when EC was given in heifers receiving only one iP4 dose and pregnancy loss was reduced by EC.

Acknowledgments: Nutricorp and Ourofino Saúde Animal.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Effects of processing method (frozen conventional, frozen sexed, and liquid sexed semen) and semen pooling (three sires) on pregnancy rates in Nelore cows undergoing FTAI

Sofía Albertini[1], Emanuele Almeida Gricio[1], Laís Ângelo de Abreu[1], Carlos Pacheco[2], Gabriel Armond Crepaldi[3], Sthefano Panazzolo[3], Evanil Pires de Campos Filho[3], Pablo Ross[4], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo, [2]Fazenda Pureza, [3]Sexing Technologies do Brasil, [4]STgenetics

This study evaluated the impact of using liquid sexed semen (LSS) and a semen pool (equal ratios of ejaculates from three Nelore sires) to improve reproductive efficiency in Nelore cows undergoing fixed-time artificial insemination (FTAI). Two experiments were conducted on a commercial farm in Santa Rita do Pardo, MS, Brazil. In Experiment 1, 310 Nelore cows (167 primiparous, 73 secundiparous, 70 multiparous) underwent FTAI with LSS processed 48 hours prior to insemination (LSS48). Semen (three individual sires or pool) was allocated based on parity. In Experiment 2, 415 multiparous Nelore cows were inseminated with the same semen (three individual sires or pool) and semen batch used in Experiment 1, processed as frozen conventional semen (FCS), frozen sexed semen (FSS), or LSS processed 72 hours prior to insemination (LSS72), with semen randomly assigned. Pregnancy diagnosis was performed via ultrasound 32 days post-FTAI. Data were analyzed using PROC GLIMMIX in SAS 9.4. In Experiment 1, no effects of parity (P = 0.11), sire (P = 0.36), or their interaction (P = 0.59) were observed for pregnancy per AI (P/AI). P/AI was: primiparous (51.5%, 86/167), secundiparous (48.0%, 35/73), and multiparous (64.3%, 45/70); Sire 1 (49.4%, 38/77), Sire 2 (64.9%, 50/77), Sire 3 (46.2%, 36/78), and semen pool (53.9%, 42/78). In Experiment 2, no sire (P = 0.11) or sire*processing method interaction (P = 0.33) was detected on P/AI. P/AI was: Sire 1 (48.1%, 50/104), Sire 2 (64.4%, 67/104), Sire 3 (55.8%, 58/104), and semen pool (57.3%, 59/103). The processing method significantly affected P/AI. Cows inseminated with FCS (66.2%, 90/136) and LSS72 (61.4%, 86/140) had higher P/AI than those with FSS (41.7%, 58/139; P = 0.0001). Given the absence of significant interactions between sire and parity or processing method in previous models, an exploratory analysis was conducted, pooling data from both experiments to assess sire effects on P/AI, with parity and semen processing method included as random effects. The semen pool resulted in intermediate P/AI values and mitigated the negative impact of the lowest-performing sire [Sire 1 = 48.6% (88/181)^{B}; Sire 2 = 64.6% (117/181)^{A}; Sire 3 = 51.7% (94/182) ^{AB}; Semen pool = 55.8% (101/181)^{AB}; P = 0.01]. These findings demonstrated that LSS72 achieves pregnancy rates comparable to FCS in Nelore cows. Additionally, semen pooling showed promise in buffering individual sire variability under field conditions. Together, these strategies may help optimize reproductive efficiency and genetic gain in beef cow-calf operations.

Acknowledgments: Sexing Technologies do Brasil, Fazenda Pureza.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Efficacy of Recombinant Equine Chorionic Gonadotropin in 7- or 8-Day Progesterone Device Protocols for Fixed-Time Artificial Insemination in Postpartum Nelore Cows

Tiago de Oliveira Carneiro[1], João Bino Wellington Luiz Alves de Souza[2], Fernanda Nunes Marqui[3], Ricardo Montalli[4], Rafael Moreira[5], Federico Randi[5], Alexandre Hênryli de Souza[5]

[1]Fazenda Bela Vista, [2]Fazenda Nelore Bino, [3]Universidade Estadual Paulista, [4]Arroba Genética, [5]Ceva Saúde Animal

This study assessed the efficacy of recombinant equine chorionic gonadotropin (reCG; Foli-Rec®, CEVA) in fixed-time artificial insemination (TAI) protocols with 7-day (D7) or 8-day (D8) intravaginal progesterone device durations in postpartum Bos indicus Nelore cows (n = 780). Conducted across two herds in São Paulo and one in Mato Grosso do Sul, Brazil, the trial involved primiparous and multiparous cows maintained on Brachiaria grazing systems with a mean body condition score (BCS) of 2.91 (1–5 scale). On day 0 (D0), cows received a 0.75-g progesterone device (Prociclar®, CEVA) and 2 mg estradiol benzoate (Benzoato HC®, CEVA). Cows were randomly assigned to device removal on D7 (n = 389) or D8 (n = 391). At device removal, cows received 1 mg estradiol cypionate (Cipionato HC®, CEVA), 150 µg D-cloprostenol, and reCG (105 IU [1.5 mL] for primiparous or 84 IU [1.2 mL] for multiparous cows). TAI and 100 µg gonadorelin administration occurred 48 hours post-device removal, using semen from 20 sires balanced across groups. Ovarian ultrasonography was performed on a subset of cows on D0, D7/D8, at TAI, and on D30 for pregnancy diagnosis. Data were analyzed using PROC GLIMMIX in SAS 9.4. Larger dominant follicles (DF) were observed at device removal and TAI in the D8 group ($P < 0.05$), but no further significant differences occurred in DF growth rate, premature ovulation, ovulation rate, double-ovulations or corpus luteum between groups. Pregnancy per AI (P/AI) was comparable between D7 (53.7%) and D8 (50.8%) groups ($P = 0.68$), with no interactions involving parity, BCS, or herd ($P > 0.10$). Sire and BCS at TAI significantly influenced fertility ($P < 0.05$). These findings indicate that reCG supports consistent fertility outcomes in TAI protocols with either 7- or 8-day progesterone device durations, offering flexibility for commercial Bos indicus beef herds.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Efficacy of Recombinant Equine Chorionic Gonadotropin in Fixed-Time Artificial Insemination Protocols for Bos indicus × Bos taurus Dairy Cows.

Rodrigo S. Belli[1], Thiago N. Marcantonio[1], Tiago de Oliveira Carneiro[2], Rafael Moreira[3], Federico Randi[3], Alexandre Hênryli de Souza[3]

[1]Fazenda Canto Porto, [2]Fazenda Bela Vista, [3]Ceva Saúde Animal

This study evaluated the efficacy of recombinant equine chorionic gonadotropin (reCG, Foli-Rec®, CEVA) at two doses compared to an untreated control in fixed-time artificial insemination (TAI) protocols for Bos indicus × Bos taurus dairy cows. Conducted in São Paulo, Brazil, the trial involved 590 lactating cows housed in compost barns, producing on average 39.1 Kg/day, comprising 391 pre-synchronized for first postpartum AI and 199 resynchronized cows. On day 0 (D0), cows were enrolled in a 2 × 3 factorial design, receiving an intravaginal progesterone device (0.75 g, Prociclar®, CEVA, n=323; or 2 g, ReproSync®, Globalgen, n=267), 2 mg estradiol benzoate (Benzoato HC®, CEVA), and 200 µg gonadorelin (Cevarelin®, CEVA). On day 7 (D7), devices were removed, and cows received 1 mg estradiol cypionate (Cipionato HC®, CEVA) and 150 µg D-cloprostenol. Concurrently, cows were assigned to one of three treatments: control (untreated, n=189), 105 IU reCG (1.5 mL, n=205), or 140 IU reCG (2 mL, n=196). On day 8 (D8), a second 150 µg D-cloprostenol dose was administered, followed by TAI and 100 µg gonadorelin on day 9 (D9). Ovarian ultrasonography was performed on a subgroup of cows (n=249) on D0, D7, D9, and D16, and on all cows on D30 and D60 post AI. Data was analyzed using PROC GLIMMIX in SAS 9.4. There was no effect of reCG or device type on the diameter of the dominant follicle 48h after device removal, ovulation rate, incidence of double ovulations or premature ovulations. In contrast, CL diameter was greater (P=0.04) for cows receiving 2 ml of reCG (24.5 mm) compared to 1.5 reCG (23.8 mm) and control (23.3 mm). Progesterone device type did not affect estrus expression, ovulation rate, or pregnancy per AI at 30 days (P/AI; P=0.94; Prociclar: 39.1%, ReproSync: 39.4%), with no interactions involving treatment, category, body condition score, corpus luteum presence on D0, breeding order, or milk yield (P>0.10). Both reCG doses significantly improved P/AI (1.5 mL: 43.6%; 2 mL: 45.9%) compared to the control (30.1%; P=0.03), with no dose-dependent differences or interactions with progesterone device type or other variables. Pregnancy loss between D30 and D60 was unaffected by treatment. These results demonstrate that reCG at either dose enhances P/AI compared to untreated controls, independent of progesterone device type, supporting its utility in TAI protocols for crossbred dairy cows.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Estradiol esters pharmacokinetics in a bovine waveless model

Túlio Vinicius Arruda Silva[1], Aline Silva Mendes Teixeira[1], Sara Adna Santos de Oliveira[2], Luiz Gustavo Bruno Siqueira[3], Ricardo Alamino Figueiredo[4], João Henrique Moreira Viana[4]

[1]Universidade de Brasília, [2]Universidade Federal de Uberlândia, [3]Empresa Brasileira de Pesquisa Agropecuária, [4]Empresa Brasileira de Pesquisa Agropecuária Recursos genéticos e Biotecnologia

Estradiol esters can be used in timed artificial insemination (TAI) and embryo transfer (TET) protocols. Most studies on exogenous estradiol pharmacokinetics, however, used a standard dose, regardless of body weight and did not take into account the potential interference of endogenous estradiol on circulating concentrations. The aim of this study was to compare serum estradiol concentrations after the injection of estradiol benzoate or cypionate using a waveless bovine model. Sound, pluriparous, non-lactating and nonpregnant Nelore cows (n=24) had their follicular waves suppressed by immunization against GnRH using two 1 mL SC doses of an anti-GnRH vaccine (Bopriva, Zoetis, Brazil), given 20-days apart, as previously described (Viana et al. Theriogenology;172:133-141, 2021), and were allocated using a randomized block-design according to body weight into three groups, which received either 2 mL saline (control group, CG), 5 mcg/Kg im estradiol benzoate (Syncrogen, GlobalGen, Brazil, EB group), or 5 mcg/Kg im estradiol cypionate (Cipion, GlobalGen, EC group). Blood samples were collected immediately before (0h) and 6h, 12h, 24h, 48h, 72h, 96h, and 120h after treatment. Plasma estradiol concentrations were analyzed by electrochemiluminescence (ECL) using a commercial kit (Elecsys Estradiol III, Roche Diagnostics GmBH, Germany). Data were analysed using the GLIMMIX procedure of SAS with a repeated statement to account for measurements over time. Results are shown as mean±SEM. There was no difference in the average body weight among groups (622.5±25.3, 623.1±27.1 and 625.9±25.1 kg in CG, EB and EC groups, respectively, $p>0.05$). The average E2 concentration was greater in groups EB and EC compared with CG ($p<0.0001$), but did not differ between EB and EC ($p=0.4558$). We observed time and time x treatment effects ($p<0.0001$) for groups EB and EC. In the EB group, there was a peak in E2 concentrations (154.9±16.1 pg/mL) at 6 h, when it was greater ($p<0.0001$) than those observed in EC group, followed by a progressive decline up to 120 h, moment when it became lower ($p=0.0003$) than in EC group. Conversely, in the EC group, E2 concentrations increased ($p=0.01$) up to 24 h after treatment reaching a plateau thereafter, with the maximum average value (38.0±2.5 pg/mL) occurring at 96 h. Most of the samples (75%) collected from the CG had E2 concentrations below the detection limit of the ECL kit used (5 pg/mL). In summary, the use of EB results in an earlier (6h vs. 24h) and 4-fold greater peak estradiol concentration, followed by lower values after 96 h, compared with EC.

Acknowledgments: Projects FAPDF 00193-00001711/2022-69 and CNPq INCT 406866/2022-8.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Estrus detection efficiency and optimal insemination timing using automated activity monitoring in semi-intensive grazing dairy cows

Isadora Zago[1], Bruno Mezzomo Pasqual[2], Rogério Ferreira[1]

[1]Universidade do Estado de Santa Catarina, [2]Datamars Livestock

Estrus detection is a crucial factor for ensuring the reproductive and productive efficiency of dairy herds. In this context, automated activity monitoring (AAM) systems have emerged as a tool to assist in estrus detection by enabling continuous observation of animals. This study aimed to assess the efficiency of estrus detection using AAM collars in cows under semi-intensive pasture-based systems, as well as the relationship between the estrus alert (issued by the system) and the timing of artificial insemination (AI) in relation to the non-return rate. A total of 3,653 inseminations were performed on 1,636 dairy cows in a semi-intensive (center-pivot irrigated) system from a single farm between May 14 and December 11, 2024. Estrus detection efficiency was estimated based on the interval between services, calculated as the service period (defined as the difference between average days open and days in milk at first service) divided by the number of service intervals per conception, which corresponds to the number of services per conception minus one. The interval between services was then applied to the equation $y = 0.0306x2 - 4.1012x + 166.62$, where y represents the estimated estrus detection efficiency and x corresponds to the interval between services. The following variables were analyzed: estrus intensity, time of estrus alert, time of AI after the alert, days in milk (DIM) at AI, interval between estrous cycles, and the rates of non-return and irregular return to estrus. Time intervals after the estrus alert were categorized as follows: less than 5 hours (very early), 5 to less than 9 hours (early), 9 to less than 20 hours (ideal), 20 to less than 24 hours (late), and 24 to 48 hours (very late). The effect of different factors on the non-return rate was analyzed using logistic regression, and class comparisons were made using contrasts. The non-return rate results indicate that the optimal time for artificial insemination is between 9 and 20 hours after the estrus alert, with non-return rates of 29.3%, 41.0%, 43.8%, 40.8%, and 35.1% for the very early, early, ideal, late, and very late categories, respectively. The ideal interval differed significantly from both the early and late intervals ($P < 0.05$). Furthermore, DIM influenced the non-return rate (estimate: 0.008; odds ratio: 1.008; $P < 0.0001$) and the irregular return rate (estimate: -0.009; odds ratio: 0.99; $P < 0.0001$). Estrus intensity, as measured by the AAM system, did not significantly affect the non-return rate. The non-return rate ranged from 36% to 43% across estrus intensity values between 40 and 100, with no significant effect observed between these variables ($P > 0.05$). The most frequent interval between estrous cycles was within the expected range of 17 to 24 days, characterizing regular cycles. The average estrus detection efficiency of the AAM system was 72.4%. In conclusion, the optimal time for AI is between 9 and 20 hours after the estrus alert, and a lower DIM at the time of AI is associated with higher rates of both regular and irregular estrus return. Additionally, the estrus intensity identified by the AAM system does not impact the non-return rate.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Evaluation of 2 ovulation induction protocols differing in the progesterone implants (previously used Repro one and new Repro one Novilhas) prior to the TAI protocol in 14-month-old Nelore heifers

Daniel Cavenague[1], Paulo Vitor Luciano Silva[2], Josimar da Silva Souza[1], Danilo Alves[1], Adson Fontes[1], Vinícius Faria[2], Moacir Marchiori Filho[3], Mauro Meneghetti[4], Alexandre Barbieri Prata[4], Carlos E. C. Consentini[4]

[1]Consulvet Reprodução Bovina, [2]JBJ Agropecuária, [3]UCBVET Saúde Animal, [4]GlobalGen Vet Science

The study evaluated two progesterone (P4) implants in the ovulation induction protocols prior to the TAI protocol: 1) "Regular" commercial implant (Repro one, GlobalGen) previously used for seven days, and 2) Smaller new implant (Repro one Novilhas, GlobalGen). A total of 1,993 Nelore heifers (14.2 ± 0.01 months old and 319.2 ± 0.64 kg of body weight; BW) were randomly allocated into 1 of 2 groups on d-24: 1) **One**: insertion of a 0.5 g P4 implant (Repro one) previously used for seven days, and 2) **OneN**: insertion of the smaller new 0.5 g P4 implant (Repro one Novilhas). On d-12, P4 implants were removed and 0.5 mg of E2 cypionate (EC) was administered. The TAI protocol was similar for both groups: d0: 1.5 mg of E2 benzoate, 0.250 mg of cloprostenol sodium (PGF), and insertion of a new 0.5 g P4 implant (Repro one Novilhas); d7: implant removal, 0.5 mg of EC, 0.530 mg of PGF, and 200 UI of eCG; d9: TAI. All injectable hormones were from GlobalGen. The pregnancy diagnoses were performed on d30 and 60 after TAI. The age (≤ 14.1 and > 14.1) and BW (≤ 316 and > 316 kg) on d-24 were divided according to the median to study their effects and interactions with treatments. Moreover, BW on d0 was also recorded. Statistical analyses were performed with SAS 9.4 (significant differences: $P \leq 0.05$). The average daily gain of BW between d-24 and d0 was greater in OneH compared to One group (0.433 ± 0.01 vs. 0.393 ± 0.01 g/d). The pregnancy per AI (P/AI) on d30 was greater for OneN than One (53.6 vs. 49.2%) and tended to be greater on d60 (50.5 vs. 46.8%). The pregnancy loss (PL) did not differ between OneN and One (4.9 vs 5.8%). There were interactions between treatments and age and BW. The OneN group resulted in greater P/AI on d30 in younger heifers ≤ 14.1 mo (53.7 vs. 47.3%), while in > 14.1 mo heifers, the P/AI did not differ between OneH and One (53.5 vs. 51.1%). In lighter heifers (BW ≤ 316 kg), OneN group resulted in greater P/AI on d30 (54.5 vs. 44.2%), while in heavier heifers (BW > 316 kg) the fertility was similar between OneH and One (52.7 vs. 54.3%). Interestingly, when studying another way of interaction, the BW affected P/AI in One (44.2 vs. 54.3%) but not in OneN group (54.5 vs. 52.7%) for ≤ 316 and > 316 kg, respectively. There was no interaction between treatments and age or BW on PL. In summary, the use of the new Repro one Novilhas in the ovulation induction protocol prior to the TAI protocol resulted in greater overall fertility than the protocol including the previously used Repro one, particularly in classes with expected lower P/IA such as younger and lighter heifers.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Evaluation of a three-day estrus synchronization protocol in sheep

Iara Bettin Foster[1], Gabriel Maggi[1], Fabiane Pereira de Moraes[1], Sergio Farias Vargas Júnior[1], Julia Nobre Blank Camozzato[1], Antônio Arosteguy Pereira do Amaral[1], Camila Rohde Brondani[2], Fernando Caetano de Oliveira[3], Rafael Gianella Mondadori[1], Bernardo Garziera Gasperin[3,1]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Universidade Federal de Santa Maria, [3]Universidade Federal do Rio Grande do Sul

The aim of this study was to evaluate the efficiency of a three-day estrus synchronization protocol by comparing it with a conventional eight-day protocol. The experiment was conducted during the breeding season (March–April) and involved 208 ewes from three different herds: Herd 1 – Corriedale (n=90); Herd 2 – mixed breeds (n=89); and Herd 3 – Corriedale × Texel crosses (n=29). Animals were randomly assigned to one of two treatment groups: 3D (n=105) or 8D (n=103). Both groups received an intravaginal device (IVD) containing progesterone (Primer PR; Agener União, São Paulo, Brazil). Ewes in the 8D group received the IVD on day -8, while those in the 3D group received it on day -3. On day 0, all devices were removed, and animals were treated with 250 µg of cloprostenol (Estron; Agener União, São Paulo, Brazil) and 300 IU of eCG (SincroeCG; OuroFino, Cravinhos, Brazil). Following treatment, ewes were exposed to fertile rams at a ratio of 1:10 for five days. Estrus was monitored during this period using chest painting on the rams. Twenty-five days after ram removal, pregnancy diagnosis was performed via transrectal ultrasonography. Data were analyzed by logistic regression using the JMP18 software and the significance level was set at 5%. Regarding estrus detection, 90.4% (95/105) of the 3D group and 93.2% (96/103) of the 8D group exhibited estrus (P=0.26), indicating no significant difference between treatments. However, a significant herd effect was observed for this parameter (P=0.002). Pregnancy rates were 52.4% (55/105) for the 3D group and 71.8% (74/103) for the 8D group (P=0.003), being significantly different among herds (P=0.0038; 55% (49/90), 77% (68/89), and 35% (10/29) for herds 1, 2, and 3, respectively). Conception rates were 57.9% (55/95) for the 3D group and 76% (73/96) for the 8D group (P=0.007), influenced by herd (P = 0.002) but not by the herd × treatment interaction (P=0.12). Based on our results, the three-day protocol negatively impacted pregnancy outcomes and conception rates, suggesting that reducing progesterone exposure to three days during the breeding season impairs fertility in estrus synchronization protocols. The authors thank FAPERGS, CNPq and CAPES for their financial support, and all the farmers who provided animals and facilities to make this study possible.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Exploring the traits of early sexual development in young Nelore (*Bos indicus*) heifers

Bruna Lima Chechin Catussi[1], Laís Ângelo de Abreu[1], Adriana Santana Croazara[2], Rubens Cesar Pinto da Silva[2], Lucas O. e Silva[1], Angela Maria Gonella[3], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo, [2]HoRa Agronegócio, [3]University of Florida

This study explored the associations among phenotypic, reproductive, nutritional, and metabolic traits and early sexual development in Bos indicus heifers. A total of 443 prepubertal Nelore heifers (11.1 ± 0.07 months old) were evaluated during a feedlot (automated feeders) period from day -70 to day 0. Measurements on day -70 (beginning of the feedlot) included body weight (BW), body condition score (BCS), cyclicity rate (presence of CL), uterus diameter (UT), largest follicle diameter (LF), and blood collection for LC/MS metabolomics (Subset of 139 heifers). On day 0 (end of the feedlot), all heifers were subjected to a fixed-timed artificial insemination protocol (FTAI) to evaluate their early sexual development. At the same time, ribeye area (REA) and rump fat thickness (RFAT) were assessed, and the residual feed intake (RFI), average daily gain (ADG), and dry matter intake (DMI) were calculated. Pregnancy was determined on day 39, and heifers confirmed as pregnant were classified as early maturing (n= 212), while those that remained open were classified as late maturing (n= 231). On day -70, no differences in BW (P = 0.30), BCS (P = 0.11), UT (P = 0.32), LF (P = 0.39), and cyclicity rate (P = 0.98) were observed between groups. On day 0, BW (P = 0.46), BCS (P = 0.82), LF (P = 0.56), and REA (P = 0.19) remained similar between groups, but EM heifers exhibited greater UT (P = 0.05), RFAT (P = 0.02) and cyclicity rate (P = 0.04). Additionally, nutritional analysis revealed higher RFI (P = 0.05) in EM heifers but no differences in DMI (P = 0.15) and ADG (P = 0.40). Metabolomic analysis on day -70 revealed a separation between LM and EM heifers with an accuracy of 0.74. Significant modulation of pathways related to amino acid and energy metabolism, including alanine, aspartate and glutamate metabolism, TCA cycle, and pyruvate metabolism. In conclusion, phenotypical traits observed on day -70 did not reliably predict early sexual development in heifers. By day 0, EM heifers demonstrated increased fat deposition and enhanced reproductive system maturation. Feed efficiency impacts the early sexual development of heifers, suggesting that selection for highly efficient animals should be balanced with reproductive traits. Additionally, metabolomic analysis provided predictive accuracy, identifying key changes in amino acids and energy metabolism. This research deepens our understanding of the mechanisms behind early maturation and suggests new avenues for selecting and breeding early maturing heifers in future genetic and breeding studies.

Acknowledgment: FAPESP 2023/17999-2.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Expression of estrus in native female sheep subjected to reproductive management in periods of the year with different thermal conditions in the Middle São Francisco

Rafaella Sanches de Jesus[1], Yali Táfeni Trindade Barbosa[2], Aline de Souza Cordeiro[2], Artur Azevedo Menezes[1,2], Mariana Fernandes Souza[1], Diane Duarte Dantas[1], Gabriel Santos Palma[1], Jairo Torres Magalhães Junior[2], Tiago Oliveira Brandão[3], Alexandra Soares Rodrigues[2]

[1]Universidade Federal da Bahia, [2]Universidade Federal do Oeste da Bahia, [3]Universidade do Estado da Bahia

This study aimed to evaluate body temperature and estrus expression in native ewes subjected to reproductive management under distinct annual thermal conditions. Twenty-eight Santa Inês ewes from the experimental farm of the Federal University of Western Bahia (Barra Campus) were managed in a semi-intensive production system. Based on meteorological data from INMET, the animals were allocated into two groups: a low-temperature period (LTP; June to August; average temperature: 28.2 ± 2.6°C; average humidity: 77.2 ± 12,9%; THI: 78.3; n = 16) and a high-temperature period (HTP; November to December; average temperature: 31,9°C± 3.1°C; average humidity: 79,7 ± 10,6; THI 84,7; n = 12). On a random day of the estrous cycle (designated Day 0), all females received an intravaginal device containing 0.33 g of P4 (CIDR®, Zoetis, São Paulo, Brazil), which remained for seven consecutive days. On Day 7, 200 IU of equine chorionic gonadotropin (eCG; Novormon®, Zoetis) and 0.125 mg of sodium cloprostenol (Lutalyse®, Zoetis) were administered intramuscularly, coinciding with the removal of the P4 device. Estrus expression was evaluated using a teaser ram at 24, 36, 48, 56, 72, and 84 hours post-device removal. Ewes were classified as "in estrus" when they accepted mating and "not in estrus" when they did not. In addition, rectal temperature was measured every 48 hours using a digital thermometer, starting at the beginning of the protocol and continuing through pregnancy diagnosis. Statistical analysis was conducted using SPSS software (version 19), with a significance level of 5%. Student's t-test was employed to compare body temperatures between groups, and the chi-square test was used to compare estrus expression rates.The average ambient temperature during the HTP was significantly higher than that recorded in the LTP (31.9 ± 3.1°C vs. 28.21 ± 2.6°C; $P<0.05$). Mean rectal temperatures were 38.7 ± 0.2°C for the LTP group and 38.9 ± 0.9°C for the HTP group, with no significant difference ($P > 0.05$). Estrus expression rates were also comparable: 93.8% (15/16) in the LTP group and 91.7% (11/12) in the HTP group ($P = 0.832$). The mean time to estrus onset was 39.2 ± 13.1 hours for LTP and 44.7 ± 10.8 hours for HTP, with no statistically significant difference between groups. In conclusion, under the conditions of this study, estrus expression and body temperature in ewes were not influenced by reproductive synchronization protocols implemented during periods of high or low environmental temperatures. These findings suggest that fixed-time artificial insemination (FTAI) protocols can be successfully applied throughout the year in this region.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Factors affecting the reproductive efficiency of the resynchronization protocol with FTAI every 28 days

Gabriel Cunha Cruz[1], Sofía Albertini[2,3,4], Emanuele Almeida Gricio[2,4], Laís Ângelo de Abreu[2], Pietro Sampaio Baruselli[2,3,4]

[1]GCRA, [2]Universidade de São Paulo, [3]University of São Paulo, [4]Animal Reproduction Department, University of São Paulo

The objective of this study was to evaluate factors influencing pregnancy per insemination (P/IA) and pregnancy losses (PL) in non-pregnant Nelore females subjected to a resynchronization protocol with 28-day intervals between inseminations (Cunha et al., 2024, Anim Reprod, 21, 3). Females were enrolled in the resynchronization protocol without prior pregnancy diagnosis at D17 (9 days of protocol length; n=3,322), D18 (8 DPL, n=8,336), or D19 (7 DPL, n=466) after the previous FTAI. All animals received a P4 device and estradiol benzoate (Heifers=1.0mg; Cows=2.0 mg). The P4 device was removed after 7, 8, or 9 DPL, coinciding with pregnancy diagnosis via B-mode ultrasonography on D26. Non-pregnant females received 0.530mg of cloprostenol, eCG (Heifers=200IU; Cows=300IU), and 1.0mg of estradiol cypionate. Chalk applied to the tailhead was used for estrus detection. Females were inseminated 48 hours after P4 device removal (D28), and this resynchronization protocol was repeated until the fifth insemination. Data were analyzed using the GLIMMIX procedure in SAS 9.4. A significant effect of resynchronization order on P/IA at 26 days was observed [Resynch2 = 45.3% (3,797/8,378)^{a}; Resynch3 = 32.5% (892/2,749)^{b}; Resynch4 = 28.9% (244/843)^{b}; Resynch5 = 17.4% (39/224)^{c}; P < .0001]. Additionally, protocol length influenced P/IA [7DPL = 42.3% (197/466)^{a}; 8DPL = 41.6% (3,469/8,336)^{a}; 9DPL = 38.2% (1,270/3,322)^{b}; P = 0.0028]. Also, the P4 device had influence over P/AI [Monodose = 39.9% (209/524)^{b}; New = 40.7% (4,332/10,633)^{b}; Usedx1 = 44.7% (114/255)^{ab}; Usedx2 = 46.7% (257/550)^{a}; P = 0.02]. Parity significantly influenced P/IA [Multiparous = 53.1% (2,019/3,801)^{a}; Primiparous = 35.8% (1,617/4,512)^{b}; Heifer = 34.4% (1,336/3,881)^{b}; P < .0001]. Animals that exhibited estrus had higher (P < .0001) P/IA compared to those that did not [48.6% (3,460/7,117) vs. 29.8% (1,510/5,067), respectively]. Moreover, pregnancy loss did not differ significantly among protocols [Resynch2 = 5.9% (204/3,446); Resynch3 = 6.0% (49/821); Resynch4 = 5.3% (13/244); P = 0.92]. The results of this study demonstrate that multiple factors influence the reproductive efficiency of non-pregnant Nelore females undergoing a 28-day resynchronization protocol. The order of resynchronization, protocol length (DPL), P4 device status, and parity significantly affected P/IA, with higher conception rates observed in earlier resynchronizations, shorter protocol durations, and with the reused P4 device. These findings highlight the importance of protocol optimization in improving reproductive performance in beef cattle under intensive resynchronization strategies.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Fertility of Native Ewes Under Reproductive Management Across Seasonal Thermal Conditions in the Middle São Francisco Region

Mariana Fernandes Souza[1], Aline de Souza Cordeiro[2], Yali Táfeni Trindade Barbosa[2], Thadeu Fernandes Tonhá[2], Artur Azevedo Menezes[1,2], Rafaella Sanches de Jesus[1], Diane Duarte Dantas[1], Jairo Torres Magalhães Junior[2], Tiago Oliveira Brandão[3], Alexandra Soares Rodrigues[2]

[1]Universidade Federal da Bahia, [2]Universidade Federal do Oeste da Bahia, [3]Universidade do Estado da Bahia

This study aimed to evaluate body temperature, fertility, and prolificacy in native ewes subjected to reproductive management under distinct annual thermal conditions. Twenty-eight Santa Inês ewes from the experimental farm of the Universidade Federal do Oeste da Bahia (Campus Barra) were managed in a semi-intensive production system with mineral and concentrate supplementation and ad libitum access to water. Based on meteorological data from the Instituto Nacional de Meteorologia (INMET), animals were assigned to two groups: the low-temperature period (LTP; June to August; n=16) and the high-temperature period (HTP; November to December; n=12). On a random day of the estrous cycle (designated Day 0, D0), all females received an intravaginal progesterone device containing 0.33 g of P4 (CIDR®, Zoetis, São Paulo, Brazil) and retained it for seven consecutive days. On Day 7 (D7), 200 IU of equine chorionic gonadotropin (eCG; Novormon®, Zoetis) and 0.125 mg of sodium cloprostenol (Lutalyse®, Zoetis) were administered intramuscularly, coinciding with the removal of the progesterone device. From Day 8 to Day 10 (D8–D10), natural mating was performed twice daily (morning and afternoon) using fertile rams. Pregnancy diagnosis was conducted by transabdominal ultrasonography 35 days after mating, using a 5.0 MHz linear transducer (Sonoscape E2V). Pregnancies were classified as single (one fetus) or twin (two or more fetuses). Rectal temperature was measured every 48 hours using a digital thermometer, from the beginning of the hormonal protocol until pregnancy diagnosis. Ambient temperature was also recorded throughout the experimental period. Statistical analysis was performed using SPSS software (version 19), with significance set at $P<0.05$. Student's t-test was used to compare body temperature between groups, and the chi-square test was used to assess differences in pregnancy and prolificacy rates. The average ambient temperature during the HTP was significantly higher than that recorded in the LTP (31.97 ± 3.05°C vs. 28.21 ± 2.65°C; $P<0.05$). However, rectal temperatures did not differ significantly between groups (38.75 ± 0.21°C in LTP vs. 38.9 ± 0.095°C in HTP; $P>0.05$). The overall pregnancy rate was 85.7% (24/28), with similar rates between groups (LTP: 87.5%, 14/16; HTP: 83.3%, 10/12; $P=0.755$). The incidence of twin pregnancies was 7.1% (1/14) in LTP and 20.0% (2/10) in HTP, with no statistically significant difference ($P=0.755$). In conclusion, body temperature, fertility, and prolificacy in native ewes were not affected by reproductive management under contrasting seasonal thermal conditions, suggesting that these animals exhibit thermal adaptability and reproductive resilience in the Mid-São Francisco region.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Follicular parameters of lactating Nelore females subjected to different times of permanence of the progesterone device in an FTAI protocol

Alexandra Soares Rodrigues[1], Aline de Souza Cordeiro[1], Yali Táfeni Trindade Barbosa[1], Thadeu Fernandes Tonhá[1], Isabela Pereira de Oliveira[1], Laysla Silva Lacerda[1], Manuella Maria Oliveira Santana[1], Artur Azevedo Menezes[2], Tiago Oliveira Brandão[3]

[1]Universidade Federal do Oeste da Bahia, [2]Universidade Federal da Bahia, [3]Universidade do Estado da Bahia

The aim of this study was to evaluate the size and vascularization of the ovulatory follicle of lactating cows subjected to FTAI protocols, with the progesterone implant lasting seven (D7) or 8 days (D8). The research took place between April and June 2024 at Fazenda Columbi, located in the Barra, Bahia, in which 20 lactating Nelore females were used, with an average of 60 days postpartum, belonging to the pluriparous and with a body condition score of an average of 2.25 (scale from 1 to 5). The farm adopted an extensive management system of raising animals on pasture, with mineral supplementation and water ad libitum. At this time, the cows were randomly divided into two experimental groups: Group P4D8 (n=10) - on day zero (D0) of the protocol, 10 cows received an new intravaginal P4 release device 1 g (Sincrogest, São Paulo, Brazil), associated with 2.0 mg of Estradiol Benzoate (Sincrodiol, São Paulo, Brazil) intramuscularly (IM); and Group P4D7 (n=10) - 10 cows were subjected to the same treatment performed in the P4D8 group, but on day one of the protocol (D1). On day eight (D8), the intravaginal P4 devices were removed from both groups, in addition to the application of 500µg of sodium cloprostenol (Sincrocio, São Paulo, Brazil), 1mg of estradiol cypionate (SincroCP, São Paulo, Brazil) and 300IU of Equine Chorionic Gonadotropin (SincroeCG, São Paulo, Brazil), IM. On day 10 (D10), to evaluate the follicular characteristics, transrectal ultrasonography was performed at a frequency of 5.0 MHz (Sonoscape S2VET, Shenzhen, China), using B mode, the image of the largest follicle was frozen to determine the diameter of the preovulatory follicle (DFOL) and the area of the follicular wall (AFOL). Using the color Doppler mode, the quantification of the vascularization area of the preovulatory follicle wall (VFOL) was performed, through a function of the device itself. The data were processed by the Statistical Package for Social Science (SPSS, version 19) to compare the differences in DFOL, AFOL and VFOL between the groups, using the Student T test ($P < 0.05$). Thus, females submitted to the P4D7 protocol had an average DFOL of 0.95±0.12 Cm and AFOL of 0.28±0.08 Cm^2, statistically similar to those belonging to the P4D8 group, which was 0.99±0.09 Cm and 0.31±0.06 Cm^2, respectively. Regarding VFOL, there was no difference ($P=0.46$) between the groups, animals that remained with the progesterone device for 7 days and 8 days presented averages of, respectively, 0.11±0.05 Cm^2 and 0.14±0.05 Cm^2. Therefore, the follicular parameters of animals submitted to synchronization protocols for FTAI with different periods of permanence of the progesterone implant (P4) (7 vs. 8 days) were not affected, suggesting that the reduction of the duration of the FTAI protocol can be performed without compromising the results in lactating multiparous females.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Genetic parameters of pregnancy loss in Nellore cattle using a large Brazilian database

Luiz Carlos Louzada Ferreira[1], Dauydson Antônio Gonzalez Cordeiro[2,1,3], Juan Cuevas de Alvarenga Martins[2], Felipe de Oliveira Pedro[4,3], Roberto Augusto de Almeida Torres Júnior[5], Joao Gabriel Nascimento Moraes[6], Alexandre Prata[7], Gilberto R Menezes[5], Ériklis Nogueira[8,5]

[1]Cia Assessoria, [2]Universidade Federal de Mato Grosso do Sul, [3]Grupo de Estudos em Tecnologia da Reprodução Animal, [4]Universidade Estadual de Mato Grosso do Sul, [5]EMBRAPA GADO DE CORTE, [6]Oklahoma State University, [7]GlobalGen Vet Science, [8]Empresa Brasileira de Pesquisa Agropecuária

Fertility traits, particularly pregnancy loss, are critical for improving reproductive efficiency in beef cattle, yet they are often overlooked in breeding programs due to their low heritability and late expression. This study aimed to estimate the genetic parameters for pregnancy loss in Nellore cattle using a large field database from Brazil, and analyzed data from 305,325 fixed-time artificial insemination (FTAI) events across 271 farms, spanning from 2015 to 2022, involving 667 bulls. The research used a Bayesian animal model that accounted for fixed effects (such as contemporaneous group and cow age) and random effects (such as direct genetic effects). The results revealed that pregnancy loss rates varied across different categories of females, with precocious heifers (heifers bred close to 15 months old) showing the highest losses (12.35%), followed by conventional heifers (9.13%). Pregnancy losses were higher in primiparous (8,28%) than multiparous (6,64%), with non-lactating cows (7,35%) showing similar results to primiparous. A notable age effect was observed, with younger females (precocious heifers) experiencing higher losses compared to older, more mature cows. Additionally, there was considerable variation in gestational loss among bulls, ranging from 4.8% to 16.3%, and a heritability estimate of 3.07% for gestational loss was found. The study suggests that despite the low heritability, the genetic selection of bulls could potentially reduce pregnancy losses in Nellore cattle, and targeted management practices for different female categories could improve reproductive outcomes. This research contributes valuable insights into genetic factors affecting pregnancy loss and can inform future breeding strategies to enhance reproductive performance in beef cattle. Further studies are needed to confirm these findings in diverse environments.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

How body weight and BCS at the start of the previous FTAI affect cyclicity at resynchronization in Nelore females

Lígia Mattos Rebeis[1], Adriel Vânio Gretter[1], Bruno Silva do Espirito Santo[1,2], Silvana Aparecida dos Santos Martins[2], Sofía Albertini[1], Pietro Sampaio Baruselli[1]

[1]University of São Paulo, [2]Agropecuária Jacarezinho

This study assessed the probability of cyclicity (CLR; presence of CL at the beginning of a resynchronization protocol) at the time of pregnancy diagnosis (30 days post previous FTAI) in relation to the body condition score (scale from 1 to 5; BCS, n=20,883) and body weight (n=19,228) on the D0 of the previous insemination in Nelore (Bos indicus) females. The study was performed at a farm located in Juruena, MT, Brazil. Categories included in the analysis were: early heifers (12-15 months), heifers (24 months), primiparous, secundiparous, multiparous, and non-lactating Nelore cows. Statistical analyses were performed using GLIMMIX of SAS 9.4. Correlations between the weight and BCS and the probability of CL were determined using Proc CORR of SAS. In Nelore early heifers, a linear trend in CLR probability (P=0.07) was observed with an increase in BCS (between 2.0 and 4.25; n=7,647). A quadratic effect (P<0.001) was observed for weight (230 to 450 kg; n=7,097). Low CLR is observed in early heifers at lower weights, gradually increasing at intermediate weights, with a smaller decrease noted at higher weights. Primiparous cows (n=3,644) showed a linear (P=0.02) BCS (2.0 to 3.75) effect on CLR and a trend (P=0.1) for weight (230 to 520kg; n=3,221). As the BCS and weight improve, the CLR also increases. Secundiparous cows exhibited quadratic effects (P=0.003) for BCS (1.5 to 3.5; n=2,185) and weight (P<0.001, 260 to 570kg; n=2,188), indicating low CLR in secundiparous at lower BCS and weights, gradually increasing at intermediate BCS and weights, with a smaller decrease at higher BCS and weights. Multiparous cows displayed quadratic BCS effects (P<0.001, 1.75 to 4.50; n=6,249) and weight effect (P=0.0001, 280 to 650kg; n=5,545), indicating low CLR in multiparous cows at lower BCS and weights, gradually increasing at intermediate BCS and weights, with a smaller decrease at higher BCS and weights. No effects were detected for 24-month heifers or non-lactating cows. Higher BCS and optimal weight at D0 were associated with greater cyclicity rate in nonpregnant heifers and cows. These results highlight the importance of BCS and weight in evaluating the cyclicity of heifers and cows after FTAI, as well as in determining strategies for introducing bulls or resynchronizing the breeding female group.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Impact of diarrhea in the first 75 days of life on productive and reproductive performance of Nellore (Bos indicus) cattle

Ana Carolina dos Santos Oliveira[1], Sofía Albertini[1], Emanuele Almeida Gricio[1], Karen Nascimento da Silva[2], Stella Morais[3], Victor Guidi Zardo[1], Viviani Gomes[2], Pietro Sampaio Baruselli[1]

[1]Animal Reproduction Department, University of São Paulo, [2]Department of Internal Medicine, College of Veterinary Medicine and Animal Science, University of São Paulo, [3]Fazenda Segredo

Diarrhea presents a significant challenge in calf rearing, increasing the mortality, treatment costs, and reducing performance. This study evaluated the impact of diarrhea in the first 75 days of life on the productive and reproductive performance of 376 Nelore (Bos indicus) calves (194 male and 182 female) born during the 2022/23 season at a commercial farm in Bataguassu, MS, Brazil. Diarrhea episodes were recorded during the first 75 days of life, and calves were classified into three groups: A = no diarrhea (n=76); B = 1 or 2 episodes of diarrheia (n=187); C = 3 or more episodes of diarrheia (n=113). Animals were weighed at birth (D0), 30 (D30), 60 (D60), 210 (D210, weaning), and 420 days (D420, yearling). The females were submitted to a FTAI protocol (n=182) at 13–14-month age, and ultrasound examinations were performed to assess pregnancy rate (P/IA) at 30 and 60 days post-FTAI and pregnancy loss. In the males (n=194), scrotal circumference was measured at D420. Data were analyzed using PROC GLIMMIX in SAS 9.4. Mortality rate was 2.5% (11/445) and did not differ between groups (P=0.68). A group*time interaction was observed for weight (P<.0001). No differences were observed at D0 (A=32.2±0.50; B=31.4±0.29; C=31.1±0.33 kg; P=0.19), however, calves in group A and B were heavier than those in group C at D30 (A=74.7±1.76A; B=70.9±0.91A; C=66.6±1.12B kg; P=0.0001), D60 (A=103.7±2.26A; B=99.5±1.30A; C=94.5±1.57B kg; P=0.002), D210 (A=181.6±3.46A; B=178.2±2.12A; C=165.7±2.48B kg; P=0.0001), and D420 (A=327.1±4.10A; B=321.5±2.62A; C=307.5±3.42B kg; P=0.0003). In females, first-service P/AI was higher in B than C group [A=44.1% (15/34) AB; B=49.4% (44/89)A; C=25.4% (15/59)B; P=0.02], but final P/AI (after 3 FTAI) did not differ [A=73.5% (25/34); B=76.4% (68/89); C=67.8% (40/59); P=0.52]. The number of services (AI) per conception was similar between groups (A=1.5±0.14; B=1.6±0.11; C=1.9±0.13 services; P=0.42). Pregnancy loss was similar across groups [A=2.94% (1/34); B=7.87% (7/89); C=6.78% (4/59); P=0.64]. In males, scrotal circumference at D420 was unaffected by group (A=27.1±0.51; B=27.8±0.30; C=27.0±0.34 cm; P=0.21). In conclusion, females with three or more episodes of diarrhea in the first 75 days of life had lower weight at 420 days, which may have affected reproductive performance, indicating a long-term impact on reproductive efficiency. These findings highlight the need for effective diarrhea prevention to optimize growth and reproductive performance in Nelore cattle.

Acknowledgment: Segredo farm.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Impact of the interval between calving and FTAI protocol on pregnancy rates and losses in Nelore cows

Bruno Silva do Espirito Santo[1,2], Lígia Mattos Rebeis[1], Silvana Aparecida dos Santos Martins[2], Emanuele Almeida Gricio[3,4], Adriel Vânio Gretter[1], Pietro Sampaio Baruselli[3,4]

[1]University of São Paulo, [2]Agropecuária Jacarezinho, [3]Universidade de São Paulo, [4]Animal Reproduction Department, University of São Paulo

The objective of the study was to evaluate the influence of days postpartum (DPP) in relation to the start of the IATF protocol, on the pregnancy rate (P/AI) and pregnancy losses between 30-120 days (PL30-120) and 120 to calving (PL120-C) in lactating, 2-year-old primiparous (PP) and multiparous (MP) Nelore cows. We used 3,238 precocious primiparous (24.5 ± 1.4 months; BCS 2.5 ± 0.18; scale 1-5) from three breeding seasons with DPP range of 21-70 days (mean of 42.5 ± 9.6) and 6,948 multiparous (67.5 ± 27.4 months; BCS 2.64 ± 0.33) with DPP range of 15-95 days (mean 46.3 ± 15.0) from a commercial farm (Agropecuária Jacarezinho, Brazil). DPP was considered the period from the day of calving to day 0 (DO) of the FTAI protocol. Pregnancy diagnoses were assessed by ultrasound after 30 days (P/AI) and at the end of the breeding season (120 days after AI) to calculate PL30-120 and at birth to calculate PL120-C. The Statistical analyses were performed using GLIMMIX of SAS 9.4. The logistic regression curves were obtained using the coefficients generated by "interactive data analysis". The pregnancy rate at the 1st IATF was 41% (1,333/3,238) for PP and 58% (4,052/6,948) for MP (P<0.01). DPP influenced the P/AI of PP (quadratic effect, P=0.002). Low P/AI is observed at lower DPP, gradually increasing at intermediate DPP, with a smaller decrease noted at higher DPP. In multiparous cows, there was a linear effect (P=0.002), indicating that the higher the DPP, the greater the probability of P/AI. There was also a difference for PL30-120 in both categories (quadratic effect; PP = P=0.01, n=1,321; MP = P=0.05, n=3,995). High PL30-120 is observed at lower DPP, gradually decreasing at intermediate DPP, with a slight increase noted at longer DPP in both categories. For the PL120-C variable, there was no significant difference for DPP in both categories (Primiparous, n=1,085, Multiparous, n=3,821). This study examines the effects of DPP on pregnancy rates and losses in Nelore cows undergoing FTAI. It concludes that a shorter postpartum period may decrease pregnancy rates and increase the likelihood of pregnancy losses.

Acknowledgments: Agropecuária Jacarezinho.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Impact of using ovulation synchronization protocols for timed AI on increasing reproductive efficiency and reducing CO2 equivalent emissions in cattle

Laís Ângelo de Abreu[1], Vanessa Romário de Paula[2], Rodrigo Faleiros[3], Gabriel Sandoval[3], Mauro Meneguetti[4], Alexandre Barbieri Prata[4], Marcelo Brunini Rodrigues[3], Milo Wiltbank[5], Pietro Sampaio Baruselli[1,6,7]

[1]Universidade de São Paulo, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]UCBVET Animal Health, [4]GlobalGen Vet Science, [5]University of Wisconsin-Madison, [6]University of São Paulo, [7]Animal Reproduction Department, University of São Paulo

Research utilizing Life Cycle Assessment (LCA) in milk and beef production in tropical environments demonstrates that factors such as herd diet, genetic composition, and reproductive efficiency significantly influence estimates of CO2 equivalent (CO_2eq) emissions. The objective of this study was to evaluate the impact of using ovulation synchronization protocols for TAI (GlobalGen) on improving reproductive efficiency, productivity, and reducing CO2eq emissions in dairy and beef production programs. The carbon footprint of milk and beef production was estimated based on the LCA. The study followed ISO 14040, 14044 and 14067 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. Regional Brazilian data were collected, including national inventories and production data, focusing on semi-confined dairy systems (Girolando) and full-cycle beef systems (Nelore). The analysis included 11,479,663 protocols commercialized by GlobalGen (annual analysis considered), of which 80% were intended for beef and 20% for dairy production. The number of cows in production were considered as a fixed value to compare the modeled scenarios: SCE-NM) system using natural mating, and SCE-TAI) system adopting TAI. For the dairy system, 595,241 cows in milk were considered (2.7 TAI/cow). The following premises were determined: age at first calving (AFC; months) of 36 (SCE-NM) and 24 (SCE-TAI), calving interval (CI; months) of 16 (SCE-NM) and 13 (SCE-TAI). For the beef system, 4,017,882 cows were considered (1.7 TAI/cow). The following premises were determined: AFC of 48 (SCE-NM) and 24 (SCE-TAI), weaning rate of 60% (SCE-NM) and 80% (SCE-TAI). Quantitative values of inputs for animal feed were estimated. Results were expressed as CO_2eq/liter of milk corrected for fat and protein content (FPCM; dairy system), and kg of beef produced corrected for kg of live weight (LW; beef system). In the dairy system, the footprint decreased from 1.44 to 1.06 kg CO_2eq/FPCM, representing a 37% reduction, along with a 25% increase in productivity. This improvement was attributed to a lower AFC, shorter CI, and genetic gains. In the beef system, emissions were reduced from 41.4 to 27.9 kg CO_2eq/LW, a 49% decrease, accompanied by a 27% increase in productivity due to higher pregnancy rates, earlier calvings, and improved genetics. The main source of emissions was enteric methane, followed by off-farm feed production and manure management. The conclusions highlight that the protocols used for TAI commercialized by GlobalGen enhance productivity and decrease emissions per unit of milk or beef produced. This supports the sustainability of Brazilian livestock production, aligning with global demands for agricultural products with a lower environmental impact.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Implementation of microencapsulated chili pepper supplements improving follicular functionality behavior in dairy cows

Artur Azevedo Menezes[1], Mónica Madrigal Valverde[2], Murilo Ramos Santiago[3], Lara Lôbo Dantas[3], Mariana Fernandes Souza[3], Rafaella Sanches de Jesus[3], Endrigo Adonis Braga Araujo[3], Juliana Reolon Pereira[4], José Esler Freitas Júnior[3], Marcus Vinícius Galvão Loiola[3]

[1]Universidade Federal do Oeste da Bahia, [2]Instituto Tecnológico de Costa Rica, [3]Universidade Federal da Bahia, [4]NutriQuest

The present study evaluates the effect of including microencapsulated hot chili pepper (MHCP) in the diet of crossbred dairy cows on ovarian morphofunctionality. A total of 24 crossbred females (Bos taurus × Bos indicus) were used in the lactating period with an open interval of 53.54±11.8 days, an age of 7.16±3.41 years, and an average daily milk production of 6.34±1.91 liter/cow/day. The cows were divided into two experimental groups: a control (CT) and an MHCP-supplemented group (CP), each receiving 1 g per day per animal of microencapsulated hot chili in a balanced feed for 42 days. On D0, an intravaginal device containing 1.0 g of progesterone (São Paulo, Brazil, DIB, Zoetis) was administered, combined with 2 mg of E2 benzoate (im) (São Paulo, Brazil, Gonadiol, Zoetis). On D8, the intravaginal device was removed, and 12.5 mg of dinoprost tromethamine (IM) was administered (São Paulo, Brazil; Lutalyse, Zoetis). Simultaneously, 1 mg of E2 cypionate (im) (São Paulo, Brazil, ECP, Zoetis) and 300 IU of eCG (im) (São Paulo, Brazil, Novormon, Zoetis) were administered. Evaluations in B mode and color Doppler Flow Power were conducted by means of ultrasonographic equipment (Sonoscape S2, Shenzhen, China) using a transrectal transducer with a frequency of 7.5 MHz every 12 h between D8 of the protocol and the moment of ovulation; 96 h thereafter, the intravaginal device was removed. The analysis was performed using the PROC MIXED procedure of SAS 9.4 ($P \leq 0.05$). For ovarian morphofunctionality evaluated by B-mode ultrasound, significant differences ($p<0.05$) were observed for follicular parameters between groups for follicular diameter parameters on D8 (CP=0.93±0.10 and CT= 0.61±0.11; p=0.01), D9 (CP=1.21 ± 0.10 and CT= 0.85 ± 0.11; p=0.01), D10 (CP=1.40 ± 0.09 and CT= 1.02 ± 0.09; p<0.001) and D11 (CP=1.43 ± 0.13 and CT= 1.06 ± 0.13; p=0.01). The area of the follicular wall and the area of the largest follicle presented significant differences between groups on D8 (CP=0.56 ± 0.08 and CT= 0.35 ± 0.08; p=0.01), D9 (CP= 0.67 ± 0.07 and CT= 0.45 ± 0.07; p<0.001) and D10 (CP= 0.83 ± 0.09 and CT=0.60 ± 0.09; p=0.04). For both the measurement results for the ovulatory follicle growth rate and time between removal of the progesterone device and ovulation, there were no differences ($p \geq 0.05$). Follicular characteristics evaluated by color Doppler ultrasound do not indicate significant differences between treatments ($p \geq 0.05$). The findings show that the inclusion of MHCP in the diet of dairy cows can enhance follicular growth during synchronization programs.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Influence of bovine neosporosis on reproductive indices in a Girolando herd

Daniela Cristina Rocha de Freitas[1], Mateus Gonçalves Costa[1], Nicolas Moreira Piedras Monnerat Caparelli[1], Thiago de Souza Vieira[1], Samuel Rodrigues Bonamichi do Couto[2], Liria Hiromi Okuda[3], Vera Lucia Teixeira de Jesus[1], Marco Roberto Bourg Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro, [2]Universidade Federal Fluminense, [3]Instituto Biológico de São Paulo

This study had as its main objective to compare the reproductive indices of Girolando cows seropositive and seronegative for N. caninum. The study included 144 Girolando cattle (cows, heifers, calves, and bull) fed a complete diet and given water ad libitum. Reproductive management was conducted via natural mating or artificial insemination. The animals were tested for N. caninum through blood samples analyzed by Indirect Immunofluorescence Assay (RIFI), which identified seropositive and seronegative animals. The reproductive indices evaluated included: number of services; SP; CI; reproductive efficiency (RE); conception rates in the 1st and 2nd services; pregnancy rates of cows with up to 2 services and at 150 days in milk (DIM) and abortion rate (%). Statistical analysis was conducted using SPSS software (version 26). Continuous variables were evaluated for residual normality and tested for homogeneity of variances. The dependent variables were expressed as means. A one-way analysis of variance (ANOVA) was also applied. The significance level was set at 5%. The study was conducted in a closed herd, and the sample was determined, based on the total number of females available during the study period that met the inclusion criteria and had complete reproductive records. No prior sample calculation was performed. However, the sample provided sufficient statistical power to detect significant differences in key reproductive indices between seropositive and seronegative animals. The incidence of N. caninum in the herd was 29.9% (43/144). Among the 144 tested samples, 67 were from multiparous cows, and upon testing for N. caninum, 23.9% (16/67) were seropositive. Reproductive data were analyzed only for multiparous females. The number of services was 2.7 ± 0.2 and 2.1 ± 0.08 (P= 0.05) for seropositive and seronegative cows, respectively; the SP was 142.3 ± 11.5 and 107.9 ± 4.8 days (P= 0.01) for seropositive and seronegative cows, respectively. Regarding the IP, seropositive animals presented 413.6 ± 10.1 days and seronegative animals 387.7 ± 5.5 days (P = 0.07), the same trend was observed in relation to the ER, which presented 91.2% ± 2.5 for seropositive animals and 96.1% ± 1.6 for seronegative animals (P = 0.07). The conception rate at the 1st service was 32.7% (16/49) for seropositive animals and 40.7% for seronegative animals (P= 0.16). For the 2nd service, the conception rate was 42.4% for seropositive animals and 60.3% for seronegative animals (P= 0.04). The pregnancy rate for animals with up to 2 services was 61.2% for seropositive animals and 76.4% for seronegative animals (P= 0.02). The pregnancy rate at 150 DIM for seropositive and seronegative animals was 69.4% and 78.0%, respectively (P= 0.1). No significant difference was observed in the abortion rate between the groups (14.3% x 12.2%). It was concluded that N. caninum seropositive Girolando cows showed compromised key reproductive indices.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Injectable Metabolic Additive Improves Fertility in Beef Cows

Kassia Gomes Galvão[1], Thallyni Chayane de Almeida Costa Borges Pereira da Silva[1], Juliano Rodrigues Grangeiro da Silva[2], Fernando Martins Dantas[2], Fernando Flores Correa Junior[2], Eliane Vianna da Costa e Silva[1], Marcos Malacco[3], Baity Leal[3], Alexandre Hênryli de Souza[3], Gustavo Guerino Macedo[1]

[1]Universidade Federal de Mato Grosso do Sul, [2]Flores Consultoria Rural, [3]Ceva Saúde Animal

Previous metabolomic findings by Nobre et al. (Animal Reproduction, 3:21, 2024) indicated that lower levels of branched-chain amino acids (BCAAs) at the onset of the timed artificial insemination (TAI) protocol were associated with reduced pregnancy rates in beef cows. This study aimed to evaluate the effect of Roboforte® (CEVA)—an injectable metabolic and energy-activating supplement rich in amino acids, calcium, phosphorus, and choline—on pregnancy per AI (P/AI) of Nelore (Bos indicus) beef cows subjected to fixed-time artificial insemination (TAI). A total of 545 cows from two commercial farms of same company, maintened in B. Brizanta pasture, with water and mineral salt supplement ad libitum, were randomly allocated equally to one of two groups: Control (C, no supplementation, n = 271), and Roboforte® (ROBO, 1 mL/20 kg LW on D0 i.m., n = 274). All animals were subjected to the same estrus synchronization protocol, which included an intravaginal progesterone device (Prociclar® 750 mg, CEVA) and 2 mg estradiol benzoate (Benzoato-HC, CEVA) on D0, followed by device removal and administration of PGF2α (Luteglan®, CEVA), estradiol cypionate (Cipionato-HC, CEVA), and eCG (Folirec 105 IU, CEVA) on D7, with GnRH (Cevarelin®, CEVA) and TAI performed on D9. Pregnancy diagnosis was performed via transrectal ultrasonography 30 days after insemination. Body weight (BW) and body condition score (BCS; 1–5 scale) were recorded on D0. Data were analyzed by proc GLIMMIX of SAS. There was no interaction of parity or farm on treatment effects (p>0.05). Animals were retrospectively classified as “Light” (377.8 ± 1.8 kg; n = 281) or Heavy (473.1 ± 2.4 kg of BW; n = 264) and Lean (2.4; n = 211) or Fat (3.3 of BCS; n = 334). Overall, PR was significantly higher in cows receiving the metabolic additive (ROBO = 43.8%) than in Control (36.0%; P = 0.030). A tendency for interaction was observed with BCS (P = 0.060) and BW (P = 0.130). Among Lean cows, ROBO significantly improved PR (40.9%) compared to C (27.4%; P = 0.040). A similar effect was detected among Light cows: ROBO (48.2%) vs. C (37.7%; P = 0.040). In contrast, no benefit was observed for Fat or Heavy animals, and no interaction with reproductive category (multiparous vs. primiparous) was found. A curvilinear analysis of predicted probabilities revealed a positive effect of ROBO in cows with low BCS or BW, especially between BCS 2.5 3.0 and BW 320 380 kg, with differences of up to 12 percentage points over Control. In conclusion, a single dose of Roboforte® administered on D0 of the TAI protocol improves PR in cows with lower body condition or weight, likely by correcting transient metabolic deficits that impair early reproductive events. These findings support the hypothesis proposed by Nobre et al. (Animal Reproduction, 3:21, 2024), suggesting that metabolic supplementation enhances fertility outcomes, and highlight the potential of Roboforte® as a targeted strategy to boost reproductive efficiency.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Luteolytic effect of administering two doses of PGF2α in lactating Bos indicus cows with a 7-day-old corpus luteum

Matheus Pedroso Vicente[1], Laís Reis Carvalho[1], Lucas Araújo Lemos[1], Rafael Resende Rabelo Silva[1], Luiz Manoel Souza Simões[1], Jose Nélio de Sousa Sales[2,1]

[1]Universidade Federal de Lavras, [2]Universidade Federal de Juiz de Fora

The objective was to describe the luteolytic effect of administering two doses of PGF2α in lactating Bos indicus cows with a 7-day-old corpus luteum (CL) induced by administering a GnRH analog. The study involved 99 lactating Nelore cows, between 30 and 40 days postpartum, with a body condition score of 2.79 ± 0.02 (on a 1–5 scale). Seven days before the start of the protocol (Day -7), all cows received 150 mg of injectable progesterone (P4). On Day 0 (D0), the cows received 2.5 mg of a new GnRH analog. After eight days (D8), 500 µg of cloprostenol (PGF; Sincrocio, Ourofino, Brazil) was administered, and on the following day (D9), all cows received 1 mg of estradiol cypionate (EC; SincroCP, Ourofino, Brazil), 500 µg of cloprostenol (PGF; Sincrocio, Ourofino, Brazil), and 300UI of equine chorionic gonadotropin (eCG; SincroeCG, Ourofino, Brazil). Doppler ultrasound exams (DP50-Power) were performed on D8 and D10 to assess the presence (D8) and vascularization (D10) of the CL. Vascularization scores of zero and one (0–1) were assigned to inactive CL, and scores from two to four (2–4) were assigned to active CL. Only cows with active CL on day 8 were included in the study. Statistical analyses were performed using the GLIMMIX procedure in SAS. A low rate of active CL 4% (n = 4/99)] was observed 48 hours after the administration of the first dose of PGF. It was concluded that the administration of two doses of PGF2α was effective in inducing luteolysis of functional CL with 7 days of development in lactating Bos indicus cows.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Meta-Analysis of cyclicity induction protocols in Nelore (Bos indicus) heifers: Sincrogest® Injetavel (injectable P4) vs. intravaginal progesterone device

Bruno Gonzalez de Freitas[1], Emanuele Almeida Gricio[2,3], Álvaro de Miranda Alves[2], Sofía Albertini[2], Marcilio Nichi[2], Igor Garcia Motta[1], Pietro Sampaio Baruselli[2,3]

[1]Ourofino Saúde Animal, [2]Universidade de São Paulo, [3]Animal Reproduction Department, University of São Paulo

A meta-analysis was conducted to compare the efficacy of two cyclicity induction protocols in Nelore (Bos indicus) heifers. Data from 2,918 diagnostic records across 10 published studies were analyzed. The protocols evaluated were: 1) iP4+EC: Administration of 150 mg injectable progesterone (iP4; Sincrogest Injetavel, Ourofino Saude Animal, Brazil) on D-24, followed by estradiol cypionate (EC) on D-12, prior to the initiation of a fixed-time artificial insemination (FTAI) synchronization protocol (D0), 2) P4D+EC: Insertion of an intravaginal progesterone device previously used during 16 days (P4D) on D-24, removed on D-12, concurrently with EC administration. Cyclicity rate, defined as the presence of a corpus luteum (CL) on D0, and pregnancy per artificial insemination (P/IA), assessed 30 days post-FTAI, were determined through ultrasonographic examination. Statistical analysis was performed using binomial logistic regression with aggregated data (counts of cyclic/non-cyclic and pregnant/non-pregnant heifers per treatment group) in R software (version 4.4.2). The P4D+EC protocol served as the reference for comparison with iP4+EC. For each study, odds ratios (ORs), 95% confidence intervals (95% CI), and P-values were calculated. Statistical significance was declared when $P < 0.05$ and the 95% CI excluded 1. The results indicated no difference in cyclicity rate between the two protocols [iP4+EC = 69.0% (1,022/1,482) vs. P4D+EC = 74.5% (1,119/1,503); OR = 1.00; P = 1.00; 95% CI (0.886, 1.127)]. Furthermore, no differences in pregnancy per AI at 30 days were observed between cyclicity induction protocols [iP4+EC = 47.4% (669/1,411) vs. P4D+EC = 46.0% (661/1,436); OR = 1.06; P = 0.41; 95% CI (0.921, 1.219)]. In conclusion, the iP4+EC protocol, with a single injection of 150 mg injectable progesterone (Sincrogest® Injetavel) on D-24, demonstrated equivalent efficacy to the P4D+EC protocol in inducing cyclicity and achieving pregnancy per AI in Nelore heifers. Given its comparable performance and simplified application, the injectable progesterone protocol represents a practical alternative for heifer breeding programs.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Metabolic and hormonal profile of pregnant and non-pregnant Bos indicus cows subjected to the timed artificial insemination protocol

Luisa Oliveira Orlandi[1], Rafael Resende Rabelo Silva[1], Laís Reis Carvalho[1], Matheus Pedroso Vicente[1], Lucas Araújo Lemos[1], Luiz Manoel Souza Simões[1], Miguel Pizzolante Bottino[2], Raphael Evangelista Orlandi[3], Marco Antônio Faria Silva[1], Jose Nélio de Sousa Sales[4,1]

[1]Universidade Federal de Lavras, [2]Matriz Genética - Veterinária Avançada, [3]Profit Max, [4]Universidade Federal de Juiz de Fora

The objective was to evaluate the metabolic (cholesterol, NEFA and glucose) and hormonal (Insulin and IGF-1) profile of pregnant and non-pregnant Bos indicus cows subjected to the timed artificial insemination (TAI) protocol. In the study, 100 Bos indicus (Nelore) primiparous (n=50) and multiparous (n=50) cows with a body condition score of 2.75 ± 0.01 (scale of 1 - 5) were used. The animals were maintained Brachiaria brizantha pastures and received mineral supplementation. On a random day of the estrous cycle (D0), the cows received 2mg of estradiol benzoate (EB; Sincrodiol, Ourofino, Brazil) and an intravaginal device containing 1g of progesterone (P4; Sincrogest, Ourofino, Brazil). Eight days later (D8), the P4 devices were removed and 1.0mg of estradiol cypionate (EC; SincroCP, Ourofino, Brazil), 0.5mg of sodium Cloprostenol (PGF; Sincrocio, Ourofino, Brazil) and 300UI of equine chorionic gonadotropin (eCG; SincroeCG, Ourofino, Brazil) were administered. Timed artificial insemination (TAI) was performed 48 hours after progesterone device withdrawal (D10). Blood samples were collected on D10 to assess serum concentration of IGF-1, cholesterol and glucose. Ultrasound examinations were conducted 30 days after TAI for pregnancy diagnosis. Subsequently, a retrospective analysis of the metabolic profile of pregnant and non-pregnant cows was performed. Statistical analyses were conducted using GLIMMIX procedure of SAS. There was no interaction between animal category (primiparous and multiparous) and reproductive status (pregnant and non-pregnant) for metabolic and hormonal analysis. The concentrations of IGF-1 (Non-pregnant = 217.6±12.5 ng/mL and pregnant = 230.3±10.8 ng/mL; P =0.47), insulin (Non-Pregnant = 21.8±4.9 µUI/mL and pregnant = 16.8±2.4 µUI/mL; P = 0.89), cholesterol (Non-Pregnant = 214.8±6.1 mg/dL and pregnant = 212.7±4.4 mg/dL; P = 0.84), NEFA (Non-Pregnant = 0.49±0.03 mmol/L and pregnant = 0.56±0.03 mmol/L; P = 0.13) and glucose (Non-pregnant = 106.5±4.3 mg/dL and pregnant = 107.0 ±3.6 mg/dL, P = 0.92) were similar between cows pregnant and non-pregnant. In conclusion, there was no difference between the metabolic and hormonal profiles analyzed and reproductive status (pregnant and non-pregnant) in suckled Bos indicus cows subjected to the ovulation synchronization protocol.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Optimizing fertility in dairy cows: GnRH- versus P4-based early resynchronization protocols

Ísis Soares da Cunha[1], Fábio de Souza Guagnini[2], Bárbara Luana Wollmeister[2], André Gustavo Cabrera Dalto[2], Ronaldo Luis Aoki Cerri[3], Gustavo Desire Antunes Gastal[4], Bernardo Garziera Gasperin[2,1], Monique Tomazele Rovani[2]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Universidade Federal do Rio Grande do Sul, [3]University of British Columbia, [4]Instituto Nacional de Investigación Agropecuaria

Early resynchronization based on progesterone (P4) and estradiol (E2) has not yet been compared with GnRH and prostaglandin (PGF) protocols in dairy cows. In Exp1, Holstein primiparous (n=179; 447 procedures) and multiparous (n=369; 860 procedures) cows were pre-synchronized 36±3 days in milk (DIM) with a 7-day P4 and E2 protocol. Synchronization started on 53±3 DIM with an 8-day P4 and E2 protocol and TAI was performed 63±3 DIM. The first resynchronization protocol started 25 days after TAI (88±3 DIM). G group (n=359) received 50µg lecirelin (GnRH; Tec-Relin, Agener União) (D0). If non pregnant, received PGF (Estron, Agener União) on D7 and D8, and GnRH on D9 afternoon. Cows without a CL were resynchronized using an 8-day P4 and E2 protocol. The GBD group (n=401) received an IVD (Sincrogest 1g P4, Ourofino), 2mg of EB (Ric-BE, Agener União, IM), and 50µg of GnRH (D0). On D7 (95±3 DIM), non-pregnant cows received PGF; and on D8 the IVD was removed, and cows were treated with PGF and EC (SincroCP, Ourofino, IM). TAI was performed on D10 (am) in both groups. Cows were resynchronized up to the sixth service or until 203±3 DIM. Cows returned to their respective groups 25 days after AI. In Exp2, follicular diameter and luteal function were evaluated in a subgroup of cows, allocated in G (n=13) and GBD (n=13) groups 25 days after AI, as described in Exp1. Presence of CL and the largest follicles were assessed by B-mode ultrasonography on D0, 7 and 10; on D7, pregnant cows were excluded from the study. Blood samples were collected on days 5 and 11 after TAI to determine serum P4. P/AI and pregnancy loss were compared using logistic regression. The effects of parity and semen type were included in the model and were excluded when not significant (p>0.2). Follicles were compared using ANOVA; CL measurements, and serum P4 concentration were compared using a Mixed Model. The significance level was set at 5%. In Exp1, P/AI was higher in G (42.6%) vs. GBD (35.4%, P=0.04), but G had more pregnancy loss (9.2% vs. 5%, P=0.02). No difference in P/AI (P=0.2) or pregnancy loss (P=0.4) was observed considering all AI after synchronization and resynchronization protocols (G: 39.3% and 8.7%, GBD: 36.2% and 7.4%). Although CL diameter increased significantly from Day 5 to Day 11 (P<0.001), no difference was observed between groups G and GBD (P=0.54). As expected, P4 concentrations increased between Days 5 and 11 in both groups (P<0.001), and group G had higher P4 concentrations than group GBD (P<0.001). In conclusion, early resynchronization protocols based on GnRH and PGF increased P/AI and serum P4 concentrations after AI compared to protocols using P4 and E2. However, they were also associated with greater pregnancy loss. No differences were found between protocols in follicular diameter or CL , suggesting similar follicular and luteal dynamics. Overall, considering all AI up to 203±3 DIM, both protocols resulted in same P/AI outcomes.

Funding: FAPERGS, CNPq, CAPES.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Performance of an Automated Activity Monitoring System for estrus detection in crossbred dairy cows under intensive or extensive management: preliminary results

Sara Adna Santos de Oliveira[1], Juaci Vitoria Malaquias[2], Ricardo Alamino Figueiredo[3], João Henrique Moreira Viana[3], Ricarda Maria dos Santos[1], Carlos Frederico Martins[4,2], Isabel Cristina Ferreira[5,2]

[1]Universidade Federal de Uberlândia, [2]Embrapa Cerrados, [3]Empresa Brasileira de Pesquisa Agropecuária Recursos genéticos e Biotecnologia, [4]Empresa Brasileira de Pesquisa Agropecuária, [5]Universidade de Brasília

The aim of this study was to evaluate the performance of an Automated Activity Monitoring (AAM) system to detect estrus behavior in lactating Holstein x Gir (n=25, average 5/8 blood share) dairy cows under different management systems. Estrus detection was carried out by visual observation (VO), adhesive patches (AP), and AAM after a timed-AI (TAI) protocol. The VO and AP were considered as reference standards. The AAM system used was originally set up for barn-confined Holstein cows. The experiment was carried out during two seasons (dry and rainy), during which the animals were managed either intensively, in a feedlot, being supplemented with corn silage, or extensively, in a rotational grazing system in Panicum maximum cv. Mombaça pasture. After receiving an AAM collar (CowMed, Brazil) and subsequent establishment of individual baselines, all cows underwent a conventional TAI protocol, consisting of the insertion of an 1g progesterone (P4) intravaginal device and injection of 2mg im estradiol benzoate on day 0 (D0), and P4 device removal, injection of 0.5 mg sodium cloprostenol, 400 IU eCG, and 1g estradiol cypionate on D8. On this day, the animals also received breeding indicator AP (Estrotect, USA) between the hip and tailhead. Data from each method (VO, AP, and AAM) were retrospectively compared using a contingency table to calculate the positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, accuracy, and Kappa value. Differences were compared using the Chi-squared or Fisher's exact tests. There was no difference ($p>0.05$) between the reference methods (VO or AP) for any of the performance endpoints analyzed. The overall performance of the AAM was similar ($p>0.05$) to both reference methods. However, when compared with both VO and AP, the AAM was characterized by a low NPV (22.2%), and therefore specificity (40.0% and 28.6%, respectively), resulting in low Kappa agreement values (0.18 and 0.10, respectively). The AAM showed lower estrus detection sensitivity under intensive than extensive management, considering both the VO (68.2% vs. 100%, $p=0.0038$) and AP (69.6% vs. 100%, $p=0.0109$) as reference methods. However, the overall accuracy of AAM was lower for cows under intensive management only when compared with VO (64.0% vs. 96.0% for intensive and extensive, respectively; $p=0.0050$). In summary, management may interfere with the performance of estrus detection using AAM systems. In the current study, the freedom of movement associated with the extensive management may have favored estrus characterization, resulting in the difference in sensitivity observed. This shall be taken into account for further adjustments of the activity-based diagnosis of estrus.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Pregnancy rate and gestational losses of early primiparous as a function of postpartum uterine environment

Dauydson Antônio Gonzalez Cordeiro[1,2,3], Luiz Carlos Louzada Ferreira[1,2], Guilherme Baroni Da Silva[4], João Victor Bezerra Machado[4,3], Wallery Caroliny Costa da Costa[4,3], Luana Gomes da Silva[1,5], Felipe de Oliveira Pedro[4,3], Ériklis Nogueira[6], Luiz Francisco Machado Pfeifer[7], Fabiana de Andrade Melo Sterza[4,1,3]

[1]Universidade Federal de Mato Grosso do Sul, [2]Cia Assessoria, [3]Grupo de Estudos em Tecnologia da Reprodução Animal, [4]Universidade Estadual de Mato Grosso do Sul, [5]Lida Produtos Agropecuárias e Serviços, [6]EMBRAPA GADO DE CORTE, [7]Empresa Brasileira de Pesquisa Agropecuária - Rondônia

The aim of this study was to evaluate the effect of the interval between calving and the start of the FTAI protocol (IP-D0) and uterine health on the pregnancy rate at FTAI (PIA) and pregnancy losses (PL). To this end, 3.738 primiparous Nelore cows were evaluated in two experiments in the center-west of Brazil. In both studies, the FTAI protocol was as follows: D0 - insertion of the first use P4 device (DIP4) (CIDR®; Zoetis, SP - Brazil) and i.m. application of 2 mg estradiol benzoate (Gonadiol®, Zoetis, SP - Brazil). On D8, DIP4 was removed followed by i.m. application of 16.8 mg PGF2α (Lutalyse®; Zoetis, SP - Brazil), 300 IU eCG (Novormon®; Zoetis, SP - Brazil) and 0.5 mg estradiol cypionate (ECP®; Zoetis, SP - Brazil). At 48-52 hours (D10), AI was performed (AI technicians and bulls were included in the statistical model). Pregnancy diagnosis (PD) occurred between 31-35 days after AI, and PL was calculated after PD 110-120 days after AI. Experiment 1 was based on retrospective data from the first FTAI of early primiparous (EP: n=1.145; 24±2.5 months; BCS-D0 2.75±0.42; IP-D0 44.69±9.9 days) and conventional primiparous (CP: n=2.317; 36±4.6 months; BCS-D0 3.0±0.48; IP-D0 49.75±12.55 days). Statistical analyses were performed using PROC GLIMMIX SAS© 9.2. The cut-off points for the analyzed variables were determined using the ROC curve. Regressions were performed using the PROC REG function, and paired samples were compared using the T-test. PIA was similar between EP (52.9%; 606/1.145) and CP (53.7%; 1.244/2.317; P=0.42). However, PL was higher in EP (10.72% vs. PC; 8.6%; P<0.001). The probability of PL decreased linearly (P=0.004) with increasing IP-D0. The ROC curve defined a cut-off for the highest PIA when IP-D0 was until 44 days for EP and 38 days for CP. It was observed that PIA was higher when IP-D0 was above the cut-off (EP 57.60% vs 48.9%; CP 55.67% vs 45.81%; P=0.001). In experiment 2, 276 EP cows were divided into 2 groups: PGF (16.8 mg i.m. of PGF2α [n=147]) and SS (16.8 mg i.m. of saline; [n=129]) on D0. On the same day, n=91 EP cows were assessed for subclinical endometritis (SE) by polymorphonuclear count (PMN) and clinical endometritis (CE) by observation of vaginal discharge (PVD). The SE ranged from 1.0% to 19%. The ROC curve showed a trend towards higher PIA (63.83% - 30/47 vs. 42.85% - 18/42) from the PMN cut-off ≤4.0% (AUC=0.61; P=0.059). Cows without CE showed a trend (P=0.09) towards higher PIA (56.3%; 40/71 vs 45.0% 9/20). PGF2α treatment reduced (P=0.0006) %PMN and PVD at D8 (P=0.04). In conclusion, the PIA of EP cows in tropical conditions is maximized at a minimum IP-D0 of 44 days, and CP 38 days, and the probability of PL decreases with advancing IP-D0. PIA tends to be lower when PMN>4%. Uterine and vaginal health may be favored using PGF2α at D0 in EP cows.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Preliminary trial for evaluating replacing the progestin-releasing intravaginal device-IVPD with hCG on reproductive outcome following a FTAI program in 22-month-old Angus heifers

Luis Ferré, PhD[1], Natalia Cerviño[2], Marcelo Rodriguez[3], Néstor Formia[4], Luzbel de la Sota[4], Michael Kjelland[5], Marcos Colazo[6], Jordan Thomas[7]

[1]Instituto Nacional de Tecnología Agropecuaria, [2]Consejo Nacional de Investigaciones Científicas y Técnicas, [3]Universidad Nacional del Centro de la Provincia de Buenos Aires, [4]Instituto de Investigaciones en Reproducción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, [5]Mayville State University, [6]Leduc Farm Animal Hospital, [7]University of Missouri Columbia

The objective of this study was to evaluate replacing a progestin-releasing intravaginal device (IVPD) with hCG on reproductive performance of heifers synchronized with the one-step pre-synchronization protocol. Angus heifers with 22±2 months, and average body condition score (BC, on a scale of 1 to 5) of 3.37±0.21, were used in this study. Prior to protocol initiation, ultrasound examination was performed to register the presence of the corpus luteum (CL) and/or the larger follicle diameter. Heifers with CL were considered cycling (CY), no-CL + follicles ≥10mm in superficial anestrous (SA) and no-CL + ≤10 mm in deep anestrous (DA). Only CY and SA females were used. Heifers were randomly allocated into two groups considering BC and physiological status. Day -17, Group 7 & 7 IVPD Synch (n = 73) received 1.0 g IVPD and 150 µg PG and Group 7 & 7 hCG Synch (n = 72) 2500 UI hCG. All groups received 10.5 µg GnRH on Day -10 and at the time of fixed-time artificial insemination (FTAI) and 150 µg PG at Day -3. The TAI was performed with a single bull and operator at 54±2 hours after Day -3. All heifers were tail painted to identify those in heat (YES= ≥50% paint erased and NO= <50% paint erased) at the time of AI. The pregnancy diagnosis was performed 35 days after FTAI by ultrasound. Fisher's LSD test was used for mean values comparisons and Pearson's Chi-Square for proportions (α = 0.05). Results for the effect of replacing a IVPD with hCG on reproductive performance of heifers synchronized with the one-step pre-synchronization protocol on the dominant follicle [DF (mm)] and CL presence (%) for 7 & 7 IVPD Synch and 7 & 7 hCG Synch were 9.7±3.7a and 86.3%a; and 9.4±3.0a (P>0.53) and 97.2%b (P=0.03), respectively. Results of the number of CL presence for 7 & 7 IVPD Synch and 7 & 7 hCG Synch were CL absent (13.7%a and 2.8%b); Single (75.3%a and 66.7%b); and Double (11.0%a and 30.6%b), respectively, with significant differences for each group (P=0.002). P/AI (%) for 7 & 7 IVPD Synch and 7 & 7 hCG Synch were 52.0%a, and 38.9%a, respectively, with no significant difference (P=0.2). P/AI (%) for 7 & 7 IVPD Synch and 7 & 7 hCG Synch for anestrus heifers were 46.3%, and 35.5%, respectively, versus those showing estrus 59.4%, and 60.0%, respectively. In conclusion, 22-month-old Angus heifers treated with a 7 & 7 protocol based on hCG, replacing the intravaginal P4 device, showed higher presence of CL compared to the standard 7 & 7 protocol. No differences in DF and pregnancy rate were observed. A tendency of higher pregnancy rate was observed in those heifers that exhibited estrus behavior.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Reducing GnRH Dose: Effects on Ovulation and Pregnancy in Nelore Cows

Samira Alves de Souza Silva[1], Gabrielly Cristina Santos Noleto[2], Ingrid Pedraça Barbosa[3], Bernardo Garziera Gasperin[4,1], Rafael Gianella Mondadori[1], Monique Tomazele Rovani[4], Luiz Francisco Machado Pfeifer[5]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Fundação Universidade Federal de Rondônia, [3]Universidade Federal do Pará, [4]Universidade Federal do Rio Grande do Sul, [5]Empresa Brasileira de Pesquisa Agropecuária - Rondônia

Three experiments were performed to compare the efficacy of 12.5 µg versus 25 µg of Lecirelin (GnRH analog) in cows treated with estradiol cypionate on ovulation and pregnancy outcomes. In Experiment 1, 37 postpartum Nelore cows (BCS 2.75-3.5) received an intravaginal progesterone device (IPD, 1.9 g P4, CIDR®, Zoetis, São Paulo, Brazil) and 2 mg i.m. estradiol benzoate (Gonadiol® Zoetis, São Paulo, Brazil) on Day 0. On Day 8, devices were removed, and all cows received 25 mg i.m. dinoprost tromethamine (Lutalyse® Zoetis, São Paulo, Brazil), 1 mg estradiol cypionate (E.C.P® Zoetis, São Paulo, Brazil), and 300 IU eCG (Novormon®Zoetis, São Paulo, Brazil). On Day 9, 34 h after IPD removal, cows were ultrasonographically evaluated and allocated according to dominant follicle diameter to receive either 25 i.m. µg Lecirelin (GnRH1_34OV, n=18; TecRelin®Agener União, São Paulo) or 12.5 µg Lecirelin (GNRH1/2_34OV, n=19). Dominant follicles were monitored by ultrasonography at 12-hour intervals until ovulation. In Experiment 2, 305 lactating Nelore cows (60 primiparous, and 245 multiparous) with ≥30 days postpartum, received the same protocol as Experiment 1 and were randomly assigned to receive either 25 µg (GNRH1_34P; n=159) or 12.5 µg (GNRH1/2_34P; n=146) of Lecirelin 34 h after IPD removal. All cows were inseminated 48 h post-device removal. In Experiment 3, 439 cows (103 primiparous, and 336 multiparous) received the same protocol as in Experiment 2, except that cows were not treated with Lecirelin on Day 9. At timed-artificial insemination (TAI), cows that exhibit estrus were inseminated without additional treatment (Estrus Group, n=248). Non-estrus cows were randomly allocated to receive either 25 µg (GNRH1_48; n=94) or 12.5 µg (GNRH1/2_48; n=97) of Lecirelin. Data were analyzed using SAS. Ovulation rate were compared by Chi-square test. The P/AI was evaluated by GLIMMIX, and the effects of category, treatments, and their interactions were considered. In Experiment 1, no difference (P=0.6) was observed on ovulation rate between GNRH1_34OV (77%; 14/18) and GNRH1/2_34OV (84%; 16/19) groups. In Experiment 2, P/AI did not differ (P=0.23) between GNRH1_34P (53%; 84/159) and GNRH1/2_34P (59%; 87/146) groups. In Experiment 3, P/AI of the cows observed in Estrus (49%; 122/248) was greater (P=0.03) than cows from GNRH1_48 (38%; 36/94) and GNRH1/2_48 (38.5%; 37/97) groups, with no difference (P=0.9) between cows from GnRH1_48 and GnRH1/2_48 groups. No effects (P>0.05) of parity, treatments, or their interaction on P/AI were observed in Experiments 2 and 3. In conclusion, fertility and ovulation rate in Nelore cows subjected to TAI protocols were equivalent when using either 12.5 µg or 25 µg of Lecirelin. These results suggests that the reduced dose of GnRH in the protocols studied represents a more cost-effective approach.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Reduction of equine chorionic gonadotropin dose through pharmacopuncture using the bai hui and hou hai acupoints in estrus induction and synchronization protocols in ewes

Tiago Oliveira Brandão[1], Gutemberg Gama Neiva Santos[1], Mateus de Almeida da Silva Câmara[1], Alexandra Soares Rodrigues[2], Laura Nicole Filipin da Costa[1], Cintia Figueiredo de Araújo[1], Aline de Souza Cordeiro[2], Yali Táfeni Trindade Barbosa[2], Marcio da Silva Chaves[1], Lucas Myrran Macedo de Oliveira[1]

[1]Universidade do Estado da Bahia, [2]Universidade Federal do Oeste da Bahia

This study evaluated the efficacy of pharmacopuncture using reduced doses of equine chorionic gonadotropin (eCG) given at the *Bai Hui* and *Hou Hai* acupoints in protocols of induction and estrus synchronization in ewes, as well as its influence on conception rates and treatment costs. Induction and synchronization of estrus are essential strategies in reproductive management; however, the high cost of hormonal treatments limits their widespread use. Pharmacopuncture, which consists of administering the drug at specific acupoints with potential neuroendocrine modulatory effects, represents a promising alternative for reducing hormone dosages. A total of 42 clinically healthy Dorper and Santa Inês ewes, including multiparous and primiparous individuals, were randomly assigned to three groups (n = 14): Group 1 (pharmacological control) received 300 IU of eCG (Sincro eCG®) intramuscularly, 0.5 mL of sodium cloprostenol (Sincrocio®) intramuscularly, and removal of the intravaginal progesterone device (Primer® PR) on Day 7; Group 2 (*Bai Hui*) received 60 IU (20% of the standard dose) of eCG given at the *Bai Hui* acupoint, 0.5 mL of sodium cloprostenol intramuscularly, and device removal on Day 7; Group 3 (*Hou Hai*) received the same protocol as Group 2, with eCG administered at the *Hou Hai* acupoint. Following device removal, the ewes were subjected to controlled natural mating at a 4:1 female-to-male ratio. Pregnancy diagnosis was performed 30 to 45 days after mating using transrectal ultrasonography (Mindray DP10, 7.5 MHz linear rectal transducer). The cost of each protocol was calculated based on the quantity of the drug used and its commercial price. Statistical analyses were performed using SPSS Statistics software, employing Pearson's Chi-square test to compare groups (significance level set at 5%) and the likelihood ratio as an additional measure. The overall conception rate was 73.8% (31/42). The control group achieved 78.6% (11/14), while both *Bai Hui* and *Hou Hai* groups reached 72.0% (10/14). No statistically significant differences were observed among groups (p = 0.872; likelihood ratio p = 0.869), indicating that eCG administration at acupoints, even at 80% dose reduction, did not impair reproductive performance. In terms of cost, pharmacopuncture protocols resulted in an 80% reduction in hormonal expenses. These findings confirm that stimulation of the selected acupoints, which are rich in nerve endings, activates neuroendocrine responses that enhance the release of GnRH and LH, increase ovarian blood flow, and support the function of the hypothalamic-pituitary-gonadal axis. Therefore, pharmacopuncture with eCG given at *Bai Hui* and *Hou Hai* acupoints proved to be an effective, safe, and economically advantageous alternative for induction and synchronization of estrus in ewes.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Removal of estradiol treatment at the end of timed-artificial insemination protocols impairs the fertility of Nelore (*Bos indicus*) cows

Patrícia R. Cavalcanti[1], Lucas O. e Silva[1], Flávia Aguiar Carrieri[1], Vinicius Louro Velame[1], Eloisa Akemi Artner Yoshimura[1], José Renato Silva Gonçalves[2], Alexandre Barbieri Prata[3], Moacir Marchiori Filho[4], Armando Leonelo[4], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture, [2]"Hildegard Georgina Von Pritzelwiltz" Experimental Station, [3]GlobalGen Vet Science, [4]UCBVET Animal Health

The study evaluated reproductive outcomes of Nelore (*Bos indicus*) cows submitted to timed-artificial insemination (TAI) protocols with or without estradiol (E2) for the 1st postpartum service. Primiparous (n=266) and multiparous (n=523) cows (52.3±0.6 d postpartum and body condition score = 2.9±0.01) were submitted to one of three synchronization protocols starting on D0: EE (n=262), administration of 2mg E2 benzoate (EB) on D0 and 1mg E2 cypionate (EC) on D7; GE (n=263), administration of 16.8μg buserelin acetate (GnRH) on D0 and 1mg EC on D7; and G0 (n=264), 16.8μg GnRH on D0 without EC on D7. In addition, all cows received an intravaginal progesterone device (IVD; 0.5g) on D0, which was removed on D7, simultaneously to administration of 1.06mg cloprostenol sodium and 300IU eCG, and tail chalk for estrus behavior. On D9, all cows received 8.4μg GnRH and TAI was performed. Ultrasound examinations were done on D, 0, 7, and 9 of the protocol and 7, 14, 30, and 60 d after TAI to assess the presence of CL, largest follicle (LF) diameter, ovulation after D0, premature ovulation (between D7 and 9), premature luteolysis (between 7 and 14 d after TAI), pregnancy per AI (P/AI), and pregnancy loss (PL). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($^{a,b}P \leq 0.05$). The presence of CL on D0 was similar among groups (EE: 29.4 [77/262] *vs* GE: 25.5 [67/263] *vs* G0: 28.8% [76/264]). Ovulation after D0 was greater in cows treated with GnRH on D0 than in EB-treated cows (GE: 57.8^{a} [152/263] *vs* G0: 54.9^{a} [145/264] *vs* EE: 12.6%^{b} [33/262]), as well as the diameter of LF on D7 (GE: 11.9±0.1^{a} *vs* G0: 12.0±0.1^{a} *vs* EE: 10.8±0.1^{b} mm) and D9 (GE: 13.5±0.1^{a} *vs* G0: 13.6±0.1^{a} *vs* EE: 12.6±0.1^{b} mm). More cows treated with EC on D7 were detected in estrus than the cows not treated with EC (GE: 62.0^{a} [163/263] *vs* EE: 55.0^{a} [144/262] *vs* G0: 37.5%^{b} [99/264]). Moreover, treatment with EC on D7 resulted in greater P/AI (GE: 52.1^{a} [137/263] *vs* EE: 55.3^{a} [145/262] *vs* G0: 37.9%^{b} [100/264]). Regardless of treatment, cows expressing estrus had greater P/AI than those that did not express (57.0 [233/406] *vs* 39.0% [149/382]). Despite the greater fertility in cows receiving EC on D7, no differences were observed among treatments on premature ovulation (EE: 3.1 [8/262] *vs* GE: 6.8 [18/263] *vs* G0: 5.7% [15/264]), ovulation after TAI (EE: 93.7 [133/142] *vs* GE: 91.7 [132/144] *vs* G0: 93.6% [132/141]), premature luteolysis (EE: 4.5 [6/133] *vs* GE: 3.0 [4/132] *vs* G0: 1.5% [2/132]), nor in PL (EE: 4.8 [7/145] *vs* GE: 6.6 [9/137] *vs* G0: 9.0% [9/100]). In conclusion, synchronization protocols initiated with EB or GnRH resulted in similar P/AI when EC was given at the time of IVD removal. However, the lack of EC treatment at the end of the protocol impaired expression of estrus and fertility, although ovulation and subsequent CL development were not affected. These results highlight the importance of E2 at the end of the synchronization protocol in postpartum Nelore cows.

Acknowledgments: CAPES, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Reproductive outcomes of Nelore (Bos indicus) heifers submitted to different doses of estradiol cypionate to induce ovulation at the end of a 8d-timed artificial insemination protocol

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

[1]Federal University of Goiás, [2]Nova Piratininga Farm, [3]GlobalGen Vet Science

The study evaluated the effect of the dose of estradiol cypionate (EC; 0.6 vs. 1.0 mg) as ovulation inducer on expression of estrus and fertility in 940 Nelore heifers (Age: ~24 mo.; BW: 312 ± 17.0 kg; BCS: 2.85 ± 0.27) submitted to a 8-d estradiol (E2)/progesterone (P4)-based timed-artificial insemination (TAI) protocol. Heifers were submitted to a ciclicity induction protocol with 150 mg of injectable P4 (Sincrogest®, Ourofino) on d-24, followed by 0.6 mg of EC (Cipion®, GlobalGen) on d-12. The TAI protocol initiated (d0) with 2 mg of estradiol benzoate (Syncrogen®, GlobalGen), 0.53 mg of cloprostenol sodium (PGF) in heifers with CL (Induscio®, GlobalGen), and insertion of a new 0.5 g intravaginal P4 implant (Repro One®, GlobalGen). On d8, concomitant with P4 implant withdrawal, heifers received another PGF treatment, 200 IU of eCG (eCGen®, GlobalGen), and were randomly assigned to receive 0.6 mg (Group: EC0.6) or 1.0 mg (Group: EC1.0) of EC. The TAI was performed on d10, and all heifers received 8.4 µg of buserelin acetate (MaxRelin®, GlobalGen) at the time of AI. In addition, heifers had their tailheads painted with chalk on d8 for later evaluation of expression of estrus on d10. For further analysis, two classes of BCS (< 3.0 and ≥ 3.0) and BW (≤ 311 and > 311 kg) were established on d0. The pregnancy per AI (P/AI) was determined by transrectal ultrasound at 30 and 150 d after AI, and all treatments were administered intramuscularly. Statistical analyses were performed using the GLIMMIX procedure of SAS 9.4 (P ≤ 0.05). The treatment with 1.0 mg of EC increased expression of estrus compared to 0.6 mg of EC (84.4 [399/473] vs. 73.9% [345/467]), in both lighter (84.0 [195/232] vs. 76.0% [150/198]) and heavier (84.0 [178/212] vs. 73.0% [178/243]) heifers, and in both BCS classes < 3.0 (81.5 [190/233] vs. 70.0% [168/240]) and ≥ 3.0 (87.1 [209/240] vs. 78.0% [117/227]). There was no effect of BW on expression of estrus, while heifers with BCS < 3.0 had lower expression of estrus (75.6 [358/473] and 82.7% [386/467]). The P/AI was similar between groups on d30 (EC0.6: 48.8 [228/467] vs. EC1.0: 44.6% [211/473]), and on d150 (EC0.6: 45.1 [211/467] vs. EC1.0: 40.6% [192/473]), as well as in pregnancy loss (EC0.6: 7.5 [17/226] vs. EC1.0: 9.0% [19/209]). No interaction was detected between treatments and BW on P/AI. Interestingly, EC0.6 and EC1.0 treatments resulted in similar P/AI in heifers with BCS < 3.0 (44.6 [107/240] vs. 50.2% [114/233]), but EC1.0 had lower P/AI in heifers with BCS ≥ 3.0 (53.3 [121/227] vs. 40.4% [97/240]). Moreover, heifers that expressed estrus had greater P/AI in both groups EC0.6 (52.5 [181/345] vs. 38.5% [47/122]) and EC1.0 (47.4 [189/399] vs. 29.7% [22/74]). In summary, despite the greater expression of estrus in heifers receiving 1.0 mg of EC, P/AI was similar between treatments, although in heifers with BCS ≥ 3.0, fertility was negatively affected.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Reproductive performance of *Bos indicus* heifers and cows managed with the Resynchronization 18 program: technical report

Ana Stela Betto[1], Giselly B. Kolling[1], Mirela Balistrieri[1], Paula R. Cortat[1], João Gabriel Gomes[1], Flavio Augusto Portela Santos[1], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil.

This report describes the use of the Resynch18 program (Cunha et al., Animal Reproduction, 1:58, 2024) as a tool to reduce the interval between timed-artificial inseminations (TAI) in *Bos indicus*. The Resynch18 program is an early resynchronization strategy that does not use Doppler ultrasound for pregnancy diagnosis. Reproductive management was conducted during the 2024/2025 breeding season (BS) in southern Brazil, under tropical conditions. Cows and heifers were kept on *Brachiaria brizantha* pastures and supplemented with mineral salt throughout the entire BS. Three categories were used: heifers (yearlings and 2 yr old), primiparous, and multiparous cows. The 1st TAI protocol was the same for all females and involved the use of an 8-d, estradiol (E2)/progesterone (P4)-based protocol. On day 18 (D18) after the first TAI, all females received an intravaginal P4 device (IVD; 1 g) and administration of E2 benzoate (1.5 mg for heifers; 2.0 mg for cows). On D26, pregnancy diagnosis was performed by B-mode ultrasound. In pregnant females, the IVD was withdrawn, and no other treatment was imposed. Non-pregnant females continued in the protocol: after IVD withdrawal, they received E2 cypionate (0.5 mg for heifers; 1.0 mg for cows), equine chorionic gonadotropin (eCG; 200 IU for heifers; 300 IU for cows), and cloprostenol sodium (PGF2α; 0.53 mg for heifers and cows). Then, a second TAI was performed on D28. All injectable treatments were IM. Four TAI were performed in that BS. Ultrasound was used to assess cumulative pregnancy (CP) and pregnancy loss (PL). Pregnancy per AI (P/AI) in heifers was 18.0% (9/50), 46.7% (21/45), 29.2% (7/24), and 0% (0/2) in the 1st, 2nd, 3rd, and 4th TAI, respectively. Among primiparous cows, P/AI was 55.2% (16/29), 38.9% (7/18), 56.2% (9/16), and 42.8% (3/7) in the 1st, 2nd, 3rd, and 4th TAI, respectively. In multiparous cows, P/AI was 46.0% (23/50), 62.8% (22/35), 42.8% (6/14), and 22.2% (2/9) in the 1st, 2nd, 3rd, and 4th TAI, respectively. PL was 0% (0/47), 2.6% (1/38), and 1.7% (1/57) for heifers, primiparous, and multiparous, respectively. At the end of the 84-d long BS, the CPs were 76.6% (36/47) for heifers, 86.8% (33/38) for primiparous, and 86.0% (49/57) for multiparous. In conclusion, the Resynch18 protocol proved to be an efficient, practical, and viable strategy for reproductive management in *Bos indicus* Nelore cattle under tropical conditions. The possibility of resynchronizing females as early as 18 d after the first TAI, combined with pregnancy diagnosis on D26 using B-mode ultrasound, allows for new AIs every 28 d and increases CP. This report reinforces the benefits of Resynch18 as another strategy to intensify breeding management in beef herds, especially in field conditions when Doppler ultrasound is not available.

Acknowledgments: Clube de Práticas Zootécnicas (CPZ) and MSD.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Timing of injectable progesterone administration prior to the TAI protocol does not interfere on the follicular diameter at the beginning of the ovulation synchronization protocol in Bos indicus cows

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

[1]Universidade Federal de Lavras, [2]Ourofino Saúde Animal, [3]Universidade Federal de Juiz de Fora

The objective was to evaluate the effect of timing of injectable progesterone administration prior to the TAI protocol (7 vs 10 days) on follicular diameter at the beginning of the ovulation synchronization protocol in Bos indicus cows. In the study, retrospective data of 1279 Bos indicus (Nelore) with a body condition score of 2.73 ± 0.01 (scale of 1 - 5) and between 30 and 40 days postpartum were used. The animals were maintained Brachiaria brizantha pastures and received mineral supplementation. The animals were pre-synchronized with P4i (150 mg; Sincrogest Injectable, Ouro Fino, Brazil) seven days (Group P4i7, n = 546) or ten days (Group P4i10, n = 733) prior to timed artificial insemination (TAI) protocol. Ultrasound examinations were conducted at the beginning of the protocol (D0) to measure the diameter of largest follicle. Statistical analyses were conducted using GLIMMIX procedure of SAS. There was no interaction between treatment presynchronization duration and BCS for follicular diameter on D0 (P = 0.71). In addition, follicular diameter on D0 did not differ between experimental groups (Group P4i7 = 12.5 ± 0.1 and Group P4i10 = 12.7 ± 0.2; P = 0.93). However, cows with low body condition score (BCS) at pre-synchronization exhibited smaller follicular diameter on D0 (BCS 2.25 = 10.7 ± 0.4c; BCS 2.5 = 11.6 ± 0.2c; BCS 2.75 = 12.9 ± 0.1b; BCS 3.0 = 13.3ab ± 1.3; BCS 3.5 = 14.4 ± 0.4a; P < 0.0001). In conclusion, timing of injectable progesterone administration prior to the TAI protocol (7 or 10 days) does not interfere on the follicular diameter at the beginning of the TAI protocol. However, cows with low BCS have smaller follicular diameter at the beginning of the ovulation synchronization protocol in Bos indicus cows.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Treatment with prostaglandin F2α at the onset of an estradiol/progesterone-based synchronization protocol in Nelore (*Bos indicus*) heifers influences reproductive outcomes

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

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil.

This study evaluated reproductive outcomes of Nelore (*Bos indicus*) heifers treated or not with prostaglandin F2α (PGF) at the onset of an estradiol benzoate (EB)/progesterone (P4)-based synchronization protocol. On D-19 (D0: onset of the protocol), 43 heifers (24.2±0.1 mo old, 383.4±3.3 kg) underwent an ovulation induction protocol. All heifers ovulated and were assigned to two groups: 1) PGF (n=22): on D0, heifers received 0.53 mg PGF and 1.5 mg EB IM, along with insertion of a 0.5 g intravaginal P4 device (IVD). On D7, the IVD was removed, and 0.5 mg estradiol cypionate IM, 0.53 mg PGF IM, and 200 IU eCG IM were administered; 2) NoPGF (n=21): heifers underwent the same synchronization protocol, without PGF on D0. Follicular dynamics were assessed daily by ultrasonography from D0 to 7, and every 8 h from D7 to 11, to determine the timing of ovulation. Blood samples were collected daily to evaluate circulating P4 concentrations. Estrus behavior was monitored 24 h/d from D7 to 11 by visual observation of standing to be mounted. Statistical analyses were done by PROC GLIMMIX (SAS 9.4; difference: P≤0.05; tendency: 0.05<P≤0.10). Circulating P4 concentrations did not differ between groups on D0 (5.1±0.3 ng/mL), but the mean circulating P4 from D1 to 7 was greater in NoPGF than in PGF group (5.2±0.4 vs 1.6±0.1 ng/mL). Synchronization rate (proportion of heifers having emergence of a new follicular wave within 5 d after D0) did not differ between groups (PGF: 77.3% vs NoPGF: 85.7%). However, time of emergence was delayed in NoPGF compared to PGF group (3.5±0.2 vs 2.4±0.2 d). The dominant follicle (DF) on D7 and 9 was larger in PGF than NoPGF group (D7: 10.7±0.4 vs 8.4±0.4 mm; D9: 13.2±0.3 vs 11.6±0.5 mm). Follicle growth rate from emergence to D7 tended to differ between groups (PGF: 1.5±0.1 mm/d vs NoPGF: 1.3±0.1 mm/d), while from D7 to 9, it was greater in NoPGF than in PGF group (1.6±0.1 vs 1.3±0.1 mm/d). Ovulation rate at the end of the protocol did not differ between treatments (PGF: 100% vs NoPGF: 94.4%). After removal of the IVD, time to ovulation was shorter (59.5±1.6 vs 70.8±2.8 h) and the onset of estrus was earlier (32.1±0.8 vs 40.1±2.0 h) in PGF- than NoPGF-treated heifers. Although no differences in estrus length were observed (PGF: 15.1±1.6 h vs NoPGF: 12.1±1.4 h), heifers from the PGF group received more mounts during estrus (29.0±3.9 vs 14.4±2.5), thereby, a shorter interval between mounts (37.5±6.9 vs 64.7±11.0 min). In conclusion, reducing circulating P4 concentrations by administration of PGF at the onset of an E2/P4-based synchronization protocol resulted in earlier emergence of a new follicular wave and larger DF, besides inducing earlier onset and greater intensity of estrus. Despite a not detectable difference in ovulation rate, time of ovulation was anticipated in heifers treated with PGF, occurring closer to the expected time of AI, which might reduce fertility.

Acknowledgments: CAPES, FAPESP#2018/03798-7, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTAI AND AI

Use of GnRH or estradiol benzoate at beginning of a resynchronization protocol starting 12 days after timed-AI in beef cattle: preliminary results

Gabrielle Ceragioli Damaceno[1], Ana Karolyne Alves Miguel[1], Samuel Volpe Souza[1], Karine Galhego Morelli[1], Isabella Rio Feltrin[1], Thiago Neder Lisboa[1], Carlos E. C. Consentini[2], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]GlobalGen Vet Science

The aim of this study was to evaluate the use of GnRH or estradiol benzoate (EB) at the beginning of an estrus resynchronization protocol and their effects on ovulation induction, new corpus luteum (CL) formation, follicular growth, and the incidence of false-positive (FP) pregnancy diagnoses. Nelore (*Bos indicus*) suckled cows (n = 199) and 2-year-old heifers (n = 88) were submitted to an estradiol/progesterone-based protocol for 1st TAI (D0). Twelve days later (D12), all animals received an intravaginal device (IVD) containing 0.5 g (ReproOne®, GlobalGen, for heifers) or 1 g (ReproNeo®, GlobalGen, for cows) of progesterone (P4), and underwent ultrasonographic evaluation. Animals were randomly assigned to one of three groups: (1) Control (no treatment); (2) EB (1 mg EB IM; Syncrogen®, GlobalGen); and (3) GnRH (16.8 µg buserelin acetate IM; Maxrelin®, GlobalGen). On D20, IVDs were removed, and all animals received 1 mg estradiol cypionate (Cipion®, GlobalGen) and 300 IU eCG (eCGen®, GlobalGen) IM. Dominant follicle and CL blood perfusion were assessed by B-mode and Doppler ultrasonography. On D22, females with non-functional CL (blood perfusion ≤25%) were classified as non-pregnant, had the preovulatory follicle measured, received 8.4 µg GnRH (Maxrelin®), and underwent the 2nd TAI. Pregnancy diagnoses were confirmed on D52. Data were analyzed using the software SAS and significance was set at $P \leq 0.05$. The GnRH group induced more new CL formation from D12 to D20 than EB and Control (27.9% [26/93], 4.1% [4/97] and 0% [0/97], respectively). More animals had functional CLs on D20 in the GnRH group (67.7% [63/93]) than in EB (49.5% [48/97]) and Control (51.6% [50/97]). There was no effect of treatments in the follicular growth rate from D20 to D22 (1.1 mm/day). The FP rate between D20 and D22 was lower in the EB group (0% [0/48]) compared to the Control (14.0% [7/50]) and GnRH (11.1% [7/63]). From D22 to D52, FP rates were similar ($P > 0.1$) among Control (14.0% [6/43]), EB (18.8% [9/48]) and GnRH (14.4% [8/56]). Pregnancy rates at the 1st TAI (43%), 2nd TAI (56%), and cumulative (71%) did not differ among groups ($P > 0.1$). In conclusion, EB administered at the beginning of the resynchronization 12 days after TAI reduced FP rates, which potentially improves the accuracy of US-Doppler when performed at the moment of P4 device removal. Although GnRH treatment at D12 induced ovulation and new CL formation in almost 1/3 of the group, it did not impact FP rates or pregnancy outcomes. Further studies with larger sample s are needed to confirm the reproductive impacts of GnRH at resynchronization.

Acknowledgments: GlobalGen Vet Science.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Cavity corpus luteum in lactating dairy cows receiving embryo transfer: impacts on fertility and pregnancy maintenance

Raphael Azevedo Lamim[1], Nicolas Moreira Piedras Monnerat Caparelli[1], Matheus de Oliveira Dini Nascimento[2], Maria Eduarda de Melo[1], Thiago de Souza Vieira[1], Luis Fernando Moroz[3], Samuel Rodrigues Bonamichi do Couto[4], Carlos E. C. Consentini[5], Roberto Sartori[2], Marco Roberto Bourg Mello[1]

[1]Universidade Federal Rural do Rio de Janeiro, [2]Universidade de São Paulo, [3]Fazenda Frankanna, [4]Universidade Federal Fluminense, [5]GlobalGen Vet Science

The objective of this study was to determine the incidence of cavity corpus luteum (CavityCL) presence at the time of embryo transfer in recipients lactating dairy cows enrolled in a commercial timed ET (TET) program, as well as to assess whether the presence of CavityCL affects pregnancy per ET (P/ET) and pregnancy loss (PL). A total of 274 healthy, high-producing (42 kg/d), between 60 and 85 days of lactation at the first ET, with a body condition score between 2.5 and 4.5 (scale of 1 to 5), multiparous Holstein cows, housed in free stall barns, were used for the analysis. In a trial period from October 2024 to April 2025. The cows underwent the following TET protocol: Day -10: 16.8 µg of buserelin acetate plus 2 mg of E2 benzoate and insertion of a 2 g P4 implant; Day -3: 0.530 mg of cloprostenol sodium (PGF); Day -2: P4 implant removal, 0.530 mg of PGF and 1 mg of E2 cypionate. On Day 7, frozen *in vitro* produced embryos were transferred by the same technician throughout the study. Moreover, ultrasonographic evaluation was performed and only recipients presenting a single CL were included in the analysis. These were classified into two groups: 1) presence of cavity CL (CavityCL; n = 127), and 2) presence of a compact CL (CompactCL; n = 147). The CavityCL was characterized by the presence of anechoic fluid within the luteal structure. Pregnancy diagnosis was performed on Day 32, and PL was assessed at 60 days. The P/ET was calculated as the proportion of pregnant cows relative to the total number of embryo transfers, while PL was defined as the absence of a fetus at 60 days among those initially diagnosed as pregnant. Statistical analyses were performed using IBM SPSS Statistics (version 26). The association between corpus luteum type and the outcomes (P/ET and PL) was assessed using logistic regression models. When necessary, post hoc comparisons were applied to evaluate differences between groups. The significance level was set at 5%. Results showed that 46% of the cows presented a CavityCL and 54% a CompactCL on Day 7 (day of ET). No significant differences were observed in P/ET between groups: CavityCL = 35.4% (45/127) and CompactCL = 35.3% (52/147). Similarly, no significant differences were found in PL: 22.2% (10/45) in the CavityCL group versus 26.9% (14/52) in the CompactCL group. These findings indicate that the presence of CavityCL at the time of ET, does not compromise the reproductive performance of recipient lactating dairy. Therefore, the presence of a CavityCL should not be considered a disqualifying criterion in recipient selection for such TET programs.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Effect of long-acting injectable progesterone on the day of ET on the reproductive efficacy of F1 Nelore x Angus recipients

Everton Santos Souza[1], Emanuele Almeida Gricio[2], Adriel Vânio Gretter[2], Ana Carolina dos Santos Oliveira[3], Sofía Albertini[4], Igor Garcia Motta[5], Bruno Gonzalez de Freitas[5], Pietro Sampaio Baruselli[4,2,3]

[1]Fazenda Santa Nice, [2]University of São Paulo, [3]Animal Reproduction Department, University of São Paulo, [4]Universidade de São Paulo, [5]Ourofino Saúde Animal

This study evaluated the effects of long-acting injectable progesterone (iP4; Sincrogest, Ourofino) on the day of embryo transfer (ET; D17) in crossbred (F1 Nelore x Angus) heifers recipients. A total of 317 24-month-old heifers [average body condition score (BCS): 3.29±0.01; Body weight: 427.76±2.05] were allocated into two groups: Control (n=159) and iP4 (n=158). The experiment was conducted at Fazenda Santa Nice (Amaporã/PR, Brazil). On D0, all females received 2 mg of estradiol benzoate, 0.52 mg of sodium clorprostenol (PGF2α), and an intravaginal progesterone device (P4). On D8, the P4 device was removed, and animals were administrated 0.5 mg of estradiol cypionate, 0.52 mg of PGF2α and 300 IU of eCG. ET was performed on D17. All females had their corpus luteum (CL) evaluated and classified on a 1-3 scale (1=small; 3=large). Heifers in the iP4 group received 300 mg of iP4 at the time of ET. Vitrified *in vitro*-produced embryos were used. Pregnancy diagnoses were performed via ultrasound on days 30 and 90 of pregnancy to assess pregnancy per embryo transfer (P/ET) and pregnancy loss. Data were analyzed using PROC GLIMMIX in SAS® 9.4. Fixed effects included treatment, CL classification, and their interaction. The variables analyzed were P/ET at 30 and 90 days, and pregnancy loss between 30 and 90 days. No interaction between treatment*CL classification was observed for the variables studied (P>0.05). There was a tendency for higher P/ET at 30 days in the iP4 group (P = 0.06), with 52.8% (84/159) in the Control group and 61.4% (97/158) in the iP4 group. This trend was consistent in recipients with CL grades 1 and 2 [Control = 55.3% (42/76) vs. iP4 = 59.2% (45/76)] and CL3 [Control = 50.6% (42/83) vs. iP4 = 63.4% (52/82)]. At 90 days, P/ET did not differ between groups [Control=41.4% (65/157) vs. iP4=46.5% (73/157); P=0.36]. Furthermore, the pregnancy loss between 30 and 90 days did not differ between the groups [Control=20.7% (17/82) vs. iP4=24.0% (23/96); P=0.60]. In conclusion, administration of 300 mg of iP4 at the time of ET tended to increase P/ET at 30 days but had no effect on P/ET at 90 days or pregnancy loss. These findings suggest that iP4 may improve reproductive efficiency in ET programs; however, due to the limited sample, further studies with larger populations are warranted.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Efficacy of recombinant equine chorionic gonadotropin in fixed-time embryo transfer protocols for Bos indicus and Bos taurus recipients

Andressa Rodrigues[1], Adriel Vânio Gretter[2], Alexandre Hênryli de Souza[3], Federico Randi[3], Rafael Moreira[3], manuele Almeida Gricio[4,5], Everton Santos Souza[6], Telcio Minaré[7], Pietro Sampaio Baruselli[4,5]

[1]Agropecuária Jacarezinho, [2]University of São Paulo, [3]Ceva Saúde Animal, [4]Universidade de São Paulo, [5]Animal Reproduction Department, University of São Paulo, [6]Fazenda Santa Nice, [7]Cepx Embriões

This study assessed the efficacy of recombinant equine chorionic gonadotropin (reCG, Foli-Rec®) at two doses (105UI and 140UI) compared to conventional eCG (300UI; Folligon®) in fixed-time embryo transfer (TETF) protocols for Bos indicus and Bos taurus recipients. The trial involved 672 animals across two regions: 260 F1 Angus heifers in Southern Brazil and 198 Nelore heifers plus 214 primiparous Nelore cows in Northeast Brazil. On day 0 (D0), synchronization began with an intravaginal device containing 750mg progesterone (Prociclar®), 2mg estradiol benzoate (Benzoato HC®), and 150µg D-cloprostenol (Luteglan®). On D8, the device was removed, and animals received 1mg estradiol cypionate (Cipionato HC®) and 150µg D-cloprostenol. Concurrently, treatments were administered: 300IU eCG (Folligon®) for the control group, or 105 IU (1.5ml) or 140IU (2ml) reCG (Foli-Rec®) for the FoliRec105 and FoliRec140 groups, respectively. Embryo transfer occurred on D17. Data were analyzed using PROC GLIMMIX in SAS 9.4. The treated-to-transferred rate were similar across treatments [Folligon=93.0% (224/241) vs. FoliRec105=85.0% (186/219) vs. FoliRec140=84.4% (178/212)], with no treatment-by-site or breed-category interactions (P = 0.79). Double ovulation rates, pregnancy per embryo transfer (P/TE) at 30 [Folligon= 46.8% (104/224) vs. FoliRec105=47% (86/186) vs. FoliRec140=55.1% (97/178)] and 60 days [Folligon=42.3% (94/224) vs. FoliRec105=39.3% (72/186) vs. FoliRec140=44.6% (78/178)], and pregnancy loss [Folligon=9.6% (14/86) vs. FoliRec105=16.3% (14/86) vs. FoliRec140=18.8% (18/97)] showed no significant differences among groups (P > 0.10), with no interactions detected. Notably, FoliRec140 exhibited a tendency for higher P/ET at 30 and 60 days compared to Folligon in primiparous Nelore cows (P = 0.07). These findings indicate that Foli-Rec® at either dose yields ovulation and P/TE outcomes comparable to Folligon®, with FoliRec140 potentially enhancing P/TE in primiparous Nelore recipients.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Estrus expression, luteal parameters, and progesterone levels in embryo recipients maintained on pasture or under confinement conditions

Celso Gabriel Ritz[1], Deborah Nakayama Yokomizo[1], Gustavo Martins Gomes dos Santos[2], Katia Cristina Silva-Santos[2], José Henrique Ayres Dias[2], Marcelo Marcondes Seneda[1], Fábio Morotti[3,1]

[1]Laboratório de Biotecnologia da Reprodução Animal (REPROA), [2]Sheep Embryo, [3]Universidade Pitágoras Unopar Anhanguera

This study aimed to evaluate the reproductive performance of recipients kept on pasture or in confinement during the fixed-time embryo transfer (FTET) protocol and the development and quality of the corpus luteum (CL). Sixty-three Brangus recipients from a commercial beef cattle farm were used, with an average body condition score (BCS) of 3.34 ± 0.03 (1-5). The experimental groups consisted of a pasture group (n = 34; diet consisting of pasture and protein-energy supplement) and a confinement group (n = 29; a diet consisting of 40% silage and 60% concentrate, average daily gain >1.2 kg/day). The recipients received a conventional FTET protocol, beginning on D0 with the insertion of the progesterone (P4) intravaginal device (1.2g Fertilcare®, MSD, Brazil) and intramuscular (i.m.) administration of estradiol benzoate (2mg Fertilcare® Sincronização, MSD) and cloprostenol (0.530mg Ciosin®, MSD). On D8, the device was removed, and animals received cloprostenol (0.530mg Ciosin®, MSD), eCG (300IU, Folligon®, MSD), and estradiol cypionate (1mg Fertilcare® Ovulação, MSD), and a tail paint for estrus detection. On D10, the estrus score was recorded, and the diameter of the dominant follicle (DF) was evaluated by transrectal ultrasonography (E2V Sonoscape®, Domed, Valinhos, Brazil). On D17, CL vascularization (diameter and area), morphology (compact and cavitary), and P4 levels (measured by chemiluminescence assay) were evaluated. Data were analyzed by ANOVA using GLM, considering group and estrus expression as fixed effects and BCS as a covariate, in addition to interactions. The Chi-square test was used to analyze association measures, and Pearson's test for correlation measures (Minitab®, P≤0.05). No differences (P>0.1) were observed between groups for DF diameter on D10 or for estrus expression. However, confined cows showed higher BCS (P=0.002) and ovulation rate (P=0.01; 55.2% vs. 23.5%) on D10. Pasture cows presented greater CL diameter (P=0.01; 21.95±0.46 vs. 19.81±0.63 mm) and area (P=0.02; 38.39±0.16 vs. 31.59±0.20 mm^2). These parameters were influenced by BCS on D0 (P<0.09, trend) and estrus score on D10 (P<0.05), but without interaction with treatments (P>0.1). CL blood flow distribution (low, medium, high) did not differ between groups (P>0.1). Recipients kept on pasture had higher plasma P4 concentrations (P=0.01; 9.39±0.69 vs. 6.68±0.50 ng/mL), which were not affected (P>0.1) by BCS, estrus expression, or interactions. CL diameter (r=0.57; P<0.0001) and area (r=0.56; P<0.0001) were moderately and positively correlated with P4 concentration, whereas estrus expression (P=0.28), ovulation (P=0.28), CL blood flow (P=0.23), and CL morphology (P=0.18) were not. Recipients in confinement exhibited earlier ovulation, smaller CLs, and reduced P4 levels, suggesting that confinement, due to increased metabolic demand, may accelerate P4 metabolism, advance ovulation, and impair luteal function. These findings highlight the importance of nutritional and reproductive management in confined recipients to enhance FTET protocol outcomes.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Evaluation of the effects of including protected fat in the diet of beef recipients

Ana Laura dos Santos Munhoz Gôngora[1], Thiago Neder Lisboa[1], José Ricardo Fonseca Miranda[1], Heitor Jose Reis Maciel[2], Isabella Rio Feltrin[1], Bruno Alves[3], Hugo Savioli Marques[3], Bruno Pietsch Cunha Mendonça[4], Osvaldo Alex de Sousa[4], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]Pontifícia Universidade Católica de Minas Gerais, [3]Agropecuária Nelore Paranã, [4]Nutricorp

The inclusion of protected fats in beef cow diets potentially improves the reproductive performance. The aim of this study was to evaluate if supplementation with protected fat enriched in omega 6 (Nutri Gordura Reprodução®, Nutricorp, Brazil) influences pregnancy rates and pregnancy losses following fixed-time embryo transfer (FTET) in recipient suckled cows. The experiment was conducted on a commercial farm (Agropecuária Nelore Paranã, Iaciara-GO). For this, 953 F1 (Angus x Nelore) cows between 21 and 60 days postpartum were used, of which primiparous n=761 (379.2 ± 2.2 kg; BCS:2.7 ±0.01) and multiparous n=192 (522.9 ± 6.9 kg; BCS: 2.9 ±0.03). All females were submitted to an eight-days P4/E2-based synchronization protocol to synchronize ovulation for FTET. Animals from 10 breeding groups were randomly split into pairs of two contemporary groups: NG group (supplementation with NutriGordura Reprodução®, 38% omega 6, 100g/day/head, added to the protein/energy supplement at 0.3% live weight), and Control group (supplementation with the same protein/energy supplement at 0.3% live weight). Eight days after beginning of the synchronization protocol (Day 8), animals started to receive the fat supplementation, which last for 62 days (Day 70). On Day 17, recipients were evaluated by transrectal B-mode ultrasonography and those bearing a well-developed corpus luteum received a fresh (n=787) or frozen (n=166) *in vitro* produced embryo. The weight and body condition score were determined. Pregnancy was confirmed by presence of an embryo or fetus with heartbeats using ultrasonography approximately on Days 40 and 70. A completely randomized design was used, stratifying groups by parity and embryo type. The data was analyzed using PROC GLIMMIX of SAS software. No significant effect (P>0.1) of fat treatment was observed on body weight and condition score, but the multiparous cows were heavier than primiparous. The rate of recipient use on Day 17 was greater (P≤0.05) in the NG group (80% [393/491]) than in the Control groups (75% [349/462]). Conception rates (pregnancy/FTET) on Day 40 were greater (P≤0.05) in recipients from the NG group than in the Control group (44% vs. 37%); whereas on Day 70, conception rate tended to be greater (P=0.09) in the NG group (34% vs. 29%). The rate of pregnancy per exposed female to the synchronization protocol on Days 40 and 70 were greater (P≤0.05) in the NG group (36% and 27%, respectively) than in the Controls (29% and 22%). The rate of pregnancy losses between Days 40 and 70 did not differ (P>0.1) between the NG (23% [38/166]) and the Control (20% [23/112] groups. In conclusion, supplementation with an enriched source of omega 6 for about two months improves the reproductive performance of suckled recipient beef cows by enhancing the rate of recipient use and the embryo survival between the FTET and day 30 of pregnancy, but does not reduce pregnancy losses between days 40 and 70.

Acknowledgments: Nutricorp and Agropecuária Nelore Paranã

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Factors affecting fertility and pregnancy loss after *in vitro* embryo transfer in beef cattle

Mirela Balistrieri[1], Lucas O. e Silva[1], Paula R. Cortat[1], Carlos E. C. Consentini[2], Douglas Gaitkoski[3], André Zaganin Braga[3], Matheus Silvério de Oliveira[3], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture, [2]GlobalGen Vet Science, [3]CPEX Embryos

The aim was to evaluate potential factors affecting fertility and pregnancy loss (PL) between 30-60 d of pregnancy in a beef cattle *in vitro* embryo production (IVP) system. Data were collected from August 2021 to February 2023 from a single embryo production company, involving donor cows from one farm and recipient cows from eight farms. Analyses were performed to assess the effects of donor/recipient category and recipient breed, semen type, and synchronization protocol length on pregnancy per embryo transfer (P/ET) and PL. Only sires with at least 100 records were included in the analysis. To improve the reliability of the analyses and avoid confounding effects, the effect of each variable was analyzed within a specific sample group. Statistical analyses were done by GLIMMIX of SAS ($^{a\text{-}b}P \leq 0.05$). The effect of donor category (Ca: calf; He: heifer; Co: cow) was analyzed within Nelore (NE) and crossbred Angus×Nelore (A×N) recipient cows. Donor category influenced P/ET at 30 d in NE (Ca: 38.5^{b} [1,073], He: 45.9^{a} [1,029], and Co: 44.2%^{ab} [925]; P=0.02) and A×N recipients (Ca: 39.9^{b} [1,030], He: 41.9^{b} [831], and Co: 48.4%^{a} [3,255]; P=0.01). However, no effect was observed on PL in NE (Ca: 12.1 [413], He: 15.0 [472], and Co: 8.6% [409]; P=0.17), nor in A×N recipients (Ca: 24.3 [411], He: 15.5 [348], and Co: 19.0% [1,576]; P=0.25). The effect of type of semen was analyzed considering only donor and recipient cows. Sex-sorted sperm was associated with greater P/ET at 30 d than conventional semen (48.9 [2,117] vs. 46.0% [2,063]; P=0.02), whereas PL did not differ (15.9 vs. 17.7%; P=0.83). The effect of protocol length (permanence of progesterone device) was analyzed within A×N primiparous recipients, considering only embryos from donor cows. The 7-d long protocol was associated with greater P/ET at 30 d than longer protocols (7-d: 64.8^{a} [335], 8-d: 52.6^{b} [950], and 9-d: 50.4%^{b} [401]; P=0.01). However, PL was similar among protocols (7-d: 10.6 [217], 8-d: 12.0 [500], and 9-d: 17.8% [202]; P=0.15). The effect of recipient category was evaluated within A×N recipient cows. Multiparous (M) and primiparous (P) recipients had greater P/ET at 30 d and lower PL than heifers (P/ET: He: 39.1^{b} [1,339], P: 54.5^{a} [1,686], and M: 58.3%^{a} [230]; P<0.01; PL: He: 31.4^{b} [524], P: 13.0^{a} [919], and M: 11.9%^{a} [134]; P<0.01). Finally, the effect of the recipient breed was evaluated considering only embryos from donor cows. A×N recipients had greater P/ET at 30 d (48.4 [3,255] vs. 44.2% [925]) but also greater PL (19.0 [1,576] vs. 8.6% [409]; P<0.01) than NE recipients. In conclusion, findings from this dataset indicated that multiple factors can influence IVP success in beef cattle, such as donor/recipient category and breed, type of semen, and protocol length. Apparently, cow recipients and shorter synchronization protocols may result in improved P/ET, while NE recipients, despite having lower P/ET at 30 d than A×N recipients, seem to have lower PL.

Acknowledgments: CNPq, CPEx, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Impact of the interval between calving and ET on reproductive efficiency in primiparous and multiparous recipients

Emanuele Almeida Gricio[1,2], Bruno Silva do Espirito Santo[3,4], Sofía Albertini[1], Pietro Sampaio Baruselli[1,3,2]

[1]Universidade de São Paulo, [2]Animal Reproduction Department, University of São Paulo, [3]University of São Paulo, [4]Agropecuária Jacarezinho

The objective of the study was to evaluate the influence of days postpartum (DPP) in relation to embryo transfer (ET), on the pregnancy per ET (P/ET) and pregnancy losses between 30-60 (PL30-60) and 60 to calving (PL60-C) in lactating F1 Angus x Nelore (Primiparous = 466; Multiparous = 590) and Nelore (Primiparous = 303; Multiparous = 2,421) recipients. The data from four breeding seasons, from recipients with a DPP range of 19-102 days (mean of 49.9±0.23), from a commercial farm (Agropecuária Jacarezinho, Brazil), were used. DPP was considered the period from the calving to D0 of the protocol for fixed time embryo transfer (FTET). Only the first service ET recipients were used for the analysis. Pregnancy diagnoses were assessed by ultrasound after 30 days (P/ET) and 60 days to calculate PL30-60 and at birth to calculate PL60-C. The statistical analyses were performed using the LOGISTIC procedure for logistic regression to model probability and GLIMMIX in SAS 9.4. There was an interaction between DPP and category for the transferred-to-treated rate (P=0.01). In lower DPP, the primiparous recipients present a lower transferred-to-treated rate than multiparous recipients. However, this difference was not observed in higher DPP. In Nelore and F1 Angus recipients, a linear effect (P<0.0001) was observed for the transferred-to-treated rate in both breeds, with no interaction between breed and DPP (P = 0.41). As the DPP increases, the transferred-to-treated rate increases.In primiparous and multiparous recipients, a linear effect (P=0.02) was observed for the P/ET at 30 days in both categories, with no interaction between category and DPP (P=0.43). As the DPP increases, the P/ET increases. In Nelore and F1 recipients, a linear effect (P=0.03) was observed for the P/ET at 30 days in both breeds, with no interaction between breed and DPP (P=0.32). As the DPP increases, the P/ET increases. In primiparous and multiparous recipients, a trend for linear effect (P=0.07) was observed for the P/ET at 60 days in both categories, with no interaction between category and DPP (P=0.95). As the DPP increases, the P/ET increases. In primiparous and multiparous recipients, no effect of DPP was observed for PL30-60 in both categories. Also, no effect of breed was observed for PL30-60 according to DPP. For the PL60-C variable, there was no significant difference for DPP in both categories and breeds. It concludes that a shorter postpartum period may decrease the transferred-to treated rate in primiparous. However, a positive linear effect of DPP was observed for both breeds. The 30-day P/ET increases according to the DPP increase in the categories and in breeds. However, the DPP did not affect the PL30-60 and PL 60-C.

Acknowledgments: Agropecuária Jacarezinho.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

In vivo embryo production using recombinant hormones (rFSH and reCG) for superovulation in Nelore (Bos indicus) donors

Adriel Vânio Gretter[1,2], Lígia Mattos Rebeis[1,2], Claudiney de Melo Martins[3], Priscila Olimpio Reis Martins[3], Nelise Kasue Samecima Prosperute[3], Sofía Albertini[1,2], Pietro Sampaio Baruselli[4,1,2]

[1]University of São Paulo, [2]Animal Reproduction Department, University of São Paulo, [3]Fertiliza Cuiabá, [4]Universidade de São Paulo

The use of recombinant hormones for ovarian superstimulation represents a significant advancement in optimizing protocols for both in vivo (SOV) and *in vitro* (IVEP) embryo production. This study evaluated the effects of recombinant follicle-stimulating hormone (rFSH; Zimbria®) and recombinant equine chorionic gonadotropin (reCG; Foli-Rec®) in superovulation protocols for in vivo embryo production in Nelore (Bos indicus) donors (n=40). Donors were submitted to a 9-handling superstimulation protocol. On D0 (a.m.), all donors received 2mg of estradiol benzoate (EB; Benzoato HC®) and a 750mg intravaginal progesterone device (Prociclar®). On D4 (a.m.), the donors were assigned, based on the ovarian follicular population count by ultrasonography, to one of three groups.: 1) rFSH group (Control; n=12) received 100µg of rFSH, 2) rFSH + reCG group (n=13) received 100µg of rFSH and 175IU of reCG on D6 (a.m.), and 3) reCG group (n=15) received 1,050IU of reCG on D4 (a.m.). All animals received two treatment with 150µg of cloprostenol (Luteglan®) on D6 (a.m. and p.m.), and progesterone devices were removed on D7 (a.m.). On D8 (a.m.), 175µg of gonadorelin (Cevarelin®) was administered. Inseminations were performed on D8 (p.m.) and D9 (a.m.). Embryo collections were conducted on D15 (n=21) and D16 (n=19). Ultrasound evaluations were performed on D4 for baseline follicular count and on D6 and D8.5 to classify follicles as small (<5 mm), medium (5–8 mm), or large (>8 mm). Data were analyzed using SAS 9.4. The model evaluated the effects of treatment group on ovulation rate (OR), recovery rate (RR), and viable embryo rate (VER). No significant differences were found among groups for OR (rFSH: 59%±6% vs. rFSH + reCG: 56%±8%; vs. reCG: 56%±10%; P>0.1). The number of larger follicles in D8.5 (>8,0mm) (rFSH: 36.1±8.2 vs. rFSH + reCG: 30±6.6 vs. reCG: 50.1±8.3; P=0.23) and the total CL at the flushing (rFSH: 18.5±3.8 vs. rFSH + reCG: 14.4±2.3 vs. reCG: 16.3±2.6; P=0.61) did not differ between groups. The RR was lower (P=0.03) in the reCG group (34%±5%) compared to the rFSH group (65%±11%), while the rFSH + reCG group (48%±8%) did not differ statistically from either. The total number of recovered structures (rFSH: 8.33±1.3 vs. rFSH + reCG: 6.25±0.84 vs. reCG: 6.6±1.27; P=0.39) and the number of viable embryo (rFSH: 4.3±0.8 vs. rFSH + reCG: 4.1±0.6 vs. reCG: 3.9±0.8; P=0.78) were similar between groups. The VER did not differ among groups (rFSH: 58% ± 8%; rFSH + reCG: 67% ± 8%; reCG: 65% ± 9%). These results indicate that reCG is effective in superstimulation protocols for Nelore donors, yielding embryo viability comparable to rFSH-based treatments, although recovery rates may be reduced when reCG is used alone. The combined treatment with rFSH and reCG did not improved the embryo production.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FTET, ET, AND SUPEROVULATION

Performance of IVEP and AI-derived dairy heifers born and raised in herds of small farms in the state of São Paulo. Preliminary results

Raquel Castellucci Caruso Sachs[1], Maurício Roberto de Souza[2], Rafael Herrera Alvarez[1]

[1]Agência Paulista de Tecnologia dos Agronegócios - Unidade Regional de Piracicaba - Secretaria de Agricultura e Abastecimento de São Paulo, [2]Instituto de Terras do Estado de São Paulo

The present study aimed to evaluate the reproductive and productive performance and economic return of dairy heifers originated from *in vitro* produced embryos (IVEP) and artificial insemination (AI). Reproductive and productive (up to the seventh month of the first lactation) data of 43 IVEP-derived F1 heifers (Gyr x Holstein) born and raised in five herds in the Pontal de Paranapanema region, SP, were compared with contemporaries AI-derived heifers (n=120) from different crosses with the Holstein breed. Data were analyzed by ANOVA using SAS 9.4. The herds had similar reproductive management, including hormonal estrus synchronization protocols to perform IATF, deferring in food management and milking hygiene. In some animals from both groups, the use of oxytocin was common practice to promote milk letdown. There was no difference in age at conception (19 ± 3 months) and age at calving (29 ± 2 months) of heifers derived from IVEP or AI ($p>0.05$). The average milk production of IVEP and AI-derived heifers was 23.2±1.53 and 16.13±1.61 kg milk/day, respectively ($p<0.05$). On average, milk production was 43.83% higher in IVEP-derived heifers. In four herds, IVEP-derived heifers produced 23.92% to 74.75% more milk than AI-derived heifers ($p<0.05$), while in one herd production did not differ between the two groups of animals ($p>0.05$). In economic terms, the observed increase in production between 5 kg milk/day and 10 kg milk/day generates an increase in revenue between R$15.00 and R$30.00/day. Considering only the increase in feed costs for this increase in production, with the other costs constant, the return on investment in genetics in the herds that increased milk production occurred through additional milk production in the period of 3 to 6 months. Based on the results obtained, it is concluded that IVEP and embryo transfer is an excellent tool for incorporating superior genetics into dairy herds of small producers, whose investment can be quickly paid back with the surplus production. On the other hand, is still under analysis the reason why superior genetics was not expressed in one of the herds studied.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Action of melatonin in bovine embryo culture under high oxygen tension in blastocyst production

Isabella Rodrigues dos Santos Oliveira[1,2], Fabiana lima rodrigues[3,2], Lucas Costa de Faria[1,2], Adriano Queiroz de Mesquita[2], Maria Tereza de Oliveira Rodrigues[1], Gabriel Antônio M. Medeiros Martins[4], Victor Carlos Mello[1], Márcio José Poças Fonseca[1], Carlos Frederico Martins[2], Sônia Nair Báo[1]

[1]Universidade de Brasília, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]Icesp, [4]Centro Universitário de Brasília

Melatonin (Mel) is an antioxidant that modulates the microenvironment where oxidative stress is one of the main challenges to embryonic viability. In this context, this study evaluated the impact of supplementation with melatonin at 10□⁹ M during the *in vitro* culture of bovine embryos under different oxygen tensions. After *in vitro* fertilization, bovine zygotes were cultured in four experimental groups: SOF with and without melatonin under high oxygen tension (high O□ with Mel/without Mel) and SOF with and without melatonin under low oxygen tension (low O□ with Mel/without Mel). On day 7, blastocyst rate, gene expression related of oxidative stress and embryo quality, mitochondrial activity (MitoTracker), and cytoplasmic lipid accumulation (Bodipy) were evaluated. The blastocyst rate was significantly higher ($P<0.05$) in the high O□ with Mel group (39.80±2.14%) compared to the control group without Mel (34.04±1.72%) and the other treatments under low oxygen tension (31.67±2.52% and 31.39±2.10%, with and without Mel, respectively). There was a significantly higher expression ($P<0.0001$) of the genes SOD2 (oxidative stress control) and KRT8 (cell metabolism and apoptosis regulation) in the high O□ with Mel treatment compared to the other groups. Additionally, the cytokine IFNT, crucial in maternal-embryo signaling, and the gene PLAC8, involved in trophoblast development, showed greater expression ($P<0.0001$) in the high O□ with Mel group compared to the control without Mel and the low O□ with melatonin group. However, the highest overall expression of these two genes was observed in the low O□ without melatonin group ($P<0.0005$). These findings demonstrate the effectiveness of melatonin in high oxidative stress environment, contributing to the expression of key genes related to embryo development and pregnancy. We also inferred that in low oxygen tension environment, melatonin does not enhance the expression of these genes, since they were expressed even in its absence, indicating an adaptive embryonic response under hypoxia. Furthermore, melatonin led to a reduction in lipid accumulation in embryos under high O□ ($P=0.0174$) compared to the control group and low O□ with Mel, possibly without affecting mitochondrial function. However, no difference in lipid accumulation was observed in the low O□ without melatonin group. These results reinforce that the effects of melatonin are dependent on oxygen conditions and oxidative stress. Mitochondrial activity was only more intense in the low O□ with Mel group, indicating a change in embryonic metabolism due to its presence. Thus, this study observed that melatonin was effective under increased O□ levels, promoting better embryo production, higher expression of genes linked to viability and maternal-embryonic signaling, as well as reduced lipid accumulation. These results suggest that melatonin is a promising strategy to improve the production and quality of bovine embryos in systems with high oxygen tension.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Breed-specific differences in commercial large-scale *in vitro* embryo production: a report on Brahman and Wagyu Cattle in Australia

Sara Yamaguishi Tomita[1], Rafael Junqueira[2], Fernando Lopes[2], Guilherme Cancado[2], Cesar Manrique[2], Adriana Rettke[2], Marcia Hemann[2], Marcelo Stiaque[2], Kim Giliam[2], Cedric Wise[2]

[1]Inventia Genetic Technolgies Pty Ltd, [2]Inventia Genetic Technologies Pty Ltd

Successful commercial *in vitro* embryo production (IVP) is influenced by various biological and technical factors, including breed-specific developmental kinetics and embryo yield. This report compares the IVP performance of Brahman and Wagyu cattle under commercial conditions in Australia, aiming to enhance our understanding of breed-related differences and improve laboratory practices. Inventia Genetic Technologies Pty Ltd (IGT), an Australian *in vitro* embryo production laboratory, has been operational since 2012. Data for comparing viable embryo production was collected from 2018 to 2024, covering all months of the year for both breeds. The Oocyte Pick-Up (OPU) numbers, total embryos produced, and average viable embryos per donor for Brahman and Wagyu, respectively, were as follows: 8,766 x 7,569; 68301 x 25277; and 7.8 x 3.3. The comparison focused on breed groups (Brahman vs. Wagyu). Donors were not categorized by age. The second parameter compared was the kinetics of embryo development at day 7. Data for this analysis was obtained from February 2025. The total number of embryos evaluated was 320 for Brahman and 221 for Wagyu. The classification of embryo development stages at day 7 was based on the International Embryo Transfer Society (IETS) manual. The *in vitro* production procedures were standardized for all breeds, with ABS serving as the media supplier, as IGT operates as an ABS franchise. To assess differences in overall embryo output between the breeds, an Independent Samples t-test was conducted. Brahman donors produced an average of 7.8 viable embryos per session (range: 7.0–8.9), while Wagyu donors averaged 3.3 (range: 2.5–3.8). Statistical analysis using an unpaired t-test confirmed this difference as highly significant ($t = 7.03$; $p = 0.0033$). A chi-square test was employed to determine whether the distribution of development stages depended on the breed. The stages of development at day 7 for Brahman and Wagyu embryos were as follows: morula (0% vs. 2.7%), early blastocyst (0% vs. 47.5%), blastocyst (51.6% vs. 35.3%), expanded blastocyst (76.5% vs. 14.5%), and hatched blastocyst (0.98% vs. 0%). The chi-squared test confirmed that these differences were statistically significant ($\chi^2 = 47.87$; $p < 0.0001$), indicating that Brahman embryos exhibit greater synchrony and speed of development. These results demonstrate that Brahman cattle show superior embryo development kinetics and higher embryo yields in commercial IVP systems compared to Wagyu. This has practical implications for laboratory optimization, donor management, and genetic program planning. Understanding breed-specific characteristics is essential for refining IVP protocols and maximizing reproductive outcomes in high-throughput embryo production systems.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Bull sensitivity to sperm sexing in bovine *in vitro* embryo production

Carlos Alonso Paco Nagaki[1], Thawan Santana Piemonte[1], Marcio Gonçalves de Paula Junior[1], Mariana Karla Francolino da Silva[1], Camilla Mota Mendes[1], Marcelo Demarchi Goissis[1], Mayra Elena Ortiz D Avila Assumpção[1]

[1]Universidade de São Paulo

The use of sexed semen is widely adopted to direct the sex of progeny during *in vitro* embryo production (IVEP). However, the sexing process can compromise sperm integrity and reduce IVEP efficiency. Although individual variability in response to cryopreservation has been described, studies evaluating the individual sensitivity of bulls to the effects of sperm sexing are limited. Objective: To retrospectively investigate the individual response of bulls to the use of sexed semen, focusing on cleavage (CL), blastocyst (BL), and embryo development (ED) rates. Methodology: Retrospective data from four commercial laboratories were analyzed. The analysis included 46 Nellore bulls, each with at least three IVEP procedures, using both conventional and sexed semen. The CL, BL, and ED rates (defined as the proportion BL/CL) were compared between the two types of semen. Statistical analyses were conducted using R software. Initially, paired t-tests identified significant differences between conventional and sexed semen in CL ($p < 0.001$), BL ($p < 0.001$), and DE ($p < 0.001$) rates. Subsequently, mixed linear models (lme4 package) with random intercept for the bull effect were used to estimate individual variability. Results: The contribution of the bull effect was more pronounced in DE (ICC = 19.8%) and CL (ICC = 8.1%), whereas it was null in BL (ICC = 0), indicating significant individual variability, particularly in cleavage and early embryo development rates. Mixed modeling allowed for respecting the paired structure of the data and quantifying individual influence. Although, on average, conventional semen showed better performance, some bulls demonstrated a lower impact of sexing on CL (n = 8), BL (n = 10), and DE (n = 10) rates. Conclusions: The results indicate that the sexing procedure negatively affects sperm functionality, with an impact on cleavage and embryonic development rates. Individual analysis of the bulls revealed differences in sensitivity to the process, highlighting the importance of specific evaluation of sires regarding the use of sexed semen in IVEP.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Cumulus oophorus and hypothermia enhance *in vitro* sperm longevity

Marcela da Silva Cordeiro[1], Bruno Porpino Homobono[2], Eduardo Baia de Souza[2], Mateus Ramos Trindade[2], Matheus Hadigo Pires Costa[2], ANDRÉ LUIZ ALVES DE SÁ[3], Patrícia de Cássia da Silva Lima[2], Priscila di Paula Bessa Santana[3], Nathalia Nogueira da Costa de Almeida[2], Simone do Socorro Damasceno Santos[2]

[1]Instituto Federal de Educação, Ciência e Tecnologia do Pará, [2]Universidade Federal do Pará, [3]Universidade Federal Rural da Amazônia

It is well established that cumulus oophorus (CO) cells play a crucial role in extending sperm viability *in vitro* by producing substances that protect them from stress and enhance their fertilizing capacity. Therefore, the aim of this study was to evaluate the effect of mature CO fragments combined with hypothermic conditions in prolonging sperm fertilization potential. For this purpose, bovine ovaries obtained from a local slaughterhouse were aspirated, and cumulus oophorus complexes (COCs) classified as grade 1 were selected and biopsied using micro-hematocrit tubes. Fragments from 10 COCs were matured in 100 µL drops of medium (Medium 199 supplemented with 10% FBS, 0.5 µg/mL FSH, 5 IU/mL hCG, 22 µg/mL pyruvate, and 50 µg/mL gentamicin) under mineral oil and incubated in a humidified atmosphere containing 5% CO_2, 20% O_2, and 75% N_2 at 38.5°C for 24 hours. To prepare the *in vitro* fertilization (IVF) drops, frozen semen straws from a single bull were thawed in a water bath at 0, 24, and 48 hours before the day of fertilization. For sperm separation, the semen was deposited on the discontinuous Percoll gradient (90% and 45%). Approximately $2.5x10^6$ spermatozoa were added to 100 µL drops of IVF (Tyrode-albumin-lactate-pyruvate medium supplemented BSA, PHE, heparin, pyruvate, and gentamicin) according to the following experimental groups: Control - IVF standard (n=160 COCs); Hipo24 - sperm maintained in IVF drops at 33°C for 24h (n=121 COCs); Hipo48 - sperm maintained in IVF drops at 33°C for 48h (n=148 COCs); HipoCO24 - sperm maintained in IVF drops at 33°C in the presence of mature CO cells fragments for 24h (n=159 COCs) and HipoCO48 - sperm maintained in IVF drops at 33°C in the presence of mature CO cells fragments for 48 hours (n=183 COCs). For fertilization, 20 COCs previously matured for 24 hours were placed in the each IVF drops. After 24 hours of IVF, presumptive zygotes were transferred to 100 µL drops of synthetic oviduct fluid medium. Embryos were evaluated for cleavage rate on Day 2 and blastocyst formation rate on Day 8 (ANOVA followed by Tukey's post-hoc test, $p < 0.05$, BioEstat 5.0). A significant difference in cleavage rates ($p < 0.05$) was observed between Control (88.10±2.22) and Hipo24 (59.61±5.48), Hipo48 (34.64±9.97), HipoCO24 (78.58±2.84), and HipoCO48 (75.09 ± 5.78), as well as between Hipo24 and HipoCO24. Regarding blastocyst formation rates, significant differences ($p < 0.05$) were observed among all experimental groups (42.48±3.64; 11.55±2.42; 0; 34.70±3.53; and 23.48±3.8, for Groups Control, Hipo24, Hipo48, HipoCO24 and HipoCO48, respectively). Although inferior to the Control, the results obtained in the HipoCO24 (78.58±2.84, 34.70±3.53) demonstrate the possibility of its use as an important strategy in IVP routines by allowing greater temporal flexibility in the IVF process, providing savings in the use of semen doses for both research and commercial laboratories.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Description of the first pregnancies in buffaloes (*Bubalus bubalis*) from *in vitro* embryo production in the state of Ceará, Brazil

Satish Kumar[1], Maiana Silva Chaves[1], Ana Flávia Bezerra da Silva[1], William Gomes Vale[1], Sebastião Tavares Rolim-Filho[2], Lydia Reis Rodrigues[3], Yeda Fumie Watanabe[4], Larissa Linhares Teixeira[1], Luciana Magalhães Melo[1], Vicente José de Figueirêdo Freitas[1]

[1]Universidade Estadual do Ceará, [2]Universidade Federal Rural da Amazônia, [3]Fazenda Laguna, [4]Vitrogen

In vitro embryo production (IVEP) has significantly transformed animal breeding and livestock management. However, due to the cost of this technique and the lack of availability of professionals for it, it is not as common as artificial insemination. In recent years, buffalo farming has increase significantly in Ceará, both numerically and in genetic quality. Given this scenario, this study aimed to provide a comprehensive overview of the IVEP and embryo transfer process and to relate laboratory practices with on-farm applications. Seven hormonally stimulated buffaloes (body condition score between 3 - 4) were selected to *cumulus*-oocyte complexes (COCs) recovery by transvaginal OPU at Laguna Buffalo farm (Paracuru-CE, Brazil). After washing and selecting, the grade I, II and III COCs were submitted to IVM for 24 h in portable incubator (LabMix, WTA, Cravinhos, Brazil) and transported to the laboratory (approximately 1 h from Laguna Farm). Oocytes were *in vitro* fertilized using a frozen straw (2 x 10^6 spermatozoa/ml) and incubated for 18 h. Afterwards, the presumptive zygotes were *in vitro* cultured for six days. All the *in vitro* processes (from IVM to IVC) were conducted in a 5% CO□, 5% O□, and 90% N□ (Eve, WTA) at 38.5°C. On day 6, the blastocysts were loaded into straw and transported to Laguna farm in LabMix. Five embryos were transferred to five recipients (one/female). It was verified the aspiration rate, time taken during the processes, amount of media used, blastocyst rate and pregnancy rate. The mean ± SEM were calculated from the total data.. The aspiration rate was 68.02% (100/147). The number of follicles aspirated from the left and right ovary were 3.57 ± 0.50 and 4.15 ± 0.47, respectively. The number of COCs recovered was 5.26 ± 0.63. The time taken per OPU was 5.42 ± 0.66 min (ranging from 3-14 min). The time to manipulate the aspirated COCs was 20.89 ± 1.54 min (ranging from 5-32 min). The grade I, II, III and IV COCs recovered were 1.64 ± 0.30, 2.07 ± 0.32, 2.56 ± 0.49 and 1.12 ± 0.12, respectively, and the percentages were 23 (23/100), 27 (27/100), 41 (41/100) and 9 (9/100), respectively. For each buffalo, approximately 5 ml of manipulation medium and 100 ml of phosphate-buffered saline for OPU and to wash the filter for recovering the COCs were used, respectively. The blastocyst rate was 60.71% (51/84), and the transferable blastocyst rate was 30.95% (26/84) on day 6 or 7, respectively. The maximum time taken from the embryo loaded into straw to transfer was 5 h. The pregnancy rate achieved from this process was 40% (2/5). This study demonstrated that IVEP and transfer from laboratory to farm is viable in buffalo breeding. To our knowledge, this is the first study describing pregnancy in buffaloes from embryos produced *in vitro* and should contribute to the increase of buffalo farming in the state of Ceará.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Does a single FSH injection prior to OPU affect oocyte quality and embryo production in cycling Gyr heifers?

Mateus Domingues Eller Teixeira[1], Natani Silva Reis[1], Ricarda Maria dos Santos[1]

[1]Universidade Federal de Uberlândia

The success of *in vitro* embryo production in bovine depends largely on the quantity and quality of oocytes recovered through ovum pick-up (OPU). Hormonal stimulation protocols with follicle stimulating hormone (FSH) have been explored to enhance oocyte yield, but simplified strategies are still under investigation. Zebu females have a greater number of follicles recruited in each follicular wave, but heifers tend to produce lower quality oocytes. Conventional protocols for ovarian stimulation before OPU usually involve multiples injections, increasing costs, animal stress and operational complexity. The hypothesis was that a single injection of FSH after the follicular wave synchronization with an injection of GnRH can effectively stimulate ovarian follicles, resulting in a greater number of good quality oocytes and, consequently, an increase in embryos production. This study aimed to evaluate the effect of a single intramuscular injection of FSH prior to OPU on oocyte quality and embryo production in cycling Gyr heifers. Nine heifers, aged 18 to 24 months, were assigned to one of two treatment groups: Control group (no hormonal stimulation) and FSH group (50 mg FSH; Folltropin-V, Vetoquinol, São Paulo, Brazil). In the FSH group, animals received 100 µg of gonadotropin-releasing hormone (GnRH) intramuscularly, followed 36 hours later by a single injection of 50 mg of FSH, diluted in saline. OPU was performed 36 hours after FSH administration. All animals underwent both treatments at least once, with a 30-day interval. A total of 23 OPUs were performed in the Control group and 13 in the FSH group. Data were analyzed using Poisson-normal models, considering treatment and sire as fixed effects, and donor as a random effect. Statistical significance was set at $P \leq 0.05$. There was no significant effect of FSH stimulation on the total number of oocytes recovered (19.89 ± 7.01 vs. 20.02 ± 9.57 for FSH and Control, respectively). Similarly, the number of grades I and II oocytes (9.33 ± 5.70 vs. 8.78 ± 5.89) and embryos classified as grade I and II (7.00 ± 5.55 vs. 5.11 ± 4.23) did not differ between treatments. In conclusion, a single FSH injection prior to OPU did not improve oocyte or embryo number and quality in cycling Gyr heifers.

Acknowledgments: CAPES; CNPq and FAPEMIG.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of chlorogenic acid addition in the *in vitro* maturation medium of ovine oocytes

Érika Karoline de Oliveira Aureliano[1], Andreza Mayara Carneiro Lima[1], Bruna Moura Silva[1], Damaris Raquel Pires dos Santos[1], Luana Kealy Pimentel de Oliveira[1], Rafael Soares dos Anjos[1], Luanna Mendes Souza Rodrigues[1], Victoria Passos Fernandes Castro[1], Mabel Freitas Cordeiro[1], Edilson Soares Lopes Júnior[1]

[1]Fundação Universidade Federal do Vale do São Francisco

The study aimed to evaluate the effect of chlorogenic acid (CGA) inclusion in the *in vitro* maturation medium (IVM) of ovine oocytes. For this purpose, oocytes were collected from ovaries obtained from a local slaughterhouse. The oocytes were then sent to IVM, where they were equally distributed into four treatment groups: CON group, composed of TCM-199 supplemented with 500 IU of penicillin, 0.5 mg of streptomycin, 1.25 µg of amphotericin B, 0.2 mM of sodium pyruvate, 10% (v/v) of fetal bovine serum (FBS) and 15 IU/mL of eCG (Sincro eCG®, Ourofino Saúde Animal, Brazil); in groups CGA1.25, CGA5, CGA10 and CGA20, oocytes were matured in the presence of IVM medium from group CON, supplemented with 1.25 µM, 5 µM, 10 µM and 20 µM of ACG, respectively. At the end of the maturation period, mature cumulus oocyte complexes (COCs) were evaluated for the presence or absence of cumulus cell expansion and the degree of expansion was classified as: non-expanded, partially expanded or fully expanded (Marei; Wathes; Fouladi-Nashta, Reproduction, 139:979-988, 2010). Some of the mature oocytes were used for IVF, while the others were used for morphological evaluations, assessment of chromatin configuration, quantification of reactive oxygen species (ROS) levels, mitochondrial activity and glutathione (GSH). After IVF, presumptive zygotes were denuded and cultured *in vitro* (IVC) for 48 h in an incubator (CO_2 Incubator – TE-399®, Tecnal, Brazil) at 38.5°C, under 5% CO_2. At the end, the number of cleaved structures and the proportion of structures at different stages of development were recorded. The following tests were used: Shapiro-Wilk to verify variances; Kruskal-Wallis, for significant comparisons and SNK, to analyze the means via SAS® software. And, to evaluate the degrees of expansion of cumulus cells and presumptive zygotes, the Chi-square test in Epi Info software (Epi Info 7.2.5, Atlanta, GA, USA, 2021). Comparisons were considered significant when $P<0.05$. Regarding cumulus cell expansion, all groups that received GCA (GCA1,25, GCA5, GCA10 and GCA20) showed greater expansion (95.3%, 97.6%, 93.5% and 91.1%) ($P<0.05$) compared to the group that did not receive the drug (CON) (82.8%). The CGA5 group exhibited a higher number of oocytes in metaphase II than the other groups (49.2%) ($P < 0.05$), except for the CGA1.25, which showed a similar result (36.4%) ($P > 0.05$). Compared with CON group, CGA1,25 and ACG5 groups demonstrated significantly lower levels of ROS, GSH and mitochondrial activity ($P<0.05$). Furthermore, it was observed that CGA1,25 and CGA5 groups promoted a higher percentage of cleaved structures. In conclusion, concentrations of 1.25 µM and 5 µM of CGA improve the *in vitro* maturation rate of ovine oocytes, reduce the levels of reactive oxygen species, GSH and mitochondrial activity, and increase the proportion of fertilized and cleaved structures, suggesting the valuable potential of CGA as an additive in the *in vitro* production of ovine embryos.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of circulating progesterone concentrations on *in vitro* embryo production in yearling Nelore (*Bos indicus*) heifers

Giselly B. Kolling[1], Rodrigo L. O. R. Alves[1], Patrícia R. Cavalcanti[1], Douglas Gaitkoski[2], Jaci de Almeida[1], André Braga[2], Rafael Araújo Braz[3], Maria Eduarda Rocha e Silva[4], Rodrigo Brüner[4], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil., [2]CPEX Embryos, [3]Norte Agro artificial insemination, [4]Tulipa Agropecuária Farm

This study evaluated the effects of distinct circulating progesterone (P4) concentrations at the time of ovum pick-up (OPU) on oocyte (COC) recovery and *in vitro* embryo production (IVP) of yearling Nelore (*Bos indicus*) heifers raised in a feedlot system. Heifers (n=383; 11.7±0.1 mo old; 304.0±2.1 kg of body weight [BW]) were randomly assigned to one of three P4 environments at OPU (D0) - Control group (CON): no treatment (n=131); Intravaginal P4 device (IVD) group: an IVD (0.5 g) inserted on D-7 and removed on D0 (n=125); and Corpus Luteum (CL) group (n=126): designed to have a functional CL at the time of OPU (D0) through an ovulation induction protocol (D-23: insertion of an IVD (0.5 g); D-16: IVD removal and 0.5 mg estradiol cypionate im; D-14: 8.4 µg buserelin acetate im). Blood samples were collected on D0 to measure circulating P4. The variables analyzed were: n of recovered COC (COCr), n of viable COC (COCv), COCv rate (%; COCv/COCr), n of cleaved COC (COCc), COCc rate (%; COCc/COCr), n of blastocysts (BL), n of viable BL (BLv), and BLv rate (%; BLv/COCr). The study also evaluated the effects of age terciles (≤11.5 mo [n=126], 11.6-13.0 mo [n=127], and ≥13.1 mo [n=129]) and BW classes (lighter [n=123], intermediate [n=122], and heavier [n=130]). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($^{a,b}P\leq0.05$; $^{A,B}0.05$). The experimental design successfully created distinct P4 concentrations at OPU (CON: 0.3±0.1^{c} vs IVD: 1.5±0.2^{b} vs CL: 4.6±0.4^{a} ng/mL). Respectively, there was no effect of P4 environment on n of COCr (32.4±1.9 vs 32.5±2.6 vs 28.0±1.7), n of COCv (25.1±1.5 vs 25.5±2.0 vs 21.8±1.4), COCv rate (74.6 vs 73.7 vs 74.3%), COCc rate (60.9 vs 63.4 vs 61.6%), n of BL (3.7±1.4 vs 4.2±1.4 vs 4.0±1.5), or n of BLv (3.2±0.4 vs 3.7±0.4 vs 4.0±0.4). However, P4 environment affected BLv rate (9.5^{b} vs 11.7^{a} vs 13.2%^{a}), considering CON, IVD, and CL, respectively. Moreover, the CON group tended to have greater n of COCc compared to CL group, but did not differ from IVD (19.6±1.6^{A} vs 19.7±1.5^{A} vs 16.8±1.1^{B}). There was no effect of age tercile on n of COCr (mean = 31.0±2.0), n of COCv (mean = 23.9±1.6) and COCv rate (mean = 74.3%), respectively. However, age terciles (≤11.5, 11.6-13.0 and ≥13.1 mo, respectively) affected the n of COCc (16.8±1.2^{b} vs 20.4±1.3^{a} vs 19.0±1.2^{ab}), COCc rate (56.7^{b} vs 64.7^{a} vs 64.7%^{a}), n of BL (3.1±0.4^{b} vs 3.9±0.4^{ab} vs 4.8±0.4^{a}), n of BLv (2.6±0.4^{b} vs 3.5±0.4^{ab} vs 4.2±0.4^{a}), and BLv rate (8.5^{c} vs 10.4^{b} vs 15.1%^{a}). There was effect of BW class only in the COCc rate (lighter: 60.8^{b} vs intermediate: 63.7^{a} vs heavier: 60.7%^{b}). In conclusion, circulating P4 derived from both the IVD and CL resulted in higher BLv rates compared to no P4. Additionally, regardless of treatment, younger heifers had lower n COCc rate and BLv, and BW only affected COCc rate, with intermediate heifers having greater values.

Acknowledgments: FAPESP#2018/03798-7, Tulipa, CPEX, Norte Agro, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of epigallocatechin-3-gallate (EGCG) on *in vitro* maturation of ovine oocytes

Andreza Mayara Carneiro Lima[1], Luana Kealy Pimentel de Oliveira[1], Érika Karoline de Oliveira Aureliano[1], Damaris Raquel Pires dos Santos[1], Rafael Soares dos Anjos[1], Brena Ferreira de Menezes[1], Neilton de Lima Oliveira Junior[1], Maria Helena Tavares de Matos[1], Mabel Freitas Cordeiro[1], Edilson Soares Lopes Júnior[1]

[1]Fundação Universidade Federal do Vale do São Francisco

The objective of this study was to evaluate the effect of including epigallocatechin-3-gallate (EGCG) in the IVM medium of ovine oocytes. To this end, oocytes obtained from slaughterhouse ovaries were subjected to IVM and allocated into four groups: control (CON), consisting of TCM-199, supplemented with 500 IU/mL penicillin, 0.5 mg/mL streptomycin, 1.25 µg/mL amphotericin B, 0.2 mM/mL sodium pyruvate, 10% (v/v) of FBS and 15 IU/mL eCG (Sincro eCG®, Ourofino Saúde Animal, Brazil); and groups EGCG25, EGCG50, EGCG100, in which oocytes were matured in the CON medium supplemented with 25 µM, 50 µM and 100 µM EGCG (Sigma-Aldrich, USA), respectively. Approximately 15 CCO were placed in 75 µL drops of IVM medium from each treatment group, under mineral oil, in 60 x 15 mm Petri dishes, and incubated in CO2 Incubator (TE-399®, Tecnal, Brazil) for 24 hours at 38.5°C, in a humidified atmosphere with 5% CO2. At the end of this period, part of the mature oocytes was used to evaluate cumulus cell expansion, chromatin configuration, and to quantify levels of reactive oxygen species (ROS), glutathione (GSH), and mitochondrial activity. The remaining oocytes were used for *in vitro* fertilization (IVF) and *in vitro* embryo culture (IVC), under the same IVM culture conditions, for periods of 20 and 48 hours, respectively. The variables cumulus cell expansion degrees, chromatin configuration, and formation of presumptive zygotes were expressed as percentages and compared using the Chi-square test in Epi Info software (Epi Info 7.2.5, Atlanta, GA, USA, 2021). The GSH, ROS and mitochondrial activity data were submitted to the D'Agostino Pearson normality test, and the Kruskal-Wallis and SNK tests using the online program SAS® OnDemand for Academics 9.04.01 (SAS 3.8 Enterprise Edition, USA, 2020). Differences were considered significant when $P<0.05$. When analyzing the cumulus cell expansion rate, no significant differences were observed. However, regarding chromatin configuration, could be observed in the metaphase I stage, that the EGCG50 group was inferior (29.0%) to the other groups; telophase I, the EGCG100 group (23.2%) was superior to the EGCG25 group (9.7%) ($P= 0.04$); and metaphase II (MII), where the EGCG50 group exhibited a greater number of oocytes at this stage (46.4%) when compared to the other groups tested. In addition, this group showed an increase in intracellular GSH levels and mitochondrial activity in the oocytes, in addition to having maintained ROS levels similar to the control group. Finally, regarding the rate of cleaved structures, the EGCG50 group demonstrated superior results (31.2%) compared to the control group (17.6%) ($P= 0.03$). In conclusion, a concentration of 50 µM EGCG increases the proportion of ovine oocytes reaching the MII stage by maintaining high levels of intracellular GSH and, consequently, decreasing ROS. Furthermore, 50 µM EGCG increases mitochondrial activity and contributes to a higher rate of ovine embryos produced *in vitro*.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of follicular wave synchronization associated with eCG on follicular diameter and *in vitro* maturation of oocytes from braford cows submitted to OPU

Larissa Thaísa Weide[1], Felipe Gustavo Garcia[1], Caroline Fernandes Possebon[1], Janislene Mach Trentin[2], Daniele Missio[3], Laura Rohde Brondani[1], Willian Tomaz Kroll[1], Matheus Gindri Wenning[1], Daniela dos Santos Brum[1], Fábio Gallas Leivas[1]

[1]Fundação Universidade Federal do Pampa, [2]Universidade Federal do Paraná, [3]Universidade Federal de Santa Maria

Hormonal treatments to control follicular waves in OPU protocols may enhance the efficiency of *in vitro* embryo production (IVEP) programs. The administration of eCG stimulates follicular growth and improves ovarian response. This study aimed to evaluate the effect of administering 800 IU of eCG combined with follicular wave synchronization on the follicular population, oocyte competence, and nuclear maturation of oocytes retrieved via OPU in Braford cows. Fifteen non-lactating, non-pregnant Braford cows (48 months old) were used. The animals were divided into three groups. In the control group (G1), the cows were aspirated on random days of the estrous cycle without hormonal treatment. In the synchronized group (G2), follicular wave synchronization was performed using 2 mg of estradiol benzoate and 5 mg of PGF2α (Dinoprost Tromethamine, Zoetis, São Paulo, Brazil) administered intramuscularly on day 0, along with an intravaginal progesterone device containing 1g of P4, which was removed on day 6. The eCG group (G3) received the same synchronization protocol as G2 with the addition of 800 IU of eCG (Novormon®, Zoetis, São Paulo, Brazil) on day 3. Prior to OPU, the total number of ovarian follicles was counted, and their diameters were measured and classified as small (<6mm), medium (6–10mm), or large (>10mm). After OPU, grade 1, 2, and 3 COCs were selected for IVM. After 24 hours, they were denuded and incubated at 39°C, with MitoTracker Green FM (MT, Molecular Probes) at a concentration of 250 nM and after 20 minutes with 10 µg/ml of bisbenzimide (Hoechst 33342, Sigma). After staining, they were individually evaluated using a fluorescence microscope. Nuclear maturation was assessed by extrusion of the first polar body, and cytoplasmic maturation was evaluated based on mitochondrial reorganization. Statistical analysis was performed using ANOVA, and differences among groups were analyzed with Tukey's test at a 5% significance level. The total number of follicles did not differ significantly among groups (G1=657; G2=616; G3=654; P=0.987), nor did the number of small follicles (G1=623; G2=597; G3=516; P=0.574). However, G3 showed a significantly higher number of medium follicles (n = 92) compared to G1 (n = 23) and G2 (n = 11; P<0.0001). Regarding the number of large follicles, G3 (n = 46) had significantly more (P = 0.025) compared to G2 (n = 8), with no significant difference from G1 (n = 11). Nuclear maturation rates (G1=33.3%; G2=40.0%; G3=33.1%; P=0.905) and mitochondrial reorganization rates (G1=59.6%; G2=52.8%; G3=49.2%; P=0.342) were similar among the groups. In conclusion, the use of 800 IU of eCG associated with a follicular synchronization protocol in Braford cows had a positive impact on follicle diameter, resulting in a higher proportion of medium follicles. However, there were no differences among groups in nuclear or cytoplasmic maturation rates, further studies are warranted to evaluate *in vitro* embryo production outcomes.

Financing: CAPES, PPGCA-UNIPAMPA, ZOETIS.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of long-acting injectable progesterone prior to ovum pick-up on *in vitro* embryo production in yearling prepubertal Nelore (*Bos indicus*) heifers

Rodrigo L. O. R. Alves[1], Douglas Gaitkoski[2], Denilson de Souza Siqueira[2], André Zaganin Braga[2], Joao Ricardo Cussolim[2], Leandro Diniz da Cunha[3], Rafael Araújo Braz[3], Maria Eduarda Rocha e Silva[4], Rodrigo Brüner[4], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture, [2]CPEX Embryos, [3]Norte Agro artificial insemination, [4]Tulipa Agropecuária Farm

The aim was to evaluate the effects of long-acting injectable progesterone (iP4) on oocyte (OC) recovery and *in vitro* embryo production (IVP) in prepubertal Nelore (*Bos indicus*) heifers raised in a feedlot system. The heifers (n = 650; 11.7 ± 1.7 mo old; 304.0 ± 42.7 kg of body weight [BW]) were randomly assigned to two treatments: no treatment prior to ovum pick-up (OPU; CON, n = 320); or administration of 150 mg iP4 im 4 to 7 d prior to OPU (GP4, n = 330). The following variables were analyzed: n of recovered OC (OCr); n of viable OC (OCv); OCv rate (%; OCv/OCr); n of cleaved OC (OCc); OCc rate (%; OCc/OCr); n of blastocysts (BL); n of viable BL (BLv); and BLv rate (%; BLv/OCr). Effects of treatment (CON vs GP4), age terciles (≤ 11.1 [n = 228], 11.2-12.8 [n = 220], and ≥ 12.9 mo [n = 202]), and BW classes (lighter [n = 206]; intermediate [n = 224], and heavier [n = 220]) were evaluated. Statistical analyses were done by PROC GLIMMIX of SAS 9.4 ($^{a,b}P \leq 0.05$; $^{A,B}0.05 < P \leq 0.10$). Only heifers with at least one recovered/produced OC/BL were included in the analyses. The outcomes were not affected by the day on which iP4 was administered, nor by any interaction between treatments and other variables. CON group tended to have greater n of OCr (32.3 ± 1.5 vs. 29.6 ± 1.4) and OCv (26.2 ± 1.2 vs. 24.0 ± 1.1) than GP4. There were no differences between CON and GP4 groups in OCv rate (78.0 vs. 77.8%), n of OCc (18.5 ± 0.9 vs. 17.1 ± 1.8), OCc rate (59.4 vs. 60.1%), n of BL (5.1 ± 0.4 vs. 5.0 ± 0.4), n of BLv (4.5 ± 0.3 for both), or BLv rate (13.9 vs. 16.5%), respectively. Regardless of treatment, there were effects of age tercile (≤ 11.1, 11.2-12.8 and ≥ 12.9 mo, respectively) on the n of OCr (24.9 ± 1.3^{b} vs. 35.5 ± 2.2^{a} vs. 32.8 ± 1.8^{a}), n of OCv (20.2 ± 1.1^{b} vs. 28.3 ± 1.7^{a} vs. 26.9 ± 1.5^{a}), OCv rate (76.7^{b} vs. 78.0^{ab} vs. 79.2%^{a}), n of OCc (14.1 ± 0.8^{b} vs. 20.1 ± 1.2^{a} vs. 19.3 ± 1.2^{a}), n of BL (3.3 ± 0.3^{b} vs. 5.3 ± 0.4^{a} vs. 6.4 ± 0.5^{a}), n of BLv (3.0 ± 0.3^{b} vs. 4.6 ± 0.4^{a} vs. 5.6 ± 0.4^{a}), and BLv rate (12.2^{c} vs. 14.7^{b} vs. 18.2%^{a}). The OCv rate did not differ among age terciles. Additionally, there were effects of BW classes (lighter, intermediate and heavier, respectively) on the n of OCr (28.0 ± 1.8^{b} vs. 33.7 ± 1.8^{a} vs. 30.9 ± 1.8^{ab}), n of OCv (22.6 ± 1.5^{b} vs. 27.4 ± 1.5^{a} vs. 24.9 ± 1.4^{ab}), OCv rate (76.7^{B} vs. 78.9^{A} vs. 78.0%^{AB}), n of OCc (16.1 ± 1.0^{b} vs. 19.8 ± 1.1^{a} vs. 17.2 ± 1.0^{ab}), and OCc rate (61.2^{a} vs. 61.2^{a} vs. 56.9%^{b}). However, BW did not affect n of BL and BLv, nor BLv rate. In conclusion, the pre-treatment with iP4 did not enhance IVP outcomes, but it tended to decrease the n of OCr and OCv. Younger heifers had the lowest efficiency across the evaluated parameters, including OC recovery, viability, cleavage and IVP. Regarding BW, lighter heifers had the lowest OC recovery, viability and cleavage, while heavier heifers had the lowest OCc rate.

Acknowledgments: FAPESP#2021/09904-6, Tulipa, CPEX, Norte Agro, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of nicotinamide on *in vitro* maturation of ovine oocytes: Preliminary results

Bruna Moura Silva[1], Luana Kealy Pimentel de Oliveira[1], Gabriela Cristina da Silva Santos[1], Jennifer da Silva Veloso[1], Érika Karoline de Oliveira Aureliano[1], Andreza Mayara Carneiro Lima[1], Damaris Raquel Pires dos Santos[1], Rafael Soares dos Anjos[1], Mabel Freitas Cordeiro[1], Edilson Soares Lopes Júnior[1]

[1]Fundação Universidade Federal do Vale do São Francisco

The aim of this study was to evaluate the effect of nicotinamide on the IVM medium of ovine oocytes. For this purpose, oocytes from ovaries obtained from a local slaughterhouse were collected and then randomly allocated into four IVM groups: the control group (CON), in which the COCs were immersed in medium containing TCM-199, supplemented with 500 IU/mL of penicillin, 0.5 mg/mL of streptomycin and 1.25 µg/mL of amphotericin B, 0.2 mM/mL of sodium pyruvate, 10% (v/v) of fetal bovine serum (FBS) (Sigma-Aldrich®, MilliporeSigma, United States), and 15 IU/mL of eCG (Sincro eCG®, Ourofino Saúde Animal, Brazil); and groups NIC1, NIC3, NIC5 and NIC7, where the oocytes were matured in the presence of IVM medium from the CON group, supplemented with 1 mM, 3 mM, 5 mM and 7 mM nicotinamide, respectively. This stage was performed in a culture incubator (CO2 Incubator – TE-399®, Tecnal, Piracicaba, Brazil), at 38.5ºC, in a humidified atmosphere with 5% CO2. After IVM, the mature COCs were evaluated for the occurrence or not of expansion and the degree of expansion of the cumulus cells as: non-expanded, partially expanded or fully expanded (Marei; Wathes; Fouladi-Nashta, Reproduction, 139:979-988, 2010). Some of the mature oocytes proceeded to the evaluation of nuclear maturation, with the different meiotic stages being analyzed. In addition, remaining matured oocytes was submitted to IVF for 20 hours and then to IVC for 48 h, and the percentage of cleaved structures and the proportion of structures in different embryonic stages were evaluated. The data were evaluated using the Chi-square test, using the Epi Info software (Epi Info 7.2.5, Atlanta, GA, USA, 2021), with a significant difference being considered when $P < 0.05$. At the end of IVM, it was possible to observe that the NIC3 and NIC5 groups presented the highest rates of cumulus cell expansion (92.6% and 97%, respectively) ($P < 0.05$). In addition, the NIC5 group presented a higher rate of fully expanded cumulus cells (62%) ($P < 0.05$) and a lower rate of partial expansion of these cells (37.9%) ($P < 0.05$). For the analysis of chromatin configuration, only the NIC5 group showed a higher rate of mature or metaphase II (MII) oocytes, when compared to the CON group (56.3% and 32.1%, respectively) ($P<0.05$). Evaluating the rate of cleaved structures, the NIC3 and NIC5 groups showed higher rates (33.3% and 37.9%, respectively) when compared to the CON group (19.8%) ($P<0.05$), however, they did not differ from each other, nor from the others added with nicotinamide. For the proportion of different embryonic stages, it was observed that the NIC5 group presented more structures with more than 8 blastomeres ($P<0.05$) when compared to the remaining treatment groups, demonstrating greater embryonic development. Given the results, it is possible to conclude that the NIC5 group improves the maturation of ovine oocytes and, consequently, the *in vitro* production of embryos.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effect of treatment with recombinant bovine somatotropin and recombinant follicle stimulating hormone on oocyte quality of heifer and cow Nelore (*Bos indicus*) donors

Felipe Perecin[1,2], Ana Carolina dos Santos Oliveira[3], Helena Fabiana Reis de Almeida Saraiva[1,2], Juliano Rodrigues Sangalli[1,4], Ana Beatriz Bossois Moura[1,4], Raissa Braido Rangel[1,4], Maria Alice de Almeida[1], Laura Gabrielli Haupenthal[1], Taísa Tavares Ribeiro[5], Pietro Sampaio Baruselli[1,3]

[1]Universidade de São Paulo, [2]University of São Paulo, [3]Animal Reproduction Department, University of São Paulo, [4]University of São Paulo, Faculty of Animal Science and Food Engineering, [5]University of São Paulo - School of Veterinary Medicine and Animal Science

The variability of oocytes germinal vesicle (GV1-GV3) stages is a critical factor in IVM systems. GV1 oocytes benefit more from pre-IVM, while GV3 oocytes perform better in direct IVM (Saraiva et al., Reproduction, 169: e230235, 2025). Herein, the effects of follicular synchronization using rBST (recombinant bovine somatotropin) or rFSH (recombinant FSH), in Nelore heifers and cows, were evaluated regarding follicle, GV stage distribution, and TZP densities. Nelore heifers (H; 10–12 months) and cows (C; 30–60 months) were assigned to: Control (H-CTL, n=5; C-CTL, n=7), rBST (H-BST, n=7; C-BST, n=7), or rFSH (H-FSH, n=7; C-FSH, n=7). On D0, animals received 150mg injectable progesterone (iP4), 0.53mg sodic cloprostenol (PGF), and estradiol benzoate (heifers: 1mg; cows: 2mg), rBST groups additionally received 325mg rBST (Posilac, Agener). On D4, rFSH groups received 100µg rFSH (Ceva, France). On D7, OPU was performed, and follicles were classified and aspirated as small (S; <5mm), medium (M; 5–8mm), or large (L; >8mm). Cumulus-oocyte complexes (COCs) were immunostained for anti-lamin A/C (SC376248, Santa Cruz, USA), DNA (Hoechst 33342, Invitrogen), and actin (ActinGreen 488 Phalloidin, Invitrogen) for GV stage and TZP analysis. Imaging was done in the Mica Widefocal Live Cell confocal microscope (Leica Microsystems, Germany; 63x), and TZP densities (TZPs/10 µm oocyte perimeter) were quantified in Fiji (NIH, USA). COCs counts by treatment, category, and follicle were analyzed using the PROC GLIMMIX in SAS 9.4. GV frequencies were compared by Chi-Square test in GraphPad Prism 8.0.1, considering median, CV, skewness, and kurtosis. TZP densities were compared by Tukey-Kramer test in PROC GLIMMIX. Significance level: 5%. In heifers CTL and rBST produced more COCs from small follicles than rFSH (H-CTL: S=106, M=2, L=2; H-BST: S=115, M=5, L=3 vs. FSH: S=21, M=26, L=54; P<0,0001). However, in cows, rBST produced more COCs from small follicles than CTL and rFSH (C-CTL: S=102, M=4, L=7; C-BST: S=222, M=6, L=2; C-FSH: S=83, M=40, L=19; P<0.0001). In cows, GV1-GV3 frequencies were similar among (P=0.7) C- CTL (GV1: 21.4%, 21/98; GV2: 44.9%, 44/98; GV3: 33.7%, 33/98), C-BST (GV1: 26.3%, 20/76; GV2: 50%, 38/76; GV3: 23.7%, 18/76), and C-FSH (GV1: 23.5%, 19/81; GV2: 46.9%, 38/81; GV3: 29.6%, 24/81). However, in heifers GV1 was enriched (P<0.0001) in H-CTL (GV1: 36.5%, 27/74; GV2: 27%, 20/74; GV3: 36.5%, 27/74) and H-BST (GV1: 56.3%, 54/96; GV2: 31.3%, 30/96; GV3: 12.5%, 12/96) vs. higher GV3 in H-FSH (GV1: 14.3%, 8/56; GV2: 21.4%, 12/56; GV3: 64.3%, 36/56; P<0.0001). TZP densities were similar among groups (H-CTL: 3.40 ± 0.21, n=32; H-BST: 3.41 ± 0.26, n=45; H-FSH: 3.16 ± 0.23, n=6; C-CTL: 3.41 ± 0.23, n=31; C-BST: 3.33 ± 0.25, n=35; C-FSH: 3.29±0.18, n=28). Heifers were more responsive to GV modulation without affecting TZPs. Predicting the enrichment of GV1 (rBST) or GV3 (rFSH) may help tailor IVM protocols.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effects of different hormonal protocols on *in vitro* embryo production in brangus donors

Camila Bortoliero Costa[1], Gustavo Martins Gomes dos Santos[2], Katia Cristina Silva Santos[2], José Henrique Ayres Dias[2], Fernando Eiras de Barros Pinto[3], Marcelo Marcondes Seneda[1], Fábio Morotti[1]

[1]Universidade Estadual de Londrina, [2]Sheep Embryo Reprodução Animal, [3]Universidade Pitágoras Unopar Anhanguera

The present study aimed to evaluate the effect of different hormonal protocols on *in vitro* embryo production (IVEP) in Brangus donors. Sixteen Brangus donors were submitted to 3 routines of OPU/IVP so that at each routine the donors were aspirated randomly (control group = without treatment); or with prior synchronization using injectable P4 (150 mg, i.m., Sincrogest® Injetável, Ourofino, Cravinhos, Brazil) associated with 2 mg of EB (Sincrodiol®, Ourofino) on D0 and OPU on D6 (P4+EB group); or injectable P4 (150 mg, i.m., Sincrogest® Injetável, Ourofino,) associated with 2 mg of EB (Sincrodiol®, Ourofino) on D0, 1.000 IU of eCG (Sincro eCG®, Ourofino) on D4 and OPU on D6 (P4+EB+eCG group). At each IVEP routine, the treatments were alternated among the animals, so that at the end of the experiment all donors received the three treatments. The data were analyzed by ANOVA using the procedure for an adjusted mixed model. In the model, the treatments (control, P4+EB or P4+EB+eCG) were considered a fixed main effect, the donors and the OPU date were random factors in the model. Furthermore, for embryonic variables, the bulls were added as a covariate in the model. For a significant effect, Tukey's test was used as a post hoc mean test. The data are presented as the mean ± standard error and/or percentage (%), with significance set at $P \leq 0.05$. An effect of the treatments was observed for the total number of oocytes (P=0.03) and viable oocytes (P=0.03) among the treated groups. The treatment with P4+EB presented higher means (35.25 ± 3.21a and 27.75 ± 3.00a, respectively), differing from the P4+EB+eCG group (26.31 ± 4.93b and 20.00 ± 4.20b), while the control group presented intermediate values (33.75 ± 6.36ab and 25.94 ± 5.53ab). No differences were observed for oocyte viability rate (P=0.27), total number of embryos (P=0.46), vitrified embryos (P=0.56) and blastocyst rate (P=0.27). For all variables analyzed, there was an effect of the animal ($P<0.05$) but no effect of the date of OPU ($P>0.1$). These effects were considered in the statistical model. It is concluded that prior hormonal synchronization with P4+EB promotes better recovery of total and viable oocytes compared to the protocol with eCG. However, it did not differ in OPU without prior synchronization for any of the monitored variables. The individual response of the donors represents a determining factor in reproductive performance during IVEP.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effects of Natriuretic Peptide C (NPPC) on Meiotic Arrest and Chromatin Configuration in Equine Oocytes

Mariana Degli Esposti[1], Ana Caroline Silva Soares[2], Nathalia Romeiro Silva[1], Perla Fleury[3], Gustavo Merlini[3], José Buratini Junior[1,3]

[1]Universidade Estadual Paulista, [2]Equinvitro/Progest Biotecnologia em Reprodução e Saúde Animal, [3]*In Vitro* Equinos

The physiology of follicular development in mares differs from that of other domestic species, and the mechanisms involved remain to be fully elucidated. Current protocols for *in vitro* maturation (IVM) of equine oocytes involve two phases: a pre-IVM phase known as "holding," which lasts from 2 to 24 hours, and the IVM phase, which typically lasts around 30 hours. During the holding phase, oocytes are maintained in a buffered medium at room temperature (22°C) to preserve them at the germinal vesicle (GV) stage by reducing metabolic activity (Lazzari et al., J Equine Vet Sci, 89:103097, 2020). Since little is known about the use of physiological maturation inhibitors in mares, this study aimed to evaluate the effect of natriuretic peptide C (NPPC), a physiological inhibitor of maturation, at different concentrations on the maintenance of meiotic arrest and GV dynamics in equine oocytes. The experiment was conducted in three replicates, each with an average of 10 cumulus-oocyte complexes (COCs) per group. COCs were obtained via ovum pick-up (OPU) and cultured in DMEM-F12 maturation medium supplemented with increasing concentrations of NPPC (0, 50, and 100 nM). Culture was carried out for 10 hours in a controlled atmosphere (5% CO□, 38.5°C). After culture, COCs were denuded and stained with Hoechst 33342 to assess meiotic stage, categorizing oocytes based on GV presence. Those retaining a GV were further classified by chromatin configuration into three stages: fibrillar (dispersed, poorly condensed chromatin), intermediate (beginning to form a single aggregate), and condensed (dense chromatin cluster) (Hinrichs et al., Biol Reprod, 48:363–370, 1993). The percentages of oocytes that maintained a GV after 10 hours in groups N0, N50, and N100 were 87%, 86%, and 95%, respectively. Of these, the proportion in the fibrillar GV stage was 7% (N0), 27% (N50), and 33% (N100); in the intermediate stage, 40% (N0), 41% (N50), and 43% (N100); and in the condensed stage, 40% (N0), 18% (N50), and 19% (N100). Oocytes that had resumed meiosis (i.e., no GV) were observed in groups N0 (7%) and N50 (5%), with 6%, 9%, and 5% of oocytes showing chromatin degeneration in groups N0, N50, and N100, respectively. No meiotically advanced oocytes were found in group N100. Although differences were not statistically significant ($p > 0.05$), the 100 nM NPPC group showed a trend toward a higher proportion of oocytes in the fibrillar GV stage ($p = 0.06$), suggesting a possible effect in maintaining chromatin in an earlier configuration. In conclusion, while most oocytes remained at the GV stage across all groups, the use of physiological inhibitors such as NPPC appears to be a promising strategy to preserve oocyte viability and chromatin integrity during *in vitro* culture. Further studies are needed to evaluate the effects of NPPC over longer culture periods and to better understand its role in maintaining meiotic arrest in equine oocytes.

Acknowledgments: FAPESP (2023/07531-3).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effects of sexed semen on mitochondrial activity, oxidative stress and *in vitro* development of bovine embryo

Leticia Zoccolaro Oliveira[1], Dayana Silva Araújo[1], Rafael Monteiro dos Santos[1], Egle Menezes de Souza[1], Eliza Diniz de Souza[2], Wasim Al Shebli[2], Naiara Zoccal Saraiva[2], Luiz Sérgio de Almeida Camargo[2], Clara Slade Oliveira[2]

[1]Universidade Federal de Minas Gerais, [2]Empresa Brasileira de Pesquisa Agropecuária

The use of sex-sorted semen for *in vitro* embryo production is an efficient strategy for controlling offspring sex. However, the sex-sorting process can induce structural and functional changes in sperm cells, potentially affecting cellular events during early embryonic development. This study aimed to investigate the effects of semen type (sexed male, sexed female, and conventional) on oxidative stress and mitochondrial activity in bovine embryos on the fifth day of culture (D5), and to evaluate blastocyst development rates on the seventh day (D7). Oocytes were collected from ovaries obtained from abattoirs and, after IVM, conventional and sexed semen from the same bull (the same semen batch from one single bull) were used. To assess mitochondrial activity and intracellular oxidative stress, Mitotracker® Red and CellROX® Green fluorescent probes were used (n=73 embryos, distributed across four replicates). Cleavage and blastocyst rates were evaluated from conventional semen (control embryos; n=137), female-sexed semen (Xembryos; n=226), and male-sexed semen (Yembryos; n=198), also across four replicates. The same IVM, IVF, and IVC protocols were applied for all experimental groups. Data were analyzed in R at 5% of significance. ANOVA was used for normally distributed variables, Kruskal-Wallis for non-normal data, and chi-square test for cleavage and embryo development rates. Embryos produced with conventional semen exhibited higher mitochondrial fluorescence intensity (29.96) compared to Xembryos (20.75; $P<0.05$), while no difference was observed between conventional and Yembryos (25.04) for this parameter. No differences were found among the three groups regarding oxidative stress levels (control=7.55, Yembryos=11.6, Xembryos=6.22; $P=0.090$). However, a higher frequency ($P<0.001$) of embryos with 16 or more cells on D5 was observed in the conventional group (41.6%) compared to Y embryos (20.0%) and X embryos (31.1%), suggesting that the use of sexed semen may impair mitotic progression during early embryonic development. On D7, however, the proportions of embryos at the initial blastocyst (control=33.3%, Yembryos=18.7%, Xembryos=16.7%; $P=0.45$), blastocyst (control=25.0%, Yembryos=25.0%, Xembryos=66.7%; $P=0.11$), and expanded blastocyst (control=41.7%, Yembryos=16.7%, Xembryos=50.0%; $P=0.37$) stages did not differ between groups, indicating similar developmental competence at this stage. In conclusion, the use of sexed semen negatively affects mitochondrial activity and the early cleavage pattern of bovine embryos but does not impair blastocyst development by the seventh day of culture. These results suggest that, although embryos derived from sex-sorted semen present early metabolic alterations, they may retain the ability to reach advanced developmental stages, reinforcing the viability of using this biotechnology in bovine production systems, provided that specific adjustments are considered in reproductive protocols.

Supported by: Fapemig and CNPq.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Effects of supplementation with rumen protected methionine in oocyte donor cows on *in vitro* embryo production and pregnancy rate

Josiane Caobianco Dias Zucoloto[1,2], Wanderley Teixeira Zucoloto[1,2], Tiago Dias Zucoloto[1], Leticia de Oliveira[1,2], Sophia Camargo Tozoni[2], Angela Maria Gonella[3], Claudia Maria Bertan Membrive[2]

[1]Laboratorio de Fertilização Central Senepol, [2]Universidade Estadual Paulista, [3]University of Florida

The objective was to evaluate the effect of oral supplementation with rumen-protected methionine (RPM; Smartamine M® – Adisseo) in the oocyte donors during the 14 days prior to follicular aspiration (OPU) for *in vitro* embryo production (IVEP) and pregnancy per embryo transfer (PET) after fixed-time embryo transfer (FTET). In Experiment 1, 25 non-lactating purebred Senepol oocytes donor cows were randomly assigned to a crossover design with two groups: Control group (1kg of feed/donor/day) and RPM group (1kg of feed + 15g of RPM/donor/day), for 14 days prior to OPU. Donors were individually fed daily. After the first OPU, a 30-day washout period was implemented before the second replicate, in which each donor received the opposite treatment. IVEP was performed using a conventional system in a commercial laboratory, and embryo production parameters were evaluated. In Experiment 2, 90 non-lactating purebred Senepol oocytes donors were supplemented (RPM group; n = 47) or not (Control group; n = 43) with RPM for 14 days before OPU. After OPU and IVEP, Day 7 of *in vitro* culture (D0 = fertilization *in vitro* day) embryos were morphologically classified according to developmental stage and transferred to previously synchronized recipients using a conventional FTET protocol (Control: n = 277 FTET; RPM: n = 245 FTET) and PET was evaluated at Day 45 of gestation. Continuous, normally distributed data were analyzed using linear mixed models, while non-normally distributed data were analyzed with generalized linear mixed models using SAS. In Experiment 1, no significant differences were observed between Control and RPM groups for most variables, except for the proportion of cleaved embryos becoming a blastocyst (Control: 32.90 ± 0.75; RPM: 43.31 ± 0.71; P = 0.05). Also, groups tended to be different when evaluating the average number of expanding hatched blastocyst per donor (Control: 0.11 ± 0.03; RPM: 0.55 ± 0.03; P = 0.09), the percentage of Grade 3 oocytes (Control: 79.39 ± 0.52; RPM: 72.79 ± 0.65; P = 0.08), and the percentage of presumptive zygotes developing to blastocysts (Control: 26.90 ± 0.59; RPM: 34.78 ± 0.68; P = 0.09). In Experiment 2, the percentage of early blastocysts was higher in the Control group (Control: 38.4% [94/245]; RPM: 23.1% [64/277]; P < 0.01), while the percentage of blastocysts was higher in the RPM group (Control: 34.3% [84/245]; RPM: 51.6% [143/277]; P < 0.01). No differences were observed in PET (Control: 36% [88/245]; RPM: 35% [97/277]). In conclusion, supplementation with RPM increased the percentage of cleaved embryos and may enhance oocyte quality, as suggested by a tendency toward fewer Grade 3 oocytes. Furthermore, RPM increased the proportion of blastocysts by Day 7 but did not affect PET. Further research is warranted to explore the effects of RPM supplementation in breeds where IVEP is less challenging than in Senepol cattle and to investigate potential implications for post-cryopreservation embryo survival.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

In Vitro Culture of Feline Embryos Using Human Media: A Promising Approach for Assisted Reproduction in Wild Felids

Ana Paula da Silva Cupello[1], Ian Figueiredo Navarezi [2,3], Maitê Cardoso Coelho da Silva[2,3], Camila Cesca Ventura[2,3], Liandra Martiliano Gaeth[2,3], Thyara de Deco Souza e Araujo[2,3], Joanna Maria Gonçalves Souza-Fabjan[1], Gediendson Ribeiro de Araújo[2,3]

[1]Universidade Federal Fluminense, [2]Universidade Federal de Mato Grosso do Sul, [3]Instituto Reprocon

IVF is a valuable assisted reproduction technique that can be implemented to conserve endangered wildlife species. In this context, the domestic cat serves as an accessible experimental model for improving reproductive biotechnologies in wild felids. This study aimed to test different culture media in domestic cat IVP. After elective sterilization, COCs were recovered from ovaries by slicing, and only structures with homogeneous cytoplasm and at least one cumulus cell layer were selected. COCs were incubated for 24 h in IVM medium (TCM 199 HEPES supplemented with 3 mg/mL BSA, 0.25 mg/mL sodium pyruvate, 1.68 µg/mL sodium lactate, 1% penicillin-streptomycin, 0.15 mg/mL L-glutamine, 0.1 mg/mL cysteamine, and 20 µg/mL FSH-LH). IVF was carried out by co-incubating COCs with $2x10^6$ sperm/mL in a 150 µL GAINTM medium (FertiPro NV, Beernem, Belgium) drop for 18 h. Spermatozoa were obtained from different ejaculate pools for each replicate through fresh epididymal collection by flushing. Presumptive zygotes were denuded by gentle pipetting and placed into one of three IVC experimental groups, where they remained for eight days: CONT (SOF containing 2% MEM essential amino acids, 1% MEM non-essential amino acids, 40 µg/mL gentamicin, 1% streptomycin, penicillin, and amphotericin B, 2.8 mM myo-inositol, 340 µM sodium citrate, 2.5% fetal bovine serum, and 5 mg/mL BSA), SAGE (Quinn's Advantage™ Protein Plus Blastocyst Medium, SAGE™, CooperSurgical Company, Trumbull, USA) and FUJI (Continuous Single Culture-NX Complete with gentamicin and HSA, Fujifilm™, Irvine Scientific, Santa Ana, USA). The first medium was prepared in the laboratory (Santos et al., Animal Reproduction, 19:e20210093, 2022), while the other two were commercial media commonly used in humans. A total of 10 IVC replicates were performed (CONT, n=177; SAGE, n=147; FUJI, n=147). During the entire incubation period, structures were kept at 38.5 °C in a 5% O_2, 5% CO_2, and 90% N_2 atmosphere. Cleavage and embryo rates were assessed on D2 and D8, respectively. The ANOVA test was used to compare the groups, and $P<0.05$ was considered statistically significant. Data are presented as Mean±SEM. No difference ($P>0.05$) was observed among the groups in cleavage rate [CONT (36±3.4%), SAGE (27±5.0%), and FUJI (26±5.2%)] or rate of embryos produced *in vitro* per total structures and per cleaved structures [CONT (11±3.4% and 26±5.9%, respectively), SAGE (13±4.7% and 36±9.9%, respectively) and FUJI (12±3.2% and 36±10.3%, respectively)]. Of note, all groups presented embryos at the blastocyst stage on D8. Overall data reported were slightly below those described by other researchers (Veraguas et al., Theriogenelogy, 146:94-103, 2020). In conclusion, the similar embryonic development efficiency observed among different media not only confirms their suitability for cat IVP, but also strongly supports their practical application in assisted reproduction programs aimed at the conservation of wild feline species.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Evaluating peroxide levels in oils for embryo culture: a comparative study of mineral and synthetic oils in IVP

Raquel Zaneti Puelker[1], Ana Caroline Silva Soares[2], Valdecir Farias Ximenes[3], Juliano Passaretti Filho[1]

[1]Botupharma - Biotecnologia e Saúde animal Departamento de Pesquisa e Desenvolvimento, [2]Progest Biotecnologia em Reprodução e Saúde Animal LTDA, [3]Universidade Estadual Paulista

Mineral and synthetic oils are commonly used to protect embryos cultured in microdroplets during IVP, helping to maintain stable environmental conditions such as pH, temperature, and osmolality, which are essential for embryo development. These oils prevent evaporation of the culture medium and ensure optimal parameters for embryonic growth (Swain, Fertil. Steril., 109:53, 2018). However, both mineral and synthetic oils may contain peroxides, which are embryotoxic and can negatively impact IVF success rates due to their direct correlation with the formation of reactive oxygen species (ROS). The concentration of peroxides in oils is influenced by factors such as ultraviolet light exposure, heat, and prolonged storage (Hughes et al., J. Assist. Reprod. 27:87–92, 2010). Therefore, determining peroxide levels in oils or synthetic oil used in embryo culture is crucial to improving IVP outcomes. Among the various methods for determining peroxide levels, the FOX method is widely used due to its sensitivity. The FOX method is based on the reduction of Fe2+ to Fe3+ in the presence of hydrogen peroxide, forming a stable complex with xylenol orange that can be detected spectrophotometrically at 560 nm (Bridi et al., Food Chem., 175:25–28, 2015). In this study, we adapted the FOX method to quantify the total peroxide content in oil samples used in embryo culture. The FOX reagent was prepared by combining ammonium iron (II) sulfate hexahydrate, xylenol orange, and sorbitol in deionized water. For oil sample analysis, light mineral oil for embryo culture (Irvine Scientific, USA) and Botufiv® OIL (Botupharma, Brazil) were selected. The oils were mixed with isopropyl myristate to improve the interaction between the apolar oil and the aqueous FOX reagent, making the mixture more homogeneous. Spectrophotometric measurements were performed using a Synergy 2 Multi-Mode spectrophotometer, with samples incubated with FOX reagent for 30 minutes before measurement. The peroxide equivalent concentrations were determined using an analytical curve and found to be below the detection limit of 0.15 nmol/g for both oil samples. The results showed that the tested samples contained detectable peroxide levels, suggesting that they are safe for use in embryo culture within the detection range of the FOX method. In conclusion, both mineral and synthetic oil, showed no detectable peroxide levels, indicating that they are free from significant contamination and the formation ROS. While mineral oil has traditionally been the preferred choice in IVP, synthetic oil is emerging as a viable alternative. With comparable results to mineral oil, synthetic oil offers a promising option for culture applications, potentially providing similar protective qualities with less risk. Further studies could explore its compatibility and benefits in various culture conditions.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Excitable temperament impairs oocyte quality and *in vitro* embryo production efficiency in nellore donors

Samuel Rodrigues Bonamichi do Couto[1], Thiago de Souza Vieira[2], Nicolas Moreira Piedras Monnerat Caparelli[2], Daniela Cristina Rocha de Freitas[2], Mateus Gonçalves Costa[2], Mellyssa Sad Rodrigues Gomes[2], Rondineli Pavezzi Barbero[2], Marco Roberto Bourg Mello[2], Felipe Zandonadi Brandão[1]

[1]Universidade Federal Fluminense, [2]Universidade Federal Rural do Rio de Janeiro

This study aimed to evaluate the influence of temperament and reactivity in Nellore females on the quantity and quality of recovered oocytes, as well as the efficiency of *in vitro* embryo production (IVEP). The working hypothesis was that stress associated with an excitable temperament induces systemic effects that compromise the efficiency of IVEP in oocyte donor females. The experiment was conducted on a commercial farm in the state of Paraná, Brazil, and involved 133 Nellore females subjected to three follicular aspiration sessions, resulting in the recovery of 3,820 oocytes. Temperament was assessed based on behavioral observations during animal restraint in a pen and upon exit, using a 1-to-4 scale as described by Cooke et al. (Journal of Animal Science, 95:1-8, 2017). Based on temperament scores, animals were categorized into two groups: adequate (calm) temperament (ADQ; score ≤ 2) and excitable (reactive) temperament (EXC; score > 2). To validate the subjective temperament assessment, blood samples were collected on the day of follicular aspiration for quantification of serum cortisol concentrations via radioimmunoassay using a commercial kit, with a detection limit of 3.12 ng/mL for the ELISA test. Recovered oocytes were morphologically evaluated according to the criteria proposed by de Loos et al. (Gamete Research, 24:197-204 2003) and subjected to a commercial IVEP protocol. Statistical analyses were performed using the GLIMMIX procedure in SAS software (version 9.4), with significance set at $P \leq 0.05$. Animals in the EXC group (n = 32) exhibited higher plasma cortisol concentrations compared to the ADQ group (n = 101) (62.5 ± 7.2 ng/mL vs. 42.2 ± 3.8 ng/mL; $P = 0.008$), confirming a physiological response to stress associated with temperament. The proportion of viable oocytes was higher in the ADQ group compared to the EXC group (79.3% vs. 75.5%; $P = 0.01$), while the EXC group had a greater proportion of degenerated oocytes (24.4% vs. 20.6%; $P = 0.01$). Cleavage rates were also higher in the ADQ group (74.9% vs. 64.7%; $P < 0.001$), as were blastocyst rates (25.4% vs. 19.2%; $P = 0.02$). Furthermore, the mean number of blastocysts produced per donor was significantly greater in the ADQ group (6.2 vs. 3.0 embryos; $P < 0.001$). In conclusion, temperament has a direct impact on oocyte quality and IVEP efficiency in Nellore females. Calm-tempered donors demonstrated superior performance in *in vitro* embryo production, indicating that temperament-based selection and behavioral management strategies may enhance the success of commercial IVEP programs.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Impact of follicular wave synchronization associated with eCG on *in vitro* embryo production in Braford cattle

Yasmim Rosa de Oliveira[1], Felipe Gustavo Garcia[1], Daniele Missio[2], Marcelo Silveira Albornoz[1], Rosana Klaus[1], Janislene Mach Trentin[3], Maria Eduarda Ávila de Araujo[1], Suzane Rossato Soares[1], Daniela dos Santos Brum[1], Fábio Gallas Leivas[1]

[1]Fundação Universidade Federal do Pampa, [2]Universidade Federal de Santa Maria, [3]Universidade Federal do Paraná

In vitro embryo production (IVEP) is a reproductive technology in cattle, and its efficiency is influenced by oocyte quality. To mitigate this limitation, follicular wave synchronization and the administration of equine chorionic gonadotropin (eCG) prior to OPU have emerged as strategies to improve the acquisition of competent oocytes and embryonic development. In this context, the objective of this study was to evaluate the effect of follicular wave synchronization, either alone or combined with 800 IU of eCG prior to OPU, on the *in vitro* blastocyst production rate. Twelve non-lactating and non-pregnant Braford cows were used, allocated into three groups: control group (G1), where females underwent OPU on a random day of the estrous cycle; Synchro group (G2), where females underwent follicular wave synchronization using an intravaginal device (IVD) containing 1 g of progesterone (P4) associated with 2 mg of estradiol benzoate (EB) and 5 mg of Dinoprost Tromethamine (Day 0) administered intramuscularly; and eCG 800 group (G3), which received the same synchronization protocol as G2, with the addition of 800 IU of eCG (Novormon®, Zoetis Brazil) administered 72 hours before OPU (Day 3). On Day 6, the IVD was removed and OPU was performed. The COCs recovered per treatment (G1 = 129; G2 = 126; G3 = 126) were incubated in maturation medium under controlled atmosphere. For IVF, semen from a Braford bull with proven fertility was used, and spermatozoa were selected through Percoll gradient centrifugation. After 24 hours of co-culture, presumptive zygotes were denuded and transferred to SOF 1 medium under mineral oil, being maintained for seven days in an incubator at 38.7 °C. On the third day of culture, uncleaved structures were removed and 50% of the medium was replaced SOF 1 medium. On the fifth day, another 50% of the medium was replaced, this time with SOF 2. Cleavage rate was assessed 24 hours after IVF, and the blastocyst rate was determined after seven days of culture. The data obtained were analyzed by ANOVA, followed by Tukey's test for group comparisons. The cleavage rate was similar among groups [G1 = 44.2% (n=57/129), G2 = 52.4% (n=66/126), and G3 = 60.3% (n=76/126); P=0.249]. Blastocyst formation showed a tendency toward improvement (P=0.075), with an increase from G1 (17.0%; n=22/129) to G2 (23.1%; n=30/126) and G3 (37.3%; n=47/126). The proportion of excellent-quality embryos was similar among groups (G1 = 45.4%, G2 = 56.7%, and G3 = 55.3%; P = 0.343), as were the number of blastocysts (P = 0.372) and Grade I embryos (P = 0.301) per donor per OPU: G1 = 1.8 and 0.8; G2 = 2.5 and 1.4; and G3 = 3.9 and 2.1, respectively. Therefore, the use of 800 IU of eCG associated with follicular wave synchronization showed a tendency to improve both the blastocyst rate and embryo quality. Thus, the results suggest that this strategy has potential to optimize OPU/IVP outcomes in Braford donor cows.

Financial Support: CAPES, PPCGA and Zoetis Brazil.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Impact of sires in genetic progress for milk production in sindhi bovines generated by *in vitro* fertilization

Lucas Costa de Faria[1,2], Ludmilla Costa Brunes[3], Adriano Queiroz de Mesquita[2], José Ricardo Sobral da Silva[2,4], Julia Carvalho de Melo[1,2], Isabella Rodrigues dos Santos Oliveira[1,2], Vinicius Nunes Antunes[5], Isabel Cristina Ferreira[6], Claudio Ulhoa Magnabosco[2], Carlos Frederico Martins[2]

[1]Universidade de Brasília, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]Universidade Federal de Goiás, [4]Centro Universitário do Planalto Central Apparecido dos Santos, [5]Universidade Católica de Brasília, [6]Embrapa Cerrados

Thi study aimed to assess the impact of bulls selected for milk production based on their predicted transmitting ability for milk production (PTA_MP), reliability (REL) and inbreeding coefficient (InC) of descendants generated by *in vitro* fertilization (IVF) in the Sindhi breed. The hypothesis is that bulls with higher PTA_MP and REL produce offspring with superior PTA_MP. Data from 86 Sindhi animals were evaluated, including 22 female embryo donors (first generation) and 64 IVF-derived descendants (second generation), resulting from the mating of 7 bulls and 34 cows. To ensure robustness in estimating genetic effects and minimize confounding from unequal progeny, only bulls with at least two offspring recorded were included. ANOVA, considering bull as a fixed effect was performed to test differences among sires, with means compared using Tukey's test ($p<0.05$). Pearson correlations between PTA_MP and REL of bulls and their offspring were also estimated using R software. The correlation between bull and offspring PTA_MP was strong and positive (0.89), as expected due to the additive genetic merit transmitted from sire to progeny. This positive relation between PTA_MP suggests that bulls with a greater genetic merit tends to produce offspring with a greater milk production potential. The correlation between REL of bulls and their offspring was weak and negative (-0.06), reflecting the greater amount of information typically available for sires compared to their often younger progeny, which emphas that accumulating more performance data increases the reliability. These results highlights the importance of considering both PTA and REL in selection decisions. Significant differences ($p<0.05$) in PTA_MP and REL were observed among sires. PTA_MP ranged from -15.82 to 190.09 kg, revealing substantial variability in the genetic potential for milk production across sires. REL varied from 3.86% to 38.87%, demonstrating considerable differences in the certainty of genetic evaluations. These magnitudes underscore the practical relevance of careful sire selection to optimize genetic gain. Notably, despite some animals showing high REL, their PTA_MP was low or even negative, reinforcing the need to evaluate both parameters concurrently when selecting bulls. The broad range observed in PTA_MP (206 kg) indicates that sire choice could lead to substantial genetic differences in offspring milk production. These findings, based on predictive genetic evaluations, provide essential guidance for reproductive decisions and genetic improvement programs aiming to enhance milk production in Sindhi cattle. However, validation through field data would be valuable to confirm the predictive accuracy and practical outcomes of these selection strategies. The strategic use of bulls with both high genetic merit and reliability has the potential to significantly advance genetic progress, thereby improving the efficiency of breeding programs employing IVF technologies.

Support: FAPDF.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Influence of carcass measurements evaluated by ultrasonography in senepol donors in the *in vitro* production of embryos

Wanderley Teixeira Zucoloto[1,2], Josiane Caobianco Dias Zucoloto[1,2], Tiago Dias Zucoloto[2], Leticia de Oliveira[1,2], Sophia Camargo Tozoni[1], Liliane Suguisawa[3], Guilherme Pugliesi[4], Angela Maria Gonella[5], Claudia Maria Bertan Membrive[1]

[1]Universidade Estadual Paulista, [2]Laboratorio de Fertilização Central Senepol, [3]DGT Brasil, [4]Universidade de São Paulo, [5]University of Florida

Carcass ultrasonography enables the evaluation of the loin eye area corrected for 100 (AOL 100), subcutaneous fat thickness corrected for 100 (SFT 100), rump subcutaneous fat score (RSFS), and marbling score (MS). The hypothesis is that these measurements in the oocytes donors influence the *in vitro* embryos production (IVEP). The aim was to evaluate the influence of AOL 100, STF 100, RSFS, and MS on IVEP using a database. The measurements of AOL 100, SFT 100, RSFS, and MS were obtained before the first ovum pick-up (OPU), and the females underwent at least three consecutive aspirations. The study involved 42 heifers (< 3 years old), totaling 253 aspirations (Database 1), and 34 cows (6 to 10 years old), totaling 217 aspirations (Database 2). IVEP was performed using a conventional system in a commercial laboratory, and embryo production parameters were evaluated on days 6 and 7 (D0 = day of *in vitro* fertilization). For each category (heifer and cow) and each measurement (AOL 100, STF 100, RSFS and MS) the median was established for the division of groups, where values below the median constituted the low group and above the median the high group. For heifers, the model considered the fixed effect of AOL 100 (Low < 16.04 and High > 16.04), SFT 100 (Low < 1.11 and High > 1.11), RSFS (Low < 7.57 and High > 7.57) and MS (Low < 3.44 and High > 3.44) classes. For cows, the model considered the fixed effect of AOL 100 (Low < 13.86 and High > 13.86), SFT 100 (Low < 1.08 and High > 1.08), RSFS (Low < 9.41 and High > 9.41) and MS (Low < 4.00 and High > 4.00) classes. Comparisons between the high and low groups for each characteristic were made using analysis of variance with the PROC MIXED procedure in SAS. AOL 100, SFT 100 and RSFS in cows and heifers had no effect on the production potential of oocytes and embryos during IVEP. The MS influenced IVEP, where heifers with high MS compared to those with low MS had a trend to lower number of unviable oocytes (6.75 ± 0.31 vs. 9.17 ± 0.53 respectively; P = 0.0515) and significantly higher cleavage rate (65.45 vs. 59.59% respectively; P = 0.0298) and embryo rate [(number of embryos produced on D6 and D7/total number of oocytes placed at maturation x 100); 20.38 vs. 13.54% respectively; P = 0.0011] and cows with high MS when compared to those with low MS had a higher number of embryos produced (7.04 ± 0.71 vs. 5.00 ± 0,55 embryos/donor/OPU respectively; P = 0.0060) and a higher rate of embryos (43.44 vs. 27.74% respectively; P = 0.0034). We conclude that in heifers, a high MS was associated with a trend to decrease in the number of non-viable oocytes and an increase in embryo production rate. In cows, a high MS also increased the number of embryos produced and the embryo rate. Future studies should investigate the effects of MS in breeds where IVEP is less challenging than in Senepol cattle and to investigate the potential implications for embryo survival after cryopreservation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

In vitro production of bovine embryos using frozen/thawed semen added with natural extract (NP) to the extender medium - Partial results

Matheus Ramos Rosin[1], Camila Keterine Gorzelanski Trenkel[1], Isabeli Pastore[1], Gabriela Gonçalves Fagundes[1], Leandro Francisco Cichoski[2], Edi Kava Kailer[1], Jonatas Cattelam[1], Dalila Moter Benvegnú[1], Joaquim Garcia[3], Adalgiza Pinto Neto[1]

[1]Universidade Federal da Fronteira Sul, [2]Sêminna Biotecnologia da Reprodução Animal, [3]Universidade Estadual Paulista

In the search for methods that lead to the viability of bovine semen, specifically for its efficient use in the *in vitro* production of embryos (IVEP), it becomes necessary studies capable of increase the quality of sperm cells in this process, considering that the process of freezing and thawing lead to injures like oxidative stress. Based on this, natural and artificial substances with potential antioxidant properties have been identified as suitable for addition to semen at the time of freezing. With the aim of optimizing *in vitro* embryo production, this study evaluated the effects of adding a natural extract (NP) with antioxidant potential at different concentrations to semen used in IVEP, therefore, ovaries were collected from slaughterhouses, the COCs aspirated was selected (Grade I, II, and III) and the IVEP was performed with two culture media, the base media and a commercial medium, used as control. Viable COCs were divided into groups and subjected to IVM for 22-24 hours in cryotubes (400µL of maturation medium covered with 125µL of mineral oil - Irvine®) and incubated. IVF was carried out in droplets and fertilized with semen from four groups containing different concentrations of the natural extract (NP: 0% control; 0.5%; 0.75% and 1%), processed and capacited with a Percoll gradient. 18 to 22 after fertilization, cumulus cells were removed from the zygotes in each group, and zygotes were cultured in a controlled atmosphere. Cleavage of each group was evaluated at 48 hours, and blastocyst rate was observed on the seventh day post-IVF. Two IVEP runs were performed, and the average results for the control group, considering the base and commercial media, were 21 and 24 total/viable COCs, 61.66% and 56.04% cleavage rate, and 48.33% and 38.35% blastocyst formation, respectively. Using semen added with 0.5% NP, means of 23 and 23.5 total/viable COCs were observed; 54.46% and 53.09% cleavage rate; and 12.28% and 16.79% blastocyst formation for base and commercial media, respectively. With 0.75% NP addition, means were 21.5 and 23 total/viable COCs; 44.08% and 51.96% cleavage rate; and 19.33% and 14.22% blastocyst formation for base and commercial media, respectively. For semen added with 1% NP, average results were 21 and 22 total/viable COCs; 30.35% and 67.85% cleavage rate; and 12.5% and 3.57% blastocyst formation rate in base and commercial media, respectively. The partial results indicate an inhibitory effect on blastocyst production rates with increasing concentrations of the natural extract added to frozen semen. Cleavage rates in the base medium at 0.75% and 1.0% NP concentrations were lower. However, blastocyst production rates remained more stable in the base medium compared to the commercial one. The inclusion of NP (0.5%, 0.75%, or 1.0%) in the semen extender tends to affect blastocyst production in both media tested (base and commercial). Nonetheless, the number of replicates needs to be increased to support this observation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

IVEP performance of pregnant and nonpregnant precocious Nellore heifers fed with a high-energy diet in a feedlot system

Felipe de Oliveira Pedro[1,2], Taynara dos Santos Santana[1], Wallery Caroliny Costa da Costa[1,2], Thais Ferreira Lima[1], Silvio André Isler[1], Aldair Félix da Silva[3], Dauydson Antônio Gonzalez Cordeiro[3], Jessica Nora Drum[4], Ériklis Nogueira[5,6], Fabiana de Andrade Melo Sterza[1,3,2]

[1]Universidade Estadual de Mato Grosso do Sul, [2]Grupo de Estudos em Tecnologia da Reprodução Animal, [3]Universidade Federal de Mato Grosso do Sul, [4]South Dakota State University, [5]Empresa Brasileira de Pesquisa Agropecuária, [6]EMBRAPA GADO DE CORTE

The *in vitro* embryo production (IVEP) system has contributed to increasing reproductive efficiency worldwide by providing multiple calves per cow. The possibility of using pregnant donors for Ovum Pick Up (OPU) is well known. Still, little is known about whether pregnancy and nutritional management can modulate the success of the technique. In this perspective, this study aimed to investigate the OPU/IVEP performance of pregnant and non-pregnant precocious Nellore heifers fed with a high-energy diet in the feedlot. The study was conducted in Anastacio, Mato Grosso do Sul, Brazil, in the 2023/2024 breeding season. Ten (10) heifers, aged 12.04 ± 0.42 months with an average weight of 300.80 ± 80.0 kg, were housed in a feedlot system in collective stalls (three pens) with food and water ad libitum. All heifers received a common diet with 1.5% of body weight (BW) of concentrate. BRS Capiaçu silage was offered as roughage. The treatment period was sixty (60) days, which starts ten (10) days after a fixed-time artificial insemination (FTAI) (9-day BE-P4 protocol), and OPU was carried out 70 days after AI. Animals were divided into 2 groups: pregnant (n=5) and non-pregnant (n=5). Subcutaneous croup fat thickness (SCFT) was measured between the ileum and ischium bones at the intersection of the Gluteus medius and Biceps femoris muscles. The measurements were taken at the beginning (d0), and end (d60) of the experiment with images collected using an ultrasound (KAIXIN®, Xuzhou Kaixin Electronic Instrument CO., Ltd., Xuzhou, Jiangsu, China) with a 17 cm linear transducer and a frequency of 3.5 MHz with bi-dimensional (B-mode) images. The performance at OPU-IVEP per group, and the influence of dietary energy, was observed on the following parameters: number of cumulus oocyte complexes recovered at OPU (COCS/OPU), cleavage rate, embryo number, and blastocyst yield on d7. The statistical analyses were conducted using the PROC Mixed and PROC Reg procedures of SAS (SAS Institute Inc., Cary, NC, USA; SAS on-demand), with a 5% significance level. Pregnant and non-pregnant heifers demonstrated similar OPU-IVEP efficiency ($P>0.05$), being respectively: number of cumulus oocyte complexes recovered at OPU (21.40 vs 23.75), cleavage rate (66.78 vs 61.55), embryo number (15.20 vs 17.33), and blastocyst yield on d7 (49.65 vs 43.30). There was a negative influence of SCFT on the number of cumulus-oocyte complexes (COCs)/OPU and number of embryos, despite the group ($P<0.05$). Greater SCFT (12.0 mm) animals had a lower number of COCs/OPU and embryos (10.0 and 5.0, respectively) compared to a moderate SCFT (6.0 mm) that had greater COCs/OPU and embryos (30.0 and 15.0, respectively). In conclusion, the OPU/IVEP performance did not differ between pregnant and non-pregnant Nellore donors on a high-energy diet, although higher SCFT had a negative effect on the number of cleavage embryos.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

IVF medium supplementation with follicular fluid and oviduct mucosa fragments modulates gene expression related to glucose, progesterone metabolism and stress in bovine blastocysts

Bruno Porpino Homobono[1], Eduardo Baia de Souza[1], Mateus Ramos Trindade[1], Omar Said Correa Mounsif[1], Leonardo Henrique da Silva Nogueira[1], João Vitor Amaral Magalhães[1], Vinícius Kauan Lobato Resende[1], Nathalia Nogueira da Costa de Almeida[1], Marcela da Silva Cordeiro[2], Simone do Socorro Damasceno Santos[1]

[1]Universidade Federal do Pará, [2]Instituto Federal de Educação, Ciência e Tecnologia do Pará

This study aimed to evaluate the effect of supplementing IVF medium with 5% bovine follicular fluid (BFF) and oviduct mucosa fragments (OMF) on the production of *in vitro* bovine embryos. BFF was collected from ovarian follicles with a diameter of 8–10 mm, while OMF were collected from bovine oviducts of non-pregnant cows at the early luteal phase by separating and squeezing the ampulla and centrifuging its contents, with 1 ul of the resulting cell pellet being used for each drop of IVF. IVF was performed by co-incubating COCs with sperm in TALP medium (6 mg/mL BSA, 2 µM de penicilin, 1 µM hipotaurin, 0,25 µM epinefrin, 10 µg/mL heparin, 22 µg/mL piruvate and 50 µg/mL gentamicin) for 18 h. *In vitro* matured oocytes (800 in total) were then distributed among the following experimental groups: control medium; medium supplemented with 5% BFF; medium supplemented with OMF; and medium supplemented with both OMF and 5% BFF. Embryos were cultured *in vitro* for 8 days. Embryo development was assessed through cleavage rates (d2) and blastocyst formation rates (d8). RNA extraction from a total of 60 embryos was conducted utilizing TRIzol® reagent (Invitrogen RNA, Life Technologies) and reverse transcription was performed to obtain cDNA using the High-Capacity Reverse Transcription® kit (Applied Biosystems, Foster City, CA, USA). The relative expression of the genes OCT4, BAX, HSP70, GLUT1, PGRMC1 and HSD3B1 was measured using real-time PCR and analyzed by the 2−ΔΔCt method. Statistical analysis was made using the SigmaPlot® (Systat Software Inc.) software and one-way ANOVA. There was no difference ($p>0.05$) among the different experimental groups in cleavage rates (85.64% ± 5.25 Control, 85.02% ± 6.30 BFF, 85.87% ± 4.96 FMO, 80.17% ± 8.34 OMF and BFF), however, the blastocyst formation rates were significantly lower in medium with both OMF and BFF (47.48% ± 2.52 Control, 40.71% ± 7.54 BFF, 46.41% ± 5.56 FMO, 30.17% ± 7.45 OMF and BFF). No significant differences were observed for OCT4 expression ($p>0.05$). When compared to control, blastocysts fertilized medium supplemented with OMF or both OMF and BFF had increased expression of the apoptosis related gene BAX ($p=0.018$ and $p=0.013$ respectively), reduced expression of the glucose transporter gene GLUT1 ($p=0.043$ and $p=0.005$) and higher expression of the progesterone receptor gene PGRMC1 ($p=0.001$ and $p<0.001$). Blastocysts fertilized in medium with only OMF had increased expression of stress (HSP70) in comparison to control ($p<0.001$). In contrast, blastocysts fertilized in medium supplemented with only BFF displayed a lower expression of stress (HSP70 [$p=0.035$]) as well as progesterone metabolism (PGRMC1 [$p=0.006$] and HSD3B1 [$p=0.027$]). In conclusion, supplementation of the IVF medium with BFF or OMF appears to modulate the embryonic expression of genes related to stress, glucose and progesterone metabolism, although the simultaneous use of both of these supplements resulted in lower blastocyst formation rates.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Oocyte maturation in COC-conditioned medium produces blastocysts with higher expression of GLUT1, HSD3B1, and PGRMC1

Matheus Hadigo Pires Costa[1], Felipe de Souza Cardoso[2], Mateus Ramos Trindade[1], Patrícia de Cássia da Silva Lima[1], Moysés dos Santos Miranda[1], Bruno Porpino Homobono[1], Eduardo Baia de Souza[1], Marcela da Silva Cordeiro[2], Simone do Socorro Damasceno Santos[1], Nathalia Nogueira da Costa de Almeida[1]

[1]Universidade Federal do Pará, [2]Instituto Federal de Educação, Ciência e Tecnologia do Pará

Conditioned media derived from various cell types have been explored as a strategy to enhance IVP and provide insights into cellular metabolism. This study aimed to evaluate the effects of COC-conditioned medium on the IVM of bovine oocytes and subsequent gene expression in blastocysts. Bovine ovaries were collected from a local slaughterhouse, COCs were cultured at a concentration of 15 COCs/mL in IVM medium (TCM-199 supplemented with FBS, FSH, LH, EGF, pyruvate, and gentamicin) under standard IVM conditions (38.5°C, 5% CO_2) for 48 hours. Aliquots (250 uL) of the conditioned medium were collected and stored for subsequent use in IVM. The maturation procedure followed the standard protocol of our laboratory, using 80 µL of standard IVM medium combined with 20 µL of conditioned medium. The Control Group (CG) was matured using only the standard IVM medium. Fertilization was performed using frozen-thawed semen from a single bull, processed using a Percoll gradient, and washed in TALP. The treated sperm was then added to fertilization drops at a final concentration of 2×10^6/mL and incubated under the same conditions as the IVM. Twenty-eight hours after fertilization, presumptive zygotes were denuded and transferred to SOF culture medium (supplemented with FBS, BSA, pyruvate, and gentamicin). Cleavage rate was assessed on Day 2 and, on Day 8, hatched blastocysts were used for gene expression analysis by TRIzol. Data were analyzed using ANOVA with SigmaPlot® version 11.0 (Tukey's post-hoc test, $p < 0.05$). No statistically differences were found in cleavage rates ($p = 0.42$) between the CG (90.17% ± 5.15) and the Treatment Group (TG; 91.77% ± 3.40), nor in blastocyst formation rates ($p = 0.51$) between CG (52.93% ± 8.80) and TG (49.94% ± 11.14). Regarding gene expression, the TG group showed th same lvel of BAX expression compared to CG ($p = 0.80$), while SOD2, an enzyme associated with oxidative stress protection, was the same in CG ($p = 0.47$). The pluripotency gene OCT4 ($p = 0.004$) and mitochondrial transcription factor TFAM ($p < 0.001$) were significantly more expressed in CG. Conversely, TG showed higher expression of GLUT1 ($p=0.02$), suggesting increased metabolic activity and significantly higher expression of HSD3B1 ($p < 0.001$) and PGRMC1 ($p < 0.001$), genes associated with the conversion of pregnenolone to progesterone and progesterone modulation. This may indicate elevated progesterone levels in the COC-conditioned medium. Taken together with the increased expression of GLUT1, these findings suggest a metabolic shift toward enhanced progesterone modulation. Although no significant improvements were observed in cleavage or blastocyst formation rates, the increased expression of HSD3B1, PGRMC1, and GLUT1 indicates a potentially more favorable metabolic environment for IVP. Nonetheless, further research is required to clarify the role of COC-conditioned medium and progesterone in enhancing IVP outcomes.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Photobiomodulation of Bovine Embryos at 72 Hours Post-fertilization

Isabella Vogler[1], Saige Summersett[1], Victoria Mueller[1], Isabella Zarzaca[1], Hannah Culler[1], Matt J. Hersom[1], Celina M Checura[1]

[1]Clemson University

Photobiomodulation has been shown to increase ATP and improve embryo developmental rates when applied during late oocyte maturation. In bovine embryos, major embryonic genome activation occurs during the 8 to 16-cell stage, marking the transition to embryonic genomic control. We hypothesize that photobiomodulation at the 8 to 16-cell embryo stage will influence the embryo metabolic activity and contribute to higher blastocyst rates compared to the control. A standard *in vitro* fertilization protocol and commercial media (Stroebech media) were used. Bovine ovaries were obtained at a local slaughterhouse, and cumulus oocyte complexes (COC) were collected by aspiration. Compacted COCs were matured, fertilized, and cultured under standard bovine *in vitro* production protocols. Cleavage rate was assessed at 72 hours post-fertilization, and embryos with ≥ 4 cells were selected. Embryos with ≥ 4 cells were randomly assigned to two groups of 50 and returned to embryo culture conditions (38.5ºC in 5% CO_2 and 5% O_2 with high humidity) in new IVC media. After an hour of equilibration, the Light group received photobiomodulation treatment (red light 660-665 nm for 10 min) and the Control group received no treatment. Blastocyst development was assessed at 168- and 192-hours post-fertilization. Blastocyst rates were calculated as a ratio of blastocysts over ≥4-cell embryos for each group. Two bulls were used, one for each replicate, for a total of 8 replicates (4 replicates/bull). At 192 hours post-fertilization, blastocysts were fixed and stained with Hoechst for cell counting. For statistical analysis, each well was considered an experimental unit. Blastocyst rates were analyzed as a model of repeated measures, with main effects of treatment, time, and bull, blocked by replicate; for blastocyst cell number, main effects were bull, treatment, and bull by treatment interaction, blocked by replicate (Mixed procedure, SAS Institute Inc.). Blastocyst rates at 168 hours were 46.1 ± 2.3 and 41.9 ± 1.0 % (mean ± SEM), and blastocyst rates at 192 hours were 53.5 ± 2.7 and 48.6 ± 2.5 % for Light and Control groups, respectively. There was a tendency ($p=0.06$) for higher embryo development rates for the Light group compared to the Control; there was a time effect ($p<0.05$), but no bull effect ($p>0.05$). Blastocyst cell numbers at 192 hours were 130.1 ± 7.5 and 146.5 ± 7.6 for Light and Control groups, respectively. There were no significant differences for treatment, bull or the interaction on blastocyst cell numbers ($p>0.05$). Further research is needed to determine the mechanism by which the photobiomodulation treatment affects the early bovine embryo metabolism and development.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Recombinant FSH (ZIMBRIA®) Improves Blastocyst Yield in Gir Cows Undergoing OPU-IVF

Sebastião Gomes da Silva[1], Thiago Figueiredo Ferreira[1], João Lucas Lobo Alves Correia[1], Gustavo Mabub[2], Rafael Moreira[2], Federico Randi[2], Alexandre Hênryli de Souza[2]

[1]GEPEC, [2]Ceva Saúde Animal

This study evaluated the efficacy of recombinant bovine follicle-stimulating hormone (rFSH, ZIMBRIA®, CEVA) on embryo production in Gir (Bos indicus) cows undergoing ovum pick-up (OPU) for *in vitro* fertilization (IVF). The trial was conducted in a commercial dairy in Paraopeba, Minas Gerais, Brazil, by BH Embriões laboratory. The experiment involved 11 non-lactating, open Gir cows with prior successful OPU-IVF outcomes. A randomized crossover design was employed, with each cow serving as her own control across two OPU sessions 41 days apart. Cows were assigned to either the rFSH group (2 mL, 100 µg rFSH, n=11) or the control group (saline, n=11), with treatments randomized within and switched between sessions. Reproductive status was assessed via ultrasonography seven days before and on the day of OPU. On day 0 (D0), rFSH-treated cows received 2 mg estradiol benzoate and a progesterone implant. On D4, cows in the rFSH group were administered 2 mL rFSH (100 µg); the progesterone implant was removed on D7, immediately followed by OPU in all cows. Semen from a single bull was used. Oocyte and embryo quality was assessed by the same treatment-blind technician. All statistical analysis were performed with the proc Glimmix in SAS (v9.4). The total number of oocytes recovered (rFSH: 13.2; control: 13.1, $P > 0.10$) and number of viable oocytes (rFSH: 9.5; control: 9.1, $P > 0.10$) did not differ significantly between groups. However, rFSH treatment significantly increased embryo production (rFSH: 5.0; control: 2.8, $P < 0.05$) and blastocyst rate (rFSH: 40.6%; control: 31.1%, $P < 0.05$). These results indicate that ZIMBRIA enhances IVF efficiency in Gir cows, supporting its potential for use in assisted reproductive technologies.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Reproductive stability of Nelore bulls under seasonal variation: retrospective analysis from 2013 to 2024 in a commercial IVF program

Thawan Santana Piemonte[1], Carlos Alonso Paco Nagaki[1], Marcio Gonçalves de Paula Junior[1], Camilla Mota Mendes[1], Álvaro de Miranda Alves[1], Marcilio Nichi[1], Mayra Elena Ortiz D Avila Assumpção[1]

[1]Universidade de São Paulo

Climate change has intensified in recent decades, with a progressive increase in average temperatures and a higher frequency of extreme events, including heatwaves and prolonged droughts. According to the National Institute of Meteorology (INMET), the summer of 2024/2025 in Brazil was the sixth hottest since 1961. This was confirmed by NASA (National Aeronautics and Space Administration), highlighting a concern for animal production, especially in tropical regions. In bovine reproduction, the effects of heat stress on females have been widely studied. However, the repercussions on sperm and embryonic performance are still poorly understood. Spermatogenesis is highly sensitive to temperature, and exposure of these animals to heat during semen collection can compromise functional aspects of sperm, even in the absence of morphological changes. Such damage can negatively impact embryonic development and pregnancy rates, even when reproductive biotechnologies are used. Given this context, this study aimed to evaluate the effect of seasonality (summer vs. winter) on the reproductive performance of Nellore bull semen used in a commercial *in vitro* embryo production (IVP) program. To this end, retrospective data for the period from 2013 to 2025 were analyzed. The analysis considered the blastocyst rates obtained from viable oocytes, as well as the pregnancy rates at 30 (P30) and 60 (P60) days after embryo transfer. As an inclusion criterion, bulls with at least three semen batches used in PIVE were selected. Seasonal classification was analyzed considering two groups: semen collected in the summer and semen collected in the winter in the southern hemisphere. Summer was defined as the period between the December solstice and the March equinox, while winter was defined as the interval between the June solstice and the September equinox. Data were tested for normality, and Student's t-test and Wilcoxon's test were applied for parametric and non-parametric variables, respectively; $p<0.05$ was considered the significance level. Data from 31 bulls were included, and there was no difference between summer and winter for any of the variables analyzed. The blastocyst rate was 35.57% (25.83–42.89) and 36.35% (26.93–45.76) ($p = 0.1931$), while the number of viable oocytes was 294.06 ± 37.96 and 377.94 ± 53.55 ($p = 0.3453$) for summer and winter, respectively. P30 rate was 44.4% (35.3–55.6) in summer and 43.6% (33.3–51.4) in winter ($p = 0.226$), and P60 was 20.95% (0–37.05) in summer and 23.3% (0–34.35) in winter ($p = 0.4752$). These results confirm the adaptability and hardiness of Nellore cattle to the tropical climate, indicating that, even in the face of possible initial seasonal variations, the controlled environment of IVF and sperm selection by density gradient contributes to the uniformity of semen quality.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Supplementation of Oleic Acid to Bovine Oocyte Maturation MediumImproves Embryo Quality Parameters

Alexandre Marcos de Melo[1], Alice Santos Cardoso[1], Clara Slade Oliveira[1], Carolina Capobiango Romano Quintão[1], Wasim Al Shebli[1], Luiz Sérgio de Almeida Camargo[1], Naiara Zoccal Saraiva[1]

[1]Empresa Brasileira de Pesquisa Agropecuária

In vitro embryo production (IVP) is a key biotechnology for improving genetic progress and reproductive outcomes in cattle. The quality of embryos produced *in vitro* is largely affected by the the maturation medium (IVM) composition, especially the presence of fatty acids that influence oocyte developmental competence. Stearoyl-CoA desaturase 1 (SCD1) converts stearic acid (SA), a saturated fatty acid, into oleic acid (OA), a monounsaturated fatty acid known to enhance oocyte viability and embryo development. This study evaluated the effects of SA and OA supplementation during IVM on bovine embryo quality, assessed by total cell number, neutral lipid content, and apoptotic index. Oocytes and cumulus-oocyte complexes were collected from slaughterhouse-derived ovaries. A total of 544 oocytes were distributed across six experimental groups: BSA control (n=92), Ethanol control (n=95), 25 µM SA (n=110), 50 µM SA (n=76), 100 µM OA (n=89), and 200 µM OA (n=82). Oocytes were matured for 24 h at 38.5 °C in a 5% CO_2 atmosphere, fertilized *in vitro* for up to 24 h, and cultured for 7 days until the blastocyst stage. Embryos were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, USA) and stained with Hoechst 33342 (Thermo Fisher Scientific, Waltham, USA), Nile Red (Molecular Probes, Inc., Eugene, OR, USA) and TUNEL (apoptosis, DeadEnd Fluorometric TUNEL System, Promega, Madison, USA). Embryos were analyzed by fluorescence microscopy (EVOS M5000) and images were evaluated using ImageJ software (NIH). Data were analyzed by one-way ANOVA followed by Tukey's test (GraphPad Prism 10; $P<0.05$). Cleavage rates ranged from 73.7% (50 µM SA) to 82.9% (200 µM OA) and the blastocyst rate was significantly higher in the 200 µM OA group (51.2%) compared to the BSA control (32.6%; $P<0.05$). The total cell number per blastocyst was significantly higher in the 200 µM AO group (152.5 ± 39.2) than in the BSA control (142.6 ± 52.6; $P<0.05$) while the average number of apoptotic cells was lower (0.05 ± 0.04 vs. 0.2 ± 0.2; respectively $P<0.05$). Lipid content was reduced in the 200 µM OA group (0.0001 ± 0,00007) compared to the BSA control (0.0002 ± 0.0002; $P<0.05$). Supplementation with 200 µM AO during IVM significantly improved bovine embryo quality by optimizing cellular dynamics, reducing apoptosis and lipid accumulation, reinforcing its potential in IVP protocols. These findings confirm previous results (Melo et al., Animal Reproduction, vol. 21, no. 3, p.111, 2024). regarding the positive contribution of AO to the competence for early development.

Financial Support: Fapemig and CNPq.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU-IVF

Use of *in vitro* fertilization as a tool for genetic improvement in Red Sindhi dairy cattle: reproductive and genetic aspects

José Ricardo Sobral da Silva[1,2], Ludmilla Costa Brunes[3], Adriano Queiroz de Mesquita[2], Claudio Ulhoa Magnabosco[2], Julia Carvalho de Melo[4,2], Lucas Costa de Faria[4,2], Otávio Augusto Costa de Faria[4], Isabel Cristina Ferreira[2], Francisco Gomes Fernandes[5], Carlos Frederico Martins[2]

[1]Centro Universitário do Planalto Central Apparecido dos Santos, [2]Embrapa Cerrados, [3]Universidade Federal de Goiás, [4]Universidade de Brasília, [5]Empresa Paraibana de Pesquisa, Extensão Rural e Regularização Fundiária

The objective of this study was to evaluate the performance of using female sexed versus conventional semen for embryo production and pregnancies, as well as verify the impact of *in vitro* fertilization (IVF) and the sires used on the predicted transmitting ability (PTA) for milk production (PTA_MP), reliability (REL), and inbreeding coefficient (F) values in Red Sindhi dairy cattle. Data from four sequential IVF, pregnancies, and animals born at the Center of Technology for Zebu Dairy Breeds from Embrapa Cerrados-DF were analyzed. The first generation consisted of 22 embryo donor females and the second generation included 61 offspring generated by IVF using semen from seven different bulls. Embryo and pregnancy rates between sexed and conventional semen were analyzed using the chi-square test, and parameters in both generations were compared using the T-test ($p<0.05$) and Pearson correlations. No difference was observed in the blastocyst rate produced with sexed semen (27.22%) and conventional semen (22.38%). Similarly, pregnancy rates did not differ (35.08% vs 43.75%, for sexed and conventional; $p>0.05$). A greater number of fertilizations involved sexed semen compared to conventional, but some sires were used with both types, possibly leading to over-representation; however, as analyses focused on group comparisons rather than individual sires, this minimized potential bias. In addition, an average increase of 65.23 kg in PTA_MP was observed in the offspring compared to the mothers, representing a 220% increase, highlighting the potential of IVF to generate descendants with higher genetic merit and accelerate genetic progress. An average reduction of 7.95 points in REL was observed in the genetic values of the offspring compared to the mothers. This decrease (63%) is justified by the younger age of the individuals and the volume of information available. Although no statistical difference was found for F between generations, a numerical reduction of 61% in the average values was observed, suggesting that selection was aimed at mating less related animals, which helps preserve genetic variability. The correlations between animals in generations 1 and 2 for PTA_MP, REL, and F were high: 0.85, 0.91, and 0.67, respectively. The high correlation for REL suggests that, although the individual accuracy of young animals was lower, the reliability pattern was maintained due to the high reliability of the sires. The moderate correlation for F indicates that the levels of inbreeding were maintained, but with greater variability, suggesting a trend toward the gradual reduction of inbreeding. IVF with sexed semen can be as effective as conventional semen while enabling targeted genetic improvement, that combined with careful selection of sires and donors with high genetic values and good reliability, might reducing the interval between generations and accelerate the animal selection process and maximize the genetic progress in zebu breeds such as Sindhi.

Financial support: FAPDF, AgroIntegra, ABCZ.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Decellularized ovarian bioscaffolds and resveratrol-loaded polymeric nanoparticles improve *in vitro* development of bovine secondary follicles

Francisco das Chagas Costa[1], Andreza de Aguiar Silva[1], SUELINE CAVALCANTE CHAVES[1], Regislane Pinto Ribeiro[1], Laryssa Gondim Barrozo[1], Venância Antonia Nunes Azevedo[1], Mariana Aragão Matos Donato[2], Josimar Oliveira Eloy[1], Alice Vitória Frota Reis[1], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará, [2]Universidade Federal de Pernambuco

This study aimed to decellularize bovine ovarian tissue (dECM) and to evaluate the effects of these three-dimensional scaffolds, combined with resveratrol-loaded polymeric nanoparticles (RLNP), on the viability, ultrastructure, and antioxidant gene expression in bovine secondary follicles cultured i*n vitro*. The RLNP were synthed by nanoprecipitation. dECM was obtained from bovine ovarian cortical fragments through three freeze–thaw cycles at –80 °C, followed by sequential incubation in 1% Triton X-100 and 0.5% sodium dodecyl sulfate (SDS) solutions for 9 hours each. dECM scaffolds were subjected to hematoxylin and eosin staining to assess cell removal, and Hoechst staining to corroborate the absence of cells and to detect residual DNA. Collagen and glycosaminoglycans (GAGs) were evaluated by Picrosirius red and Alcian blue staining, and quantified with Fiji-ImageJ software. Scanning electron microscopy was performed to analyze scaffold microarchitectural post-decellularization and after 12 days of culture in TCM199+ (without RLNP) to assess temporal structural changes. Bovine secondary follicles (150–200 µm) were isolated and cultured for 12 days in either a two-dimensional system (100 µL drops of TCM-199^{+}) or a three-dimensional system using dECM scaffolds. The 3D medium was supplemented with 0.02, 0.2, or 2.0 µM RLNP, blank nanoparticles (to assess nanoparticle-specific effects), or unencapsulated resveratrol (to compare with the nanoencapsulated form). Follicular viability was evaluated in 40 follicles/treatment (4 replicates of 10 follicles) using calcein-AM/ethidium homodimer-1 staining with fluorescence quantification via Fiji-ImageJ. Ultrastructure was analyzed in 12 follicles/treatment (4 replicates of 3 follicles) by transmission electron microscopy. Gene expression of *CAT, SOD, GPX1*, *PRDX6*, and *NRF2* was assessed by qRT-PCR in 40 follicles/treatment (4 replicates of 10 follicles). To minimize handling, follicular diameter was not measured at the end of the culture. Quantitative data were analyzed by unpaired t-tests or one-way ANOVA, followed by Tukey's test ($P < 0.05$). Hematoxylin-eosin and Hoechst staining confirmed effective cell removal. Collagen, GAGs, and ECM ultrastructure were preserved after decellularization. Collagen remained stable until day 12 of *in vitro* culture, whereas GAGs remained stable until day 10, with subsequent reduction and network disorganization from day 12 onward. Follicles cultured 3D system showed increased viability, further enhanced by 0.02, 0.2 or 2.00 µM RLNP. At 0.02 µM RLNP, follicles exhibited intact zona pellucida, and-well-preserved oocyte membrane, and organelles. RLNP also downregulated antioxidant gene expression. In conclusion, the decellularization protocol effectively removed cellular content and preserved extracellular matrix structure. Moreover, a 3D culture system combined with 0.02 µM RLNP supported follicular development and ultrastructure, as well as downregulated antioxidant gene.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Effect of a PTEN inhibitor on the *in vitro* culture of goat ovarian tissue

Joisyleide Gonçalves Da Costa Pinto[1], Vitoria Louise Pinho Mateus[1], Regina Lucia dos Santos Silva[2], Istefani Moreira Mota[1], Gabriela Cristina da Silva Santos[1], Alane Pains Oliveira do Monte[1], Ana Paula Ribeiro Rodrigues[3], Maria Helena Tavares de Matos[1]

[1]Fundação Universidade Federal do Vale do São Francisco, [2]Universidade Federal do Piauí, [3]Universidade Estadual do Ceará

This study aimed to evaluate the effect of bisperoxovanadium (bpV), an inhibitor of phosphatase and tensin homolog deleted on chromosome-10 (PTEN), on follicle survival, activation, cell proliferation, the immunostaining of tumour necrosis factor-α (TNF-α), and DNA fragmentation following the *in vitro* culture of goat ovarian tissue. Additionally, the potential involvement of the PI3K/Akt/FOXO3a pathway in the actions of bpV was analyzed. Ovarian fragments were cultured for 1 (experiment 1) or 7 (experiment 2) days in control medium (α-MEM+), consisting of α-minimum essential medium supplemented with 10 ng/ml insulin, 5.5 µg/mL transferrin, 5 ng/mL selenium, 2 mM glutamine, 2 mM hypoxanthine, 1.25 mg/mL bovine serum albumin (BSA), 50 µg/mL ascorbic acid, and 50 ng/mL epidermal growth factor (EGF), or in α-MEM+ with 1.5, 15, or 150 µM bpV (Sigma Aldrich Chemical; MO, USA). After 24 hours, the medium was replaced with one lacking bpV, and the culture was maintained for six more days. The evaluated endpoints included follicular morphology and activation. In experiment 2, additional parameters assessed were cell inflammation (anti-TNF-α), granulosa cell proliferation, cytoplasmic exclusion of p-FOXO3a from oocytes, and DNA fragmentation. Data on normal follicles, activation, PCNA-positive cells, nuclear exclusion of p-FOXO3a, and DNA fragmentation were analyzed using the Chi-square test. Differences were considered significant at $P<0.05$. Experiment 1 showed that after one day of culture, the group with 1.5 µM bpV maintained the percentage of normal follicles (71.33%) similar to that of the fresh control (78%; $P>0.05$) and enhanced survival compared to α-MEM+ (49.33%), 15 µM (59.33%) and 150 µM bpV (52.67%, $P<0.05$). In addition, all bpV concentrations increased follicular activation compared to α-MEM+. However, there was no significant difference in primary follicles between cultured treatments. After a 7-day culture in experiment 2, all groups showed a reduction in the percentage of normal follicles compared to the fresh control (72%; $P<0.05$). Only the 1.5 µM bpV group (8%) maintained a similar percentage of normal follicles to that observed in the α-MEM+ group (15.33%; $P>0.05$). All groups promoted a massive follicular activation compared to the fresh control ($P<0.05$), with no differences among the treatment groups themselves ($P>0.05$). The 1.5 µM bpV group promoted granulosa cell proliferation and nuclear exclusion of p-FOXO3a, while reducing TNF-α expression and DNA fragmentation compared to α-MEM+. In contrast, the 150 µM bpV group showed a higher percentage of degenerated primary follicles and increased DNA fragmentation compared to α-MEM+ ($P<0.05$). In conclusion, after 24 hours, 1.5 µM bpV maintains follicular survival and enhances primordial follicle activation. After 7 days, 1.5 µM bpV induces massive activation and cell proliferation through the PI3K/Akt/FOXO3a pathway. However, higher bpV concentration results in increased DNA fragmentation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Effect of eugenol on the *in vitro* culture of sheep secondary follicles

Milena Menezes de Araújo[1], Istefani Moreira Mota[1], Joisyleide Gonçalves Da Costa Pinto[1], Luanna Mendes Souza Rodrigues[1], Anna Thaylla Venceslau França[1], Joãozito Liandro de Oliveira Junior[1], Regina Lucia dos Santos Silva[2], Gabriela Cristina da Silva Santos[1], Maria Helena Tavares de Matos[1], Alane Pains Oliveira do Monte[1]

[1]Fundação Universidade Federal do Vale do São Francisco, [2]Universidade Federal do Piauí

This aim of this study was to evaluate the effect of eugenol on the morphology, growth, glutathione (GSH) and reactive oxygen species (ROS) levels, and mitochondrial activity of *in vitro* cultured sheep secondary follicles. Secondary follicles (250-300 µm) were isolated from ovine ovaries by microdissection and individually cultured for 12 days in 100 µL medium drops under mineral oil. The standard control medium consisted of α-MEM supplemented with 50 ng/mL ascorbic acid, 2 mM glutamine, 2 mM hypoxanthine, 10 ng/mL insulin, 5.5 µg/mL transferrin, 5 ng/mL selenium, and 3 mg/mL bovine albumin serum (BSA). To evaluate the effect of eugenol, follicles were cultured in either the control medium (α-MEM) or α-MEM supplemented with 10, 20, or 40 µM eugenol (Sigma Aldrich Chemical; St. Louis, MO, USA). The evaluated endpoints included follicular morphology, antrum formation, follicular diameter, percentage of fully grown oocytes (diameter ≥ 110 µm), GSH, ROS, and mitochondrial activity levels. Data on normal follicles, antrum formation, and percentage of fully grown oocytes were analyzed using the Chi-square test. Data on follicular diameter, GSH, ROS and mitochondrial activity levels were analyzed using the ANOVA and Tukey test. Differences were considered significant at $P<0.05$. After 12 days of culture, there were no significant differences in follicular survival among the treatment groups ($P>0.05$). However, follicles cultured with 20 µM eugenol showed significantly greater antrum formation than those cultured with other treatments ($P < 0.05$). Follicular diameter increased after culturing with 10 or 20 µM eugenol compared to culturing in α-MEM alone or with 40 µM eugenol ($P < 0.05$). The presence of 20 µM eugenol in the culture medium resulted in the highest percentage of fully grown oocytes and the lowest ROS concentrations ($P < 0.05$). Furthermore, mitochondrial activity increased in follicles cultured with 10 µM eugenol. In conclusion, 20 µM eugenol improved antrum formation, follicular and oocyte growth, increased GSH levels, and reduced ROS levels following *in vitro* culture of sheep secondary follicles.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Evaluating mitochondrial potential in bovine cumulus cells as a predictor of oocyte developmental competence

Marilin Ivask[1], Mariani Fiorenza[1], Matheus Andrade Chaves[1], Helena Fabiana Reis de Almeida Saraiva[1], Flávio Vieira Meirelles[1], Lawrence Charles Smith[1,2]

[1]Universidade de São Paulo, [2]Université de Montreal

Oocyte competence, essential for assisted reproduction success, may be influenced by metabolic activity in surrounding cumulus cells. Our previous studies have indicated that the mitochondrial transcriptional patterns in cumulus cells vary among competent and incompetent cumulus-oocyte complexes. This study aimed to evaluate whether mitochondrial membrane potential (MMP) in bovine cumulus cells, assessed with JC-1 staining and flow cytometry, correlates with the developmental competence of individual oocytes. Bovine oocytes were matured individually 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% CO_2 for 18 h. In four independent experiments, cumulus cells from individual oocytes (n=87) with a visible polar body and normal cytoplasm morphology were stained with 2 µM JC-1 and 10 µg/mL Hoechst33342 for 30 min and analyzed using CytoFLEX flow cytometry system (Beckman Coulter). The corresponding oocytes were parthenogenetically activated for individual developmental assessment. Cleavage (CL%) and blastocyst (BL%) rates were evaluated on day 3 and 8, respectively. In two additional experiments, MMP was assessed from cumulus cells collected from groups of oocytes fixed after aspiration (immature) (n=200) or following 18 h (n= 412) or 24 h (n=180) maturation using 9-10 biological replicates per group. The corresponding oocytes were stained for mitochondria and cortical granule distribution using 0.4 µM MitoTracker DeepRed and 1 µg/mL *Lens culinaris* and imaged with Leica MICA confocal microscope (Leica Microsystems). Data were analyzed using Kruskal-Wallis test with Dunn's multiple comparisons and Spearman's correlation (GraphPad Prism 10), with $P<0.05$ considered significant. The mean CL% and BL% were 78.2% (68/87) and 34.5% (30/87), respectively. JC-1 red/green ratios showed no significant correlation with cleavage ($r = -0.039$) or blastocyst formation ($r = 0.048$). The patterns of mitochondria and cortical granule distribution observed in our study did not consistently reflect the cytoplasmic maturation changes reported in the literature. These preliminary findings suggest that, under the conditions tested, MMP in cumulus cells may not reliably indicate oocyte competence. The lack of consistent variability in cytoplasmic maturation markers highlights the complexity of oocyte maturation and the need for further research to identify effective non-invasive predictors of oocyte quality.

Acknowledgments: FAPESP 2021/09886-8 (São Paulo Excellence Chair, SPEC); FAPESP 2023/08037-2.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Single-dose recombinant FSH is as efficient as porcine FSH in commercial superovulation protocols in Bos taurus cows and heifers

Monique Mazzarollo Frata[1], Wagner Marques de Lima[2], Monique Tomazele Rovani[3], Rogério Ferreira[4], Alexandre Hênryli de Souza[5], Rafael Gianella Mondadori[1], Fabiane Pereira de Moraes[1], Helena Leites Freitas[1], Vilceu Bordignon[6], Bernardo Garziera Gasperin[1]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Biotec Serviços de Apoio à Pecuária LTDA, [3]Universidade Federal do Rio Grande do Sul, [4]Fundação Universidade do Estado de Santa Catarina, [5]Ceva Saúde Animal, [6]McGill University

Superovulation (SOV) for in vivo embryo production requires eight decreasing doses of porcine FSH (pFSH) due to its short half-life. Recently, a long-acting recombinant FSH (rFSH) has been launched, allowing for SOV with a single treatment. This study aimed to compare SOV outcomes in commercial ET programs using eight doses of pFSH versus a single administration of rFSH. In Exp. 1, 569 SOV procedures were evaluated under commercial conditions using either pFSH (n=291; 203 cows and 88 heifers; Pluset, Biogénesis Bagó, Curitiba, Brazil) or rFSH (n=278; 239 cows and 39 heifers; Zimbria, Ceva Animal Health, São Paulo, Brazil). In Exp. 2, four SOV procedures with rFSH (40- to 60-day intervals) were performed in a subset of cows (n=12). All the cows that received FSH treatment were evaluated, including those that did not respond to SOV. For the commercial dataset (Exp. 1), distribution differences between groups were assessed using the Kolmogorov-Smirnov test. In Exp. 2, data were analyzed using mixed models for repeated measures. Statistical analyses were performed using JMP Pro 18 software ($P < 0.05$ was considered as significant). In Exp. 1, data are presented as medians and ranges (min–max) and, in Exp. 2, as average ± standard error. No significant differences were observed in Exp. 1 between pFSH- and rFSH-treated cows for the total number of recovered structures (TS; pFSH: 10 (0 - 46), rFSH: 11 (0 - 39); P=0.2), degenerated embryos (DG; pFSH: 2 (0 - 18), rFSH: 1 (0 - 24); P=0.07), and transferable embryos (TE; pFSH: 5 (0 - 23), rFSH: 5 (0 - 25); P=0.51). However, the number of unfertilized oocytes (UF; pFSH: 0 (0 - 26), rFSH: 0 (0 - 31); P= 0.05) and proportion of UF relative to TS (pFSH: 0.0% (0 - 100), rFSH: 4.5% (0 - 100); P=0.01) were higher in the rFSH group. Conversely, the proportion of DG relative to TS was higher in the pFSH group (pFSH: 25.0% (0 - 100), rFSH: 18.4% (0 - 100); P=0.01). The proportion of TE relative to TS was consistent at 60% for both treatments (P=0.89). Similarly, Exp. 2 showed no significant differences across the four repeated SOV sessions with rFSH regarding TS (12.6±2, 14.2±2, 12.9±2, 15.2±2, respectively; P=0.64) and TE (7.4±1.5, 10.9±1.5, 8.3±1.5, 8.2±1.5, respectively; P=0.41). In conclusion, data from the present study suggest that a single administration of rFSH provides SOV outcomes comparable to those achieved with eight administrations of pFSH in commercial ET programs. Furthermore, SOV outcomes remained consistent across four repeated treatments using rFSH.

Financial support: FAPERGS, CNPq, CAPES and Ceva.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Thymol enhances growth, antrum formation, and viability of *in vitro* cultured bovine secondary follicles

Regislane Pinto Ribeiro[1], Danisvânia Ripardo Nascimento[1], Venância Antonia Nunes Azevedo[1], Ernando Igo Teixeira de Assis[1], Pedro Alves Aguiar Barroso[1], Andreza de Aguiar Silva[1], Francisco das Chagas Costa[1], SUELINE CAVALCANTE CHAVES[1], José Jackson do Nascimento Costa[1], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará

The aim of this study was to assess the effects of adding different concentrations of thymol to the culture medium on the in vitro growth and viability of bovine secondary follicles after 18 days of culture. For this, secondary follicles (~0.2mm) were mechanically isolated from ovaries and cultured in an incubator with 5% CO2 at 38.5ºC for 18 days, in TCM-199 medium, supplemented with eCG (4 IU/mL), insulin (10 µg/mL), transferrin (5.5 µg/mL), selenium (5 ng/mL), ascorbic acid (50 µg/mL), BSA (3.0 mg/mL), glutamine (2 mM), hypoxanthine (2 mM), penicillin (100 IU), streptomycin (0.1 mg/mL), and HEPES (0.05 mM). Follicles were cultured in TCM-199+ alone or supplemented with thymol at 1, 10 and 100 µg/mL, and evaluated at days 0 and 18 of culture using an inverted microscope (Nikon, Eclipse TS 100, Japan) and an image capture system (NIS Elements 2.4 Software). Follicular diameters were measured only in morphologically normal follicles. The average of two perpendicular measurements of the outer layer of the thecal cells was used to determine follicular diameter (µm). Antrum formation was characterized by the appearance of a translucent cavity within the granulosa cell layers. Ten independent replicates were performed, with a total of 30 follicles cultured per treatment. Follicles were incubated in 100 µL drops of TCM-199 containing 4 mM calcein-AM and 2 mM ethidium homodimer-1 (EthD-1) at 37°C for 15 minutes. Following incubation, the follicles were washed with TCM-199 and analyzed under a fluorescence microscope. Follicle morphology was evaluated before and after culture. Viability was assessed by measuring the fluorescence intensity of calcein-AM (live cells) and EthD-1 (dead cells), quantified using ImageJ software. The staining intensity was determined by measuring the pixel intensity in the follicular area after background subtraction. Follicular diameter and fluorescence intensity data for viability were analyzed by Kruskal-Wallis test, while antrum formation was analyzed using the chi-square test. Differences were considered significant when $P<0.05$. The results showed that the follicles had a significant growth after 18 of culture, when compared to day 0, but no effects of thymol was observed. The mean diameters of follicles after 18 days of culture were TCM-199+ (248.8 ±5.74), 1 µg/mL thymol (254.4 ±7.05), 10 µg/mL thymol (258.1±7.11) and 100 µg/mL thymol (250.1±5.82). However, follicles cultured with 10 and 100 µg/mL thymol had a higher rate of antrum formation than those cultured in the control group ($P<0.05$). The viability analysis showed that 10 µg/mL thymol increase fluorescence intensity for calcein compared to other treatments and control group ($P<0.05$). No significant differences were observed in EthD-1 fluorescence intensity, suggesting no change in cell mortality. In conclusion, the presence of 10 µg/mL thymol in the culture medium increased antrum formation and fluorescence intensity related to follicular viability for 18 days.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS AND OOGENESIS

Transcriptome modulations on bovine oocytes from non-stimulated dominant follicles

Lais Barbosa Latorraca[1], Antonio Galvao[2], Julieta M. Dauguero[1], Gavin Kelsey[2], Trudee Fair[1]

[1]University College Dublin, [2]Babraham Institute

During bovine folliculogenesis, different genes are activated as the oocyte grows, following the onset of transcription in secondary follicles. Cytoplasmic pathways are predominantly activated during the mid-growth phase, while nuclear pathways become more prominent toward the end of the growth phase (Latorraca et al., *BMC Genomics*, 25:335, 2024). Once the oocyte reaches the fully grown stage, transcription is inhibited, and the oocyte undergoes final maturation within a dominant follicle (DF). Although the acquisition of oocyte competence has been extensively studied, most investigations have relied on *in vitro* systems or superovulation protocols to collect oocytes near the maturation phase. The present study aimed to characterize transcriptomic changes from immature fully grown oocytes (group >120 µm from Latorraca et al., *BMC Genomics*, 25:335, 2024) to those collected *in vivo* from DFs without the use of superovulation. To achieve this, the estrous cycles of 26 crossbred nulliparous beef heifers (primarily Limousin and Charolais crosses) were synchronized to control the timing of ovulation. On day 0, heifers received an intramuscular injection of a GnRH analogue along with the insertion of a progesterone-releasing intravaginal device (PRID). On day 7, luteolysis was induced with an intramuscular injection of a $PGF_2\alpha$ analogue, followed by PRID removal on day 8. Estrus detection was performed every 6h on days 8 and 9. A second GnRH dose was administered to a subgroup of animals 36h after PRID removal to induce an LH surge. Heifers were slaughtered on day 9 (24h before ovulation) and on day 10 (19–23h after estrus detection and ~2h before ovulation, in the subgroup receiving a second GnRH dose). Oocytes were retrieved from DFs and subjected to single-cell genome and transcriptome sequencing. RNA sequencing data from bovine oocytes larger than 120 µm in diameter (14 samples) (GSE249434) were analyzed alongside those from DF-collected oocytes (11 samples). Differentially expressed genes (DEGs) were identified using the EdgeR package, with raw p-values adjusted using the Benjamini–Hochberg method. DEGs were defined as genes with a false discovery rate (FDR)<0.05 and a fold change>1.5. KEGG pathway enrichment analysis of DEGs was performed using ShinyGO 0.82 (FDR<0.05). A total of 1,039 DEGs were upregulated in >120 µm oocytes, while 790 DEGs were upregulated in DF-derived oocytes. GO analysis revealed an enrichment of genes related to oxidative phosphorylation in DF oocytes, supporting previous findings that highlight pyruvate utilization as a key energy source during oocyte maturation. Notably, DF oocytes showed higher expression of *CENPE*, *FIGLA*, and *OOSP*2, whereas *NLRP5*, *NOBOX*, and *XBP1* were more highly expressed in 120 µm oocytes. In conclusion, following transcriptional arrest in fully grown oocytes, only a limited number of DEGs were detected during the peri-maturation period. These findings underscore the critical role of oxidative phosphorylation in energy production during the final stages of oocyte maturation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Effect of injectable minerals (FOSFOSAN®) on bull semen quality and its relationship with seminal plasma redox modulation

Luis Armando Contreras Méndez[1], Abraham López Oliva[1], Berenice Zúñiga Lovera[1], Erick Andrés González Hernández[1], Carlos Jesús Cardoso Carmona[1], Jose Manuel Lopez Hernandez[1], Laura Denise López Barrera[1], Roberto Díaz Torres[1], Patricia Ramírez Noguera[1], Pablo Luna Nevárez[2]

[1]Universidad Nacional Autónoma de México, [2]Instituto Tecnológico de Sonora

In cattle nutrition, dietary mineral deficiencies can negatively impact the reproductive performance of breeding animals. These deficiencies are largely attributed to the low mineral content in the forage consumed by cattle. Essential minerals such as: Se, Cu, Mg, K, and P play a crucial role in the total antioxidant capacity of various body tissues. This study aims to elucidate the cellular-level effects of injectable mineral supplementation in cattle, with emphasis on its impact on gamete quality. The study was conducted during the autumn season in Hidalgo, Mexico, using thirteen young, crossbred Zebu x Angus bulls, aged 16±2 months with an average weight 390-460 kg. The animals were divided into two groups: Control (C, n =7) and Supplemented (F1, n=6). Semen samples were collected on days 0, 3, 7, 14, 28, and 56 by electroejaculation. On day 0, the F1 animals were injected with a multi-mineral supplement (Fosfosan®, Virbac), 10 mL, i.m., and control animals received 10 mL saline solution injection. Semen quality parameters were evaluated using a portable computer-assisted sperm analysis (CASA) system (iSperm®), including total counting of sperm (TSC), motility (MOT), progressive motility (PR), curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), straightness (STR), and linearity (LIN). In addition, visual assessments were conducted to determine the percentage of live sperm (LIV), normal sperm morphology (NSM), primary abnormalities (PA), and secondary abnormalities (SA). Biochemical analyses carried out on the seminal plasma (SP) included fluorometric estimation of reactive oxygen species (ROS) and spectrophotometric measurements of reduced glutathione (GSH), oxidized proteins (OP), lipid peroxidation (TBARS), and the enzymatic activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Statistical analyses were performed using ANOVA and Fisher LSD multiple comparison test at a 95% confidence level, using Statgraphics Centurion 15. The results demonstrated a modulatory effect of Fosfosan® on redox balance of the SP, leading to a increase ($P < 0.05$) in GSH, GPx, and SOD. In the semen quality evaluation, statistically significant differences ($P < 0.05$) were found in TSC, NSM, and PA. Significant effect ($P < 0.05$) were observed in day 7 for VAP and VSL, while TSC was modified on day 28. Given the diverse array of cellular events precipitated by oxidative stress, biochemical parameters were subjected to temporal analysis. This approach was necessitated by the observation that certain effects exhibited no statistical significance within shorter, day-based measurement intervals. These findings suggest a potential benefit of injectable mineral supplementation in young bulls, promoting significant cellular and biochemical changes in ejaculates that are associated with changes in the oxidative stress status of the semen, and leading to an improvement in sperm quality.

Acknowledgements: Virbac Mexico.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Elevated Temperature Disrupts *in vitro* Epididymal Function and Extracellular Vesicle Release in Bulls

Ana Beatriz Bossois Moura[1], Juliano Rodrigues Sangalli[1], Raissa Braido Rangel[1], Laura Gabrielli Haupenthal[1], Maria Alice de Almeida[1], Helena Fabiana Reis de Almeida Saraiva[1], Juliano Coelho Da Silveira[1], Maíra Bianchi Rodrigues Alves[2], Felipe Perecin[1]

[1]Universidade de São Paulo, [2]Universidade Estadual Paulista

As climate change intensifies, impaired scrotal thermoregulation poses increasing risks to bull fertility. The epididymis, essential for sperm maturation and environmental sensing, may undergo molecular disruptions under heat stress. However, the effects of elevated temperature on epididymal epithelial cells remain poorly understood, especially regarding information transfer to gametes and potential consequences for offspring. In this context, the present study aimed to investigate the effects of elevated temperature on bovine cauda epididymal epithelial cells (EECs), with emphasis on the expression of the heat shock protein HSP70 and the release of extracellular vesicles (EVs), after 48 hours of heat exposure. For this purpose, epithelial cell lines derived from the cauda epididymis of adult Nelore bulls (n=3) were cultured (5% CO_2; 32°C) in Principal Cell Medium (PCM). After the third passage, cells were seeded at approximately 5 µg/cm² and divided into two groups, maintained under the following conditions for 48 hours: heat stress (HS: 5% CO_2; 38.5°C) and control group (CO: 5% CO_2; 32°C). Following the treatment, HSP70 protein levels (1:2000; sc-66048; Santa Cruz Biotechnology, Dallas, USA) were evaluated in EECs by Western blotting. Additionally, HSP70 transcript expression was analyzed using RT-qPCR (QuantStudio, Thermo Fisher Scientific, Waltham, USA). Culture media from each group underwent sequential centrifugation and ultracentrifugation for the isolation of EVs, which were subsequently characterized and quantified using Nanoparticle Tracking Analysis (NTA). Statistical analyses were performed using R software, with the Shapiro-Wilk test for normality, followed by ANOVA and t-test for mean comparisons at a 5% significance level. The results showed a significant (p=0.046) increase in HSP70 protein expression in the HS (0.322 ± 0.117) compared to the CO (0.138 ± 0.076), indicating a cellular response to heat stress. Despite the increase in HSP70 protein levels, no difference was observed in its transcript expression (p=0.939), possibly due to post-transcriptional regulatory mechanisms. Regarding EVs, there was no significant difference in particle diameter between groups (CO: 145.67 ± 5.97 nm; HS: 131.23 ± 3.41 nm; p=0.104), although a significant reduction in particle concentration was observed in the HS ($3.07\times10^8 \pm 1.83\times10^7$ particles/mL) compared to the CO ($3.85\times10^8 \pm 1.18\times10^7$ particles/mL; p=0.023). These findings suggest that *in vitro* acute heat exposure impairs the function of EECs, affecting the production of EVs, structures fundamental for communication with sperm. Furthermore, the results highlight the complexity of the cellular response to heat stress and underscore the need for further studies to elucidate the underlying molecular mechanisms, which may impact not only bull fertility but also the transmission of information with potential repercussions on embryonic development.

Grants: CAPES-001; FAPESP 2021/08759-2; CNPq 3080.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

In vitro interaction between bovine sperm and oviductal extracellular vesicles

Laura Gabrielli Haupenthal[1], Ana Beatriz Bossois Moura[1], Maria Alice de Almeida[1], Raissa Braido Rangel[1], Juliano Coelho Da Silveira[1], Felipe Perecin[1], Maíra Bianchi Rodrigues Alves[2]

[1]Universidade de São Paulo, [2]Universidade Estadual Paulista

The interaction between sperm and oviductal extracellular vesicles (Ov-EVs) occurs during *in vivo* fertilization but not when performing assisted reproduction techniques. The aim here is to investigate the *in vitro* interaction of Ov-EVs with sperm in cattle. For that, a *pool* of bovine oviductal fluid (OF) was formed from 10 oviducts ipsilateral to the dominant follicle (10.65±1.53 mm) obtained *post-mortem*. The OF *pool* was processed to isolate the Ov-EVs by ultracentrifugation (119.700g/70min, 4°C). The Ov-EVs were characterized for morphology by transmission electron microscopy, specific EV markers by flow cytometry, and diameter and concentration by nanoparticle tracking analysis. Once characterized, Ov-EVs were stained with PKH67 and incubated with sperm isolated from cryopreserved semen of five Nelore bulls in Talp-Sperm medium to test the Ov-EVs-sperm interaction following the conditions: EVs-sperm incubated for 30 minutes with 2,000 EVs/sperm or 4,000 EVs/sperm and EVs-sperm incubated for 60 minutes with 2,000 EVs/sperm or 4,000 EVs/sperm. Control sperm samples were incubated without EVs but with equivalent concentrations of free PKH67, for each treated group. After incubation, each sample was centrifuged twice at 500g/5min, and the pellet was resuspended in Talp-Sperm medium to reach a final concentration of $2.5x10^6$ sperm/mL. The samples were then incubated with 0.5 µL of Hoechst 33342 0.05mg/mL for 10 minutes/37°C. Subsequently, sperm samples were analyzed by flow cytometry (CytoFLEX®; Beckman Coulter) and fluorescence microscopy (Thunder Imager 3D Assay®; Leica). The Ov-EVs-sperm interaction was considered by assessing the median fluorescence intensity per event of PKH67 after analyzing 10,000 events in Hoechst-positive-staining by flow cytometry and through the visualization of green fluorescence of PKH67 in sperm by microscopy. Data were compared by analysis of variance using SAS® considering the effects of group, time, concentration and interactions, considering $P \leq 0.05$ as significance level. Ov-EVs were efficiently isolated and displayed a cup-shaped form, with the detection of Alix, CD63, and CD81 specific markers, diameter of 157.4±7.0 nm, and concentration of $10.7x10^9 \pm 0.32x10^9$ particles/mL. There were no effects of time, concentration, and interactions regarding EVs/sperm interaction (measured by Hoechst-PKH67 co-staining). However, higher ($P<0.0001$) median fluorescence intensity per sperm was identified in the EVs group (0.087 ± 0.0065^a a.u.) compared to the Control group (0.050 ± 0.0014^b a.u.). Additionally, PKH67-positive-staining was detected only in sperm exposed to Ov-EVs mainly in sperm head and midpiece. In conclusion, *in vitro* interactions between Ov-EVs-sperm were evident after 30 or 60 minutes of incubation with 2,000 or 4,000 EVs per sperm. The influence of Ov-EVs on sperm fertilization potential is yet to be investigated. CAPES Finance code 001; FAPESP 2021/08759-2; 2021/09886-8; 2024/13164-6; CNPq 308014-2021-9.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Evaluation of sperm miR-7 and miR-204 expression associated with P/AI and pregnancy loss in bulls

Juan Cuevas de Alvarenga Martins[1], Lucas Petitemberte de Souza[2], William Borges Domingues[2], Vinicius Farias Campos[2], Roberto Augusto de Almeida Torres Júnior[3], Igor Matheus Amaral Gauna Zenteno[1], Ériklis Nogueira[3]

[1]Universidade Federal de Mato Grosso do Sul, [2]Universidade Federal de Pelotas, [3]EMBRAPA GADO DE CORTE

MicroRNAs (miRNAs) are small RNA molecules that function by regulating gene expression. A systematic review (De Souza et al., Research in Veterinary Science, v. 166, 2024) indicated potential predictors. Altered expression of miR-7 has been reported in the spermatozoa of infertile hybrid animals (Bos taurus × Bos grunniens), where it affects key genes (MYRFL, FANCA, INSL3, USP9X, and SHF) involved in molecular pathways essential for spermatogenesis and embryogenesis. Additionally, elevated miR-7 levels have been identified in sperm samples from men with varying profiles of idiopathic infertility. miR-204 is involved in the regulation of spermatogenesis by targeting genes such as SIRT1, which plays a role in the proliferation of spermatogonial stem cells. Reduced expression of this miRNA is associated with Sertoli cell-only syndrome, a condition linked to infertility, highlighting its role in the maintenance of spermatogenesis. This study aimed to evaluate changes in the expression of sperm miR-7 and miR-204 associated with P/AI and pregnancy loss in bulls used for fixed-time artificial insemination (FTAI), with field data. In total, 29 bulls used in FTAI were selected from a database based on their field performance and grouped according to top or bottom 10% pregnancy rates and gestational loss frequencies. Total RNA were extracted from cryopreserved semen doses following a protocol registered with the Brazilian National Institute of Industrial Property (INPI), and the concentration and quality of total RNA were assessed via spectrophotometry using a NanoDrop instrument (ThermoFisher, USA). From the extracted total RNA, cDNA was synthed using the commercial TaqMan™ Advanced miRNA cDNA Synthesis Kit (ThermoFisher, USA), followed by qPCR assays with TaqMan™ probes specific to miR-7 and miR-204. Samples were analyzed in duplicate to obtain an average Ct value for each sample. The 2ˆ-ΔΔCT method was used to assess changes in miRNA expression, and statistical differences between groups were evaluated using a Student's t-test, with a significance threshold of $p < 0.05$. RNA extraction yielded an average concentration of 103.68 ng/µl and an average purity (A260/A280) of 1.76. A significant increase in miR-7 expression was observed in sperm samples from bulls with low pregnancy rates compared to those with high pregnancy rates, but no effects on the expression of pregnancy loss group were observed. Regarding miR-204, a reduction in expression was observed in the low pregnancy group compared to high pregnancy, but only among animals with high gestational loss rates. miRNAs associated with spermatogenesis and embryogenesis, such as miR-7 and miR-204 evaluated in this study, are being investigated as potential predictors of male reproductive capacity. Furthermore, the development of biotechnological tools that link sperm miRNA profiles to high fertility traits holds significant economic interest for optimizing reproduction in cattle farming.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Exogenous microRNA delivery to bovine sperm: a novel strategy to modulate fertility potential

Maria Alice de Almeida[1], Matheus Andrade Chaves[1], Cibele Maria Prado[1], Laura Gabrielli Haupenthal[1], Ana Beatriz Bossois Moura[1], Raissa Braido Rangel[1], Felipe Perecin[1], Juliano Coelho Da Silveira[1], Maíra Bianchi Rodrigues Alves[2]

[1]Universidade de São Paulo, [2]Universidade Estadual Paulista

Sperm-borne microRNAs (miRNAs) are increasingly recognized for their role in fertilization competence and early embryonic development. In this context, the targeted delivery of exogenous miRNAs represents a promising strategy to modulate sperm functionality. This study investigated the efficiency of a liposome-based co-incubation protocol for delivering a synthetic miRNA (prd-miR-39) to bovine sperm. Cryopreserved semen from five Nellore bulls (Bos Indicus) was thawed (37 °C, 30 s), and motile sperm were selected using a Percoll® gradient (45% and 90%). Selected sperm (10×10^6 sperm/mL) were then co-incubated with anionic (LAni; negatively charged) or cationic (LCat; positively charged) liposomes at concentrations of 1,000, 10,000, and 100,000 liposomes per sperm for 5, 15, and 30 min at 38.5 °C under 5% CO_2 and high humidity. The control group (Ctrl) consisted of sperm incubated without liposomes under the same conditions. Sperm viability was assessed via nanoflow cytometry (CytoFLEX®) using Hoechst 33342 (0.5 mg/mL) and propidium iodide P4170 (0.5 mg/mL) to quantify the percentage of non-viable cells (NV%) to establish the optimal condition to test miR-39 delivery via liposome. Thus, sperm (5×10^6 sperm/mL) were co-incubated for 5, 15, or 30 min at 100,000 liposomes per sperm with the following treatments: LAni without miR-39 (LAni-Ctrl); LCat without miR-39 (LCat-Ctrl); LAni encapsulating miR-39 (LAni-39); LpCat encapsulating miR-39 (LCat-39); and Free miR-39 without liposomes (F-39). Uptake efficiency was assessed by qPCR (QuantStudio) through the detection of miR-39 in sperm cells. Data were analyzed using ANOVA (SAS) considering the factors (time, concentration, and liposome type) and their interactions, with significance set at $p\leq0.05$. In both experiments, no interaction ($p\geq0.05$) was found between factors. No significant differences in NV%, were observed across liposome concentrations ($p=0.18$) or liposome types ($p=0.06$). However, co-incubation time significantly affected ($p<0.0001$) sperm viability, with the lowest NV% observed at 5 min (34.61±0.94%) compared to 15 min (41.89±1.20%) and 30 min (49.93±1.27%). Thus, the concentration of 100,000 liposomes per sperm was used for the subsequent experiment. Sperm co-incubated with LCat-39 exhibited significantly higher miR-39 uptake ($p<0.0001$) compared to both LAni-39 and F-39, regardless of the co-incubation time ($p=0.70$). In conclusion, liposome concentration does not affect sperm viability, although incubation for 15 or 30 minutes impairs it. Cationic liposomes at 100,000 per sperm are the most efficient miRNA carriers, independent of incubation time. Thus, a 5-minute co-incubation with 100,000 cationic liposomes is an effective strategy for exogenous miRNA delivery. These findings support the development of miRNA-based approaches in assisted reproduction, with ongoing studies exploring miRNA transfer from sperm to embryo.

Grants: CAPES: 001; FAPESP: 2021/06645-0.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Identification of brain-derived neurotrophic factor (BDNF) and its receptor in ram spermatozoa

Hymerson Costa Azevedo[1], Matheus Batista de Oliveira[2], Waldecy de Lucca Junior[2], Cristiane Bani Corrêa[2], Julio Constantino Jerí Molina[2]

[1]Empresa Brasileira de Pesquisa Agropecuária, [2]Universidade Federal de Sergipe

This work aimed to identify the brain-derived neurotrophic factor (BDNF) and its receptor (Tropomyosin receptor kinase B – TrkB) in ram spermatozoa. Six adult Santa Inês rams (n = 6) were selected, and their ejaculates were collected using an artificial vagina. The ejaculates were diluted with an extender based on glycine-egg yolk-milk with glycerol to achieve a final sperm concentration of 400 × 10^6 sperm/mL. The diluted semen was packed into 0.25 mL straws, sealed, and cooled for 1.5 hours at 0.30°C/min (minitube refrigerator®; Minitube, Tiefenbach, Germany) to reach 5°C. Straws were frozen in nitrogen vapor (-110°C for 11 min) before storage at -196°C, then thawed (40°C for 20 seconds) and maintained at 37°C for sperm evaluation.. Thawed semen samples were analyzed for the detection of BDNF and TrkB in sperm using immunofluorescence techniques (Li et al., Theriogenology, 77:636-643, 2012). Smears were prepared by placing 15 µL of semen (100 × 10^6 sperm/mL) onto glass slides and fixing them with methanol at -20°C for 20 minutes. The fixed samples were washed with 0.3% Triton X-100 solution (Aldrich, St. Louis, USA) and permeabilized with phosphate-buffered saline (PBS) for 5 minutes at room temperature. Samples were divided into three groups for treatment with: primary anti-BDNF antibody (2 µg/mL; Abcam, Cambridge, UK); primary anti-TrkB antibody (8 µg/mL; Abcam, Cambridge, UK); and bovine serum albumin (Sigma-Aldrich, St. Louis, USA) as a negative control. They were incubated at 4°C in the dark for 24 hours and washed three times with PBS at 60 rpm/5 minutes. After 24 hours, all groups were incubated with the secondary antibody goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (1:1000; Alexa Fluor® 488; Thermo Fisher Scientific, Waltham, USA) at 4°C. The samples on the slides were rewashed, mounted with 60 µL of mounting medium, covered with coverslips, and air-dried for 1 hour. Fluorescence was analyzed at 400× magnification using a confocal microscope (Olympus, Tokyo, Japan). A total of 200 spermatozoa in each sample were evaluated based on fluorescence as follows: positive staining (green fluorescence) indicated antibody binding to BDNF or to TrkB; the absence of fluorescence indicated negative binding. The interpretation of the data was based exclusively on descriptive statistics. The immunofluorescence allowed for the identification of BDNF and its receptor TrkB in ram spermatozoa. Both BDNF and its receptor were detected in 100% of the spermatozoa from all rams evaluated. It was observed that spermatozoa showed positive BDNF and TrkB fluorescence in the acrosomal regions. The identification of BDNF and its receptor in ram spermatozoa represents a significant step toward understanding the complex molecular mechanisms underlying sperm function. These findings lay the groundwork for future investigations into the role of neurotrophic factors in male fertility, with potential implications for sheep breeding programs.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Immunolocalization of AMH in epididymis of buffalo fetuses

Simone do Socorro Damasceno Santos[1], Maria Eduarda Pompeu de Macedo[1], Anelise de Sarges Ramos[1], Bruno Porpino Homobono[1], Leonardo Henrique da Silva Nogueira[1], Aluizio Otávio Almeida da Silva[1], Eduardo Baia de Souza[1], Maria Auxiliadora Pantoja Ferreira[1], Marcela da Silva Cordeiro[2], Nathalia Nogueira da Costa de Almeida[1]

[1]Universidade Federal do Pará, [2]Instituto Federal de Educação, Ciência e Tecnologia do Pará

It is known that immature sperm leaves the testicle and acquires motility and the ability to fertilize during its passage through the epididymis, originated from the Wolffian ducts, is a long, coiled tubular organ that connects the efferent duct of the testicle to the deferent duct. Anatomically, it is divided into an initial segment, head, body and tail, with distinct characteristics and functions. Among the events that occur in the epididymis, we can mention the release and absorption of fluids, ions, antioxidants and even exosomes known as "epididymosomes". Anti-Mullerian hormone (AMH) is a protein belonging to the superfamily of the Transforming Growth Factor (TGF-β), together with inhibin, activin and GDF-9, which acts during gonadal development and has stimulating or inhibiting effects on the division and differentiation of germ cells and helps regulate fertility, also having an important function in sexual differentiation by inducing the regression of the Mullerian ducts in the male fetus. Although most members of this superfamily have diverse functions, the action of AMH is restricted to the reproductive organs, being produced in Sertoli cells and granulosa cells of several species, with no studies in the literature on its presence and action in the epididymis. This study aimed to evaluate the presence of AMH in the epididymis of buffalo fetuses. Epididymides of buffalo fetuses aged 6 (n=3) and 7(n=3) months (48-79cm Crown-Rump Length - CRL) were collected at a slaughterhouse, fixed in 10% formalin for 24h, processed for conventional histology and embedded in paraffin. Immunolocalization of AMH was performed on deparaffinized 5 µm histological sections using anti-AMH antibody (SC 28912) according to the manufacturer's instructions, counterstained with 3,3'-diaminobenzidine (DAB) and stained with hematoxylin and eosin (H.E) and the negative control was performed without the use of the primary antibody (AMH). The AMH immunolocalization analyzes were performed in an Eclipse Ci-E photomicroscope (Nikon Corporation, Tokyo, Japan) coupled to a NIKON DS-Ri1 digital camera (Nikon Corporation, Tokyo, Japan) and NIS-Elements Basic Research software - NIKON Version 4.0. Intense immunostaining was observed in the cytoplasm and on the apical surface of the epididymal epithelium and in the interstitial space of the epididymal duct in all samples of both fetal ages analyzed. To our knowledge, this is the first report of the presence of AMH in the epididymis of buffalo fetuses, and due to the importance of AMH in sexual differentiation, which through paracrine actions drives the differentiation of the male internal genitalia, it is suggested that further studies on the action and importance of AMH in sexual differentiation and the development of the male tubular genitalia are necessary, especially in buffalo fetuses.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

In silico modeling of BSP1 interactions with phospholipids of the bovine sperm membrane: a quantum and molecular dynamics approach

Arlindo de Alencar Araripe Moura[1], Valdir Ferreira de Paula Junior[2], Denise Damasceno Guerreiro[1], Valder Nogueira Freire[1]

[1]Universidade Federal do Ceará, [2]Universidade Estadual do Ceará

Binder of sperm proteins (BSP1, BSP3, BSP5) are major components of the bovine seminal plasma, play roles in sperm capacitation and fertilization, and have empirical associations with fertility parameters. BSP1 contains fibronectin type II (Fn2) domains that bind to sperm plasma membrane (PM). The present study evaluates the molecular structure of bovine sperm PM phospholipids and their interactions with BSP1. The work includes a computational PM model, focused on quantum biochemistry of BSP1-lipid bilayer interactions, and molecular mechanisms involved in membrane phospholipid properties and BSP1 Fn2 domains. To evaluate sperm BSP1/PM interactions, we developed a decentralized workflow using CHARMM-GUI and constructed 11 homogeneous membranes, each with a different phospholipid (100-Å length), subjected to all-atom molecular dynamics (AA-MD) simulations (100 ns). In parallel, coarse-grained molecular dynamics simulations were performed for 5 µs. Simulations were conducted using the GROMACS software. The structure of BSP1 dimer was simulated using AA-MD (100 ns) to identify the BSP1 conformation with the lowest-energy. This conformation was combined with each of the 11 membrane types to generate empirical BSP1/PM systems, subjected to AA-MD simulations (200 ns). Model analysis used built-in GROMACS and MOSAICS software. The lowest-energy snapshots of BSP1/PM systems were analyzed through quantum energy calculations and Dmol³ algorithm. As result, we obtained 11 membrane models with single phospholipids, stabilized in the liquid phase and reduced deuterium order parameters, indicating higher fluidity. Density of the membrane models displayed uniform distribution without major changes in area per lipid (APL) or bilayer thickness. Lateral lipid diffusion, at 100 ns simulation, demonstrated uniform and PM homogeneous motion. In contrast, palmitoyloleoylphosphatidylcholine (POPC)/BSP1 plasma membrane had differences in the parameters of deuterium order, lipid density, APL and lateral diffusion of lipids in comparison with homogeneous models without BSP dimer. We observed stabilization of BSP1 dimer at the membrane interface along the z-axis, with no occurrence of jumps or edge effects throughout the simulation period. Our analyses elucidated the interactions between BSP1 and sperm membrane phospholipids, revealing that BSP1 induces structural remodeling of the membrane, altering lipid order, density, and lateral diffusion. These modifications potentially increase membrane fluidity and permeability, features that are essential for the destabilization of the sperm membrane during capacitation. By uncovering the quantum and molecular-scale energy of BSP1 interactions with phospholipid bilayers, this study provides mechanistic insights into how BSP1 facilitates cholesterol and phospholipid efflux, and our understanding of BSP1 role in priming sperm cells for successful capacitation and fertilization.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

In vitro capacitation of collared peccary spermatozoa using different agents and its effect on motility parameters

Alexsandra Fernandes Pereira[1], Maria Valéria de Oliveira Santos[1], Lhara Ricarliany Medeiros de Oliveira[1], Antonia Beatriz Mendonça Pereira[1], Ana Lívia Rocha Rodrigues[1], Luana Grasiele Pereira Bezerra[1], Romário Parente dos Santos[1], Moacir Franco de Oliveira[1], Alexandre Rodrigues Silva[1]

[1]Universidade Federal Rural do Semi-Árido

The acquisition of rapid and vigorous sperm motility during *in vitro* capacitation is crucial for successful IVF outcomes. However, this step still requires optimization in collared peccaries (Pecari tajacu, Linnaeus, 1758) to establish efficient IVF protocols aiming at species conservation. This study evaluated the effects of different capacitating agents on sperm motility and capacitation over time. Semen samples were collected by electroejaculation from eight anesthetized, captive collared peccaries. After washing by centrifugation (100×g for 3 min, three times) in TALP medium, spermatozoa were incubated with the following treatments containing TALP medium supplemented with: 10 µg/mL heparin (TH group); a combination of 10 µM hypotaurine, 20 µM penicillamine, and 2 µM epinephrine (PHE; TPHE group); and both agents combined (TH-PHE group). TALP medium alone served as the control. Samples were incubated at 38.5 °C with 5% CO_2 and analyzed after 1, 3, and 6 h. Sperm motility and kinetics were assessed using computer-assisted semen analysis, while capacitation status was determined via chlortetracycline staining, classifying sperm as intact, capacitated, or acrosome-reacted. Data were analyzed using mixed-effects models, considering treatment, time, and their interaction. When significant effects were verified in the F test of the analysis of variance, the Tukey test was used to compare means ($P < 0.05$; GraphPad Software). Interaction was observed in motility, rapid sperm and capacitation. Motility parameters generally declined over time across treatments. After 3 h, total motility was significantly higher ($P = 0.0049$) in the TH (57.7%) and TH-PHE (56%) groups compared to TALP (42%) and TPHE (38%). Amplitude of lateral head (ALH) increased in all groups at 3 h, with TH (6.5 µm) and TPHE (7 µm) maintaining higher values than TALP (5.1 µm) at 6 h ($P = 0.0119$). No significant differences were observed in other kinetic parameters. At 3 h, the TH group showed a higher percentage of rapid sperm (53.7%) than TALP (39.3%) and TPHE (34.6%), while TH-PHE (50.1%) showed similar values ($P = 0.0171$). The TH group also had a higher ($P = 0.0155$) proportion of slow sperm (8.3%) compared to TALP (5.3%) and TPHE (4.6%). Regarding capacitation, TALP and TH treatments resulted in the highest rates at 3 h, whereas TPHE and TH-PHE groups reached peak capacitation as early as 1 h, indicating that PHE accelerates the process. No significant differences were observed among treatments for acrosome reaction. In conclusion, heparin enhances sperm motility and capacitation after 3 h, while PHE-containing media promote faster capacitation, observable within 1 h of incubation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Lipidomic profile of cryopreserved spermatozoa is related to sperm capacitation events and fertility of Nellore bulls

Alexandre da Rocha Bozzi[1], Laura Nataly Garcia-Oliveros[1], Thais de Oliveira Cardoso Silva[1], Fernanda Baatsch Nascimento[1], Maíra Bianchi Rodrigues Alves[2], Daniel Silva Antonelo[3], Juliano Coelho Da Silveira[1], Felipe Perecin[1], Rubens Paes de Arruda[1], Eneiva Carla Carvalho Celeghini[1]

[1]Universidade de São Paulo, [2]Universidade Estadual Paulista, [3]Lipid Marker Omics Science

The induction of sperm capacitation in bovine triggers essential biochemical and structural changes that are critical for fertilization, and the magnitude of these alterations can cause variations in the sperm lipid profile and impact bull fertility. This study aimed to understand the relationship between sperm lipidomic profile, sperm capacitation events and bull fertility. For this purpose, cryopreserved semen batches from Nellore bulls of known fertility at fixed-time artificial insemination and classified as having high fertility (HF, 54.3±1% pregnancy rate, n=10) and low fertility (LF, 41.5±2.3% pregnancy rate, n=8) were evaluated immediately after thawing (non-capacitated condition) and after *in vitro* induction of sperm capacitation. According to the response to sperm capacitation, the bulls most responsive to capacitation were chosen from HF (HFHR; n=5, as control) and those with lower responsiveness from LF (LFLR; n=4, reduced fertility due to capacitation failure). Sperm analyses included motility (CASA, Hamilton Thorne IVOS® II), phosphatidylserine translocation (Annexin V-FITC), acrosome reaction (FITC-PSA), and lipid membrane disorder (Merocyanine 540) by flow cytometry (BD Accuri C6), as well as lipidomic analysis by UPLC-HRMS (Q-Exactive Orbitrap). Bulls from the HFHR group showed higher ($P<0.05$) values of total and progressive motility, rapid cells, curvilinear velocity (VCL) and straight-line velocity (VSL) than LFLR. Capacitation increased phosphatidylserine translocation and acrosomal reaction in both groups. Lipidomic profiling revealed 50 differentially abundant lipids ($P\leq0.05$; $FC\geq1.2$), with 42 lipids overabundant in LFLR and 10 in HFHR. In the HFHR group, the most relevant lipids based on VIP score (VIP>2.0) included DHAP(O-18:0), Leukotriene C4, Guaiacylglycerol 1-glucoside, Estriol-16-Glucuronide, and Palmitic acid, suggesting active energy metabolism and membrane protection. In the LFLR group, key lipids included 2-Methyl-3-ketovaleric acid, Sphingosine 1-phosphate, and Undecanoylcholine, which may reflect degenerative processes. While PCA failed to separate the groups ($P=0.076$), PLS-DA analysis revealed partial discrimination ($R^2=0.97$), highlighting qualitative differences in lipidomic patterns. The exclusive presence of DHAP(O-18:0) in HFHR bulls suggests preserved ether lipid biosynthesis via a remnant peroxisomal pathway. The greater abundance of structural phospholipids such as phosphatidylcholines, phosphatidylglycerol and ceramides in HFHR bulls may contribute to increased membrane stability and resilience. In contrast, higher levels of lysophospholipids and phosphatidylserine in LFLR bulls are possibly linked to cryopreservation-induced membrane damage. In conclusion, *in vitro* capacitation reveals distinct lipidomic profiles and functional responses between HFHR and LFLR bulls, supporting the use of lipidomic as a promising tool for the identification of fertility biomarkers related to sperm capacitation in bovine.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Post-Thaw Nanoparticle Sorting of Bull Sperm Improves Cryopreservation Outcomes

Emily Nold[1], Abigail L. Zezeski[2], Lauren E. Hamilton[3], Peter Sutovsky[3], Thomas W. Geary[2], Karl Kerns[1]

[1]Iowa State University, [2]United States Department of Agriculture – Agricultural Research Service, [3]University of Missouri Columbia

The spermatozoon glycocalyx serves as the primary interface with the female reproductive tract and oocyte vestments, consisting of a complex extracellular network of glycans and glycoproteins essential for sperm maturation, motility, and fertilization. Our previous work demonstrated that nanopurification of sperm prior to cryopreservation using lectin PNA-conjugated magnetic nanoparticles enriched sperm quality, allowing for a 50% reduction in sperm numbers per insemination dose without compromising pregnancy rates. While this approach improved the selection of high-potential sperm before freezing, cryopreservation itself remains a significant stressor that induces sperm damage, including membrane destabilization, mitochondrial dysfunction, and oxidative stress. Our previous studies applying nanopurification post-thaw demonstrated promising results in selectively removing cryodamaged sperm with lectin PNA and ubiquitin targets. Building on this foundation, we are expanding post-thaw nanopurification by incorporating a broader range of lectin targets to refine selection strategies to enhance sperm quality and functionality for lectins that select other poor phenotypes. Cryopreserved bull semen was subjected to nanopurification using lectin-conjugated magnetic nanoparticles targeting distinct sperm surface glycans, including PNA (galactose), UEA1 (fucose), and LBA (N-acetylgalactosamine). Each lectin was tested individually and in combination across a range of concentrations to optimize nanopurification conditions. Post-thaw sperm motility, morphology, and concentration were assessed before and after nanoparticle sorting using an AndroScope Computer Assisted Semen Analysis system. In addition, sperm vitality, plasma membrane integrity, acrosome integrity, and mitochondrial membrane potential were evaluated using conventional flow cytometry. Findings suggest that post-thaw nanopurification selectively enriches for sperm populations with improved motility ($p < 0.001$), vitality ($p < 0.0001$), membrane integrity ($p < 0.01$), and mitochondrial function ($p < 0.0001$), effectively removing the sperm with detrimental effects of cryodamage. Future work will include IVF trials utilizing post-thaw, nanopurified bull sperm to assess fertilization success and early embryonic development. These studies will provide critical insights into the role of sperm surface glycans in cryopreservation resilience and their potential utility for improving fertility outcomes in bovine artificial insemination and IVF programs. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-36300 from the USDA National Institute of Food and Agriculture.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Pregnancy rates following insemination with bovine heterospermic semen cryopreserved using SpermVital® technology

André Maciel Crespilho[1], Mariana Karla Francolino da Silva[2], Marcelo Sant'Ana Borges[3], Fride Berg Standerholen[4], Heidi Spång[4], Ingrid Vikoren Mo[4], Marja Mikkola[4]

[1]Central Bela Vista, [2]Universidade de São Paulo, [3]Universidade Estadual Paulista, [4]SpermVital

SpermVital® (Hamar, Norway) is a patented semen preservation technology in which sperm cells are immobilized within an alginate-based gel extender and cryopreserved. Upon insemination, the gel gradually dissolves in the reproductive tract, enabling a slow and sustained release of viable sperm cells. This mechanism may prolong the fertile lifespan of the sperm cells and reduce dependence on precise insemination timing. This study aims to evaluate the effect of the SpermVital® sperm immobilization technology on conception rates in cows undergoing FTAI. For the study multiparous (n=635), primiparous (n=81), and nulliparous (n=27) crossbred beef cows from three different farms located in the states of Mato Grosso, São Paulo, and Paraná, Brazil, were enrolled in a fixed-time artificial insemination protocol. The hormonal treatment included an intravaginal progesterone-releasing device (1g; first or second use) and 2.0 mg of estradiol benzoate administered on Day 0 (D0). On D8 or D9, progesterone devices were removed, and all cows received a single dose of prostaglandin F2α (0.150 mg of sodium cloprostenol), 300 IU of eCG, and 1.0 mg of estradiol cypionate i.m. All cows were inseminated 48-52 hours after progesterone withdrawal using heterospermic frozen semen from Angus (n=4) or Nelore (n=5) bulls, where split ejaculates were cryopreserved either with a conventional commercial extender (CV, n=356; Optidyl®, IMV, France) or with an immobilizing alginate-based gel extender (SV, n=387; SpermVital®). Pregnancy diagnosis was performed by ultrasound scanning 60 to 80 days after FTAI. Data were analyzed using the GLIMMIX procedure of SAS®, evaluating the main effects of semen preservation method, cow parity and age, body condition score (BCS) at FTAI, farm, duration of progesterone treatment (8 or 9 days), bull breed, and their interactions ($P<0.05$). BCS did not influence ($P=0.7847$) pregnancy per AI (PAI). Neither the bull breed, duration of progesterone treatment, nor the farm affected PAI. However, nulliparous cows had higher PAI (72.2%) than multiparous cows (58.0%). In addition, PAI was influenced by cow age ($P=0.0494$), with rates of 61.9%, 59. 5%, and 50.2%, for cows <40 months, 48–64 months, and >64 months, respectively. The semen cryopreservation method affected PAI (CV=53.9%, SV=60.6%; $P=0.0226$), with cows aged 48–64 months showing a higher PAI when inseminated with SV semen (65.7%) compared to CV (52.9%; $P=0.0127$). In conclusion, the SpermVital® technology improved the pregnancy per AI in beef cows, representing a promising strategy to increase pregnancy rates in beef cows subjected to FTAI protocols.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Proteomic profile of ovine spermatozoa associated with semen cryopreservation

Denise Damasceno Guerreiro[1], Wallisson Bruno de Morais Pacheco[2], Iran Aguila Maciel[1], Suane Silva Alves dos Santos[2], Sheheryar Sheheryar[1], Laís Lacerda Brasil de Oliveira[1], Maria Elisabete Amaral de Moraes[1], Arlindo de Alencar Araripe Moura[1]

[1]Universidade Federal do Ceará, [2]Universidade Estadual do Ceará

This study aimed to evaluate the associations between the sperm proteome and semen freezability in Morada Nova rams. Twelve ejaculates were obtained from 12 rams at 3-day intervals (144 samples). Semen was evaluated before and after cryopreservation for sperm kinetics (AndroScope®, Minitube, Germany) and other parameters. Viability was evaluated using eosin-nigrosin stain, considering non-stained cells as viable and stained cells as non-viable (200 cells/sample). DNA integrity was analyzed using the sperm chromatin dispersion test. Post-thaw reduction in total sperm motility (TM) was the criterion for definition of rams with high (HF) and low semen freezability (LF). Average TM in fresh and frozen-thawed semen was 90.4 ± 3.2% and 56.5 ± 19.2%, respectively. Rams with HF (n=5) had lower decrease in TM after cryopreservation (25.8 ± 5.8%) compared to LF rams (n=7; 39.4 ± 3.9%; $p<0.05$). Sperm progressive motility was higher in HF (60.3 ± 6%) than in LF rams (42.3 ± 9.8%) after thawing, but with no statistical significance ($p>0.05$). Changes in sperm viability after cryopreservation varied from 81 ± 1.9% to 36.6 ± 12.8% in HF and from 78.4 ± 4.5% to 38.7 ± 13.1% in LF rams. DNA integrity decreased from 93.6 ± 3.4% to 65 ± 8% in HF and from 96.6 ± 2.1% to 66.4 ± 13.2% in LF rams, but without statistical difference ($p>0.05$). For proteomic analysis, fresh semen was treated with protease inhibitor and centrifuged (700 G, 15 min). Extracted sperm proteins were trypsin digested, desalted, and analyzed by LC-MS/MS. Spectral data were processed using PatternLab based on *Ovis aries* UniProt database to identify differentially expressed proteins (DEP; $p < 0.05$). Functional enrichment was performed using the STRING platform. A total of 1,368 and 1,481 proteins were identified in HF and LF rams, with 125 and 238 exclusive proteins, respectively. In addition to the unique proteins in each group, quantitative analyses defined a set of DEPs based on fold change values. HF rams showed overexpression of sperm proteins such as TUBB, HSP90B1, chaperones, AGA and MIX23, related to structural stability and stress response. In comparison with LF rams, HF animals had decreased expression of SDHA, AK88, CSNK2A2 and proteins of the flagellar axoneme, associated with mitochondrial activity and structural reorganization. Gene ontology analysis of DEPs revealed that upreguled sperm proteins in HF rams were linked to immune response and proteolysis regulation, while downregulated sperm proteins in HF animals were associated with spermatogenesis and flagellar motility. In conclusion, sperm proteome profiles differ between HF and LF rams, with HF sperm proteins related to structural stability and lower expression of mitochondrial and flagellar proteins, possibly indicating higher susceptibility to cryodamage.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Quality of ram semen supplemented with brain-derived neurotrophic factor (BDNF)

Matheus Batista de Oliveira[1], Julio Constantino Jerí Molina[1], Hymerson Costa Azevedo[2]

[1]Universidade Federal de Sergipe, [2]Empresa Brasileira de Pesquisa Agropecuária

Brain-derived neurotrophic factor (BDNF) possesses antioxidant properties that may protect spermatozoa from oxidative stress, thereby enhancing sperm viability and function. This study aimed to evaluate the effect of different concentrations of BDNF on the quality of ram semen. Semen was collected from Santa Inês rams (n = 5) using an artificial vagina, and only ejaculates with total motility ≥ 70% and vigor ≥ 3 were included. Samples were exposed to BDNF at concentrations of 0, 10, 20, 40, and 80 µg/L (Sigma-Aldrich, St. Louis, USA) and incubated for 5 minutes (T5) and 30 minutes (T30) to assess thesperm quality parameters. Samples were analyzed at for kinetic parameters using computer-assisted sperm analysis (CASA), as well as for the percentage of spermatozoa with functional and intact plasma membrane, using respectively the hypo-osmotic swelling test (HOST+) and a combination of propidium iodide and SYBR Green fluorescent stains (PMI+; Sigma-Aldrich, St. Louis, MO, USA) by epifluorescence microscopy. Additional analyses included the absence of oxidative stress (OS−; CellROX™ Green, Thermo Fisher Scientific, Waltham, USA) and high mitochondrial membrane potential (MMP+; JC-1, Thermo Fisher Scientific, Waltham, USA) by epifluorescence microscopy. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Hartley's test. The parameters were analyzed using the SAS MIXED procedure with linear mixed effect models fit by REML, that included the fixed effects of concentration, incubation time, and their interaction, with the ram considered as a random effect. The difference in means between groups was assessed by the Tukey's test. Data that remained non-normally distributed after transformation were analyzed using the Kruskal-Wallis test followed by Dunn's test. BDNF concentration influenced ($P < 0.05$) semen quality, with the 10 µg/L concentration showing the highest percentages of HOST+ (74.80 ± 27.80), PMI+ (76.80 ± 2.82), MMP+ (75.80 ± 5.94), and OS− (68.40 ± 6.05). No significant effect ($P > 0.05$) of BDNF concentration was observed on sperm kinetic parameters. BDNF exposure time influenced ($P < 0.05$) kinetic parameters, with T5 showing higher values for total motility (TM: 72.31 ± 18.56%), progressive motility (PM: 36.17 ± 25.17%), curvilinear velocity (VCL: 158.92 ± 20.21 µm/s), straight-line velocity (VSL: 94.11 ± 20.75 µm/s), average path velocity (VAP: 105.99 ± 24.20 µm/s), linearity (LIN: 59.66 ± 7.49), wobble (WOB: 66.39 ± 7.87%), and hyperactivation (HYPER: 5.73 ± 5.24%) compared to T30. The other parameters—straightness (STR), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), rapid sperm percentage (RAP), HOST+, PMI+, OS−, and MMP+ were not affected ($P > 0.05$) by BDNF exposure time. The interaction of 10 µg/L BDNF with T30 resulted in a higher ($P < 0.05$) HOST+ value compared to other combinations. It is concluded that the addition of 10 µg/L BDNF improves ram semen quality.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Testicular shape and hemodynamics of the supratesticular artery in bulls

Andressa Guidugli Lindquist-Vargas[1], Victor Hugo Gonçalves Galdioli[1], Lukas Gaudencio Bronoski[1], Flavio Antonio Barca Junior[2], Fábio Morotti[1], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina, [2]Universidade Norte do Paraná

This study evaluated whether testicular shape (TS) influences testicular hemodynamics in the supratesticular artery (SA). The hypothesis was that TS affects arterial blood flow dynamics due to differences in thermoregulation efficiency. The experiment was conducted on a farm in Londrina, PR, Brazil, during a dry day in January 2024. The average environmental temperature was 28.5±2.1°C, relative humidity 60.4±7.3%, black globe temperature 29.9±2.2°C, and the Temperature-Humidity-Globe Index (THI) was 78.6±2.2, indicating mild heat stress. Nelore bulls (n=40; mean age 15.9±2.1 months; mean weight 493±59.5kg), raised under semi-extensive conditions and clinically healthy, were evaluated. Bulls were evaluated while restrained in a natural standing (quadrupedal) position, without sedation/tranquilization. Testicular biometric measurements were taken using a caliper, recording the length and width of both testes. Testicular shape was determined by the width-to-length ratio and classified as elongated (≤0.5), moderately elongated (0.51–0.625), elongated oval (0.626–0.750), moderately oval (0.751–0.875), or spherical (>0.875). Doppler images were acquired using a Sonoscape® A6V ultrasound system with a linear transducer (7.5 MHz). The transducer was positioned distal to the spermatic cord, and scanning was initiated in B-mode. After identifying the SA, color Doppler and pulsed-wave (PW) Doppler modes were used to capture at least four cardiac cycles. Images were frozen, and one full cycle was manually delineated to calculate parameters: mean velocity (MV, cm/s), peak systolic velocity (PSV, cm/s), end-diastolic velocity (EDV, cm/s), pulsatility index (PI), and resistive index (RI). Data were analyzed using ANOVA after verifying assumptions (P≤0.05). Sixteen bulls had elongated testes and 24 had moderately elongated testes. No animals exhibited elongated oval, moderately oval, or spherical shapes, consistent with the literature for Zebu breeds. No significant differences (P>0.1) were found between groups for PSV (18.9±5.5 vs. 22.0±6.2 cm/s), EDV (9.4±3.7 vs. 11.0±4.5 cm/s), MV (14.0±4.2 vs. 16.7±4.7 cm/s), PI (0.52±0.26 vs. 0.49±0.21), or RI (0.50±0.15 vs. 0.50±0.17). The absence of differences in the testicular hemodynamics may relate to anatomical characteristics of Zebu cattle, such as longer and thinner vessels in the pampiniform plexus, enhancing countercurrent heat exchange. Additionally, the elongated TS of Nelore bulls favors testicular thermoregulation and efficient testicular blood flow due to the greater surface area and specific vascular anatomy, promoting stable hemodynamics regardless of TS. Favorable bioclimatic conditions during the study likely minimized challenges to testicular thermoregulation, contributing to the similar hemodynamic profiles observed between groups. In conclusion, under the conditions of the present study, TS (elongated or moderately elongated) did not affect testicular hemodynamics in the SA of Nelore bulls.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

MALE PHYSIOLOGY AND SEMEN TECHNOLOGY

Use of Red Cushion® as an alternative to conventional centrifugation for the preservation of sperm integrity in dogs – preliminary results

Larissa Araújo Stábile[1], Álvaro de Miranda Alves[1], Marcio Gonçalves de Paula Junior[1], Antonio Carlos Dertonio Donato[2], Camilla Mota Mendes[1], Marcelo Demarchi Goissis[1], Marcilio Nichi[1], Mayra Elena Ortiz D Avila Assumpção[1]

[1]Universidade de São Paulo, [2]Mirai - Centro de Diagnóstico Veterinário

Centrifugation of semen is a common step in assisted reproductive technology (ART) protocols for the removal of seminal plasma or sperm separation. However, it is associated with structural damages, such as alterations to the cell membrane, as well as reduced sperm fitness, especially in more sensitive species like dogs. Centrifugal force can remove cholesterol from the plasma membrane, causing osmotic imbalances, leading to premature capacitation and early acrosome reaction (AR) before contact with the oocyte. Cholesterol loss increases membrane fluidity and permeability, while osmotic stress and calcium influx activate signaling pathways that drive capacitation. Red Cushion® is a high-density colloidal medium initially developed for use in equines, designed to cushion spermatozoa during sedimentation and mitigate the deleterious effects of centrifugal force. This study hypothesized that Red Cushion® reduces centrifugation-induced sperm damage, preserving structural integrity and preventing premature capacitation in canine spermatozoa. For experimental design, 5 stud dogs (English Bulldogs) were used. A single ejaculate from each stud was collected and split into two groups: control (semen diluted in TL-Semen medium) and treated (semen with Red Cushion® at a 1:5 ratio). Both groups were centrifuged at 2500 × g for 5 minutes. Analyses included sperm concentration, computer-assisted sperm motility analysis (CASA), evaluation of plasma and acrosomal membrane integrity (FITC/IP), mitochondrial membrane potential (JC-1) assessed by flow cytometry, and sperm capacitation status (CTC) by fluorescence microscopy. Data were analyzed using SAS software after verifying variance normality and residual homogeneity. Differences between treatments were evaluated using a paired t-test, with a significance level of 5%. The results showed that the Red Cushion® group presented a higher percentage of capacitated spermatozoa (CTC2; 65.43 ± 2.23 vs. 48.00 ± 2.52; p= 0.0011) and fewer cells with reacted acrosomes (CTC3; 14.40 ± 4.85 vs. 33.33 ± 4.98; p= 0.0233) compared to the control group. Additionally, sperm centrifuged with Red Cushion® also showed a higher percentage of intact acrosomes with damaged plasma membrane (35.27 ± 4.58 vs. 25.40 ± 5.69; p= 0.0307). No other significant differences were found between groups. These preliminary results suggest that Red Cushion® helps to reduce premature capacitation (CTC3) and preserve acrosomal integrity, supporting sperm functionality in dogs. However, minor plasma membrane lesions were still observed, likely due to sedimentation dynamics or osmotic stress. Overall, Red Cushion® shows promising improvements in reproductive biotechnologies in dogs, although further optimization of the protocol is needed.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Antioxidant status of oocytes from crossbred dairy cows with different antral follicle counts

Nadja Gomes Alves[1], Clara Emmanuelly Mota Martins[1], Lorena Lima Firmino[1], Esther Abihail Fuente Arévalo[1], Luiz Gustavo Bruno Siqueira[2], Miller Pereira Palhão[1], Renato Ribeiro de Lima[1], Bruno Campos de Carvalho[2]

[1]Universidade Federal de Lavras, [2]Empresa Brasileira de Pesquisa Agropecuária

The antral follicular count (AFC) in cows has been linked to oocyte quality. This study aimed to evaluate oocyte quality in cows from different genetic groups with high or low AFC and to determine whether oocyte quality correlates with antioxidant status. Twenty-two crossbred Holstein × Gyr lactating cows, primiparous and multiparous, were assigned to groups: G1 (1/2 + 5/8 HG, n = 9) and G2 (3/4 + 7/8 HG, n = 13). Within each group, cows were classified as having low (G1: n = 6; G2: n = 8) or high AFC (G1: n = 3; G2: n = 5). Before each OPU session, follicular wave emergence was synchronized using a progesterone-releasing intravaginal device, estradiol benzoate, and sodium cloprostenol (D0). Five OPU sessions were performed on D5 for COCs recovery, and three sessions were conducted on D9 to collect follicular fluid (FF) from the dominant follicle. Viable COCs were subjected to IVM for 22–24 hours. Mature COCs were then denuded and stained with fluorescent dyes to assess mitochondrial activity (Mito-Tracker Red CMxRos) and reactive oxygen species (ROS) production (2',7'-Dichlorofluorescin diacetate). Fluorescence intensity was measured using Image J software. Additionally, relative activities of antioxidant enzymes (glutathione peroxidase [GPx], superoxide dismutase [SOD], and catalase) were measured in FF using the Bradford assay. Data were analyzed using the GLIMMIX procedure (SAS v9.4), considering cow and OPU as random effects, genetic group and AFC within group as fixed effects, and body condition score, milk yield, and days in milk as covariates. Least square means were compared using the F-test, with significance set at $P < 0.05$. AFC was lower in low- compared with high-AFC cows (G1: 15.54±1.53 vs 26.81±3.61 and G2: 11.39±1.05 vs 24.60±2.53; $P<0.01$). The number of recovered COCs was lower for low- than for high-AFC cows (G1: 5.49±01.00 vs 10.43±2.59 and G2: 2.65±0.48 vs 8.08±1.55; $P<0.05$). Viable COCs did not differ between G1 (65.33±5.56%) and G2 (69.63±7.27%) or between low- and high-AFC cows ($P>0.05$). Mitochondrial activity and ROS production were higher in oocytes from low-AFC G1 cows compared to high-AFC G1 cows (mitochondrial activity: 19.56±6.17 vs. 7.11±2.76 AU; ROS: 38.98±10.59 vs. 5.04±1.68 AU; $P<0.05$). Similarly, in G2 cows, ROS levels were higher in oocytes from low- versus high-AFC animals (26.52±8.19 vs. 9.01±2.36 AU; $P < 0.01$). Relative GPx activity in FF was lower in G1 than in G2 cows (0.0185±0.0016 vs. 0.0258±0.0018 U/mg protein/mL FF; $P<0.001$) but did not differ between low- and high-AFC cows ($P>0.05$). SOD and catalase activities in FF did not vary between groups or AFC categories ($P>0.05$). In this study, AFC was not associated with oocyte morphological quality in either genetic group. However, increased mitochondrial activity and/or ROS levels without corresponding changes in FF antioxidant enzyme activity suggest an oxidative stress condition in oocytes from low-AFC cows.

Financial support: FAPEMIG APQ 01442-17.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Application of butyrolactone I for inducing meiotic arrest during pre-maturation and its impact on meiotic resumption in bovine oocytes

Priscila Helena dos Santos[1], Francisco Thiago Vieira Oliveira[1], José Enrico Monteiro Lo Buono[1], Fernanda Schneberger dos Santos[1], Lorena Calixto Munhoz[1], Flávio Vieira Meirelles[1], Lawrence Charles Smith[2], Cláudia Lima Verde Leal[1]

[1]Universidade de São Paulo, [2]Université de Montreal

The application of oocyte prematuration systems (PIVM) has been proposed to delay spontaneous meiosis resumption in order to improve synchronization of nuclear and cytoplasmic maturation in cumulus-oocyte complexes (COCs). Butyrolactone I (BLI), a cyclin-dependent kinase inhibitor (CDKI), is known to induce meiotic arrest and can be employed in such systems. This study aimed to evaluate the efficacy of BLI in inducing meiotic arrest and the distribution of GV stages (GV1, GV2, GV3) during PIVM at 9, 12, 18, and 24 hours. COCs were aspirated from abattoir-derived ovaries and cultured (38.5°C, 5% CO_2) in TCM-199 medium supplemented with 0.2 mM sodium pyruvate, 50 µg/mL gentamicin, and 0.1% PVA (25 COCs/group; five replicates), with or without 10 µM BLI (B10 and CNTL groups, respectively). A fresh (non-PIVM) control group (0h) was also included. At each time point during PIVM, oocytes were collected, denuded, fixed, stained with Hoechst 33342, and subjected to immunofluorescence using anti-lamin antibody to detect nuclear envelope integrity. Meiotic status was assessed via fluorescence microscopy and classified as GV1, GV2, GV3, or non-GV (GVBD, MI, anaphase I, telophase I) stages. Data were analyzed using one-way ANOVA and Tukey's test (JMP, SAS Institute), with significance set at $P < 0.05$. After 9, 12, and 18 hours of PIVM, B10-treated oocytes retained high GV stage rates (98.40±1.60%, 76.83±9.20%, and 72.94±17.28%, respectively) similar to the 0h control (100%, $P>0.05$) and significantly higher than the CNTL group (9h: 48.20±6.18%, $P=0.0001$; 12h: 23.43±5.37%, $P=0.0014$; 18h: 22.76±5.01%, $P=0.0001$). In the 9h B10 group, GV3 predominated (68.30±4.80%) over GV1 (14.93±3.58%) and GV2 (15.16±3.06%). At 12h, GV substage proportions were more evenly distributed (GV1: 16.16±5.41%, GV2: 23.08±5.48%, GV3: 36.83±9.06%), with 23.16±9.19% of oocytes resuming meiosis ($P=0.3356$). A similar profile was observed at 18h (GV1: 17.74±8.81%, GV2: 17.47±8.23%, GV3: 37.73±18.74%, non-GV: 27.05±17.28%; $P=0.1095$). However, at 24h, meiotic resumption predominated (66.20±12.50% non-GV vs. 33.80±12.50% GV). In conclusion, 10 µM BLI effectively maintains meiotic arrest up to 18 hours, with enrichment in the GV3 substage, suggesting potential for synchronizing oocyte development. However, by 24 hours, the arresting capacity of BLI diminishes. These findings support the use of BLI for PIVM protocols aimed at delaying spontaneous meiosis resumption. Studies are underway to use such a strategy to associate different factors related to oocyte development, prior to IVM, to better understand their roles, and to later improve oocyte competence in *in vitro* production systems.

Financial support FAPESP grant #202109886-8; PHS 2023/16602-1 postdoctoral fellowship #2021/09886-8.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Assessment of chromatin configuration and *in vitro* oocyte development in female pigs of varying age

Thais Naomi Gonçalves Nesiyama[1], Juliano Rodrigues Sangalli[1], Edna de Fátima Elias Macule[2], Erick Marlon Pereira[1], Helena Fabiana Reis de Almeida Saraiva[1], Lawrence Charles Smith[3], Vilceu Bordignon[4], Flávio Vieira Meirelles[1]

[1]University of São Paulo, Faculty of Animal Science and Food Engineering, [2]University of São Paulo - School of Veterinary Medicine and Animal Science, [3]Université de Montreal, [4]McGill University

Oocyte *in vitro* maturation (IVM) in swine is highly variable, because ovaries are sourced from gilts (prepubertal) and sows (reproductively retired). This study compares oocyte competence from donors by assessing chromatin configuration at 0 and 22 h of IVM, maturation rates at 46 h, and blastocyst development at D7. Cumulus-oocyte complexes (COCs) were collected from slaughtered females, selected, and incubated in TCM-199B supplemented with 20% porcine follicular fluid (PFF; sows), 0.91 mM sodium pyruvate, 3.05 mM D-glucose, 0.57 mM L-cysteine, 10ng/ml EGF, 5 IU/ml hCG, 10 µg/ml eCG, 1 mM cAMP, and 20 µg/ml gentamicin at 38.5°C in 5% CO_2. After 46 h, control (CTL) and control-negative (CTL–; without PFF) COCs were evaluated by polar body extrusion. Mature oocytes were parthenogenetically activated using 15µM ionomycin (5min), 200 µM TPEN (15min), and 7.5 µg/ml cytochalasin B (4h) in PZM-3, cultured for 7 days at 38°C in 5% CO_2, 5% O_2. For immunocytochemistry COCs at 0 and 22 h of IVM were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 (5min, 25°C), blocked with 1% BSA/0.1% Tween 20 (1h, 25°C), incubated overnight with anti-fibrillarin antibody, and Alexa Fluor 488 as a secondary antibody and counterstained with Hoechst 33342. Chromatin classification was performed once by confocal microscopy (MICA, Leica, GER) and analyzed by ImageJ (FIJI-NIH, USA). According to (Pan et al., Biology of Reproduction, 6:99 1149-58, 2018) germinal vesicle and IVM chromatins were classified as nonsurrounded nucleolus (NSN), surrounded nucleolus (SN), partly NSN (pNSN), partly SN (pSN), condensed NSN (cNSN), prematurely condensed NSN (cpNSN), prematurely condensed SN (cpSN), re-decondensation configuration (RDC1/2), and early diakinesis (ED). IVM rates at 46 h were higher ($p<0.05\%$) in the PFF supplemented group: (468/631) 74.1% ± 1.8 vs. (80/149) 53.7% ± 2.5 in gilts, and (287/344) 80.2% ± 3.1 vs. (62/90) 69.2% ± 5.0 in sows. Blastocyst rates from gilt oocytes were (42/127) 33.1% ± 4.2 in CTL and (5/97) 5.4% ± 2.4 in CTL-. Data for sows is still under analysis. The frequencies of chromatin configuration at 0h (n=25) and 22h (n=12) of IVM for gilts, 0h (n=19) and 22h (n=20) of IVM for sows, were respectively (0 gilt, 22 gilt, 0 sow, 22 sow): NSN (4%, 0%, 5,26%, 0%), SN (8%, 25%, 42,11%, 40%), pNSN (4%, 25%, 15,79%, 5%), pSN (16%, 16,67%, 10,53%, 15%), cNSN/cpNSN (48%, 8,33%, 15,79%, 15%), cpSN (12%, 8,33%, 5,26%, 20%), RDC1 (4%, 8,33%, 0%, 0%), RDC2 (4%, 0%, 5,26%, 0%), and ED (0%, 8,33%, 0%, 5%). The cpNSN/cNSN pattern showed a difference in frequency between 0 and 22 h of IVM in gilts ($p<0.05$). Unlike the study of Pan et al. (2018), we used cAMP, which promotes chromatin decondensation and increases the proportion of normal chromatin (SN and cpSN). Based on that, PFF supplementation improved IVM outcomes in both donors compared to gilt oocytes cultured without PFF.

Financed by: CAPES-001 and FAPESP (21/09886-8; 23/12977-0), Sta Rosa Meatpacking.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Assessment of the stability of liposomes in relation to their composition, and lamellarity and under *in vitro* culture parameters of bovine embryos

Alexandre Mendes de Almeida Junior[1], Bruna Alves Martins[1], Isabelle Thaine Martelli[1], Pedro Henrique Benites Aoki[1], Marcelo Fábio Gouveia Nogueira[1]

[1]Universidade Estadual Paulista

In cattle, the *in vitro* embryo production (IVEP) represents a crucial biotechnology for the reproduction and genetic improvement of herds, given the global economic importance of livestock. However, IVEP still faces significant challenges, such as the low production rate and the quality of embryos, which are still distinct from those derived in vivo. In this maternal environment there are lipid vesicles (i.e., extracellular vesicles) interacting with the embryos - as carriers of signaling molecules. Thus, we developed synthetic liposomes with potential applications as carriers of signaling molecules to improve IVEP outcomes. Liposomes stabilities were evaluated under two storage conditions: (i) at 4ºC and (ii) in a controlled environment for *in vitro* culture (i.e., 38.5º C, 5% CO2, and maximum humidity) over 0, 24, 48, and 72 h. Three models of liposomes - i.e., with different s (large or small) and lamellar configurations (uni- or multilamellar) - were produced using the lipid film hydration method, followed by resuspension in ultrapure water or IVC medium (IVC1, BotuPharma®, Botucatu, SP) supplemented with 2.5 % fetal bovine serum. Preliminary experiments were designed to characterize both pure 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) vesicles and DPPC/cholesterol vesicles (70:30 mol%), through dynamic light scattering and zeta potential measurements. All measurements were performed in triplicate, with significant changes defined by non-overlapping 95% CI, and are presented as mean ± SD. The data revealed concentration-dependent aggregation, where small unilamellar vesicles (SUVs) of DPPC at 3mM exhibit significant increase in mean diameter from 89.8±0.1 nm to 192.8±4.8 nm after 48 h of storage at 4ºC; whereas lower concentrations showed less pronounced changes (e.g., 1mM: 124.2±10.0 nm to 83.9±6.0 nm; and 2mM: 110.3±1.1 nm to 148.9± 8.2 nm under the same conditions). Additionally, the zeta potential of DPPC SUVs shifted from positive to negative values at 3 mM (from +5.2 mV to -14.8 mV at 48 h), suggesting lipid reorganization on the vesicle surface. However, cholesterol incorporation enhanced molecular packing within the bilayer, improving stability and promoting lipid rearrangement to larger diameters. This effect was evident when comparing pure DPPC multilamellar vesicles (MLVs) (554.3±24.5 nm) with DPPC/cholesterol MLVs (931.2±86.4 nm). Notably, cholesterol-containing SUVs exhibited reduced aggregation at high concentrations (3 mM), with diameters decreasing slightly from 172.9±2.2 nm to 127.6±4.7 nm, and no significant change in zeta potential over 48 h at 4°C. This stabilized configuration remained reproducible under IVC condition, showing small diameter variations even after 72 h of incubation. Thus, cholesterol-stabilized liposomes are optimal for delivering signaling molecules in bovine IVEP. This study was financed, in part, by FAPESP.

Grant Numbers #2024/12714-2; 2024/03887-0; 2025/00626-4; 2022/02189-2 and 2021/11747-6.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Association of subcutaneous fat, rump fat, and longissimus dorsi muscle area with reproductive outcomes in Nelore (*Bos indicus*) heifers raised in a feedlot system

Sebastian Albavi[1], Giselly B. Kolling[1], Rodrigo L. O. R. Alves[1], Mirela Balistrieri[1], Lucas O. e Silva[1], Rafael Araújo Braz[2], Rodrigo Brüner[3], Leonardo de França e Melo[4], Paulo S. A. Moreira[5], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture, Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil., [2]Norte Agro artificial insemination, [3]Tulipa Agropecuária Farm, [4]Federal University of Goiás, [5]Federal University of Mato Grosso

This study evaluated associations between carcass traits and reproductive performance in yearling Nelore (*Bos indicus*) heifers raised in a feedlot system submitted to timed-artificial insemination (TAI). A total of 245 heifers (12.7 ± 1.3 [10.7 to 15.4] mo of age; 295.4 ± 40.4 [194.0 to 429.0] kg), underwent carcass ultrasound evaluations, 7 d prior to the onset of the TAI protocol (D0) and 120 d after TAI. In both moments, the following parameters were assessed: subcutaneous fat thickness (SCFT-7, SCFT120), rump fat thickness (RFT-7, RFT120), and longissimus dorsi muscle area (LMA-7, LMA120). For each trait, the difference between measurements at 120 and -7 d was calculated (ΔSCFT, ΔRFT, ΔLMA). Reproductive outcomes evaluated were the presence of a CL on D0, pregnancy per AI (P/AI) at 30 and 60 d, and pregnancy loss (PL; 30–60 d). Data were evaluated by PROC GLIMMIX of SAS 9.4 (difference: $P \leq 0.05$; tendency: $0.05 < P \leq 0.10$). The presence of a CL on D0 was positively related to all the carcass traits evaluated on D-7. Heifers with CL on D0 (n = 104) had greater LMA-7 (50.9 ± 0.5 vs 48.0 ± 0.5 mm); SCFT-7 (3.13 ± 0.05 vs 2.86 ± 0.04 mm), and RFT-7 (5.25 ± 0.06 vs 4.96 ± 0.04 mm) than those without CL (n = 141). Regarding P/AI at 30 d, a tendency was observed for a positive association with RFT-7. Pregnant heifers (n = 104) had greater RFT-7 (5.15 ± 0.06 vs 5.03 ± 0.05 mm) than non-pregnant heifers at 30 d (n = 141). However, there was no association between P/AI at 30 d and LMA-7 or SCFT-7. Nonetheless, P/AI at 60 d was positively associated with all traits on D-7. Heifers pregnant at 60 d (n = 90) had greater LMA-7 (50.3 ± 0.6 vs 48.6 ± 0.4 mm), SCFT-7 (3.08 ± 0.05 vs 2.91 ± 0.04 mm), and RFT-7 (5.20 ± 0.06 vs 5.02 ± 0.04 mm) than the non-pregnant (n = 155). Moreover, PL was negatively associated with the traits evaluated on D-7. Heifers with greater SCFT-7 and RFT-7 were less likely to experience PL, and heifers with greater LMA-7 tended to be less likely to experience PL. Regarding the traits evaluated at 120 d, heifers confirmed as pregnant at 60 d had greater SCFT120 (3.72 ± 0.06 vs 3.52 ± 0.05 mm) and RFT120 (6.41 ± 0.08 vs 6.10 ± 0.07 mm) than non-pregnant heifers. However, LMA120 did not differ between them (55.2 ± 0.6 vs 54.4 ± 0.4 mm; respectively). There were no associations between ΔLMA or ΔSCFT and the probability of pregnancy at 60 d, but ΔRFT was positively associated with P/AI at 60 d. Heifers confirmed as pregnant at 60 d had greater ΔRFT than those non-pregnant (1.22 ± 0.05 vs 1.04 ± 0.04 mm). Finally, no associations were observed between ΔLMA, ΔSCFT or ΔRFT and the probability of PL. In conclusion, heifers with greater LMA and fat deposition before TAI were more likely to be cyclic, conceive, and maintain pregnancy. Fat-related traits, especially RFT, were consistently associated with fertility, suggesting their potential as indicators of reproductive efficiency.

Acknowledgments: CAPES, Tulipa, Norte Agro, GlobalGen.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Behavioral patterns and artificial intelligence for early identification of energy balance status in dairy cows

Taiomar Ruthes Mascarello[1], Daniele Missio[2], Isadora Zago[1], Rogério Ferreira[1]

[1]Fundação Universidade do Estado de Santa Catarina, [2]Universidade Federal de Santa Maria

At the beginning of lactation, dairy cows face several metabolic challenges, with negative energy balance (NEB) being one of the main factors that compromise the productive and reproductive efficiency of herds. Early identification of animals with high NEB can minimize these impacts. This study aimed to characterize the behavioral pattern of cows with high and low NEB and to develop a predictive model for their early detection in the postpartum period. For that, the concentration of non-esterified fatty acids (NEFA) was measured on days 7, 14 and 21 postpartum in 80 Holstein-Friesian cows. Animals with NEFA concentrations higher than 0.6 mmol/L on at least one of the experimental days were classified as high NEB, while the others were categorized as low NEB. Rumination, feeding, idleness and standing time behavior were monitored in the first 21 days postpartum using automated monitoring collars (Active Tags; Tru-Test, Datamars SA, Lamone, Switzerland). Monitoring was carried out to identify distinct behavioral patterns between groups from the first four days after calving, and to distinguish cows with high and low NEB. Cows with high NEB had lower ($P<0.05$) mean rumination time (479±6min/24h) and feeding time (385±4min/24h) and idleness (510±5 min/24h) compared to low NEB (510±5, 368±5, and 479±6min/24h, respectively). The standing time did not differ between the groups ($P>0.05$). Based on these results, a potential development of predictive technology has been developed using neural networks, in which the rumination, feeding and idleness data from the first 4 days postpartum were used as predictors. The model was able to classify animals with high or low NEB with 100% accuracy. We conclude that cows with different NEB status have distinct behavioral patterns and that the developed model can be an efficient tool for the early identification of cows with high NEB, based on behavioral variables obtained by automated activity monitors.

Financial support: CAPES, CNPq, FAPERGS, and FAPESC.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

B-glucan supplementation in the prepartum did not affect uterine involution or cytological endometritis in dairy cows - Preliminary results

Danielle dos Santos Cinelli Pinto[1], Keren Hapuque[2], Gabriella Barreto Esteves[3], Milena Carvalho Coelho[4], Luisa Oliveira Orlandi[1], Robson Barducci[5], Davi Glanzmann[4], Marcelo Porto Bemquerer[2], Humberto de Melo Brandão[2], Bruno Campos de Carvalho[2]

[1]Universidade Federal de Lavras, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]Centro Universitário Presidente Antônio Carlos Juiz de Fora, [4]Universidade Federal de Juiz de Fora, [5]Biorigin

The β-glucan has anti-inflamatory and anti-oxidant effects and improves immunity in transition cows. It was evaluated the effects of β-glucan supplementation in transition period on uterine involution and cytological endometritis occurence in Holstein cows. Fifty-two cows housed in a windtunneled compost barn system were supplemented for 28 consecutive days starting at 40 days pre-calving. The cows were divided homogeneously, according to parity and expected calving date, into four experimental groups (n=13 per group) that received the following daily doses of pure β-glucan : 0 mg/kg body weight/day (G1), 36 mg/kg (G2), 60 mg/kg (G3), 84 mg/kg (G4). β-glucan (Macrogard®, Biorigin, São Paulo, Brazil) was mixed in ground corn in different proportions according to the groups, so that each animal received 0.545 g of the mixture per kg of body weight (BW) (equivalent to 300 g of mixture for a cow of 550 kg BW). The mixture was offered individually in headlock on feed alley. The cows were fed a balanced diet based on corn silage, concentrate and anionic mineral mixture. After calving, the cows received a balanced diet based on corn silage and concentrate. Milking was performed three times a day. Cows were submitted to endometrial cytology at 7 and 30 days postpartum by cytobrush technique. With the aid of an insemination sheath, a cytological brush was introduced into the body of the uterus and a slide was prepared. The slide was stained by the rapid panopticon technique and read under immersion microscopy (1000X). At least 300 cells were counted and samples with more than 6% polymorphonuclear cells were considered cytological endometritis. At 7, 18 and 30 days postpartum, the cows were submitted to transrectal ultrasonography, and the cross-section uterine diameter of the pregnant and non-pregnant horns were measured, immediately after their bifurcation. The data were analyzed by PROC GLIMMIX, in SAS v. 9.4, considering the effects of experimental group, parity and day postpartum. Gamma distribution was used to adjust the uterine diameter data and binomial distribution to adjust cytological endometritis (CE) data. No group or parity effect ($p>0.05$) was observed on the occurrence of cytological endometritis. At seven days postpartum, 36.54% (19/52) of cows presented CE and at 30 days postpartum 38.00% (19/50) of the cows were diagnosed with CE. No group or postpartum day effect was observed on the diameter of the uterine horns. The uterine horn where the previous pregnancy was established had a mean diameter of 2.91±0.13 cm; 1.98±0.06 cm and 1.74±0.05 cm, respectively, at 7, 18 and 30 days postpartum. Regarding the non-pregnant horn, a smaller diameter ($p<0.05$) was observed for primiparous cows (1.72±0.07 cm) compared to multiparous cows (1.96±0.06 cm). The supplementation of dairy cows with β-glucan for 28 days in the pre-partum period did not reduce the occurrence of cytological endometritis, as well as did not interfere with uterine involution.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Boosting reproductive and productivity performance with early-born Nelore heifers after different protocols to induce ovulation before timed-AI

Aline Santos da Silva Guarim[1], Amanda Guimarães da Silva[1], Pedro Antônio Pádua Andrade[1], Otacílio Jarcem Escobar Junior[2], Igor Garcia Motta[3], Rogério Fonseca Guimarães Peres[2], Isabella Rio Feltrin[1], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]Agropecuária Nelore Paranã, [3]Ourofino Saúde Animal

We aimed to evaluate the reproductive and productive performance in Nelore heifers born in three birth periods of the calving season, that were submitted to different protocols to induce ovulation prior timed-AI (TAI) using injectable progesterone (iP4; Sincrogest, Ourofino Saúde Animal, Cravinhos-SP, Brazil). Nelore heifers, from 10 to 17 months of age, born at the onset (August to October 2021, n=574), middle (November and December 2021, n=558), or end (January and February 2022, n=588) of the calving season were used. Within each birth period, heifers were randomly split to receive one of three treatments: control (without ovulation induction before the TAI; n=576); 1P4 (150 mg of iP4 on 12 days before beginning of TAI protocol]; n=571); and 2P4 (150 mg of iP4 on 24 and 12 days before beginning of TAI protocol; n=573). Heifers were submitted to an E2/P4-based protocols for three TAIs within 107 days. Heifers were monitored through reproductive, calving, and weaning seasons. Data were analyzed by PROC MIXED and GLIMMIX of SAS. There was no interaction between treatment and birth period affecting any variable (P>0.05). For the first TAI, the P/AI was greater in group 2P4 [28 %] than in the 1P4 and in the control groups [23% and 21%]. Heifers born at the onset of the calving season had the greatest P/AI [30%], while those born at the end had the lowest (17%; P<0.01). For the second and third TAIs, no difference in P/AI was observed between treatment groups (P>0.05), but the P/AI was greater in heifers born at the onset than those born in the middle or at the end of the calving season. Cumulative pregnancy rate after the third TAI was unaffected by treatment (P=0.6) but differed (P<0.01) among birth period [67% A, 50.3%B and 37%C, for onset, middle and end respectively]. The calving and weaning rates did not differ (P>0.05) among treatment groups or birth periods. The weaning weight was not affected by treatment group (P=0.6); however, it differed (P<0.01) among birth periods [173.4±2.1A, 164.4±2.2B and 165.0±2.4B kg, for onset, middle and end respectively]. The kilogram of weaned calf per inseminated heifer was not affected by treatment (P=0.8); however, it differed (P<0.01) among birth periods [76.4±3.4 A, 52.0±3.4B and 39.0±3.3C kg of weaned calf/heifer, for onset, middle and end respectively]. In conclusion, administering two doses of injectable P4 before the TAI protocol enhanced first-service P/AI but did not influence subsequent reproductive or productive outcomes. In contrast, the use of heifers born at the onset of calving season resulting in the age of 15 to17 mo at the reproductive season improved both reproductive success and productivity at weaning, underscoring the long-term importance of birth period on herd performance.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Conceptus-Induced Changes in Endometrial Gene Expression Between Days 15 and 20 of Pregnancy in Bovine: Preliminary results

Ana Karolyne Alves Miguel[1], Isabella Rio Feltrin[1], Karine Galhego Morelli[1], Gabrielle Ceragioli Damaceno[1], Aline Santos da Silva Guarim[1], Thiago Neder Lisboa[1], Juliano Coelho Da Silveira[1], Claudia Maria Bertan Membrive[2], Mario Binelli[3], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]Universidade Estadual Paulista, [3]University of Florida

Considering the crucial role of maternal immune adaptation during early gestation, we hypothesize that early pregnancy success relies on precise regulation of endometrial immunity. The aim was to evaluate the endometrial gene expression modulation in the ipsi- and contralateral uterine horns by the bovine conceptus between 15 and 20 days of pregnancy. Nelore heifers (n=56, 24-64 months old; 434±96 kg) underwent an 8-days E2/P4 synchronization protocol. On D10, heifers were randomly assigned to the Artificial Insemination (AI; n=45) or Simulated Procedure (SHAM, n=11) groups, and AI was designed as Day 0 (D0). Color Doppler ultrasonography was used to determine ovulation (D2) and luteal blood perfusion (D15 and D20). Heifers were slaughtered on D15 (AI, n=13; SHAM, n=6) or D20 (AI, n=13; SHAM, n=4), and the uterine lumen was flushed with 30 mL of PBS solution. To standardize the time of luteolysis, D20 SHAM group received a P4 device (1.0 g, Sincrogest®, Ourofino) on D15 and 526 µg of cloprostenol sodium (Sincrocio®, Ourofino) on D17. The integrous conceptus, if present, was recovered from the uterine flushes on D15 (n=7) and D20 (n=10). Endometrial samples were collected from three regions of the ipsilateral (anterior, ANT; medial, MED; and posterior, POST) and one region of the contralateral (anterior) uterine horns. Gene expression was analyzed by qPCR for interferon-stimulated genes (*ISGs; ISG15* and *RSAD2*) and immune response-related genes (*IL1β, TNFα, TGFβ1, NKκβ* and *TLR4*). Data were analyzed by ANOVA in SAS, with a difference at $P<0.05$ and trend at $0.05>P<0.1$. For both ISGs, a significant group-by-time interaction was observed, with expression in the AI group increasing 24- and 92-fold for *ISG15*, and 18- and 70-fold for *RSAD2* on D15 and D20, respectively, compared to SHAM group. A significant region effect showed greater expression in ipsilateral horn by 1.6-, 1.6- and 1.2-fold for *ISG15*, and 1.6-, 1.5- and 1.4-fold for *RSAD2* in the ANT, MED and POST regions, respectively, compared to the contralateral horn. A group-by-region-by-time interaction trend for *RSAD2* revealed greater expression in all ipsilateral regions in AI *vs.* SHAM group on D15, and in both horns on D20. For *TGFβ1*, only a 2.3-fold greater expression in SHAM than AI group on D20 was observed. For *NFκβ*, expression was 22- and 81-fold higher in AI than SHAM on D15 and D20, respectively. No significant differences were found for *IL-1β*, *TNFα*, and *TLR4*. In conclusion, the conceptus modulates endometrial gene expression predominantly in the ipsilateral uterine horn as early as Day 15, with effects extending to the contralateral horn by Day 20. A conceptus-driven immune modulation through *ISG15, RSAD2*, and *NFκβ* occurs; however, the downregulation of an anti-inflammatory cytokine (*TGFβ1*) by the conceptus suggests further investigation is needed to clarify the Th1/Th2 balance during early pregnancy.

Acknowledgments: FAPESP (#2024/16341-6, #2022/14936-7), INCT (#406866/2022-8).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Conceptus-induced changes in the luteal gene expression at 20 days after insemination

José Ricardo Fonseca Miranda[1], Ana Karolyne Alves Miguel[1], Isabella Rio Feltrin[1], Ana Laura dos Santos Munhoz Gôngora[1], Karine Galhego Morelli[1], Aline Santos da Silva Guarim[1], Gabrielle Ceragioli Damaceno[1], Juliano Coelho Da Silveira[1], Claudia Maria Bertan Membrive[2], Guilherme Pugliesi[1]

[1]University of São Paulo, [2]Universidade Estadual Paulista

This study investigated how the conceptus modulates *corpus luteum* (CL) blood perfusion and gene expression on day 20 of pregnancy. Nelore heifers (n=34) were synchronized using estradiol benzoate and progesterone (P4) device on D−10. On D0, heifers were assigned to artificial insemination (AI) or to a simulated procedure (Cycling). To standardize luteolysis, cycling heifers received a P4 device on D15 and a PGF2α analogue on D17. CL blood perfusion (CLBP) was evaluated by Doppler ultrasonography on D7, D15, D19, and D20. On D20, all Cycling heifers and inseminated heifers with active CL (>25% CLBP) were slaughtered (n=18). Reproductive tracts were collected, CLs dissected, and uteri flushed with PBS for conceptus recovery. Animals were classified as: Pregnant (n=10; filamentous conceptus), non-pregnant (n=4; no conceptus), and Cycling (n=4). All CL samples were analyzed by qPCR to evaluate gene expression of *ISG15* (conceptus signaling), *END-1* (angiogenesis), and *AKR1B1* (prostaglandin synthesis). Additionally, Pregnant heifers were categorized as low (≤50%, n=4) or high (≥60%, n=4) CLBP to assess the association between CLBP and gene expression. Correlations between CLBP and gene expression were tested. Data were analyzed using SAS PROC MIXED, with significance set at $P<0.05$. A pregnancy-by-time interaction was observed for CLBP and CL area. The CLBP remained constant from D7 to D20 in Pregnant heifers but declined on D19 and D20 in Non-pregnant and Cycling animals. The CL area remained stable in Pregnant and Non-pregnant groups but decreased on D19 and D20 in Cycling heifers. Pregnancy status significantly affected *ISG15* and *AKR1B1* expression. The *ISG15* expression was 42-fold higher in Pregnant heifers compared to Cycling heifers, while the non-pregnant group did not differ. The *AKR1B1* expression was 72- and 82-fold higher in Pregnant heifers than in the Cycling and Non-pregnant groups, respectively. The *END-1* expression was not influenced by group or CLBP. No significant correlations between CLBP and gene expression were found, as well as no significant effect of CLBP was observed for any gene analyzed. In conclusion, conceptus signaling extends beyond the uterus, inducing *ISG15* expression in the CL on day 20. There was no evidence of conceptus-mediated modulation of luteal angiogenesis or a relationship between CLBP and angiogenic gene expression, as *END-1* was unaffected. The elevated *AKR1B1* expression in Pregnant heifers suggests a role of the conceptus in intraluteal prostaglandin synthesis or P4 metabolism.

Acknowledgments: FAPESP (#2024/16341-6, #2022/14936-7), INCT (#406866/2022-8).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Decoding Parturition Disorders: Epigenetic Profiling of Italian Mediterranean Buffaloes Placentas

Ana Beatriz dos Reis Bartoli[1], Aldcejam Martins da Fonseca Junior[2], Domenico Vecchio[3], Barbara Degli Uberti[3], Mario Orrico[4], Thays Antunes[1], Patricia Kubo Fontes[1], Ariella de Oliveira Azevedo[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC, [2]Université Laval, [3]Istituto Zooprofilattico Sperimentale del Mezzogiorno, [4]Istituto Zooprofattico Sperimentale Piemonte

Parturition disorders, such as dystocia and retained placenta, are major reproductive challenges in domestic species—particularly in cattle and buffaloes—leading to economic losses and reduced animal welfare. Recent studies suggest that epigenetic regulation plays a key role in pregnancy success and fetal development, but the specific mechanisms involved in parturition disorders in buffaloes remain poorly understood. In this context, the present study aimed to characterize the epigenetic profile of placentas from buffaloes presenting parturition disorders, with the objective of identifying molecular alterations potentially associated with impaired reproductive outcomes. For this purpose, cotyledons from the fetal portion of placentas of Italian Mediterranean buffaloes with parturition disorders (n=3) and eutocic controls (n=3) were collected within two hours after expulsion. Samples were either preserved in RNAlater™ for molecular analysis or fixed in 4% paraformaldehyde for histological procedures. Immunostaining was conducted to detect the histone modification H3K27me3, and fluorescence intensity was quantified using ImageJ software. The expression levels of key epigenetic regulators and imprinted genes—TET3, DNMT1, DNMT3a, H19, and H1BP3—were quantified via RT-qPCR, using PPIA as a reference gene. Fluorescence data were analyzed using descriptive statistics and the Mann-Whitney test, while gene expression comparisons were performed with Student's t-test, both using GraphPad Prism software (significance threshold set at $P < 0.05$). Immunofluorescence analysis for H3K27me3 revealed a significant difference between the two groups ($P < 0.0001$), with placentas from eutocic births displaying greater variability in fluorescence intensity (coefficient of variation: 86.99%) compared to those from parturition disorder cases (63.67%). No significant differences were found in the expression levels of TET3, DNMT3a, and H1BP3 ($P = 0.0546$, $P = 0.6065$, and $P = 0.1669$, respectively). However, significant differences were observed for H19 and DNMT1 expression ($P = 0.0490$ and $P = 0.0330$, respectively), both of which were downregulated in the parturition disorder group. H3K27me3 distribution appeared homogeneous in fluorescence intensity on affected placentas and heterogeneous on controls, suggesting a disruption in epigenetic patterning. The reduced expression of H19, a maternally imprinted gene, suggests impaired epigenetic reprogramming during gestation, potentially leading to disrupted maternal imprinting. The concurrent downregulation of DNMT1, essential for maintaining DNA methylation at imprinted loci, supports this hypothesis. These findings advance understanding of molecular mechanisms in buffalo parturition disorders and may guide improved reproductive management.

Acknowledgments: FAPESP (2024/16287-1).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Delivery of exogenous sperm microRNAs does not restore embryo quality in low IVP fertility bulls, while their inhibition compromises ICM cells in high IVP fertility bulls

Thais Rose dos Santos Hamilton[1], Camilla Mota Mendes[2], Larissa Araújo Stábile[2], Felipe Eduardo Luedke[2], Juliano Coelho Da Silveira[3], Marcelo Demarchi Goissis[2], Mayra Elena Ortiz D Avila Assumpção[2]

[1]Universidade Estadual Paulista, [2]Universidade de São Paulo, [3]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos

Different bulls exhibit high or low *in vitro* fertility. Sperm RNAs play roles in early embryo development through the action of non-coding RNAs, such as microRNAs, which may influence bull fertility. Four microRNAs were identified exclusively in sperm from high IVP fertility bulls (Hamilton et al., Reprod Fertil Dev, 33:157, 2021); and led to an increase in blastocyst and development rates in low IVP fertility bulls following zygote microinjection with microRNAs mimics (Hamilton et al., Reprod Fertil Dev, 35:155–156, 2023). We hypothed that these microRNAs could improve embryo quality in low IVP fertility bulls. Two functional experiments were conducted using six Nellore bulls—three with high and three with low IVP fertility—retrospectively selected from commercial IVP procedures (n = 7000, 2016–2018). In the first experiment, termed rescue, IVPs were performed using low IVP fertility bulls. Zygotes were microinjected 18 hours after IVF with 5 to 7 pL of a mixture containing mimics of the four microRNAs (100 nM; miRCury LNA and miScript mimic®, Qiagen – mimic group). In the second experiment, termed proof of principle, high IVP fertility bulls were used. Zygotes were microinjected as above with inhibitors of the same four microRNAs (100 nM; miRCury LNA and miScript inhibitor®, Qiagen – inhibitor group). Control zygotes in both experiments were microinjected with negative control mimic or inhibitor molecules (scramble groups). Six IVP manipulations were performed per bull, with 30 oocytes microinjected per experimental group (mimic, inhibitor, or scramble) in each replicate. Blastocysts from each bull and experimental group were collected, fixed in 4% paraformaldehyde for 15 minutes, and stored in PBS with 1% PVP at 4°C. Immunofluorescence protocol was used for double immunostaining: Caudal Type Homeobox 2 (CDX2; polyclonal anti-CDX2 from rabbit, 1:100; Abcam®, Cambridge, UK) to label the trophectoderm (TE), and Sex-Determining Region Y-Box Protein 2 (SOX2; polyclonal anti-SOX2 from goat, 1:100; R&D Systems®, USA) to label inner cell mass (ICM). Appropriate secondary polyclonal antibodies were used. The ICM:TE ratio was calculated. Hoechst 33342 bisbenzimide probe (10 µg/mL, Thermo Fisher®, Texas, USA) was used to assess total cell number in blastocysts. Image analysis was performed using ImageJ-Fiji software (NIH, Bethesda, USA). Statistical analyses were carried out by JASP 0.19.1. No differences were observed in rescue experiment. In proof of principle experiment, a lower number of ICM cells was observed in inhibitor compared to scramble group (46.37±5.45; 67.77±6.90; p = 0.037). No differences were observed in total cell number, TE cell number, or ICM:TE ratio. Results suggest that the delivery of exogenous sperm microRNAs was not able to improve blastocyst quality in low IVP fertility bulls. However, the inhibition of these sperm microRNAs reduced the number of ICM cells in blastocysts from high IVP fertility bulls.

Funding: 2018/03871-6.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Dose-dependent effects of punicalagin on oocyte maturation and early embryonic development *in vitro* in cattle

Laryssa Gondim Barrozo[1], Efigenia Cordeiro Barbalho[1], Nicole Prado Welter[2], Livia Agostini Xavier Vitor[2], F rancisco Das Chagas Costa[1], Sueline Cavalcante Chaves[1], Vitor Braga Rissi[2], André L.f. Goetten[2], Marcos Henrique Barreta[2], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará, [2]Universidade Federal de Santa Catarina

Antioxidants have been highlighted for their ability to reduce oxidative stress during oocyte culture and embryonic development. Punicalagin, a polyphenol extracted from pomegranate (*Punica granatum*), has antioxidant properties and its effects depend on concentration. This study aimed to evaluate the effects of punicalagin on oocyte maturation and bovine embryonic development *in vitro*. Ovaries (n = 300) from cyclic cows were punctured and cumulus-oocyte complexes (COCs) were selected from medium antral follicles (3-8 mm). COCs were subjected to in vitro maturation (IVM) for 22 h at 38.5 °C, 5 % CO2, in TCM 199 medium with Earle's salts and L-glutamine (Sigma, St. Louis, USA), supplemented with 0.2 mM pyruvate, 5.0 µg/ml LH, 0.5 µg/ml FSH, 10% fetal bovine serum (FBS), 100 IU penicillin, and 50 µg/ml streptomycin. Three experiments were performed. In experiment 1, COCs (n = 420) were matured with punicalagin (1.0, 10.0, and 100.0 µM) and then assessed for nuclear maturation with Hoechst 33342. In experiment 2, COCs (n = 420) were matured with punicalagin and *in vitro* fertilization was performed in FERT-TALP medium (Nutricell, Campinas, Brazil), using sperm selected via Percoll gradient. After 20 h, zygotes were cultured for seven days in SOF medium (Nutricell) without punicalagin. In experiment 3, COCs (n = 420) were matured without punicalagin, fertilized and zygotes were cultured for seven days in SOF medium (Nutricell) with punicalagin (1.0, 10.0 and 100.0 µM), under an atmosphere of 5% CO2, 5% O2 and 90% N. Cleavage rates, blastocysts and cell number per embryo were evaluated after experiments 1 and 2. The experiments were repeated three times and statistical analyses were performed with GraphPad Prism v.10, using the chi-squared and Fisher's exact tests and differences were considered significant when $p < 0.05$. Punicalagin did not affect meiotic progression (73.65 ± 15.07%). However, 100.0 µM significantly compromised cleavage (56.14 ± 6.37%) and blastocyst (6.12 ± 1.63%) rates compared to control (80.52 ± 6.93% and 27.21 ± 7.64%), while 1.0 µM (67.90 ± 9.70% and 13.58 ± 3.35%) and 10.0 µM (65.06 ± 6.89% and 16.87 ± 3.73%) did not differ statistically from control. Similar results were observed in Experiment 3, both for cleavage (68.96 ± 5.82%) and blastocyst (18.56 ± 5.54%). The mean number of cells per blastocyst (84.02 ± 13.17) in both experiments tended to decrease with increasing concentration. It is concluded that punicalagin has dose-dependent effects, with possible cytotoxicity at high doses. These findings reinforce the importance of dose standardization for the safe use of antioxidants during oocyte *in vitro* culture.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Dynamics of Interferon Signaling and Immune Regulation in the Bovine Endometrium During Successful and Failed Pregnancies

Isabella Rio Feltrin[1], Samuel Volpe Souza[1], Thiago Neder Lisboa[1], Gabrielle Ceragioli Damaceno[1], Ana Karolyne Alves Miguel[1], Amanda Guimarães da Silva[1], Lucas Carnio de Siqueira Branco[2], Thiago Fernandes Amaral[3], Mario Binelli[4], Guilherme Pugliesi[1]

[1]Universidade de São Paulo, [2]University of Missouri Columbia, [3]Genus ABS, [4]University of Florida

Given the pivotal role of maternal immune adaptation in early gestation, we hypothesize that the successful establishment of pregnancy relies on the fine-tuned regulation of endometrial immune responses and that disruptions in this process result in pregnancy failure. Thus, the regulation of endometrial–conceptus communication during early pregnancy and its association with pregnancy loss were investigated in beef cattle. Estrous cycles of mature heifers were synchronized, followed by either a SHAM procedure (n=15) or embryo transfer (n=71) on Day 7 (Day 1 = ovulation). A single endometrial luminal epithelial cells were collected from each animal via cytobrush for gene expression analyses by qPCR, and luteal function was assessed via color-Doppler ultrasonography on Days 6, 15, and 20. Animals were retrospectively categorized into five groups (n=6–8/group): Cycling (simulated embryo transfer), EL15-20, EL20-25, EL25-30 [embryo losses detected based on spontaneous luteolysis of the CL(≤ 25% blood perfusion) on Days 20, 25, or 30, respectively], and Pregnant (confirmed pregnancy by Day 30). Data were analyzed by ANOVA in SAS, and a significant difference was considered when P <0.05. For corpora lutea (CL) and blood perfusion, there were significant effects of Group, Time, and Group by Time interaction. Both CL characteristics increased between Day 6 and Day 15 in heifers from all groups, but heifers from the Cycling and EL15-20 groups had a reduction of CL area and blood perfusion on Day 20 compared to the other groups. Endometrial ISG15 and RSAD2 expression was upregulated in EL20-25, EL25-30, and Pregnant groups on Days 15 and 20, indicating early conceptus-induced immune activation in heifers with maintained CL. The IRF2 was selectively downregulated on Day 15 in the Pregnant group. On Day 20, Pregnant animals showed increased IFNG, IL6, and IL10 expression, reflecting a balanced immune modulation supportive of pregnancy, whereas IL1B and IL8 were upregulated in EL20-25 and EL25-30 groups, indicating a pro-inflammatory state associated with pregnancy loss. The TGFB1 expression increased on Day 20 in the Cycling and EL15-20 groups. The TLR2 expression increased between Days 6 and 20 in Pregnant, EL20-25, and EL25-30 groups, while TLR4 was dysregulated across all groups except Pregnant. This study provides the first in vivo characterization of the uterine environment at two developmental milestones after embryo transfer (Day 15 and 20). Collectively, these findings indicate that the conceptus stimulates early immune activation, characterized by the induction of ISGs, IL6, IL10, IFNG, and TLR2, as early as Day 15 in recipients with sustained luteal activity, independent of pregnancy outcomes. In contrast, the dysregulated expression of IL1B, IL8, and TLR4 appears to be associated with early pregnancy failure.

Acknowledgments: FAPESP (#2022/14936-7), CAPES, INCT Reprodução Animal (#406866/2022-8).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effect of addition of the specific activator of peroxisome proliferating factor delta (L165041) on embryonic development

Jesús Alfonso Sánchez Viafara[1], Matteo Duque Rodriguez[2], Felipe Correa Monsalve[2], Luciana Echeverri Taborda[3], Daniel Abreu Acosta[4], Rodrigo Urrego[4]

[1]centro De Desarrollo Tecnológico Del Cesar, [2]Politécnico Colombiano Jaime Isaza Cadavid, [3]Universidad Nacional de Colombia, [4]Universidad CES

This study aimed to evaluate if adding L-165041 (PPARδ selective agonist) to the culture medium influences embryonic development. Activation of the PPARδ transcription factor may decrease the apoptotic index, and increase cell proliferation, which improves the development and quality of embryos produced *in vitro*. It was hypothesized that the addition of L-165041 to the culture medium enhances development. Bovine cumulus-oocyte complexes-COCs (n = 1004) were matured *in vitro* (IVM) for 24 h at 38.5°C in a 5% CO2 atmosphere, in microdroplets of 100 µL of IVM medium covered with mineral oil. At the end of the maturation period, *in vitro* fertilization (IVF, day 0) was carried out for 18–22 h at 38.5°C in 5% CO2 atmosphere and saturated humidity. Presumed zygotes were allocated to one of three treatments: (1) control: standard culture medium, synthetic oviduct fluid medium (SOFaa), along with 2.5% FBS, 22.0 µg/mL of pyruvate, 0.025 g/mL of BSA, and 83.4 µg/mL of amikacin (n= 337), or (2) standard culture medium with DMSO (0.008%) (n=333) or (3) with 1µM of L-165041 (n= 333). Supplements were added to the medium on Day 1 with medium change covered with mineral oil. L-165041 was added to the culture medium at a final concentration of 1 µM diluted in dimethylsulfoxide (DMSO). The final concentration of DMSO used as a diluent for L-165041 was 0.008%, and this same concentration was added to the DMSO group. The *in vitro* culture (IVC) was carried out for 7 days at 38.5°C at 5% CO2 and 5% O2; embryos were cultured in 100 µL droplets of medium covered with mineral oil. The cleavage rate was assessed on day 3 and was determined as a ratio of the numbers of cleaved oocytes to the total numbers of matured oocytes. The blastocyst production rate was evaluated on day 7 (D7) and determined as the ratio of the number of blastocysts produced to the total number of cleaved embryos. The cleavage rate (control 63.6 ± 13.6%, L-165041 59.7 ± 10.4%, DMSO 61.5% ± 11.1%) and blastocyst production (control 42.7% ± 16.5, L-165041 43.3 ± 19.2%, DMSO 37.4 ± 18.9%) were not affected (P > 0.05) by the addition of L-165041 to the IVC medium. In conclusion, 1 µM of L-165041 had no impact on embryo development at this specific concentration.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effect of FTAI bull fertility on *in vitro* embryo production in cattle

Taynara dos Santos Santana[1], Wallery Caroliny Costa da Costa[1,2], Mariane Gabriela Cesar Ribeiro Ferreira[1], Felipe Gabriel Barbosa de Oliveira[1], Jorge Luis Torres Garcia[1,3,4], Wilian Aparecido Leite da Silva[1], Thais Ferreira Lima[1], Ériklis Nogueira[5,4], Fabiana de Andrade Melo Sterza[1,6,2]

[1]Universidade Estadual de Mato Grosso do Sul, [2]Grupo de Estudos em Tecnologia da Reprodução Animal, [3]Universidad de los Llanos, [4]EMBRAPA GADO DE CORTE, [5]Empresa Brasileira de Pesquisa Agropecuária, [6]Universidade Federal de Mato Grosso do Sul

Studies have linked bull fertility to embryonic development. Additionally, the kinetics of embryonic development are known to be associated with embryo quality. However, information on how paternal factors influence embryonic development in cattle is limited. This study aimed to evaluate the impact of bulls categorized as having high or low fertility at FTAI on *in vitro* embryo production (IVEP) performance. Oocytes were obtained from ovaries collected at a slaughterhouse, and only those classified as grades 1 and 2 were selected. *In vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC) were performed using a commercial medium (Cenatte®) in an incubator at 38.5 °C with a controlled atmosphere of 6% CO_2, 6% O_2, and maximum humidity. The bulls came from commercial breeding centers and were classified according to their average fertility rates obtained in fixed-time artificial insemination (FTAI) programs with BIF accuracy above 24%. This classification used a Bayesian animal model that accounted for fixed effects, such as contemporaneous group and cow age, as well as random effects, such as direct genetic effects. Bulls with a pregnancy rate of at least 55% were defined as having high fertility (HF), while those with a rate of less than 40% were classified as having low fertility (LF). Five bulls were selected from each group and tested in three IVEP routines for a total of 15 routines. In each routine, the bulls' semen was evaluated and adjusted to an insemination dose of 2x106/mL. The routines consisted of three groups: a high-fertility bull (HF), a low-fertility bull (LF), and a control bull, whose blastocyst production in our lab was known and stable. The cleavage rate and initial development kinetics of the embryos were assessed on the fourth day after IVF (D4). The embryos were classified as "slow" (4–6 cells) or "fast" (eight cells or more). The blastocyst rate was assessed on day seven (D7). A statistical analysis was performed using the GLIMMIX procedure with a 5% significance level. A total of 1,500 grade 1 and 2 oocytes were used. Of those, 740 were fertilized with semen from the HF group, and 760 were fertilized with semen from the LF group. The cleavage rate was 52.3% (386/740) in the HF group, with 66.8% (194/290) of the embryos being slow and 33.1% (96/290) being fast at D4 ($P>0.05$). The LF group had a cleavage rate of 45.6% (354/760), with 73.6% (162/220) slow and 26.4% (58/220) fast ($P > 0.05$). The blastocyst rate on day 7 was 28.1% and 28.3% for the HF and LF groups, respectively ($P > 0.05$). There was a significant positive correlation between initial development kinetics and blastocyst rate ($r = 0.62$, $p < 0.0002$). The results showed that bulls classified as high or low fertility based on FTAI pregnancy rates do not affect the initial development kinetics of *in vitro*-produced embryos. However, evaluating initial development kinetics at D4 provides a more accurate prediction of D7 production.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effect of pharmacological activation of the mTOR pathway on the first cell differentiation in bovine embryos

Mirela Soares Vilioti[1], Beatriz Cartaxo Ragnelli[1], Francieli Perroni Berling[1], Camilla Mota Mendes[1], Mayra Elena Ortiz D Avila Assumpção[1], Marcelo Demarchi Goissis[1]

[1]Universidade de São Paulo

The initial development of the embryo is marked by the first cellular differentiation process, which defines the inner cell mass (ICM) and the trophectoderm (TE). The mTOR signaling pathway (mammalian target of rapamycin) acts as an integrator of signals related to metabolism and influences this differentiation process in mice . Therefore, our hypothesis was that the mTOR pathway acts positively in the TE differentiation in bovine embryos. The aim of this study was to stimulate the mTOR pathway using the agonist MHY1485 during embryonic culture and assess effects on gene expression and cell allocation. *In vitro*-produced bovine embryos were subjected to treatments at 90h post insemination (hpi). Embryos were divided into Control, DMSO (vehicle - 0.13% v/v), and MHY (MHY1485) groups. Initially a dose-response test using 1µM, 2µM and 4µM MHY1485 was conducted in 4 replicates to assess blastocyst rates and apoptosis incidence at 186 hpi using the CaspACE FITC-VAD-FMK In Situ Marker Kit (Promega). The effects of the doses on blastocyst rate and apoptosis incidence were evaluated using linear regression and comparison of means. The 2 µM dose of MHY1483 was selected as the highest dose that did not harm blastocyst rates (p=0.003).Using the 2 µM MHY1485 dose, embryos were divided into the three aforementioned groups. First, embryos were collected at the morula stage at 138 hpi for gene expression analysis in pools of 5 to 7 embryos in 5 replicates. Relative Q-RT-PCR was performed to observe the response of GATA3, CDX2, YAP, TFAP2C, SOX2, and G6PD. Then, blastocysts at 186 hpi were harvested, the zona pellucida was removed, and they were fixed in 3.8% formaldehyde for immunofluorescence to detect expression of GATA3 and YAP1. Images were obtained using a laser scanner confocal microscope. We obtained TE cell count (GATA3+), total cell count (Hoechst 33342) and YAP1 nuclear fluorescence intensity. Statistical analysis was performed considering treatments as independent variables. Q-RT-PCR data were analyzed using a linear mixed model with pre-planned comparisons. Cell count and fluorescence data were analyzed by ANOVA and Tukey's post-hoc test. There was no difference in morula gene expression analysis. There was no difference in total or ICM cell count. The DMSO group presented lower TE cell count compared to the Control and MHY groups ($p<0.05$). The control group presented higher nuclear YAP1 intensity than the MHY group, which was in turn higher than the DMSO group ($p<0.001$). Thus, we can conclude that treatment with 2 µM of MHY1485 did not alter the expression of genes related to cellular differentiation in bovine embryos at the compact morula stage. Treatment with MHY1485 did not act positively on the TE of bovine embryos compared to the control group, although it promoted a rescue in the number of TE cells, which was negatively influenced by the vehicle (DMSO).

Financial support by: FAPESP 23/06051-8, PIBIC and USP-PUB.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effect of residual feed intake on reproductive traits and pregnancy outcomes in Nellore heifers

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

[1]Instituto de Zootecnia, [2]Universidade Estadual Paulista

Feed efficiency, assessed through residual feed intake (RFI), has been regarded as a promising strategy for reducing production costs. However, due to uncertainties regarding the relationship between RFI and reproductive performance, further studies are needed to explore this interaction. The breeding value for RFI (BV-RFI) has been used as a robust tool, as it accounts for fixed and random effects, including the additive genetic effect, allowing for the identification of superior individuals and the advancement of genetic breeding programs. In the present study, the breeding values for RFI (Kg DMI/day) were derived from the genetic evaluation of 2,621 animals with record phenotypes. This study aimed to evaluate whether reproductive traits associated with fertility in Nellore heifers are influenced by RFI. A total of 199 genotyped Nellore heifers (Bos indicus), with a mean age of 17 months and average body weight of 250 kg, were evaluated and classified into two groups: 94 with negative BV for RFI and 105 with positive BV for RFI (as a deviation from the BV-RFI average of heifer' birth year). RFI was estimated using the BLUPF90 software suite, based on a combination of genomic and phenotypic information from ancestors and collateral relatives. Seven ultrasonographic evaluations were performed at 28-day intervals, during which the following phenotypes were measured: vulvar biometry (anogenital distance, vulvar length, and vulvar width), antral follicle count (AFC), ovarian diameter, uterine pixel intensity, and pregnancy rate. A total of 172 heifers were subjected to fixed-time artificial insemination (FTAI) during the breeding season, with 81 classified as BV RFI-negative and 91 as BV RFI-positive. Pregnancy rate was assessed 30 days later. Insemination was performed using fresh semen from bulls previously selected according to the genetic breeding program criteria of the Instituto de Zootecnia. Additionally, rump fat thickness (RFT), longissimus muscle area (LMA) and serum concentrations of IGF-1, leptin, and anti-Müllerian hormone (AMH) were measured. Data were subjected to repeated measures analysis of variance using the PROC MIXED procedure of the SAS software. Statistical significance was considered at $P<0.05$. No differences were observed between RFI groups for AFC, diameter of the largest follicle, uterine echogenicity and echotexture, RFT, LMA and hormone concentrations. However, higher values were observed in heifers with negative BV-RFI for vulvar length ($P<0.0001$), anogenital distance ($P=0.0027$), and mean ovarian diameter ($P=0.01$). There was no difference in pregnancy rate between heifers with negative and positive BV-RFI values (32.10% [26/81] vs. 29.67% [27/91], respectively; $P = 0.40$). Selection for feed efficiency does not compromise fertility in Nelore heifers, indicating that it can be implemented without impairing reproductive function.

Acknowledgments: CAPES for the scholarship granted and FAPESP (Process nº. 2022/08182-0).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effects of exogenously-induced increased GnRH pulse frequency on LH release profile in ovariectomized *Bos taurus* and *Bos indicus* heifers under elevated progesterone concentrations

Lucas O. e Silva[1,2], Sarah West[2], Viviana Garza[2], Jessica Sustaita-Monroe[2], Leslie King[2], Haley Sellers[2], Hayden Feist[2], George A. Perry[3], Roberto Sartori[1], Rodolfo C. Cardoso[2]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture, [2]Texas A&M University, [3]Texas A&M AgriLife Research and Extension Center

This study tested the hypothesis that increased GnRH pulse frequency, induced exogenously by administration of either Kisspeptin (Kp) or GnRH, enhances pituitary responsiveness, amplifying the GnRH-induced LH surge in heifers under elevated circulating progesterone (P4) concentrations. Additionally, LH pulse and surge outcomes of *Bos taurus* and *Bos indicus* heifers were compared under these conditions. Pubertal Hereford (n = 6) and Brahman (n = 8) heifers were previously ovariectomized (OVX) and received 2 sc estradiol (E2) implants to maintain low physiological E2 concentrations (~3 pg/mL), then enrolled in a cross-over 2×3 factorial arrangement design. On d-5, all heifers received 2 intravaginal P4 devices (1.38 g), kept until the end of that replicate. On d0, blood samples were collected every 15 min over 12 h through a jugular catheter. During the first 8 h (**pulse period**; 0 to 480 min), heifers from both genetic groups received one of three experimental treatments, administered iv every 60 min: Control = saline solution; **Kp** = 0.4 µg/kg of body weight (BW) of murine Kp; and **GnRH** = 0.005 µg/kg of BW of gonadorelin acetate. Still on d0, at 480 min, all heifers received 100 µg of gonadorelin acetate im (**GnRH challenge**) to induce an LH surge. Blood sampling continued every 15 min for an additional 4 h (**surge period**; 480 to 720 min). Statistical analyses were performed by GLIMMIX of SAS ($^{a\text{-}b}P \leq 0.05$). At the beginning of the pulse period, circulating P4 concentrations (3.6 ± 0.1 ng/mL) were similar among treatments (P = 0.44) and genetic groups (P = 0.18). Kp and GnRH treatments effectively induced a high LH pulse frequency, despite the elevated P4 concentrations. Regardless of the genetic group, 75.0% (84/112) of the Kp treatments and 87.5% (98/112) of the GnRH treatments resulted in detectable LH pulses. LH pulse frequency was greater (P < 0.01) in Kp- and GnRH-treated groups than in Control (6.1 ± 0.6^{a} vs 7.0 ± 0.4^{a} vs 2.3 ± 0.5^{b} pulses/8 h, respectively). Nevertheless, neither endogenous nor induced LH pulse outcomes differed between genetic groups. The subsequent GnRH-induced LH surge was not enhanced by increased GnRH pulse frequency (P = 0.23), however, it was consistently reduced in Brahman compared to Hereford heifers (surge peak: 6.2 ± 0.9 vs 9.0 ± 1.2 ng/mL; P = 0.05). In conclusion, this study provides new insights into the neuroendocrine regulation of LH secretion under suppressive P4 conditions. The increased GnRH pulse frequency was insufficient to enhance pituitary responsiveness in OVX heifers under elevated P4 concentrations, suggesting that rising E2 concentrations may be an essential mediator of this mechanism. Although LH pulsatile secretion was similar between *Bos taurus* and *Bos indicus* females, differential pituitary responsiveness to a GnRH ovulatory stimulus may contribute to differences in reproductive function between these genetic groups.

Acknowledgments: FAPESP 2021/09924-7, 2022/12348-0, USDA-NIFA2018-67015-27595.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effects of liposome-encapsulated α-pinene on *in vitro* oocyte maturation and embryo development in bovine

Venância Antonia Nunes Azevedo[1], Maria Alice de Almeida[2], Matheus Andrade Chaves[2], Luca Angi de Souza[3], Luiziana Cavalcante Costa Fernandes Crisóstomo[1], Felipe Perecin[2,4], Flávio Vieira Meirelles[2,4,5], Josimar Oliveira Eloy[1], Juliano Coelho Da Silveira[2,4,6], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará, [2]Universidade de São Paulo, [3]Universidade de São Paulo - Faculdade de Zootecnia e Engenharia de Alimentos, [4]University of São Paulo, [5]University of São Paulo, Faculty of Animal Science and Food Engineering, [6]Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo

Oocyte maturation represents a crucial phase in embryo production; however, *in vitro* conditions are often associated with elevated levels of reactive oxygen species (ROS). Thus, strategies to reduce ROS are essential to improve oocyte quality during IVM. This study investigated the effects of liposome-encapsulated α-pinene (Lip-α-pinene) on bovine oocyte IVM and subsequent embryonic development. COCs were matured *in vitro* (n = 40-50/group) for 22-24 h in maturation medium (TCM199+) alone or supplemented with Lip-blank (empty liposomes) or 0.01, 1.0, and 100.0 µg/mL Lip-α-pinene (14 replicates). After IVM, nuclear maturation rates were assessed, and mature oocytes underwent parthenogenetic activation (ionomycin + 6-dimethylaminopurine), followed by IVC for days 7. Cleavage (day 3) and blastocyst rates, as well as total cell number (day 7) were evaluated. ROS and GSH levels were quantified in matured oocytes (n = 45-50/group) using H2DCFDA and CellTracker Blue probes, respectively, while ultrastructure was assessed by transmission electron microscopy in matured COCs (n = 5/group), and lipid content was analyzed in oocytes and embryos (n = 45–50/group) using BODIPY 493/504. The mRNA levels of superoxide dismutase (*SOD*), catalase (*CAT*), peroxiredoxin 6 (*PRDX6*), glutathione peroxidase 1 (*GPX1*), kelch-like ECH-associated protein 1 (*KEAP1*), nuclear factor erythroid 2-related factor 2 (*NRF2*), inositol requiring enzyme-1 (*IRE1*), kinase RNA-like endoplasmic reticulum kinase (*PERK*) and transcription factor 6 (*ATF6*) were assessed from 4 pools of oocytes/group (n = 20 oocytes/pool). Data were analyzed using Chi-square, ANOVA (Tukey), and Kruskal-Wallis tests ($P < 0.05$). Nuclear maturation was not affected by Lip-α-pinene ($P > 0.05$); however, 1.0 µg/mL Lip-α-pinene preserved ultrastructure of zona pellucida and organelles of cumulus cells and oocyte. This group also showed reduced lipid accumulation compared to the control ($P < 0.0299$), Lip-blank ($P < 0.0455$) and 100.0 µg/mL Lip-α-pinene ($P < 0.0398$). ROS levels were also reduced compared to control ($P < 0.0334$) or with Lip-blank ($P < 0.0386$) and 100.0 µg/mL Lip-α-pinene ($P < 0.0216$). However, Lip-α-pinene did not affect GSH levels ($P > 0.05$). This was accompanied by an increase of *NRF2* (2.5 fold, $P < 0.0242$), *SOD* (2.0 fold, $P < 0.0472$) and *PRDX6* (3.5 fold, $P < 0.0319$) levels compared to control. Furthermore, 1.0 (71.16 ± 0.95) and 100.0 µg/mL (72.16 ± 1.95) Lip-α-pinene increased cleavage rates compared to the control (59.08 ± 1.46), while blastocyst rates and lipid content were not affected ($P > 0.05$). Lip-α-pinene [1.0 ($P < 0.0208$) and 100.0 µg/mL ($P < 0.0143$)] increased the number of cells per blastocyst compared to the control. In conclusion, 1.0 µg/mL Lip-α-pinene enhances the antioxidant capacity of bovine oocytes during IVM by reducing oxidative stress, lipid accumulation and preserving ultrastructure. It also increases the level of *NRF2*, *SOD*, and *PRDX6* transcripts.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Effects of small extracellular vesicles during *in vitro* maturation on oxidative stress parameters in bovine oocytes and cumulus cells

Priscila Assis Ferraz[1], Fernanda Schneberger dos Santos[1], Alessandra Bridi[2], José Roberto Quirino de Oliveira[1], Lorena Calixto Munhoz[1], Ludimila Cardoso Zoccal Janini[1], Priscila Helena dos Santos[1], Juliano Coelho Da Silveira[1], Cláudia Lima Verde Leal[1]

[1]Universidade de São Paulo, [2]2UNOESC - Universidade do Oeste de Santa Catarina

Fetal bovine serum (FBS) is one of the most widely used sources of macromolecules in culture media during *in vitro* embryo production and contains small extracellular vesicles (sEVs) that carry lipids, mRNAs, and miRNAs, which may influence intercellular communication and different functions in target cells. This study aimed to evaluate the impact of sEVs from FBS on oxidative stress in bovine oocytes during *in vitro* maturation (IVM). sEVs were isolated from FBS by -exclusion chromatography and ultracentrifugation. Cumulus-oocyte complexes (COCs) were collected from 3–6 mm follicles and subjected to IVM under three treatment conditions for 22 hours at 38.5°C with 5% CO_2: (1) complete FBS (cFBS), (2) sEV-depleted FBS (dFBS), and (3) dFBS supplemented with sEVs (dFBS + sEVs). After IVM, cumulus cells (CCs) were removed and maturation was assessed by the extrusion of the first polar body (PB). Part of the oocytes was collected to determine reactive oxygen species (ROS) levels using CellROX® Orange following the manufacturer's instructions, and images were captured using a MICA Leica wide-field microscope. Fluorescence intensity (FI) was quantified using ImageJ software. Another set of the oocytes and their corresponding cumulus cells (CC) were collected for RNA extraction using RNAzol® Reagent. The expression of antioxidant genes (GPX1, GPX4, SOD1 and SOD2) was assessed by RT-qPCR and normalized against reference genes (PPIA, ACTB, RPL15). Statistical analysis was conducted using one-way ANOVA, with significance set at $P < 0.05$. The rates of matured oocytes showing extrusion of the 1st PB varied from 74 to 78%, with no difference between the groups ($P > 0.05$). ROS levels were similar in oocytes from the cFCS (17118745 ± 4044075) and cFCS + sEVs (16250457 ± 3831768) groups ($P > 0.05$), and both were significantly higher than those observed in the dFCS group (12065478 ± 2383537; $P = 0.0001$). The expression of antioxidant-related mRNAs in oocytes and CCs after IVM did not show significant differences ($P > 0.05$). Nonetheless, in oocytes, SOD1, SOD2 and GPX4 consistently exhibited highest expression levels across all treatments, while in CC, the antioxidant genes GPX1, GPX4 and SOD1, were the most expressed in all groups. In conclusion, the results indicate that the depletion of EVs from FBS neither impairs nor enhances nuclear maturation, and similarly, the reintroduction of EVs does not significantly alter this process. However, EV-depleted serum was associated with reduced oxidative stress in oocytes, while supplementation with isolated EVs led to an increase in ROS levels, suggesting a potential relationship between FBS-derived EVs and intracellular oxidative status. As antioxidant genes did not vary among treatments in oocytes and CC, the transfer of these mRNAs from sEVs to cells does not seem to be linked to the changes observed in ROS levels, suggesting other genes and/or molecules may be involved.

Financial support: FAPESP grant 2021/06760-3 and 2023/01524-5; PAF postdoctoral fellowship 2024/05583-9.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Follicular fluid-derived extracellular vesicles improve mitochondrial function and redox balance in bovine oocytes during *in vitro* maturation

Fernanda Schneberger dos Santos[1], Priscila Assis Ferraz[1], Alessandra Bridi[2], José Roberto Quirino de Oliveira[1], Lorena Calixto Munhoz[1], Ludimila Cardoso Zoccal Janini[1], Juliano Coelho Da Silveira[1], Cláudia Lima Verde Leal[1]

[1]Universidade de São Paulo, [2]2UNOESC - Universidade do Oeste de Santa Catarina

Extracellular vesicles present in follicular fluid (FF) have emerged as important mediators of cell-to-cell communication within the ovarian follicle, potentially influencing oocyte maturation and developmental competence. By transporting bioactive molecules such as proteins, lipids, and RNAs, these vesicles may regulate mitochondrial function and oxidative balance—key elements in determining oocyte quality. This study aimed to investigate the effects of extracellular vesicles derived from follicular fluid (ffEVs) on cytoplasmic maturation, oxidative status, and antioxidant gene expression in bovine oocytes. Follicular fluid (FF) and cumulus-oocyte complexes (COCs) were collected from 3–6 mm follicles aspirated from slaughterhouse ovaries. ffEVs were isolated by -exclusion chromatography followed by ultracentrifugation (100,000 × g, 70 min, 4°C), and fetal calf serum (FCS) was EV-depleted by ultracentrifugation (100,000 × g, 18 h, 4°C). COCs were matured for 22 h at 38.5°C in 5% CO_2 in TCM199 supplemented with glutamine, pyruvate, gentamicin, EGF, FSH, and 10% EV-depleted FCS (Control) or 10% EV-depleted FCS with ffEVs (ffEV group). Post-IVM, denuded oocytes were stained with MitoTracker Orange and CellROX to assess mitochondrial activity and reactive oxygen species (ROS) levels by confocal microscopy (MICA Leica). Additional oocyte and cumulus cell samples were stored at −80°C for RT-qPCR analysis of antioxidant-related transcripts; ffEVs were also profiled for the same transcripts. Data were analyzed using Mann-Whitney U and Wilcoxon tests ($\alpha = 0.05$; four biological replicates). A total of 124 oocytes were analyzed for mitochondrial activity (Control: n = 54; ffEV: n = 70), and 143 oocytes for ROS levels (Control: n = 67; ffEV: n = 76). ffEV-treated oocytes exhibited significantly higher mitochondrial fluorescence intensity ($P < 0.05$), indicating enhanced mitochondrial activity and suggesting improved cytoplasmic maturation. ROS levels were significantly lower in ffEV-treated oocytes compared to controls ($P < 0.05$), suggesting a more favorable redox environment. Despite the confirmed presence of antioxidant transcripts (SOD2, GPX1, SOD1, GPX4) in ffEVs, no significant differences in transcript abundance were observed in oocytes or cumulus cells post-treatment. The most expressed transcripts included SOD2, SOD1, and GPX4 in oocytes, and GPX1, GPX4, and SOD1 in cumulus cells. These results indicate that ffEVs may enhance mitochondrial function and redox balance through mechanisms beyond direct mRNA transfer, possibly involving post-transcriptional or protein-level regulation. Collectively, our findings support the role of ffEVs as modulators of oocyte quality during *in vitro* maturation, with potential applications in improving assisted reproductive technologies.

Financial support: Capes - Finance code 001; FAPESP (SPEC Grant # 2021/09886-8; AR Grant #2021/06760-3; Grant # 2023/01524-5).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Formation, standardization, and toxicity of small unilamellar vesicles in *in vitro* culture of bovine embryos

Isabelle Thaine Martelli[1], Melissa Nishida Hirano[1], Alexandre Mendes de Almeida Junior[1], Isabela Batista de Castro Nunes[1], Malu Maria Felix Manzieri[1], Thamirys Cardoso do Nascimento Barbosa[1], Maria Giovana de Souza[1], Pedro Henrique Benites Aoki[2], Marcelo Fábio Gouveia Nogueira[1]

[1]Department of Biological Sciences, São Paulo State University (UNESP), School of Sciences, Humanities and Languages, Assis, [2]Department of Biotechnology, São Paulo State University (UNESP), School of Sciences, Humanities and Languages, Assis

Currently, *in vitro* embryo production (IVEP) is an assisted reproductive technology widely used in livestock, particularly in cattle, with Brazil leading it besides USA. However, this technique faces challenges, making it essential to develop new strategies that bring laboratory culture conditions closer to maternal conditions. In this context, liposomes emerge as an interesting approach for IVEP applications, serving as carriers for supplements and molecules targeted to embryonic cells. Several factors influence liposome stability, including composition, , lamellarity, surface charge, ambient temperature, and the surrounding medium. Among these parameters, phospholipids such as phosphatidylcholines (PC) and phosphoinositides (PI) stand out as promising components for vesicle formation in bovine embryo's culture medium (IVC medium). This study aimed to validate the formation and assess the stability and toxicity of small unilamellar vesicles (SUVs) co-cultured with bovine embryos IVP. SUVs' formulation was tested with cholesterol (37.5%), dipalmitoylphosphatidylcholine (DPPC; 60%), and phosphoinositides PI(4,5)P2 or PI(4)P (2.5%) at concentrations of 1, 2, and 3 mmol/L under IVC conditions (38.5°C, 5% CO and maximum humidity). The vesicles were produced using lipid film hydration technique, with homogenized diameter achieved via sonication and extrusion. SUVs stability was analyzed at 0, 24, and 48 h (incubation time) using dynamic light scattering (DLS). Finally, toxicity was assessed based on blastocyst production (on day 7 of culture; D7) in embryos co-cultured with SUVs during 48 h (from D5 to D7) and control group (i.e., without SUVs). The statistical analyses were performed using Minitab online, and the data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Three replicates were performed, and the results demonstrated that the concentrations of 1 to 2 mmol/L PI(4,5)P2 (41.28% ± 6.33 and 36.6% ± 4.40, respectively) and 1 mmol/L PI(4)P (25.5% ± 2.86) were not embryotoxic ($p>0.05$), whereas higher doses of 2 to 3 mmol/L for PI(4)P (18.0% ± 2.15 and 12.7% ± 2.26, respectively) and 3 mmol/L for PI(4,5)P2 (12.7% ± 2.26) did decrease blastocyst yield compared to control (27.8% ± 2.99 and 45.96% ± 3.15, respectively). The SUVs remained stable for 48 hours (average of 350.0 nm) in comparison to 0 h (average of 371.9 nm), under incubation conditions, demonstrating potential for applications in reproductive biotechnology. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Processes Numbers #2024/12714-2; 2024/03887-0; 2022/02189-2; 23/17455-2 23/06884-0 and 2021/11747-6.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Functional role of prostaglandin $F_2\alpha$ in luteal regression after induced pregnancy loss in cattle

Natalia Picoli Folchini[1], Muhammad Saad[1], Ellie Kidwell[1], Paulo Mielli Bonacim[1], Lillian Ehresmann[1], Rafael Reis Domingues[1], Michael Kleinhenz[2], Ky Pohler[2], Álvaro Garcia-Guerra[1]

[1]The Ohio State University, [2]Texas A&M University

Pregnancy loss after classical maternal recognition of pregnancy (MRP) decreases reproductive efficiency in cattle. Furthermore, evidence suggests that a substantial amount of pregnancy loss is attributed to luteolysis. Induced conceptus demise invariably results in luteolysis within a predictable timeframe and thus provides for a model to investigate the mechanisms underlying corpus luteum (CL) maintenance/regression beyond MRP. It is still unclear, however, whether PGF2α mediates luteal regression after conceptus demise. Thus, the objective was to test the hypothesis that inhibition of PGF2α secretion would delay luteolysis after conceptus demise, while replacement of PGF2α would cause luteolysis. Pregnant non-lactating crossbred cows (n=33) aged 4.8±3.1 years and with a BCS of 5.7±0.7 were randomly assigned into one of three groups: Control (n=10), Flunixin meglumine (FM, n=12), and Flunixin meglumine+PGF2α (FM+PGF, n=11). On day 35 of gestation (study day 0), conceptus demise was induced through intrauterine administration of 7.2% hypertonic saline. From days 5 to 10 (treatment period), cows received intrauterine treatments every 8h: Control cows received 10 mL phosphate-buffered saline (PBS), FM cows received 240 mg flunixin meglumine (a cyclooxygenase inhibitor), and FM+PGF cows received 240 mg flunixin meglumine. Additionally, on day 7, four intrauterine pulses of PBS or 0.5 mg of PGF2α were administered every 8h to cows in the FM and FM+PGF groups, respectively. Blood samples were collected twice daily to assess progesterone (P4). Circulating PGF2α metabolite (PGFM) was assessed on bihourly samples collected from Control and FM cows during 8h on day 6 and from samples collected before and after (10 min) the first pulse on day 7 from FM and FM+PGF cows. Cows (n=6) with luteolysis identified before the onset of treatment (i.e., day 5) were removed from analysis. Basal (average of the three lowest samples) and maximum PGFM concentrations were greater ($P<0.05$) for Control (66.3±19.6 and 171.8±48.5 pg/mL, respectively) than FM-treated (9.2±2.7 and 11.0±2.0 pg/mL, respectively) cows. Interval from conceptus demise to luteolysis was longer ($P<0.05$) for FM (12.6±0.4 d) than Control (7.6±0.4 d) cows, and for FM+PGF (9.7±0.8 d) than Control cows. The percentage of cows undergoing luteolysis during the treatment period (days 5 to 10) was 100% (8/8) and 0% (0/9) for Control and FM cows, respectively. Seventy percent (7/10) of FM+PGF cows had luteolysis due to PGF administration. Circulating PGFM before the PGF pulse did not differ (11.5±0.1 pg/mL; $P>0.10$) between FM and FM+PGF cows, whereas 10 min after the pulse, PGFM was greater for FM+PGF (625.2±102.5 pg/mL) than FM (9.8±0.7 pg/mL) cows. In summary, inhibition of PGF2α secretion prevented luteolysis, while replacement of PGF2α caused luteolysis in most cows, suggesting that PGF2α is responsible for luteal regression after conceptus demise during the second month of gestation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

How can blood calcium concentration and oral supplementation affect uterine health in dairy cows?

Jerbeson Hoffmann da Silva[1], Natália Ávila de Castro[2], Clério Antonio Hoefle[3], Camila Cupper Vieira[4], Luiz Carlos Kreutz[5], André Gustavo Cabrera Dalto[1], Paulo Bayard Dias Gonçalves[4], Monique Tomazele Rovani[1], Rogério Ferreira[3]

[1]Universidade Federal do Rio Grande do Sul, [2]Universidade Federal de Pelotas, [3]Fundação Universidade do Estado de Santa Catarina, [4]Universidade Federal de Santa Maria, [5]Universidade de Passo Fundo

The impact of blood calcium (Ca) on uterine health in dairy cows was assessed. In Exp1, serum Ca within 24h post-calving (n=26) was correlated with polymorphonuclear leukocyte (PMN) count at 34-40 days postpartum (DPP). In Exp2, 47 cows classified as normocalcemic (Ca≥8.5mg/dL) or hypocalcemic (Ca<8.5mg/dL) were assigned to either receive oral Ca formate (Bayer, Pfaffenhofen, Germany) at 6 and 30h postpartum or no treatment, and the effects on subclinical endometritis (SE) incidence were evaluated. In Exp3, five multiparous Holstein cows in a 2x2 crossover design were assigned to either normocalcemic (Control) or induced subclinical hypocalcemia (iSCH) treatment. The iSCH group received 5% Na2EDTA intravenous infusion at 500 mL/h for 45 min, while control cows received 0.9% NaCl. Ionized Ca (iCa), total Ca (tCa), and Mg concentrations were measured before treatments (M0), at 15 min (M1), 45 min (M2, end of infusions), and 3h after treatments (M3). Glucose, insulin, NEFA, and BHB levels were determined at M3. After infusions (M2), all animals received an intrauterine challenge with 300µg LPS (Sigma Aldrich, St. Louis, MO). Endometrial biopsies were performed at M3 to determine the expression of IL-6, CXCL8, TNF, glucose-insulin receptors and insulin-related genes. Data were analyzed using linear regression in Exp1, two-way ANOVA and logistic regression in Exp2, and one-way ANOVA in Exp3. Normality was tested using Shapiro-Wilk test. The significance level was set at P<0.05. All analyses were performed using JMP software (JMP Statistical Discovery LLC, Cary, NC). In Exp1, PMN count was affected by blood Ca concentration within the first 24h postpartum (P≤0.01). In Exp2, Ca formate treatment, regardless of blood Ca concentration, reduced PMN count and SE incidence between 34-40 DPP (P<0.05). For each increase of 1 mg/dL in blood Ca, SE incidence decreased by 22%. In Exp3, subclinical hypocalcemia was effectively induced; however, endometrial expression of IL-6, CXCL8, and TNF did not differ between groups 3h after the LPS challenge. The iSCH group showed significantly reduced serum BHB concentrations compared to Control cows. No differences were observed in serum glucose, insulin or NEFA concentrations, or in the endometrial expression of INSR, IRS1, IGF1, SLC2A1, and SLC2A3. In conclusion, PMN infiltration and SE incidence at 34-40 DPP relate to blood Ca immediately post-calving. Ca formate supplementation decreases endometrial PMN infiltration, thereby reducing SE incidence. Transient iSCH does not significantly alter endometrial inflammatory cytokine expression or adversely affect uterine insulin signaling within 3h of an intrauterine LPS challenge. This study provides insights into the role of blood calcium concentration and oral supplementation during the immediate postpartum period on uterine health. We thank INCT-Reprodução Animal, FAPERGS, CNPq, and CAPES for financial support.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Impact of Fluazuron, an antiparasitic compound, on *in vitro* fertilization and early embryo development in cattle

Anabella Andrea Campagna[1], Mariana Carolina Fabra[1], Maria Eugenia Sella[1], Cecilia Cristina Furnus[1], Ana Cristina Carranza-Martin[1,2], Noelia Nikoloff[1,2]

[1]Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (IGEVET, UNLP-CONICET), [2]Facultad de Ingeniería y Ciencias Agrarias (FICA-UCA)

In cattle, fertilization occurs more than 85% of cases; however, a significant proportion of the resulting embryos fail to develop to term (Diskin et al., Reproduction in Domestic Animals, 43:260-7, 2008; Wiltbank et al., Theriogenology, 86:239-53, 2016). Studies demonstrated that veterinary pesticide can altered gametes, reproductive process and cause genotoxic and cytotoxic damage in different cellular types and organisms (Carlsson et al., Aquatic Toxicology, 126:30-41, 2013; Anchordoquy et al., Environmental Science and Pollution Research, 26(3):2998-3005, 2019; Nikoloff, et al., Environmental Science and Pollution Research, 28(23):29188-29199, 2021). Fluazuron (FLZ) is a widely veterinary pour-on antiparasitic, applied along the dorsal midline of cattle during the breeding season to control ticks. The FLZ is not extensively metabolized and remains in tissues as 90% unchanged fluazuron, which may prolong systemic exposure during the critical stage of early embryo development (WHO, WHO Food Additives, 71, 2015). Previous toxicological study showed that 50 µg FLZ/ml negatively affect the bovine embryo development when added to the IVM medium (Campagna et al., Theriogenology, 227:92-101, 2024). Thus, the aim of this study is to evaluate the effect of FLZ added to IVF medium, analyzing the formation of pronucleus (PN) and bovine embryos development. Oocytes were collected from slaughterhouse-derived ovaries and subjected to three consecutives stages of IVP: IVM, IVF and IVC under controlled laboratory conditions. The IVF medium (TALP supplemented with BSA, penicillamine, hypotaurine and heparin sulphate) was treated with 0.5% of DMSO (control) or 50 µg FLZ/ml. After 18 h, PN formation was evaluated (1 PN, 2 PN, or ≥ 3 PN). Embryo cleavage was analyzed 48 h post-IVF. Blastocyst development was recorded on days 7 and 8, and hatching rates were determined on days 9 and 10 of culture. Data were analyzed as a generalized linear model with the GLIMMIX procedure (SAS Institute), statistical significance was set at $P<0.05$. Results showed that: The PN rate did not differ between DMSO and FLZ($P>0.59$). The percentage of cleavage did not differ between treatments ($P=0.95$). However, FLZ decreased the percentage of day 7 blastocysts ($P=0.004$), and total blastocysts ($P=0.002$) compared with control, and finally, the percentage of hatching blastocysts decreased when FLZ was added compared with control ($P=0.001$). It is important to clarify that our study used an acute exposure with a high concentration of FLZ. Such exposures may result from industrial accidents or significant environmental contamination events. However, there is no data available on the effects of low-dose cumulative concentrations resulting from successive applications in bovines. Given the economic relevance of cattle reproduction, further studies need to be conducted to elucidate the mechanism by which FLZ impairs embryonic development.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Increased negative energy balance alters the molecular signature of uterine fluid extracellular vesicles and influences embryo metabolism *in vitro*

Juliana Germano Ferst[1], Juliano Coelho Da Silveira[1], Constantine A. Simintiras[2], Patrick Lonergan[3]

[1]Universidade de São Paulo, [2]Louisiana State University, [3]University College Dublin National University of Ireland

Dairy cows frequently experience negative energy balance (NEB) during the post-calving period, which significantly impacts reproductive tissues and leads to economic losses due to reduced availability of animals for the next breeding season. Extracellular vesicles (EV) are key mediators of intercellular communication and modulate reproductive processes. Previous studies from our group identified that extracellular vesicles from uterine fluid (UF-EV) collected from cows with Low (LNEB) or High NEB (HNEB) at 60 days post-calving (DPC) carry miRNAs involved in metabolic regulation, as miR-199a-3p, miR-145, miR-497, and miR-22-3p. Based on these findings, the objective of this study was to investigate whether UF-EV influence embryonic metabolism *in vitro*. Immature cumulus-oocyte complexes (COC) were aspirated from slaughterhouse-derived ovaries and matured in groups of 50 for 24 h in 500 µl of commercial maturation medium (IVM medium; Stroebech Media) at 38.8°C under 5% CO2 in air. *In vitro* fertilization (IVF) was performed by co-incubating matured COC with 1 × 10^6 density-gradient-separated spermatozoa from a proven-fertility bull. At 20 h post-insemination (Day 1; IVF = Day 0) presumptive zygotes were denuded and cultured in groups of 25 in 50 µl droplets of commercial culture medium (IVC medium; Stroebech Media) supplemented with 2.5% UF-EV from LNEB or HNEB cows until Day 7. On Day 7, blastocysts were harvested from each treatment group (Control – without EV; LNEB-UF-EV or HNEB-UF-EV) and cultured for an additional 24 h in groups of 15 in 500 µl of IVC medium supplemented with the same UF-EV treatment (six replicates, 2.411 COC in total). Cleavage and blastocyst rates were recorded on Days 3 and 7, respectively. After 24 h of extended culture (Day 8), the medium was collected for metabolomic analysis performed by mass spectrometry using a Waters Synapt XS ESI-Q-IMS-TOF system coupled to a Waters Acquity Premiere UPLC. Single-factor analyses were performed using MetaboAnalyst 6.0, applying an unpaired t-test with $P < 0.1$, fold-change 1.0, assuming equal variance. Cleavage and blastocyst rates were unaffected by UF-EV treatment. A total of 621 metabolites were identified. Metabolites, such as hexyl glucoside, 2-phenylethanol and methylajoene, were more abundant in UF-EV-only media, suggesting that EV can carry metabolites. In embryo-culture media treated with HNEB-UF-EV, stearoyl lactic acid levels was increased compared to LNEB group. Enrichment analysis of the metabolites detected in the embryo-culture media treated with HNEB-UF-EV revealed pathways related to fatty acid metabolism, energy production and protein synthesis. These findings suggest that embryo metabolism is negatively modulated by UF-EV from HNEB donor animals, contributing to our understanding of how metabolic stress alters the uterine environment and embryo development.

Support: FAPESP grants 2023/15072-9 and 2021/06645-0.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Influence of early development kinetics and the presence of fetal bovine serum in the culture medium on bovine embryo production

Wallery Caroliny Costa da Costa[1,2], Christopher Junior Tavares Cardoso[3], Bianka Drawert[4], Taynara dos Santos Santana[1], Ralf Poehland[4], Fabiana de Andrade Melo Sterza[1,5,2]

[1]Universidade Estadual de Mato Grosso do Sul, [2]Grupo de Estudos em Tecnologia da Reprodução Animal, [3]Assistência técnica e gerencial em bovinocultura de corte, [4]FBN Leibniz Institute, [5]Universidade Federal de Mato Grosso do Sul

The *in vitro* production of bovine embryos is directly influenced by the composition of the culture medium. This includes components such as fetal bovine serum (FBS), glucose, amino acids and growth factors. All these factors can modulate development kinetics and blastocyst rates. This study accurately compared blastocyst production and lipid content between embryos classified as fast (≥ 4 cells) and slow (< 4 cells) 41 hours after *in vitro* fertilisation (IVF - 22 hours) with a semen from a single Holstein bull Blues 240297, cultured in commercial media supplemented (SFB - BR= Cenatte) with SFB 5% (Cripion FB0012) or not (NSFB- UK = BIOSCIENCE). Ovaries of Holstein females were obtained ia local slaughterhouse. Blastocyst rate was calculated on D7. The expanded blastocysts (D7 and D8) were submitted to fluorescence microscopy. Mitochondrial activity (MitoTracker® Orange), lipid content (Bodipy) and cell number (Hoechst 33342) were assessed. The embryos were kept in MitoTracker 40 min 1 µl Stock + 2 ml PBS + 0.4% BSA, OrangeBodipy 10 min 2.3 µl Stock + 1 ml PBS + 0.4% BSA, then the blastocysts were left overnight in PAF, 500µl, after they were fixed in PBS 3 ml + 2.5 µg Hoechst/ml, for image acquisition. Data obtained by Fluorescence analysis were analyzed using ImageJ, and the data was statistically processed using the GLIMMIX procedure in SAS. This experiment was performed in 8 replicates. The results showed no statistically significant difference ($p>0.05$) in cleavage rates between NSFB (n= 1115; 85.33%) and SFB (n=1133; 84.13%), nor in blastocyst rates between fast (n= 224; 48.14%) and slow (n= 275; 34.43%) early cleavage embryos. There was no interaction ($p>0.05$) between kinetics and culture medium. Fast embryos (n=188) showed higher ($p<0.05$) mitochondrial activity and lower lipid accumulation compared to slow embryos (n= 263). In NSFB medium, there was higher mitochondrial activity (39,696 ± 2,450.13 vs. 30,660 ± 2,450.13; Pr > F < 0.0001) and higher cell number (169.41 vs. 166.09; Pr > F < 0.0001). SFB resulted in higher lipid content (27,474 ± 1,809.56 vs. UK; Pr > F < 0.0001). It is possible to obtain better quality embryos by selecting early cleavage embryos. These findings suggest that selecting early-cleavage embryos can improve embryo quality. Additionally, using culture media without fetal bovine serum may optimize lipid metabolism and enhance embryo quality.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Interaction between sperm and uterine fluid extracellular vesicles from high- and low-fertility heifers impacts sperm quality, fertilization ability, and early embryonic development

Raissa Braido Rangel[1], Ana Beatriz Bossois Moura[1], Laura Gabrielli Haupenthal[1], Maria Alice de Almeida[1], Mateus José Sudano[2], Guilherme Pugliesi[1], Rui Machado[3], Juliano Coelho Da Silveira[1], Felipe Perecin[1], Maíra Bianchi Rodrigues Alves[4]

[1]Universidade de São Paulo, [2]Univerdade Federal de São Carlos, [3]Empresa Brasileira de Pesquisa Agropecuária, [4]Universidade Estadual Paulista

The aim here was to evaluate the effects of sperm interaction with uterine extracellular vesicles (UF-EVs) derived from high-fertility (HF) and low-fertility (LF) heifers on sperm quality, IVF outcomes and early embryonic development. Canchim heifers previously classified as HF (n=4) or LF (n=4), based on 3 consecutive embryo transfer cycles in which achieved pregnancy or failed to establish pregnancy in all cycles, respectively, were selected. UF-EVs were collected by uterine flushing on the day of artificial insemination after estrous synchronization, with all heifers showing proper estrogenic responses. EVs were isolated by ultracentrifugation and characterized based on size, morphology, and the expression of EV-specific markers. Sperm from 5 bulls selected based on similar blastocyst rates were incubated for 30 minutes with UF-EVs at a concentration of 4,000 EVs/sperm in TALP medium. In the control group (CO), sperm were incubated under identical conditions without EVs. After incubation, sperm quality was assessed through Computer-Assisted Sperm Analysis for sperm kinetics and flow cytometry for functional parameters including plasma and acrosomal membrane integrity, lipid peroxidation, cholesterol efflux, and membrane lipid disorder. In addition, sperm were used to IVF of in *vitro*–matured oocytes, and the presumable zygotes were cultured for 7 days. IVF rates and blastocyst cell numbers were evaluated. Statistical analyses were performed using SAS software, with the Chi-square test for IVF rates and ANOVA followed by Tukey's test for blastocyst cell numbers and sperm parameters ($P<0.05$). A significant difference was observed in the proportion of sperm with damaged plasma and acrosomal membranes (DPDA) (HF:49.26±2.90^{a}; LF:38.01±2.78^{b}; CO:35.33±1.20^{b}; p=0.008). No differences were observed in first cleavage rates (HF:28.28±5.30; LF:24.49±4.34; CO:31.57±4.78; p=0.07), cleavage rates (HF:33.84±3.93; LF:29.85±3.83; CO:37.63±4.30; p=0.05), or blastocyst cell numbers (HF:169.20±6.48; LF:157.30±8.27; CO:158.60±8.8; p=0.51). However, differences were detected in second polar body extrusion (HF:33.82±3.80^{a}; LF:21.70±2.84^{b}; CO:28.16±3.44^{ab}; p=0.002) and blastocyst rates (HF:14.65±3.61^{b}; LF:13.01±2.69^{b}; CO:19.95±3.28^{a}; p=0.02). These findings indicate that HF-EVs induce the highest levels of DPDA in sperm while simultaneously enhancing fertilization capacity, suggesting a mechanism of sperm selection mediated by UF-EVs, which corresponds to the selective role of the uterus. Despite enhancing fertilization rates, EVs-treated groups exhibited lower blastocyst rates compared to the CO. This result may reflect a molecular imbalance in the sperm induced by EV treatment, which potentially changes the sperm cargo and, consequently, impacts paternal contribution and embryonic development. Further studies investigating the UF-EV and zygote miRNA profiles are in progress to elucidate the mechanisms of sperm-EV molecular communication. CAPES 001; FAPESP 2024/05151-1.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

In vitro embryo performance of bulls classified by field fertility in FTAI programs

Jorge Luis Torres Garcia[1,2,3], Taynara dos Santos Santana, Wallery Caroliny Costa da Costa[1,4], Micheline Feitosa de Castro, Marcelo dos Santos Cleto[1], Wilian Aparecido Leite da Silva, Mariane Gabriela Cesar Ribeiro Ferreira, Rafaela Lira dos Santos Silva, Ériklis Nogueira, Fabiana de Andrade Melo Sterza

[1]Universidade Estadual de Mato Grosso do Sul, [2]Universidad de los Llanos, [3]EMBRAPA GADO DE CORTE, [4]Grupo de Estudos em Tecnologia da Reprodução Animal

The influence of bull fertility on *in vitro* embryo production (IVEP) in cattle remains poorly understood. This study evaluated blastocyst development using semen from bulls with high (HF) and low (LF) fertility, classified based on pregnancy rates (PR) in fixed-time artificial insemination (FTAI) programs. The bulls used were sourced from commercial breeding centers and classified according to their average fertility rates in FTAI programs, with BIF accuracy above 24%. Fertility classification was performed using a Bayesian animal model that accounted for fixed effects (e.g., contemporary group and cow age) and random effects (e.g., direct genetic effects). Bulls with PR ≥ 55% were designated as high fertility (HF), whereas those with PR ≤ 40% were classified as low fertility (LF). A total of 1,500 oocytes were recovered from ovaries collected at a slaughterhouse, and only those graded as 1 or 2 were used. *In vitro* maturation, fertilization, and culture were conducted using a commercial medium (Cenatte®) in an incubator maintained at 38.5 °C under a controlled atmosphere of 6% CO_2, 6% O_2, and maximum humidity. Five bulls were selected for each fertility group and assessed across three replicates, totaling 15 experimental routines. Semen was processed using a Percoll gradient and evaluated for motility, vigor, and sperm concentration. The insemination dose was standardized to 2 × 10^6 sperm/mL. All assessments were conducted by a trained technician. The average post-thaw sperm motility was 73.6% in the HF group and 69% in the LF group (P = 0.4715). The median sperm vigor score was 3 for both groups. On day 7 (D7), the blastocyst rate in the HF group was 28.1%, with 67% classified as early blastocysts (BI) or blastocysts (BL), and 33% as expanded (BX) or hatched blastocysts (BE). In the LF group, the blastocyst rate was 28.3% (P > 0.05), with 58% BI/BL and 42% BX/BE (P = 0.3155). Under the conditions of this study, bull fertility as determined by FTAI programs—considering post-thaw motility above 69% and a vigor score of 3—did not significantly affect blastocyst yield or developmental kinetics. These findings suggest that bulls with lower reproductive performance in FTAI programs may still be suitable candidates for IVEP protocols.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Maintenance of the bovine corpus luteum of pregnancy after interferon-tau: Novel evidence for a role of Pregnancy-Associated Glycoproteins (PAGs)

Livia Martino Duarte[1], João Paulo Nascimento Andrade[2], Pedro Leopoldo Jerônimo Monteiro Junior[3], Jackson Ford Gille[1], Autumn Rutha Joy[1], Marcelo S. El Azzi[1], Dimitra Varelis[1], Alexander Koops[1], Alysia Vang[1], Milo Wiltbank[1]

[1]University of Wisconsin-Madison, [2]West Texas A&M University, [3]University of Florida

Interferon-tau (IFN-τ), an early embryonic signal, maintains bovine corpus luteum (CL) from D14–21 of gestation, by suppressing PGF pulses. After D21, PGF pulses mount, yet the CL is maintained through pregnancy by undefined mechanisms. Because PAGs surge as IFN-τ wanes and the physiological role(s) of PAGs are not defined, we tested if PAGs maintain the CL when given during IFN-τ period (D14-20) and early PAG period (D21 on). Open Holstein heifers (expt 1: n=12, expt 2: n=25) were synchronized with cloprostenol 500 µg i.m. (PGF; Parnell, KS, USA) followed 2d later by GnRH, 200 µg i.m. (Parnell) and insertion of an intravaginal progesterone (P4) implant (CIDR; Zoetis, NJ, USA); another PGF was given at 5 and 6d with CIDR removed at last PGF. Heifers ovulated spontaneously (designated D0) as determined by ultrasound. Blood flow to CL was done daily with color Doppler, and P4 was monitored daily by 125I radio-immunoassay and PAGs by BioPRYN Flex ELISA. The PAGs used in these experiments were isolated from cotyledons collected at parturition from Holstein cattle and purified through multiple protein- exclusion steps. In expt 1, heifers were assigned to intrauterine infusions (ipsilateral horn) every 48h from D14 to 24: saline control (n=4), Low-PAG (0.635 mg of PAGs; n=4), or High-PAG (1.3 mg of PAGs; n=4). In expt 2, heifers received daily infusions (D14–D24 after ovulation) of bovine serum albumin 2 mg (control, ipsilateral; n=8) or 2 mg PAGs in uterine horn contralateral (ContraLat; n=9) or ipsilateral (IPSI; n=8) to CL (test if PAGs act locally or systemically). Blood was collected every 12 h (D14–24) or daily (D25-30). Linear models including treatment, day, block, and their interaction were fitted in RStudio; residuals were assessed, Box–Cox transformations applied when needed, and pairwise contrasts generated with Tukey's HSD. In expt 1, mean PAGs were undetectable in Control, 0.63 ±0.4 ng/mL in Low-PAG, and 3.7±1.8 ng/mL in High-PAG. Both PAG-treated groups maintained greater P4 versus Control ($P<0.0001$), with luteolysis delayed to D22 (Low-PAG) and D23 (High-PAG) versus D17 (Control; $P<0.0001$), and no difference between Low and High groups. In expt 2, circulating PAGs from D14.5 (12h post-infusion) to D19 (day before mean luteolysis day in ContraLat group) did not differ between IPSI (3.8±1.6 ng/mL) and ContraLat (3.3±1.7ng/mL), but were undetectable for Control ($P<0.0001$). IPSI heifers exhibited prolonged CL lifespan (D25) compared to ContraLat (D20) and Control (D16; $P<0.0001$). These findings indicate that PAGs can prolong the CL lifespan even in the absence of IFN-τ. Thus, intrauterine infusion of PAGs maintains the bovine CL through a local mechanism (ipsilateral but not contralateral) that is likely not systemic (ContraLat and IPSI had similar circulating PAGs). This represents a novel action of PAGs that is of potentially great physiological importance, although the precise mechanism(s) activated by PAGs remain to be determined.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Melatonin supplementation at distinct IVP stages improves bovine embryo development and quality

Hallya Beatriz Sousa Amaral[1], Ana Caroline Chaves Vall Nicolás[1], Laryssa Ketelyn Lima Pimenta[1], José Eduardo Vieira Chaves[2], Margot Alves Nunes Dode[1,3]

[1]Universidade de Brasília, [2]Universidade Federal de Goiás, [3]Empresa Brasileira de Pesquisa Agropecuária

Melatonin has well-documented benefits when supplemented during IVM or IVC, however, its continued exposure throughout the IVP process remains poorly understood. Given its antioxidant and metabolic regulatory properties, this study aimed to elucidate the effects of melatonin on embryo production and quality and to determine the most effective supplementation strategy to optimize IVP results in bovine embryos. Bovine oocytes were collected from slaughterhouse ovaries and allocated into four experimental groups: Control (without melatonin), IVM + Mlt (melatonin added during maturation), IVC + Mlt (melatonin added during culture) and IVM/IVC + Mlt (melatonin added during both periods). Melatonin was added at a concentration of 10-9 M. Cleavage rate (D2) and blastocyst rate (D6 and D7) were recorded. On D7, blastocysts were assessed for reactive oxygen species (ROS) using H2DCFDA (Fidelis et al., 2020, DOI: 10.1155/2020/6046013), lipid content using BODIPY 493/503 (Faria et al., 2021, DOI: 10.1071/RD20254), mitochondrial activity using MitoTracker Deep Red (Faria et al., 2021, DOI: 10.1071/RD20254), and total cell number using Hoechst staining 33342 (Fidelis et al., 2020, DOI: 10.1155/2020/6046013). Data were analyzed using the Chi-square, ANOVA, or Kruskal-Wallis tests ($p \leq 0.05$). Blastocyst rates on D7 were significantly higher ($p < 0.05$) in the IVM + Mlt (43%, 659/1295) and IVC + Mlt (44%, 677/1533) groups compared to the Control (35%, 481/1356) and IVM/IVC + Mlt (40%, 556/1150) groups. Embryos in all melatonin-treated groups exhibited faster developmental kinetics than the Control group, with higher rates of blastocysts expanded and hatched on D7 ($p < 0.05$). In addition, all treated groups had significantly lower levels of ROS and lipid content, as well as increased mitochondrial activity ($p < 0.05$). However, no differences were observed in the total number of cells between the groups ($p > 0.05$) (Control = 158.04 ± 8.34, n = 23; IVM + Mlt = 160.96 ± 8.35, n = 24; IVC + Mlt = 161.83 ± 10.93, n = 23; IVM/IVC + Mlt = 164.23 ± 11.45, n = 22). These findings indicate that melatonin improves the environment *in vitro*, reducing oxidative stress and lipid accumulation, while also improving mitochondrial function, thereby improving embryo development and quality. Thus, melatonin supplementation during IVM, IVC, or both represent a promising strategy to optimize bovine IVP outcomes.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Melatonin Supplementation During Pre-Maturation: Effects on Oxidative Stress and Developmental Competence of Bovine Oocytes

Laryssa Ketelyn Lima Pimenta[1], Nayara Ribeiro Kussano[2], José Eduardo Vieira Chaves[3], Hallya Beatriz Sousa Amaral[1], Maurício Machaim Franco[2], Margot Alves Nunes Dode[1,2], José Felipe Warmling Sprícigo[3]

[1]Universidade de Brasília, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]Universidade Federal de Goiás

Low oocyte competence and oxidative stress limit the success of IVP by impairing maturation. We hypothesized that melatonin supplementation during pre-IVM could enhance redox balance and improve oocyte competence. COCs were divided into three groups: control (24 h of IVM), pre-IVM (6 h of pre-IVM + 24 h of IVM), and pre-IVM+MTn (6 h of pre-IVM with 10^{-9} M melatonin + 24 h of IVM). Immature and mature oocytes were assessed for meiotic progression using Hoechst 33342 staining, intracellular reactive oxygen species (ROS) levels using H2DCFDA. A total of 1752 COCs were subjected to IVP; cleavage and blastocyst rates were evaluated on D2 and D7, respectively. Furthermore, 4 pools of 15 expanded blastocysts were used to assess relative mRNA expression of embryo quality-related genes (PLAC8, KRT8, PRDX6, and SLC2A3); 4 pools of cumulus cells (from 20 COCs each) and 4 pools of 20 oocytes were analyzed for the expression of antioxidant-related genes: CAT, SOD1, SOD2, GSS, and NFE2L2 in oocytes; and SOD1 and SOD2 in cumulus cells, using qPCR. Statistical analyses were performed by ANOVA or t-test for parametric data and by Kruskal–Wallis or Mann–Whitney tests for nonparametric data. The relative expression of each gene was calculated using the ΔΔCt method with efficiency correction. Embryo development was assessed by the chi-square test. Pre-IVM, with or without melatonin, did not affect nuclear maturation, as most oocytes reached the metaphase II stage after 24 h of IVM (control= 17/18, 94.4%; pre-IVM= 9/9, 100%; pre-IVM+MTn= 12/12, 100%). ROS levels increased during IVM in all groups (before IVM: control= 25, pre-IVM= 26, pre-IVM+MTn= 27; post-IVM: control= 27, pre-IVM= 23, pre-IVM+MTn= 16). However, this increase was significantly greater ($p \leq 0.05$) in the pre-IVM+MTn group after 24 h compared to control. Regarding gene expression, no differences were observed in cumulus cells ($p > 0.05$). However, in oocytes, GSS and NFE2L2 gene expression at the end of maturation was lower in both pre-IVM groups ($p \leq 0.05$), irrespective of melatonin supplementation. Notably, only the melatonin-exposed group exhibited reduced SOD2 and CAT gene expression compared to control. Despite these effects, the pre-IVM+MTn group showed significantly higher ($p \leq 0.05$) cleavage (75.0%) and blastocyst (30.8%) rates on D2 and D7, respectively, compared to the control (66.0% and 25.8%). However, these rates were similar ($p > 0.05$) to the pre-IVM group without melatonin (79.4% and 35.9%). Moreover, a reduction ($p \leq 0.05$) in PLAC8 gene expression was observed in blastocysts derived from melatonin-treated oocytes compared to the control and pre-IVM groups. These findings indicate that although pre-IVM improves blastocyst development, melatonin supplementation during this period does not confer additional benefits to oocyte competence or embryo development.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Metabolic and epigenetic crosstalk: glutamate-mediated dna demethylation in *in vitro* embryos

João Vitor Alcantara da Silva[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC

Despite advances, bovine IVP efficiency is hindered by epigenetic and metabolic dysregulation. During early embryonic development, global DNA methylation levels decrease through the action of Ten-Eleven Translocation (TET) enzymes, creating a permissive environment for transcriptional activation at the 8-16 cells stage. Evidence suggests that TET enzyme activity is dependent on intracellular levels of alpha-ketoglutarate, which acts as a cofactor in the demethylation process. Glutamate (Glu), a non-essential amino acid, can be converted into alpha-ketoglutarate. In this study, we investigated the relationship between Glu metabolism and DNA methylation during early bovine embryonic development. Embryos were produced *in vitro* using standard protocols. Following fertilization, presumptive zygotes were randomly assigned to one of four groups based on Glu supplementation: 0 mM (no supplementation), 0.16 mM (low), 0.32 mM (physiological), and 0.50 mM (high). Embryos were cultured in Embryonic Culture System 50 (ECS50) medium, supplemented with 4 mg/mL BSA and without glutamine until Day 7. Embryos were collected on Day 3 (cleavage rates) and Day 7 (blastocyst rates) of *in vitro* culture to assess global DNA methylation levels using immunostaining with an anti-5-methylcytosine monoclonal antibody. A total of 25 embryos per group and stage were analyzed via fluorescence microscopy and image analysis was performed using Fiji software. Data were analyzed using ANOVA, with mean ± SEM and significance set at $P < 0.05$ (0 mM vs. treatment groups). On Day 3, embryos in the 0 mM group exhibited significantly lower levels of DNA methylation compared to all supplemented groups (0.16 mM: 14.3±0.6 and 20.3±1.3, respectively, p=0.0007; 0.32 mM: 16.6±0.7 and p=0.0246; 0.50 mM: 19.8±0.7 and p<0.0001), pointing to a connection between Glu metabolism and methylation regulation. On Day 7, reduced DNA methylation was observed exclusively in the inner cell mass (ICM) and trophectoderm of embryos cultured with 0.16 mM Glu vs 0 mM (ICM: 17.1±2.6. and 40.4±2.5, respectively, p=0.0043; trophectoderm: 16.1±0.6 and 32.8±2.0, respectively, p<0.0001), indicating that low Glu levels may enhance TET enzyme activity, but its absence may enroll compensatory mechanisms to promote the de novo DNA methylation. Cleavage rates did not differ among the groups (0.16 mM: 66.3±1.3 and 59.7±2.7, respectively, p=0.3254; 0.32 mM: 63.4±4.3 and p=0.8411; 0.50 mM: 71.1±3.0 and p=0.5648). However, blastocyst rates were significantly higher in embryos cultured with 0.32 mM and 0.50 mM Glu (0.32 mM: 10.1±3.4 and 32.0±5.4, respectively, p=0.0223; 0.50 mM: 33.4±4.5 and p=0.0223), suggesting that disruptions in DNA methylation during EGA may compromise embryo viability. In conclusion, Glu metabolism may influence DNA methylation during early bovine embryonic development, suggesting its potential relevance for improving epigenetic regulation in IVP systems.

Supported by: FAPESP (24/20178-3, 21/11747-6).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Metabolic changes in oocytes exposed to negative energy balance environment during oocyte maturation

Ana Beatriz Florencio[1], Patricia Kubo Fontes[1], João Vitor Alcantara da Silva[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC

High-producing dairy cows face reproductive challenges due to Negative Energy Balance (NEB), which affects the quality of oocytes and embryos. NEB decreases glucose availability and increases the levels of non-esterified fatty acids (NEFAs) and β-hydroxybutyrate (BHB) in follicular fluid, possibly compromising cellular metabolism and causing oxidative stress. This also reduces the oviduct's ability to support embryos, resulting in a lower blastocyst rate and increased apoptosis. In this scenario, the objective of this work is to investigate the metabolic effects on oocytes exposed to NEB during *in vitro* maturation. Our hypothesis is that oocyte maturation under stress conditions induced by the NEB-conditioned environment compromises mitochondrial functionality, impacting oxidative stress production and meiotic progression. For this purpose, oocytes were collected and cultured in maturation medium (n= 50 oocytes/group). The experimental groups were formed: Control (DMEM (low glucose) supplemented + 4% BSA + 0.007% ethanol) and NEB (DMEM (low glucose) + 4% BSA + 230 µM palmitic acid, 280 µM stearic acid, 210 µM oleic acid, and 4 mM BHB), incubated at 38.5°C and 20% CO_2). Thus, the oocytes were cultured for 22-24 hours in the 5-well plate system. After incubation, the oocytes were subjected to metabolic analyses: levels of Reactive Oxygen Species (CellROX® Green, Invitrogen); Mitochondrial Membrane Potential (MitoTracker® Red CMXRos, Invitrogen). In both tests quantification was performed by the number of pixels in ImageJ-FIJI. Meiotic progression was assessed by staining with Hoechst-33345 and fixation with 4% paraformaldehyde. Data were analyzed by ANOVA and Tukey test using GraphPad Prism software, with a significance level of 5%. As result, ROS levels showed a significant increase in the NEB group compared to the control group (p =0.0002). NEB group presented greater mitochondrial activity, but with high variability, which may suggest mitochondrial dysfunction or adaptation to stress (p=0.001). As expected, NEB group showed a reduced maturation rate to the MII stage. It is concluded that NEB environment significantly compromises oocyte quality, as evidenced by lower maturation rate to the MII stage, increased levels of ROS and increased variability in mitochondrial activity. These findings suggest that NEB-induced metabolic stress negatively affects oocyte competence, likely through mechanisms related to oxidative stress and mitochondrial dysfunction.

Supported by: FAPESP (2024/04153-0).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Oocyte germinal vesicle (GV) stage profile influences the efficacy of a long-term pre-IVM with C-type natriuretic peptide and sildenafil

Helena Fabiana Reis de Almeida Saraiva[1], Priscila Helena dos Santos[1], Luca Angi Souza[1], Juliano Coelho Da Silveira[1], Cláudia Lima Verde Leal[1], Felipe Perecin[1], Flávio Vieira Meirelles[1], Lawrence Charles Smith[2,1]

[1]University of São Paulo, Faculty of Animal Science and Food Engineering, [2]Université de Montreal

The oocyte's developmental stage is critical for *in vitro* embryo production outcomes. Our goal was to evaluate the synergistic effect of C-type natriuretic peptide (CNP) and sildenafil (SDF) on causing meiotic arrest (pre-IVM) and in subsequent resumption during IVM. In Exp1, we tested the meiotic arrest efficacy of CNP-SDF by exposing COCs for different periods, i.e. 12, 15, and 18h in TCM199 with pyruvate (0.2 mM), BSA (0.4%), and gentamicin (50 µg/mL) (BM) plus CNP (100 nM), SDF (100 µM), rhFSH (10-4 IU/mL), estradiol (500 ng/mL), progesterone (50 ng/mL), and androstenedione (50 ng/mL), at 38°C in 5% CO_2. Immature COCs served as controls (CT). GV profiles and meiotic arrest rates were assessed by anti-lamin A/C and DNA immunostaining. Data from six replicates were clustered by the GV profile (P-) observed in CT: Gaussian or P1 (25% GV1, 43% GV2, 32% GV3, n=47), GV2-enriched or P-2 (4% GV1, 71% GV2, 25% GV3, n= 24), and GV3-enriched or P-3 (2% GV1, 44% GV2, 54% GV3, n=40). In Exp2, we tested CNP-SDF (15h) in a GV2-enriched profile (P-4; GV1 30%; GV2 61%, GV3 9%, n=43) and a GV1-enriched profile (P-5; GV1 52%, GV2 29%, GV3 19%, n=31). COCs were (a) submitted to IVM in BM plus AREG (100 ng/mL), rhFSH (10-2 IU/mL), IGF1 (10 ng/mL), progesterone (150 ng), and estradiol (50 ng/mL) at 38°C in 5% CO2, (M-P groups) or (b) pre-matured for 15h before IVM (Pm-P groups). Meiotic arrest efficacy was evaluated as in Exp1. COCs were collected at 12, 15, and 18h of IVM and assessed for first polar body extrusion (PBE). MII and non-MII oocytes were immunostained for α-tubulin/DNA to assess chromatin configurations: GVBD, PMI, MI, AI, TI, and MII. Meiotic arrest and PBE rates, and chromatin configuration frequencies were compared by Chi-square test (α=0.05). Exp1: CNP-SDF efficiency varied by GV profile. P-1 showed higher ($p<0.05$) arrest rates: 100% at 12h (20/20), 82% at 15h (31/38), and 74% at 18h (29/39). P-2 and P-3 showed lower ($p<0.05$) values compared to P-1: 50% (10/20), 25% (4/16), and 36% (14/39) for P-2, 46% (26/57), 41% (11/27), and 29% (13/45) for P-3 at 12, 15 and 18h, respectively. Exp2: arrest rates were 38% (11/29) in P-4 and 81% (17/21) in P-5. At 12h IVM, PBE was higher ($p<0.01$) in Pm-P-4 and Pm-P-5 (50% and 41%) than in M-P-4 and M-P-5 (4% and 0%). No differences in PBE were observed at 15h (~40-60%) or 18h IVM (~70-75%). At 12h IVM, M-P groups showed earlier chromatin stages (70-75% PMI, 10-25% MI), whereas Pm-P groups exhibited advanced configurations (36-44% MII). By 15 and 18h, profiles were similar. Chromatin/spindle abnormalities were low in M-P-4 (12h: 10%; 15h: 9%; 18h: 7%) but increased in M-P-5 (12h: 0%; 15h: 17%; 18h: 33%, all in MII). Pm-P-4 showed 31%, 21% and 0%, and Pm-P-5 had 25%, 17% and 13% of chromatin abnormalities at 12, 15, and 18h. Pre-IVM with CNP-SDF altered meiotic kinetics but did not affect MII rates. This approach may help reduce chromatin/spindle abnormalities in GV1 oocytes across IVM.

Funding: FAPESP (2021/09886-8).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Oviductal cells modulate global DNA methylation dynamics in bovine embryos during *in vitro* co-culture

Patricia Kubo Fontes[1], Ana Beatriz dos Reis Bartoli[1], Ana Beatriz Florencio[1], Thays Antunes[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC

Preimplantational embryos undergo dynamic changes in their epigenetic profile, including DNA methylation. In this regard, the 5-methylcytosine (5mC) profile has been proven to be suboptimal in *in vitro*-produced embryos (IVP) compared to *in vivo*-derived ones (IVD). Since environmental factors affect the epigenetic reprogramming, IVP embryos likely experience deviation due to the absence of the maternal milieu. Herein, to mimic the embryo-maternal communication, we used the Oviductal Magnetic Spheroid (OMS), a 3D oviductal *in vitro* model developed by our research group, and evaluated the 5mC profile in embryos cultured with (co-culture) or without (embryo-only) the OMS. As follows, *in vitro* matured cumulus-oocyte complexes (bovine ovaries from a slaughterhouse) were fertilized and denuded. The presumptive zygotes (PZ) were divided as co-culture (15 PZ + 3 OMS/well) or embryo-only (15 PZ/well) for culture at 38.5°C, 5% CO_2, 5% O_2, and high humidity. The co-culture lasted for four days (the same period that native embryos stay inside the oviduct), followed by three days without OMS until the blastocyst stage. Four independent replicates were analyzed at 30 hours post-insemination (hpi) (initial cleavages), 96 hpi (morula), and 216 hpi (blastocyst stage) to assess embryo yield production (presented as percentage, %) and 5mC level (immunofluorescence analysis processed using ImageJ-1.54p, shown as arbitrary unit, au). Data were analyzed using GraphPad Software (version 8), with a t-test or Wilcoxon test (for parametric or non-parametric data, respectively), $P<0.05$ was the significance level. As a result, co-culture *versus* embryo-only conditions presented similar cleavage (61±19 *vs.* 68±14%, $P>0.05$) and morula (40±13 *vs.* 50±16%, $P>0.05$) rates, but a lower blastocyst rate (25±14 *vs*. 32±13%, $P<0.01$), yet the hatching rate was similar (82±22 *vs.* 68±27%, $P>0.05$). The 5mC levels were lower in embryos from the co-culture *versus* embryo-only conditions at 30- (17±5 [n=12] *vs.* 34±17au [n=13], $P<0.0001$) and 96-hpi (16±10 [n=15] *vs.* 37±37au [n=16], $P<0.0001$), but higher at 216-hpi (expanded blastocysts only), either in the inner cell mass (ICM, 26±14 [n=9] *vs.* 14±4au [n=10], $P<0.0001$) and in the trophectoderm cells (TE, 30±14 [n=9] *vs.* 14±4au [n=10], $P<0.0001$). Interestingly, the 5mC level through time of culture showed a progressive pattern in the co-culture condition (30hpi = 96hpi < ICM < TE, $P<0.05$), whereas the embryo-only condition showed a decreasing pattern (30hpi > 96hpi = ICM = TE, $P<0.05$). In conclusion, the presence of OMS during IVP of bovine embryos interferes with the embryonic DNA methylation dynamics, corroborating the knowledge that the epigenetic reprogramming is sensitive to maternal environmental conditions. With further evaluation of the efficiency of this co-culture system, we might be able to apply it as a tool to elucidate the IVP embryo requirements for promoting an IVD-like epigenetic profile.

Supported by: FAPESP (21/11747-6, 20/02500-4).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Recombinant eCG effectively promotes final follicular growth and ovulation in anestrous ewes

Julia Nobre Blank Camozzato[1], Gabriel Maggi[1], Fabiane Pereira de Moraes[1], Cesar Andrei Marques da Silveira[2], Fernando Caetano de Oliveira[2], Monique Tomazele Rovani[2], Rogério Ferreira[3], Felipe Zandonadi Brandão[4], Arnaldo Diniz Vieira[1], Bernardo Garziera Gasperin[1]

[1]Fisiopatologia e Biotécnicas da Reprodução Animal - UFPel, [2]Universidade Federal do Rio Grande do Sul, [3]Fundação Universidade do Estado de Santa Catarina, [4]Universidade Federal Fluminense

This study aimed to evaluate the effects of recombinant eCG (reCG) at a dose similar to that used for estrus synchronization in cattle, on fertility in ewes during the anestrus to transition period, as a potential substitute for conventional purified eCG. In Experiment 1, conducted during September (anestrous season), 27 ewes received intravaginal devices (IVD) with progesterone (Primer-PR, Agener União, São Paulo, Brazil) for 7 days and were allocated to control (n=9), eCG (n=9) or reCG (n=9) groups. At IVD removal, all animals received 250 µg cloprostenol (Estron; Agener União, São Paulo, Brazil), while eCG and reCG groups were also treated with 400 IU eCG (SincroeCG; Ourofino, Cravinhos, Brazil) and 105 IU reCG (Foli-Rec; Ceva, Paulínia, Brazil) respectively. Follicular dynamics (FD) evaluation was performed at 24, 36 and 48 h after IVD removal. Ovulation (OV), CL area (CLA) and CL blood perfusion (CLP) were assessed via transrectal ultrasound 7 days after IVD removal. Luteal function was assessed 7 and 12 days after IVD removal by serum P4 concentration. In Experiment 2, 441 ewes were treated with similar protocol to evaluate estrus (ER), pregnancy (PR) and conception (CR) rates in five commercial farms during early December to mid-January (transition to breeding season). Statistical analyses were performed in JMP18 software (α=0.05) using ANOVA (FD, CLA, CLP), logistic regression (OV, ER, PR and CR) or non-parametric tests (P4) with comparisons by Tukey, Student's T or Wilcoxon tests. Results in Experiment 1 showed that eCG (1.07±0.27 mm/day) and reCG (1.05±0.27 mm/day) had higher follicular growth compared to controls (0.01±0.27 mm/day) (P<0.05). Ovulation rates were higher in eCG (100%; 9/9) and reCG (88.9%; 8/9) versus control (0%) (P<0.001). On day 7 after IVD removal, all ewes (9/9) from control group and one ewe (1/9) from reCG group showed serum P4 below luteal levels (<1 ng/mL). CL area (mm2) and perfusion (%) did not differ between eCG (56.62±9.86 mm2; 13.6±6.24%) and reCG (55.58±9.89 mm2; 10.66±3.67%) groups. Serum P4 concentrations of animals with a single CL after treatment were higher in eCG (n=5; D7=7.05±1.21 ng/mL; D12=8.82±1.74 ng/mL) than reCG group (n=6; D7=3.38±0.33 ng/mL; D12=4.59±0.39 ng/mL) (P<0.001). In Experiment 2, ER was higher in eCG (93.45%) than in control (76.32%) (P<0.001), with reCG showing intermediate results (87.58%). Treatment did not affect PR (control=43.75%; eCG=48.8%; reCG=48.32%; P=0.82) or CR (control=57.65%; eCG=52.6%; reCG=55.81%; P=0.72). In conclusion, reCG effectively promotes follicular growth and induces ovulation in ewes during seasonal anestrus, representing an alternative to purified eCG. However, during the transition to the breeding season, both reCG and eCG had no effect on pregnancy and conception rates by natural mating compared to control. The authors thank FAPERGS, CNPq and CAPES for their financial support and to all the farmers for providing access to animals and facilities.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Reduction of sperm-oocyte interaction time: Short IVF impacts the rate of polyspermy and expanded blastocyst in cattle

Victória Marques[1], Maria Julia Aparecida Daniel[1], Thalis de Melo Oliveira[1], Lindsay Unno Gimenes[1], Maíra Bianchi Rodrigues Alves[1]

[1]Universidade Estadual Paulista

Conventional *in vitro* fertilization (IVF) usually involves 16 to 20 hours of gamete co-incubation. However, prolonged IVF may increase polyspermy, lead to the accumulation of reactive oxygen species (ROS), and impair embryo development. This study aimed to determine the minimum co-incubation time required to maintain adequate embryo development in cattle. For that, sperm from five Nellore bulls (*Bos indicus*) were selected by Percoll® gradient (45%/90%) and used at a concentration of $1x10^6$ sperm/mL to fertilize 20–25 *in vitro* matured *cumulus*-oocyte complexes (COCs) for 2, 4, 6, 8, 10 or 16 hours. The presumptive zygotes were cultured until day-7 of development, during which the first cleavage (28hpi, hours post insemination), D4-cleavage (96hpi) and blastocyst (168hpi) rates were evaluated. All incubations were performed with 5% CO_2 in air, 38.5 °C, and humidified atmosphere. Blastocysts were classified by stage (early, blastocyst, expanded, hatching) and cell number was quantified by Hoechst 33342 staining using ImageJ®. Fertilization and polyspermy rates were determined at 12 hpi through pronuclear (PN) formation analysis after the zona pellucida removal and Hoechst staining. Analyses were performed by fluorescence microscopy (Olympus IX70). Data were analyzed using chi-square test using the SAS (Statistical Analysis System), with significance at $P<0.05$. The rate of first cleavage was significantly lower after 2h (7.59%^{c}, 18/237) and 4h (16.31%^{b}, 38/233) of IVF compared to 6h (28.82%^{a}, 66/229), 8h (33.33%^{a}, 75/225), 10h (32.09%^{a}, 73/228), and 16h (30.94%^{a}, 69/223). For D4-cleavage, 8h (60.44%^{a}, 136/225) and 10h (58.33%^{a}, 133/228) presented similar results to 16h (63.68%^{a}, 142/223), whereas lower rates were found in 6h (39.30%^{b}, 90/229), 4h (24.46%^{c}, 57/233), and 2h (12.66%^{d}, 30/237). No blastocysts developed in 2h. Blastocyst rates were similar among 6h (23.58%^{a}, 54/229), 8h (31.56%^{a}, 71/225), 10h (28.51%^{a}, 65/228), and 16h (28.25%^{a}, 63/223). Regarding cell number, 6h (123.04±9.22^a), 8h (120.16±6.95^a), 10h (106.28±6.44^{ab}), and 16h (100.64±5.28^{ab}) did not differ, but 4h (75.76±3.92^b) resulted in fewer cells ($P=0.0006$). Expanded blastocyst rates were higher ($P=0.0096$) in 8h (33.80%^{a}, 24/71) compared 16h (14.29%^{b}, 9/63). Fertilization rates were similar between 8h (53.33%^{a}, 64/120) and 16h (62.99%^{a}, 80/127), while polyspermy was higher after 16h (22.05%^{a}, 28/127) than 8h (9.17%^{b}, 11/120) ($P=0.0065$). The results indicate that the conventional IVF duration of 16 hours triggers higher rates of polyspermy. In contrast, reducing the IVF period to 8 hours preserved developmental kinetics and blastocyst cell number, while also promoting a higher percentage of expanded blastocysts at day-7 of development. Thus, shortening the IVF duration to 8 hours represents a viable strategy to optimize the efficiency of IVEP (*In vitro* embryo production) protocols in cattle.

Acknowledgment: PROEX-CAPES, 144/2024 PROPE-UNESP.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Temporal influence of the sphingosine-1-phosphate (S1P) on cell differentiation in bovine embryos

Francieli Perroni Berling[1], Camilla Mota Mendes[1], Mayra Elena Ortiz D Avila Assumpção[1], Marcelo Demarchi Goissis[1]

[1]Universidade de São Paulo

Cell lineages are specified after embryonic genome activation, differentiating into inner cell mass (ICM) and trophectoderm (TE), which develop into the fetus and placenta, respectively. Treatment with sphingosine-1-phosphate (S1P) from 90 hours post-insemination (90 hpi - D4) to 186 hpi (D8) increased the ICM cell number and led to reduced expression of transcription factor *TFAP2C* (Berling et al., Anim. Reprod, 21(3): 139, 2024), which is known to interact with ICM and TE genes in the mouse. Thus, it is important to study the roles of S1P to further understand the dynamics of cell differentiation in cattle. The hypothesis of this study is that S1P initially regulates ICM genes and later regulates TE genes. The objective was to characterize the S1P period of influence on the expression of genes related to cell differentiation. To this end, the dynamics of gene expression related to the first cell differentiation were analyzed after treatment with 200 nM S1P at three different treatment windows, with a control group (IVF) and a vehicle group (NaOH, 37.5 µM). Embryos were cultured *in vitro* using KSOM medium with amino acids, without glucose and fetal bovine serum, in 4-well plates without mineral oil. Embryos were exposed to S1P at different times (T): D4 to D6 (90 to 138 hpi – T1) and collected at the compact morula stage on D6 (T1-MO) or collected at the blastocyst stage at D8 (T1-BL); D6 to D8 until reaching the blastocyst stage (T2). The mRNA was extracted from 4 samples (6-9 pooled embryos) and converted into cDNA. Based on quantitative qRT-PCR data, genes related to the ICM (*SOX2*, *OCT4*) and to the TE (*TFAP2C*, *GATA3*, *CDX2*, *YAP1*) were analyzed, with *GAPDH* and *H2A* used as reference genes for normalization. Data were analyzed using a linear mixed model, considering treatments as independent variables and gene expression as the dependent variable and $p<0.05$. In T1-MO, there was an increase in *TFAP2C* expression in the S1P group compared to the IVF and NaOH groups; *GATA3* was increased in both S1P and NaOH groups compared to IVF; *YAP1* increased in NaOH compared to IVF. For T1-BL, there were increases in *CDX2* and *YAP1* in the S1P group compared to IVF. However, at T2 there was no difference for any of the genes analyzed. There was also no difference in *OCT4* and *SOX2* expression in any of the groups. In conclusion, S1P treatment modulated TE-related genes at the D4-D6 developmental window, while ICM genes were unaffected.

Financial support from: FAPESP 24/14065-1 and 23/06051-8.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

The effect of mitochondrial *Pink1* gene deletion in reproductive outcomes

Ana Rita Tavares Krause[1], Rachel Braz Arcanjo[1], Sergio Antonio Garcia Pereira Junior[1], Daniel de Lima Cometta[1], Marcos Roberto Chiaratti[1]

[1]Universidade Federal de São Carlos

Mutations in the mitochondrial serine/threonine protein kinase *Pink1* (PTEN induced putative kinase 1) cause early onset of Parkinson's disease. When functional, this protein accumulates in damaged mitochondria and initiates mitophagy to remove dysfunctional organelles. Mouse models with a global deletion of the *Pink1* gene have mitochondrial dysfunction, increased production of reactive oxygen species and an imbalance in calcium homeostasis. Despite homozygous knockout mutant mice be viable and fertile, little is known about the effects of this deletion on reproductive outcomes. A series of in vivo, followed by *in vitro* experiments were performed to investigate the effects of this mutation in fertility and embryo development of mutated females and males, using *Pink1* (-/-) knockout males as founders (strain #017946, The Jackson Laboratory; Bar Harbor, ME, USA). DNA analysis from digested ear punches was used to establish the *Pink1* genotype and quantitative real-time PCR results from liver and germinal vesicle oocytes were used to confirm it, at the RNA level. Binary logistic regression was used to compare embryo development rates between genotypes. Cleavage rates of zygotes produced in vivo and cultured *in vitro* among female mice (C57BL/6, 8 weeks old) with the global knockout of *Pink1* (*Pink1* -/-, n = 6), heterozygous (*Pink1* +/-, n = 6) and wildtype (*Pink1* +/+, n = 3), bred to wildtype males were not different (67 vs. 62 vs. 79%, respectively; P = 0.1). Blastocyst rates were higher for heterozygous when compared to homozygous knockout female mice (76.5 vs. 50%, P = 0.04), but were not different between homozygous knockout and wildtype (66%, P = 0.2) and between heterozygous and wildtype female mice (P = 0.5). Cleavage rates of zygotes produced in vivo and cultured *in vitro* from female wildtype mice (C57BL/6, 9 weeks old), bred to male mice (C57BL/6, 8 weeks) having the global knockout of *Pink1* (*Pink1* -/-, n = 4), or heterozygous (*Pink1* +/-, n = 4) or wildtype (*Pink1* +/+, n = 1), were higher for the heterozygous than the homozygous knockout (80 vs. 54%, P = 0.001) or the wildtype (49%, P = 0.0009), but were not different between the homozygous knockout and wildtype (P = 0.8), while blastocyst rates were not different among the genotypes (74% homozygous knockout, 68% heterozygous and 92% wildtype, P = 0.1). This preliminary data indicates that global knockout of the *Pink1* gene may not be deleterious for female or male reproduction, but more experiments using a higher number of animals and investigating oocyte/sperm mitochondrial function may help to understand any negative effect of the mutation on the gametes. This work was supported by FAPESP – Thematic Project (2023/02226-8) and ARTK was supported by FAPESP – Post-Doctoral Fellowship (2024/0727-2).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

The impact of post-ovulatory aging in cellular and morphological responses of cumulus-oocyte complex and oocytes

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

[1]Universidade Federal de São Paulo

The phenomenon of oocyte aging is recognized as a key factor in female fertility. Post-ovulatory aging (POA), defined by the time-dependent decline in oocyte quality between ovulation and fertilization, compromises fertilization rates, developmental competence, and reproductive outcomes. Previous studies in our lab indicated that POA adversely affected mitochondrial integrity, as evidenced by elevated oxidative stress and mitochondrial dysfunction. Therefore, this study used the bovine model to evaluate cellular and morphological responses to POA in cumulus-oocyte complexes (COCs) and denuded oocytes. Bovine ovaries were collected from a slaughterhouse and processed for COC isolation. Selected COCs were allocated into three groups: Immature (0 h IVM), Mature (22 h IVM), and Aged (40 h prolonged IVM). Apoptosis and cumulus cell dispersion in intact COCs were assessed using TUNEL assay (Experiment 1; N=3 replicates) and area quantification (Experiment 2; N=4 replicates), respectively. Meiotic progression was evaluated by Hoechst 33342 staining in Mature and Aged denuded oocytes (Experiment 3; N=5 replicates). Morphological parameters were analyzed in denuded oocytes to assess zona pellucida thickness, perivitelline space area, and cytoplasmic volume (Experiments 4–6; N=3 replicates). Sperm binding to the zona pellucida 1 hour after IVF was quantified in Mature and Aged denuded oocytes using Hoechst 33342 (Experiment 7; N=3 replicates). Fluorescent sample analysis and image acquisition were performed under a fluorescence microscope (Zeiss Axio Imager A2) and Zen Pro software. Area sample analysis and image acquisition were performed under an inverted microscope (Zeiss Primo Vert) and Axio Vision software. Morphological parameters and sperm binding assay were quantified using Image J software. Statistical analyses included Wilcoxon for non-parametric and ANOVA or T test for parametric data (SAS User's Guide, 1989). Apoptosis rates were significantly increased in cumulus cells from aged COCs (P=0.0024 vs. Immature; P=0.0154 vs. Mature). Nevertheless, aging induced (P=0.0008) an increase in cumulus cell dispersion as compared to Mature COCs. Examination of meiotic progression revealed no significant differences between mature and aged oocytes. Oocyte morphological parameters demonstrated an increase in the thickness of the zona pellucida (P=0.0112), perivitelline space area (P<0.0001), and reduced cytoplasmic volume (P<0.0001) in both Mature and Aged oocytes compared to Immature ones, with no differences between the Mature and Aged groups. Lastly, an increased sperm binding to the zona pellucida was evidenced in Aged oocytes compared to the Mature treatment (P<0.0001). In summary, POA induced distinct effects on intact COCs and denuded oocytes, including cell death activation, cumulus dispersion, and increased sperm binding. These findings imply an impact on fertilization microenvironments, although effects on embryonic competence remain undetermined.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY AND PHYSIOLOGY OF REPRODUCTION

Whole-Genome Enzymatic Methyl Sequencing Identifies Health Outcomes in Weaning Calves

Aldcejam Martins da Fonseca Junior[1], Mohamed Oudihat[1], Camila Bruna de Lima[1], Marc-André Sirard[1]

[1]Université Laval

Health and resilience of dairy calves are fundamental determinants of herd productivity, influencing survival rates, growth trajectories, and lifetime lactation performance; however, reliable early-life predictors, besides pedigree records and parental genomic evaluations remain scarce, limiting the driven identification of susceptible calves and the implementation of targeted management or selection strategies. In this study we applied whole-genome enzymatic methyl sequencing (EM-seq) to peripheral blood DNA from 48 Holstein-Friesian heifer calves before weaning (30–60 days of age), n = 12 per group: Resilient (Control), early growth deficit (BMI), bovine respiratory condition (RESP) or diarrhea (DIAR), to identify differentially methylated cytosines (DMCs) and regions (DMRs) associated with postnatal health outcomes. Following DNA sequencing, reads were trimmed and aligned to the bovine reference genome. Methylation calls were done with MethylKit (v.3.21). Differentially methylated cytosines (DMCs) were those with $q < 0.01$ and methylation difference (meth.diff) > 25%. Differentially methylated regions (DMRs) were filtered with $q < 0.05$ and meth.diff > 15%, using 500 bp sliding windows (250 bp overlap). Promoter regions (±3 kb from TSS) were annotated with ChIPseeker (v.3.20). The number of DMCs found across groups was respectively BMI:133,613; RESP:104,767; DIAR:107,161, while the number of DMRs detected within conditions were 4,074 for BMI; 2,817 for RESP and 3,024 for DIAR. Analysis of mean methylation differences across DMRs revealed a robust signature for all conditions. Besides, hierarchical clustering distinctly grouped the BMI profile from RESP and DIAR and exhibited methylation patterns differing from control and BMI. Gene ontology results from the unique genes related to DMRs in promoter's regions for each group (nBMI=860; nRESP=536; nDIAR=540) revealed distinct enriched biological processes across conditions. In BMI, top terms included cell adhesion, monoatomic cation transport and glycoprotein metabolism. RESP was characterized by glycoprotein and carbohydrate derivative biosynthesis, cell adhesion and glycosylation pathways. DMRs from DIAR enriched neurogenesis, neuron differentiation, chemical synaptic transmission and ion transport, highlighting neuronal and adhesion-related signatures. These findings demonstrate that, high resolution methylation profiling is a promising tool on the identification of health conditions in calves. The identification of a robust health DMR signature underlies the potential of targeted methylation markers at weaning to improve genomic selection programs and the precision dairy farming.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS AND STEM CELLS

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS AND STEM CELLS

Birth of prolactin receptor-edited Angus calves produced through the electroporation of *in vitro*-fertilized zygotes using CRISPR/Cas9 technology

Luiz Sérgio de Almeida Camargo[1], Carolina David Vieira[2], Hyago Passe Pereira[1], Diana Rangel de Lemos[3], Eliza Diniz de Souza[1], Daniele Ribeiro de Lima Reis[1], Naiara Zoccal Saraiva[1], Clara Slade Oliveira[1], Carolina Capobiango Romano Quintão[1], Luiz Gustavo Bruno Siqueira[1]

[1]Empresa Brasileira de Pesquisa Agropecuária, [2]Universidade Federal de Juiz de Fora, [3]Fundação Universidade Federal de Viçosa

CRISPR/Cas9 technology is a valuable tool for cattle precision breeding. The guide (sgRNA) and Cas9 can be injected into zygote's cytoplasm; however, it is timing consuming and can impair embryo viability. We have shown that electroporation can be used to deliver sgRNA and Cas9 effectively, promoting gene editing in bovine embryos without affecting their *in vitro* viability (Camargo et al. 2020 Front Genet 11: 570069). In this study, our objective was to assess whether *in vitro*-fertilized and electroporated embryos could successfully generate gene-edited Angus calves. The target genomic region chosen for editing was the exon 10 of the prolactin receptor (PRLR) gene (XM 024981208.2), which harbors mutations associated with short and sleek hair coats in cattle (SLICK phenotype). Such phenotype enhances the animals' ability to regulate body temperature under environmental conditions of high heat and humidity. *In vitro* matured oocytes were *in vitro* fertilized for 8 h and presumptive zygotes were electroporated with sgRNA+Cas9 together with a single-stranded oligonucleotide to introduce a stop codon between the positions 20:39,099,273-275. Sixteen grade I and II biopsied blastocysts at day 7 post-fertilization were transferred to synchronized recipients and pregnancy diagnosis performed on day 26 after embryo transfer. Biopsies collected from blastocysts and blood cells collected from calves were submitted to DNA extraction, PCR amplification of the target region and Sanger sequencing. Indels and knock-in rates were determined by DECODR program. The pregnancy rate was 37.5% (6 out of 16), with five recipients carrying embryos which biopsies exhibited indels ranging from 16.5% to 57% and knock-in between 0 and 24%. Those five recipients were housed in an isolated area and delivered five calves (three males and two females). Two calves (male #5401 and female #5402) displayed the expected phenotype. Sanger sequencing revealed indel rates of 74.8% and 83.6% and knock-in rates of 16% and 60.8% for calves #5401 and #5402, respectively. Although the sequencing data indicates that the animals are mosaic, these genetic editing are correlated with the calves' distinct phenotype, characterized by their short and sleek coat. Interesting, calf #5400 exhibited indel and knock-in rates of 50.1% and 22.1%, respectively, but displayed a less pronounced SLICK phenotype. Calves #5403 and #5404 displayed hairy coats, which denote the wild-type phenotype, with no indels detected after Sanger sequencing. In conclusion, electroporation of *in vitro* fertilized zygotes with CRISPR/Cas9 can be used to successfully produce PRLR-edited calves displaying the SLICK phenotype.

Acknowledgments: CNPq, Fapemig, SEBRAE, Associação Brasileira de Angus, Casa Branca Agropastoril and Central Embryo Ltda.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS AND STEM CELLS

Creating a mouse model of mitochondrial DNA mutation using somatic cell cytoplasm transfer

Rachel Braz Arcanjo[1], Ana Rita Tavares Krause[1], Sergio Antonio Garcia Pereira Junior[1], Daniel de Lima Cometta[1], Marcos Roberto Chiaratti[1]

[1]Universidade Federal de São Carlos

Mitochondrial DNA (mtDNA) mutations can result in severe and incurable human diseases, such as the mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes syndrome (MELAS). Embryo microinjection and fusion of cytoplasts with mutant mtDNA into embryonic stem cells are some of the methods available to study these syndromes, however, animal models of these diseases are limited. Gene editing of mtDNA has particular challenges compared to nuclear gene editing, including the delivery of the editing guide, the mtDNA repair machinery, and its replication pattern. Further, the tools currently available for mtDNA editing are more efficient and easily applied in somatic cells than in preimplantation embryos. Therefore, the present work aims to investigate whether previously edited somatic cells can be used to generate embryos, and subsequently, a mouse model carrying the mtDNA mutation. Two cell lines carrying the same mtDNA mutation (edited in a NIH3T3 background) at different levels, one considered as high level of mutation (HM, 92%) and the other as low level (LM, 37%), were enucleated using a gradient of Ficoll PM 400 (Cytiva, Uppsala, Sweden) centrifuged at 57,400 x g for 1 hour at 31°C. The resulting cytoplast, packing the mitochondria and other organelles, was used for fusion with pronucleated zygotes, obtained from C57BL/6Unib mice mating. Five cytoplasts from cells with high or low levels of mtDNA mutation were injected into the perivitelline space of the zygote, followed by electrofusion for incorporation of the mutant mitochondria into the embryo. For controls, one group of zygotes was injected with HM cytoplasts without electrofusion (FC), and an additional group was not injected (NC). Zygotes were cultured for 96 hours, after which the blastocysts (N = 4-6 per group) were individually collected for analysis of mutation levels using quantitative PCR. Data were statistically analyzed by ANOVA and Tukey post-hoc test, and $P < 0.05$ was considered significant. The mutation level detected in the blastocysts was not significantly different between groups HM (5.4% ± 1.41) and LM (2.7% ± 0.43) ($P = 0.17$), although only the group HM was significantly higher than the NC (0.4% ± 0.05) ($P < 0.01$). The group FC (7.3% ± 0.89) had a mutation level similar to the HM ($P = 0.40$), differing from groups LM and NC ($P < 0.01$ and 0.001, respectively). MtDNA mutation was successfully detected in the blastocysts, at comparable levels, regardless of the mutation level of the cell line used for transference of mitochondria. The mutation level in all groups was lower than the cell lines, due to the small number of mtDNA delivered compared to the hundreds of thousands of copies already present in the zygote. Future experiments will focus on increasing this percentage and performing embryo transfer in order to obtain a mtDNA mutant pup. This work was supported by FAPESP – Thematic Project 2023/02226-8 and Post-Doctoral Fellowship 2024/07675-8 (RBA).

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS AND STEM CELLS

Desing of gRNA for SRY gene knockout by CRISPR-Cas9 system in bovine fetal fibroblasts

Ana Carolina de Seni Silva[1], Reginaldo Aparecido Vila[1], Flávio Vieira Meirelles[1], Tiago Henrique Camara De Bem[1], Ester Silveira Ramos[1]

[1]Universidade de São Paulo

The sex-determining region Y (SRY) gene plays a crucial role in initiating male gonadal differentiation. However, its potential contribution to the differential development of bovine XY embryos during the pre-implantation period in *in vitro* conditions remains unclear, particularly its influence on the expression of other Y-linked genes involved in male development. Given the efficacy of CRISPR-Cas9 in mammalian gene editing, this study represents a preliminary step to knockout the SRY gene in bovine fetal fibroblasts using CRISPR-Cas9, aiming to assess alterations in the expression of Y-specific genes. In silico design of guide RNAs (gRNAs) targeting the SRY gene was performed for use with Cas9-GFP. The SRY genomic regions from *Bos taurus* (NC_082638.1) and *Bos indicus* (NC_032680.1) were aligned using CLUSTALW to identify conserved sequences. gRNA candidates were predicted using CRISPOR and CHOPCHOP, prioritizing high specificity and minimal off-target effects based on MIT specificity scores and Doench efficiency scores. The selected 20-nt target sequence was complementary to the HMG box region on the forward strand of the SRY gene, with a 49% MIT specificity score, 63% predicted efficiency, and no self-complementarity. Bovine fetal fibroblasts were isolated from skin samples of male and female fetuses obtained from slaughterhouse-derived uteri from crossbred *Bos taurus* × *Bos indicus.* Tissues were enzymatically digested with collagenase (1 mg/100 µL) and cultured in DMEM-F12 medium supplemented with 10% FBS and 0.5% antibiotics at 38.8°C, high humidity, and 5% CO_2. After the first passage, genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen). PCR and qPCR analyses confirmed the presence of the SRY gene only in male fibroblasts and testicular tissues, while β-actin served as a positive control. A total of 27 potential DNA break repair outcomes and 33 off-target regions were predicted for the selected gRNA. The targeted 149 bp region of the SRY HMG box was amplified and confirmed by 2% agarose gel electrophoresis. qPCR revealed early and strong amplification of SRY in male fibroblasts. No significant differences were observed between CRISPOR and CHOPCHOP regarding the efficiency predictions for the gRNA. These findings support the feasibility of targeting the bovine SRY gene using CRISPR-Cas9 *in vitro*. Future steps include functional validation of the gRNA in male fibroblast DNA to confirm knockout efficiency and assess off-target activity via DNA sequencing. This results will provide a basis for investigating the role of SRY in regulating Y-linked gene expression during early bovine development.

Funding: CNPq, CAPES, FAPESP, FAEPA.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

CLONING, TRANSGENESIS AND STEM CELLS

Impact of oxygen tension on *in vitro* maturation of cloned swine embryos

Ligiane Leme[1], Georgina Hasteinreiter[1], Tainah Moraes[1], Débora Rodrigues[1], Priscila Teixeira Silva[1], Thiago Mantovani[1], Guilherme de Lima Meskauckas[1], Luciano Brito[1], Silvano Mario Atilio Raia[1], Ernesto Goulart[1]

[1]Universidade de São Paulo

Creating an optimal *in vitro* cell culture environment requires careful simulation of all essential components, especially the gaseous atmosphere. While it is well-established that low-oxygen tension during embryo culture improves developmental outcomes, little is known about its effects during *in vitro* maturation (IVM), particularly in cloned swine embryos. Considering the species-specific sensitivity of pigs to *in vitro* conditions, this study aimed to determine the most suitable oxygen environment during the two oocyte IVM phases (IVM1, first 22 hours and IVM2, final 18 hours) to produce cloned embryos. Oocytes retrieved from slaughterhouse-derived ovaries were selected and matured in IVM medium under three different atmospheric systems: High (H) — both IVM1 and IVM2 under high O_2 tension; Low (L) — both IVM1 and IVM2 under low O_2 tension, and Mixed (M) — IVM1 under high O_2 tension (H) and IVM2 under low O_2 tension (L). After maturation, oocytes were evaluated for the presence of the first polar body (MII stage) and allocated either for parthenogenetic activation (PTN, control) or for somatic cell nuclear transfer (SCNT). Embryos were cultured in PZM medium, under low O2 tension atmosphere, and assessed for cleavage and blastocyst development on Day 7 (D7). Maturation rates were similar between M (70.62%, 767/1086) and H (73.80%, 817/1107), while L resulted in a significantly lower rate (59.49%, 586/985, P<0.0001). Cleavage rates in PTN embryos did not differ across IVM conditions — M: 89.15% (304/341), H: 93.52% (101/108), L: 92.08% (93/101). Cleavage rates in SCNT embryos were higher in M (62.96%, 578/918) compared to H (53.89%, 201/373) and L (54.90%, 185/337, P<0.05), with no statistical difference between H and L. On D7, PTN blastocyst rates were greater in H (72.22%, 78/108) and L (68.32%, 69/101), with no difference between them, while M (57.77%, 197/341) was lower than H but comparable to L. Blastocyst rates for SCNT embryos differed significantly between M (24.07%, 221/918) > L (17.21%, 58/337), but both were similar to H (20.11%, 58/337) condition. High or low-oxygen seem to improve post-IVM development in PTN embryos, while a mixed strategy enhances blastocyst yield for CLN embryos. These results empha the importance of fine-tuning oxygen levels during assisted reproduction, demonstrating a significant impact on the developmental competence of cloned swine embryos.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

A comprehensive library of heparin and gelatin binding proteins from seminal plasma of six mammalian species

Eduardo Rodrigues Pessoa[1], Fágner Cavalcante Patrocínio dos Santos[1], Jorge André Matias Martins[1], Arabela Guedes de Azevedo Viana[2], Renner Philipe Rodrigues Carvalho[2], Fábio Roger Vasconcelos[1], Denise Damasceno Guerreiro[1], Tiago Antônio de Oliveira Mendes[2], Mariana Machado Neves[2], Arlindo de Alencar Araripe Moura[1]

[1]Universidade Federal do Ceará, [2]Fundação Universidade Federal de Viçosa

Heparin-binding (HBPs) and gelatin-binding proteins (GBPs) participate in cell signaling, extracellular matrix remodeling and immune modulation. In seminal plasma (SP), these proteins contribute to sperm capacitation, oxidative stress protection, fertilization, being also related to male fertility. This study aimed to decipher the HBPs and GBPs atlas of SP from Bos indicus (n=30 animals), Ovis aries (n=20), Capra hircus (n=8), Oryctolagus cuniculus (n=18), Equus caballus (n=5) and Sus scrofa (n=12). Samples were collected within one week (for each species) from same breed, reproductively sound animals. SP proteins were fractionated by liquid chromatography using heparin and gelatin columns and analyzed in Orbitrap QExactive mass spectrometer (Thermo Scientific, USA). Protein identification used MaxQuant and UniProt databases, with further bioinformatic analyses (Venn diagrams, heatmaps, and protein abundance quantification). There were 678, 583, 635, 334, 589 and 131 proteins identified in cattle, rams, goats, horses, rabbits and swine SP, respectively. Predominant HBPs in cattle were CLU, spermadhesin-1, C-C motif chemokine 2, serine protease inhibitor, TIMP-2; rams: protein LEG1 homolog, CLU, acrosin, hyaluronidase, betamannosidase; goats: bodhesin-2, CLU, deoxyribonuclease, betahexosaminidase, serpine 2; equine: carbohydrate-binding protein AWN, lactotransferrin, CLU; rabbits: annexin, ubiquitin B, among others; swine: PSP-I, fibronectin, spermadhesin AWN, carbohydrate-binding protein AQN-3, SP-II. Abundant GBPs in cattle were spermadhesin-1 and Z13, CLU, TIMP-2; rams: beta-mannosidase, carboxypeptidase Q, CLU, ACE; goats: hyaluronidase, CLU, bodhesin-2; horses: protein S100, lactotransferrin, cysteine-rich secretory protein 3, CUB domain-containing protein, lipocalin 2; rabbits: annexin, galectin, hemoglobin subunit zeta; swine: fibronectin, spermadhesin AWN, AQN-3, PSP-I, PSP-II. Seminal plasma GBPs represented 3% in cattle, 1% in goats and equines, 2% in rabbits, 23% in swine. CLU was the only GBP found in all species. HBPs reached 65% (cattle), 67% (rams and goats), 96% (equines), 47% (rabbits) and 14% (swine) of the seminal plasma. Proteins with affinity for both heparin and gelatin were 23% (cattle), 3% (rams), 5% (goats), 2% (equines), 8% (rabbits) and 20% (swine). Only 12 proteins were present in all species: alpha-mannosidase, CLU, ACE, acrosin, nucleobindin-1, TIMP-2, carboxypeptidase, lipocalin 2, histone H4, beta-hexosaminidase. Some GBPs were conserved across species, such as CLU. Unique proteins included different types of spermadhesins and Leg1, making the species-specific GBP and HBP proteome atlas. Spermadhesins play roles in sperm protection, motility, and fertilization, while LEG1 function in reproduction remains unknown. Knowledge of biochemical and functional diversity of SP proteins will support the improvement of assisted reproductive technologies.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Anionic and cationic liposomes as nanocarriers in reproductive biotechnology: characterization and biological assessment in bovine granulosa cells and *in vitro*-produced embryos

Matheus Andrade Chaves[1], Bruna Lopes Cardoso[2], Cibele Maria Prado[1], Maria Alice de Almeida[1], Mariani Fiorenza[1], Samantha Cristina de Pinho[1], Flávio Vieira Meirelles[1,3], Felipe Perecin[1], Juliano Coelho Da Silveira[1]

[1]Universidade de São Paulo, [2]Genus ABS, [3]University of São Paulo, Faculty of Animal Science and Food Engineering

Liposomes (LPs) are lipid-based nanocarriers with broad biotechnological applications, especially in delivering bioactive molecules, such as nucleic acids, for gene therapy. This study characterized anionic and cationic LPs and evaluated their biocompatibility with bovine granulosa cells (GCs) and *in vitro*-produced embryos. Anionic liposomes (AniLip) were composed of anionic phospholipids and cholesterol (80:20 mol%), while cationic liposomes (CatLip) included anionic and cationic phospholipids and cholesterol (27:53:20 mol%). Both LPs were produced using ultrasonication followed by extrusion and characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), atomic force microscopy (AFM), transmission electron microscopy (TEM), and nanoflow cytometry. The LPs showed diameters <150 nm, PDI <0.15, spherical morphology, and zeta potentials around –40 mV (AniLip) and +50 mV (CatLip). Rhodamine-PE (1 mol%) labeling did not affect vesicle physicochemical properties. Cytotoxicity was evaluated by MTT assay: GCs (1 x 10^4 cells/well; n = 15) were incubated at 5% CO_2 and 37 °C for 24 h and then treated with LPs (2 x 10^4 to 2 x 10^6 LPs/cell). After 9 h, 22 µL of MTT solution (5 mg/mL) was added to each well, followed by a 3 h incubation. The resulted formazan crystals were then dissolved in 200 µL of DMSO, and absorbance was measured at 570 nm using a microplate reader. Viability (%) was calculated regarding the negative control (no LPs added). AniLip showed no cytotoxicity at any dose. CatLip maintained high viability only at 2 x 10^4 LPs/cell, indicating a dose-dependent response. RT-qPCR of GCs treated with 2 x 10^4 LPs/cell showed no significant changes in the expression of genes related to lipid metabolism (*SREBF1*, *CPT1B*), oxidative stress (*SOD1*, *NRF2*), apoptosis (*BCL2*, *BAX*, *XIAP*), or steroidogenesis (*CYP17A1*, *HSD3B1*) (n = 6). For embryo experiments, bovine embryos were produced via *in vitro* fertilization (IVF) across six independent replicates (n = 6). AniLip or CatLip (5x10^7 LPs/embryo) were supplemented during specific time windows of *in vitro* culture (Day 2 and Days 3–4 – feeding). A total of 350–400 embryos per group were monitored. Cleavage rates were unaffected by treatment. Blastocyst development was evaluated on Day 7, and selected embryos (n ≈ 30 per group) were stained with Hoechst 33342 and BODIPY to assess nuclear count and lipid droplet accumulation, respectively, via confocal microscopy. AniLip-treated embryos showed similar developmental progression, nuclear counts, and lipid content compared to the control group (no LPs added). CatLip-treated embryos, however, remained predominantly arrested at early blastocyst stages, and displayed fewer nuclei and reduced lipid area. Statistical analysis was performed using ANOVA followed by Tukey's test with a 5% significance level in SAS software. These findings confirm the biocompatibility of AniLip in reproductive models and highlight the potential of CatLip for gene delivery applications, although its effects are dose-sensitive and require further optimization for safe use in embryo biotechnology.

Funding: FAPESP 21/06645-0, 22/01433-7, 22/01505-8, 22/01236-7.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Association of trophectoderm biopsy and vitrification in IVF bovine embryos: effects on cryosurvival, cell number and oxidative stress

Dayana Silva Araújo[1,2], Rafael Monteiro dos Santos[1], Wasim Al Shebli[2], Naiara Zoccal Saraiva[2], Luiz Sérgio de Almeida Camargo[2], Leticia Zoccolaro Oliveira[1], Clara Slade Oliveira[2]

[1]Universidade Federal de Minas Gerais, [2]Empresa Brasileira de Pesquisa Agropecuária

Although embryo biopsy and vitrification are widely established techniques in human assisted reproduction, their combined physiological effects in bovine *in vitro*-produced embryos remain poorly understood, particularly concerning oxidative stress and cellular integrity. This study aimed to evaluate the effects of trophectoderm biopsy, followed by vitrification, on cryosurvival, oxidative metabolism, and total cell number in *in vitro*-produced bovine embryos. A total of 138 grade 1 blastocysts (144–168 hpi) were randomly allocated into Biopsy (B) and Control (C, non-biopsied) groups (n = 67–71 per group, obtained in 3 replicates). Micromanipulation for trophectoderm cell removal was performed on each blastocyst in the Biopsy group, and all embryos were vitrified immediately after the procedure. Warming was performed according to the Embrapa VD protocol. Embryos were cultured for 48 h and stained with MitoTracker™, CellROX™, and ThiolTracker™ to assess mitochondrial activity, oxidative stress, and intracellular thiol content, primarily associated with reduced glutathione (GSH), respectively. Nucleated cells were stained with Hoechst 33342 for total cell count, and images were analyzed using ImageJ software. The data were processed using R software considering $P < 0.05$. Biopsied embryos expanded at higher rates than Control at 0 h (just after warming) (B: 33.8% vs. C: 13.2%; $P < 0.01$), whereas the Control group showed greater re-expansion at 4 h (B: 74.3% vs. C: 88.2%; $P < 0.05$). At the later evaluations, no difference was present between groups (24 h, B: 90.5% vs. C: 92.1%; $P = 0.959$; 48 h, B: 86.5% vs. C: 93.4%; $P = 0.253$). Biopsy affected oxidative metabolism, as evidenced by higher mitochondrial activity (B: 43.00 ± 2.00 vs. C: 32.60 ± 2.15; $P < 0.001$), increased production of reactive oxygen species (B: 32.20 ± 1.69 vs. C: 26.90 ± 2.31; $P < 0.001$), and increased intracellular thiol content (B: 22.29 ± 0.91 vs. C: 17.41 ± 0.61; $P < 0.001$). For total cell count, higher cell numbers were observed in the Biopsy group (B: 216.68 ± 10.55 vs. C: 186.82 ± 9.21; $P < 0.05$). In conclusion, trophectoderm biopsy does not affect cryotolerance of vitrified bovine embryos but induces physiological changes consistent with increased mitochondrial activity, oxidative stress, and cellular proliferation, likely reflecting a compensatory response to cellular injury caused by cell removal. However, increased cell numbers and thiol content were observed, suggesting a positive effect of biopsy on recovery after vitrification – possibly related to zona pellucida removal. These findings indicate that trophectoderm biopsy, even when combined with vitrification, does not compromise early embryonic development, supporting the feasibility of combining the application of both techniques in routine IVF procedures.

Supported by: Fapemig and CNPq.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Astaxanthin exerts a protective effect on *in vitro*-produced bovine embryos cryopreserved by the direct transfer (DT) method – preliminary results

Thalis de Melo Oliveira[1], Nelise Kasue Samecima Prosperute[1], Amanda Bianchini Rosa[1], Victória Marques[1], Laís Engler[1], Maíra Bianchi Rodrigues Alves[1], Lindsay Unno Gimenes[1]

[1]Universidade Estadual Paulista

The direct transfer (DT) method is a commercial technique widely used for cryopreserving bovine embryos, due the elimination of cryoprotectant removal during thaw and embryo evaluation. However, the low temperatures involved in the freezing process can trigger oxidative stress due to an imbalance between the production and elimination of reactive oxygen species (ROS), impairing embryo viability. Among the antioxidants tested to mitigate such damage, astaxanthin (AST), a carotenoid with potent antioxidant activity, stands out. Therefore, the aim of this study was to evaluate the antioxidant potential of AST when supplemented in the DT cryopreservation medium for bovine embryos *in vitro* produced. Ovaries were collected from slaughterhouses and aspirated to obtain Grade I and II oocytes, which were used for *in vitro* embryo production. On the seventh day of *in vitro* culture, expanded blastocysts were cryopreserved using DT medium (Vitrogen, Cravinhos, SP, Brazil) in six experimental replicates, with AST supplementation at three different concentrations: 0 nM (Control, n = 24), 1 nM (n = 33), 0.5 µM (n = 29), and 1 µM (n = 27). After 24 hours of storage in cryogenic tanks, embryos were thawed according to the manufacturer's instructions, cultured for 24 hours in SOF medium, and subsequently assessed for re-expansion and hatching rates. Additionally, embryos were stained using CellROX and Hoechst fluorescent probes (Thermo Fisher Scientific, Massachusetts, USA) and mounted on slides following the protocol described by Rocha-Frigoni et al. (Reproduction, Fertility, and Development, 26:797–805, 2014). Fluorescence images were captured using an Olympus BX60 microscope and AxioVision software, and fluorescence intensity was measured by using ImageJ software. Embryos negative for CellROX were used to correct embryo autofluorescence. Re-expansion and hatching rates were analyzed using the Chi-square test with Fisher's exact correction, while ROS levels were evaluated by ANOVA (RStudio), with Tukey's post-hoc test and significance at $p < 0.05$. No differences were observed in re-expansion and hatching rates among the Control (54.2%), 1 nM (36.4%), 0.5 µM (41.4%), and 1 µM (37.0%) groups (P = 0.5602). A significant reduction in relative fluorescence intensity was observed in embryos treated with 1 µM AST (5.79%a ± 1.88) compared to the Control group (15.66%b ± 2.13; P = 0.01). AST 0.5 µM and AST 1 nM groups showed no significant differences from either the control or 1 µM groups (AST 0.5 µM: 14.16%ab ± 2.39; AST 1 nM: 9.75% ab ± 2.14). In conclusion, in the conditions of this preliminary study, the supplementation with 1 µM astaxanthin in the DT medium reduced ROS levels in *in vitro*-produced bovine embryos post-cryopreservation.

Acknowledgments: PPGCVet, LABOR, FAPESP (Grant: 2021/13932-5), and PIBIC-CNPq.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Cell-free DNA in spent culture media: possible marker for embryo development and quality in cattle

Nayara Ribeiro Kussano[1,2], Gabriela de Oliveira Fernandes[1], Maurício Machaim Franco[1], Margot Alves Nunes Dode[3,1]

[1]Empresa Brasileira de Pesquisa Agropecuária, [2]Fundação de Apoio à Pesquisa do Distrito Federal, [3]Universidade de Brasília

Oocyte and embryo quality is the central factor influencing the outcome of *in vitro* embryo production systems. However, quality evaluation is mainly limited to an assessment of morphological criteria. Therefore, there is a need to identify non-invasive markers to improve the accuracy of selection. This study aimed to evaluate if quantification of cell-free DNA (cfDNA) in maturation (IVM) and culture medium(ICV) can be used as a marker for oocyte competence and embryo quality. To do that, two experiments were carried out. Experiment 1 evaluated the amount of cfDNA in maturation media containing oocytes with high and low capacity to produce embryos *in vitro*. Two groups were formed according to the IVP outcomes: a group in which oocytes gave rise to blastocysts (EMB) and a group that did not form blastocysts (NEMB). The oocytes were matured, fertilized, and cultured individually until day 8 (D8) of development, and the maturation medium was stored for later analysis. In experiment 2, the cfDNA was quantified in spent culture medium from expanded blastocysts that that did (P) or did not (NP) establish pregnancy after transfer. *In vitro* produced embryos that were at morula stage at D5 were cultured individually until D7, when culture medium was collected and the blastocysts were transferred to recipient cows. Pregnancy was confirmed 60 days after embryo transfer. cfDNA was extracted from 15 µL of both media as reported by Kussano et al.,2024 (10.1371/journal.pone.0298316). Then, the number of copies of ART2 and Bov-tA, were used to quantify cfDNA in IVM and IVC by qPCR. Those sequences are short interspersed nuclear elements (SINEs) found in ruminants that are equivalent to ALU used in humans. A total of 30 IVM and IVC medium samples obtained from each group EMB/NEMB and P/NP were analyzed by qPCR. Data of the cfDNA quantification were compared using Mann–Whitney U test, GraphPad Prism 9 (GraphPad Software, San Diego, California USA). Initially, the level of cfDNA quantified by ART2, was higher in the IVM medium in the NEMB than in the EMB group. While for Bov-tA, the levels of cfDNA were similar between groups (P>0.1). In the second experiment, when we compared the quantification of cfDNA from spent culture medium (D5-D7), from P and NP groups medium no differences (P>0.01) were observed in the level of ART2. Conversely, Bov-tA did not amplify in any of the samples. It can be concluded that lower levels of the ART2 in IVM medium may indicate a greater potential of the oocytes to develop into an embryo.

Financial Support: FAP-DF, Embrapa, CAPES and CNPq.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Characterization of extracellular vesicles from bovine oviduct explant and sperm co-cultures under distinct endocrine milieu

Paula R. Cortat[1], Camila Azzolin de Souza[2], Mirela Balistrieri[1], Alessandra Bridi[3], Fernanda Schneberger dos Santos[2], José de Oliveira Carvalho[4], Juliano Coelho Da Silveira[2], Roberto Sartori[1]

[1]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Brazil., [2]Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, [3]University of Western Santa Catarina - Campus Xanxerê, [4]Department of Veterinary Medicine, Federal University of Espirito Santo (UFES)

This study aimed to characterize extracellular vesicles (EVs) in co-cultures of bovine oviduct epithelial cells (BOEC) and spermatozoa (S). We hypothesized that EVs could be isolated from small volumes of conditioned medium and that their release would be influenced by S presence and endocrine milieu. *Bos indicus* cows (n=7) were synchronized to be on diestrus (14-d old corpus luteum [CL]; high P4; n=3) or pre-ovulatory phase (dominant follicle [DF] ≥10 mm; high E2; n=4), confirmed by ultrasound and circulating hormone concentrations assessments. The oviductal isthmus was collected and cultured in TCM-199 supplemented with 10% depleted fetal bovine serum and antibiotics. Explants (EXP) formed after 24h were analyzed by morphology and sperm binding capacity. A 2×2 factorial design evaluated the effects of S presence (with or without) and endocrine environment (CL or DF), resulting in four groups: CL+S, CL, DF+S, and DF. An additional group (S-only) was used. All replicates (n=4) were performed on the same day. EXPs were incubated with 1×10^5 motile sperm/mL for 0.5 or 24h based on previous laboratory studies. Conditioned media (~100 µL) were collected and centrifuged to isolate EVs using Exoquick-TC on a 1:1 ratio and incubated overnight at 4°C. After incubation, samples were resuspended in PBS and analyzed by nanoparticle tracking (NTA) for size and concentration, and by flow cytometry for CD81 (EV marker) and calnexin (negative marker) to confirm EV isolation in a pool for each group. Statistical analyses were done by GLIMMIX of SAS ($P\leq0.05$). No interaction was observed between S presence and endocrine milieu for EV size or concentration at either 0.5 or 24h. Subsequently, the main effects of S and endocrine milieu were analyzed. The S presence and endocrine milieu did not affect EV size. At 0.5h, EV concentration was higher in groups with than without S ($7.9\times10^9\pm0.5\times10^9$ vs $6.0\times10^9\pm0.8\times10^9$; P=0.02) but did not differ at 24h. Despite no difference at 0.5h, at 24h, CL groups (CL+S and CL) tended to have more EVs than DF groups (DF+S and DF; $7.4\times10^9\pm1.0\times10^9$ vs $5.6\times10^9\pm0.7\times10^9$; P=0.08). Finally, the effect of time was evaluated within each group, and no differences were detected, including in S group. To evaluate EV isolation efficiency, flow cytometry showed CD81-positive events/µL as follows: CL+S: 85.6, CL: 16.9, DF+S: 36.6, DF: 29.9, S: 9.0, and PBS 2.2. In contrast, calnexin detection was CL+S: 8.8, CL: 3.7, DF+S: 1.3, DF: 5.8, S: 1.7, and PBS: 1.4. In conclusion, this study successfully isolated EVs from small volumes of culture medium. Sperm presence increased EV concentration at 0.5h, and the luteal endocrine environment was associated with higher EV concentrations at 24h. These findings lay the groundwork for future molecular investigations into how endocrine cues and gamete interactions individually influence EV profiles and potentially modulate sperm function within the oviductal environment.

Acknowledgments: FAPESP#2022/01433-7, CAPES, CNPq, ST Repro.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effect of Different Protocols for Cryopreservation of Skin Fibroblasts from Sindi Dairy Bull

Fabiana lima rodrigues[1,2], Isabella Rodrigues dos Santos Oliveira[3,1], Julia Carvalho de Melo[3,1], José Ricardo Sobral da Silva[1,4], Isabel Cristina Ferreira[1], Carlos Frederico Martins[1]

[1]Embrapa Cerrados, [2]Centro universitário Icesp, [3]Universidade de Brasília, [4]uniceplac centro universitário

The establishment of a germplasm bank using somatic cells is an important step prior to the use of genetic material in the bovine cloning process. Considering that each freezing curve temperature has specific implications for cell viability and functionality, the objective of this study was to evaluate the effect of three freezing protocols for fibroblasts from a high-genetic-merit dairy Sindi bull intended for cloning: 1. Pre-freezing at -80°C for 24 hours followed by immersion in liquid nitrogen (LN2); 2. Pre-freezing at -20°C for 24 hours followed by immersion in LN2; 3. Cooling to 5°C for 1 hour, gradual freezing in LN2 vapor for 20 minutes, and storage in LN2. Fibroblasts were isolated from skin biopsies cooled at 5°C for 24 hours using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% fetal bovine serum (FBS) and antibiotics. After reaching cell confluence, fibroblasts were diluted in a freezing medium (DMEM, 20% FBS, and 10% DMSO), loaded into 0.25 ml straws, and frozen according to the three proposed treatments. The experiment was conducted in triplicate, and after thawing at 36°C for 30 seconds, cell viability was measured using 0.4% Trypan Blue dye (1:1 v/v). Material from three straws per treatment was cultured in 5% CO2 and 38.5°C to observe the cell confluence time. Results were evaluated using ANOVA and Tukey's test at a 5% significance level. Fibroblasts cryopreserved using treatment 3, with initial cooling at 5°C and a freezing curve in LN2 vapor showed the highest cell survival compared to the other treatments (96.72±0.57%, 85.03±1.76%, and 66.30±7.70% for treatments 3, 1, and 2, respectively; $P<0.05$). Treatment 1, with pre-freezing at -80°C, resulted in a higher number of viable cells compared to treatment 2, with pre-freezing at -20°C ($P<0.05$). Cells from treatments 1 and 3 reached confluence in 25 cm² flasks within seven days, which did not occur in treatment 2, where cells covered approximately 50% of the flask bottom. These results indicate that cooling of the straws in a refrigerator followed by pre-freezing in LN2 vapor allowed for effective intracellular water replacement with cryoprotectant solution, causing less damage to fibroblasts than the other treatments. The protocol involving initial pre-freezing at -80°C has been a classic method for fibroblast cryopreservation with good results, as also observed in this study; however, it requires access to an ultra-low freezer. Thus, protocol 3 can be considered a viable alternative method, as it is not only effective but also faster, more practical, and more cost-efficient, because requiring only a standard refrigerator, a styrofoam box, and LN2. The authors would like to thank the Fundação de Apoio à Pesquisa do Distrito Federal-FAPDF, Embrapa Cerrados, Agropecuária Cerrado and Fazenda Asa Branca for support.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effect of mechanical dispersion on the *in vitro* culture of fresh and cryopreserved testicular fragments from prepubertal sheep

Carmen Patricia Huayhua Llacho[1], Anna Clara Accioly Ferreira[1], Bruna Novaes Costa[2], Julyane Silva Durães Pacheco[2], Gaby Judith Quispe Palomino[3], Rensson Homero Céliz Ygnacio[4], Lucy Vanessa Sulca Ñaupas[1], Ana Paula Ribeiro Rodrigues[1]

[1]Universidade Estadual do Ceará, [2]Universidade de Brasília, [3]Universidade Federal do Ceará, [4]Universidad Científica del Sur

Cryopreservation followed by *in vitro* culture (IVC) of testicular fragments represents a promising strategy for preserving the fertility of prepubertal boys, endangered species, and animals of high genetic value, such as sheep. Mechanical dispersion may promote more uniform nutrient perfusion throughout the fragment, improving IVC outcomes. Therefore, this study evaluated the effect of mechanical dispersion on the IVC of fresh or frozen testicular fragments from prepubertal sheep. A total of 16 fragments of 5 mm³ obtained from each pair of testicles (n = 5) were randomly distributed into two groups: fresh and frozen. Fresh fragments, whether intact (IF) or mechanically dispersed (DF), were either immediately fixed ($_{F}$IF and $_{F}$DF) or subjected to culture ($_{CF}$IF and $_{CF}$DF). Frozen fragments, intact or dispersed, were thawed and either immediately fixed ($_{FT}$IF and $_{FT}$DF) or cultured ($_{CFT}$IF and $_{CFT}$DF) under the same conditions as the fresh fragments. The cryopreservation method used was uncontrolled slow freezing with MEM-HEPES base medium + 20% FBS + 20% DMSO. IVC was performed on 1.5% agarose gel at 34°C and 6% CO_2 for 10 days. Fragments were subjected to gene expression analysis by RT-PCR (*OCT4, C-KIT, CASPASE-3,* and *BAX/BCL2*) and ultrastructural evaluation by transmission electron microscopy (TEM). Gene expression data were analyzed using two-way ANOVA followed by Bonferroni post-hoc test, considering treatment (intact or dispersed) and culture time (uncultured or 10 days). Results were considered significant when $p < 0.05$. The results showed that *OCT4* expression was similar across fresh and cultured fragments, regardless of being intact or dispersed. C-KIT and CASPASE-3 expression showed no differences between $_{F}$DF and $_{CF}$DF or between $_{FT}$DF and $_{CFT}$DF, whereas $_{CF}$IF and $_{CFT}$IF presented a significant reduction ($p < 0.05$) compared to $_{F}$IF and $_{FT}$IF, respectively. The BAX/BCL2 ratio was higher ($p < 0.05$) in both fresh and frozen cultured fragments, regardless of being intact or dispersed. Regarding TEM analysis, showed that $_{F}$DF preserved Sertoli cells organization and exhibited normal mitochondrial morphology. The $_{CF}$DF group exhibited tubular disorganization, darkened nuclei, and swollen mitochondria. $_{FT}$DF group showed overall structural preservation, though with cytoplasmic vacuoles and dense mitochondria, indicating moderate damage. The $_{CFT}$DF group exhibited marked degeneration, with loss of tubular organization and swollen mitochondria, although the basal membrane remained intact. In conclusion, mechanical dispersion of fragments resulted in more stable gene expression of markers related to germ cell differentiation and apoptosis regulation. However, ultrastructural analysis revealed adequate preservation of testicular structure after cryopreservation, while cultured fragments showed degeneration, indicating that the IVC protocol requires further optimization.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effect of the IFIOT maturation system on chromatin conformation, cytoplasmic organelle configuration, and reactive oxygen species levels in vitrified immature bovine oocytes

José Eduardo Vieira Chaves[1], Nayara Ribeiro Kussano[2], Laryssa Ketelyn Lima Pimenta[3], Venâncio Augusto Oliveira Silva[1], Ana Caroline Chaves Vall Nicolás[3], Alice Caroline Souza e Castro[1], Hallya Beatriz Sousa Amaral[3], Leonardo de França e Melo[1], Margot Alves Nunes Dode[2], José Felipe Warmling Sprícigo[1]

[1]Universidade Federal de Goiás, [2]Empresa Brasileira de Pesquisa Agropecuária, [3]Universidade de Brasília

Cryopreservation of cumulus oocyte complexes (COCs), when combined with reproductive biotechnologies, optimizes the use of high value females, and allows the preservation of valuable breeds and species. However, cryopreservation remains challenging due to its detrimental effects on COCs viability and the low blastocyst yield from vitrified bovine oocytes. This study aimed to assess whether *in vivo* maturation via intrafollicular immature oocyte transfer (IFIOT) would support biological pathways potentially compromised during vitrification. COCs obtained from abattoir ovaries were randomly assigned to six treatment groups: (1) fresh COCs immature (F-IMA); (2) vitrified COCs immature (V-IMA); (3) fresh COCs *in vitro* matured (F-IVP); (4) fresh COCs *in vivo* matured by IFIOT (F-IVD); (5) vitrified/warmed COCs and *in vitro* matured (V-IVP); and (6) vitrified/warmed COCs and *in vivo* matured by IFIOT (V-IVD). Vitrification and warming followed the Cryotop® methodology, and IFIOT was performed according to the IFIOT-Embrapa® protocol. Chromatin conformation was evaluated by lacmoid staining in groups F-IVP (n=69), F-IVD (n=72), V-IVP (n=83), and V-IVD (n=59) by optical microscopy; cytoplasmic ultrastructure was analyzed by transmission electron microscopy (TEM) in groups F-IMA, V-IMA, F-IVP, F-IVD, V-IVP, and V-IVD (n=5 each); and reactive oxygen species (ROS) levels were quantified with the H2DCFDA probe in groups F-IMA and V-IMA (n=30 each), F-IVP (n=25), F-IVD (n=12), V-IVP (n=22), and V-IVD (n=20) using epifluorescence microscopy. Statistical analyses were performed using the chi square test for chromatin evaluation, and ANOVA followed by Tukey's test for the assessment of ROS content (SAS; $P \leq 0.05$). Chromatin evaluation showed similar MII rates in fresh groups (F-IVP=89.8 %; F-IVD=90.2 %) and significantly lower rates in vitrified groups (V-IVP=45.7 %; V-IVD=69.4 %), with V-IVD higher than V-IVP ($P < 0.05$). Chromatin abnormalities were lower ($P < 0.05$) in fresh groups (F-IVP=5.7 %; F-IVD=5.5 %) and higher in vitrified groups (V-IVP=33.7 %; V-IVD=18.6 %), with V-IVD lower than V-IVP ($P < 0.05$). TEM revealed structural differences between fresh and vitrified oocytes. COCs in the IMA F group exhibited more cortical granules and homogeneously distributed mitochondria, whereas V-IMA showed larger vacuoles. After maturation, fresh groups (F-IVP and F-IVD) had fewer vacuoles and uniformly distributed organelles, whereas vitrified groups (V-IVP and V-IVD) displayed clustered organelles near the plasma membrane, irregular mitochondria, and more vacuoles. Regarding ROS content, F-IMA showed higher ROS levels ($P \leq 0.05$) than V-IMA; after maturation, F-IVP had higher ROS levels ($P \leq 0.05$) than F-IVD, V-IVP, and V-IVD, which were similar. In conclusion, while IFIOT improved nuclear maturation and decreased chromatin abnormalities in vitrified oocytes, it was not effective in preventing cytoplasmic organelle damage or lowering ROS levels.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effect of three cryoprotectant solutions on the cryopreservation of spermatozoa recovered from the epididymis of Cervus elaphus post-mortem

Julia Carvalho de Melo[1,2], Betânia Pereira Borges[3], Lesley Diego Santiago Freire[4], Juliana Gonçalves da Silva[4], Carolina Gonzales da Silva[5], Gabriel Antônio M. Medeiros Martins[6], Carlos Frederico Martins[2]

[1]Universidade de Brasília, [2]Embrapa Cerrados, [3]Jardim Zoológico de Brasília, [4]Centro Universitário de Desenvolvimento do Centro, [5]Instituto Federal de Educação, Ciência e Tecnologia Baiano, [6]Centro Universitário de Brasília

The recovery of spermatozoa from the epididymis of animals that die suddenly is an alternative to enhance germplasm banks and allow future use for the propagation of wild animals. In this context, the present study evaluated the protective potential of three cryoprotectant media for the freezing of epididymal spermatozoa from Cervus elaphus (Red Deer): 1. Botubov® (20% egg yolk); 2. Criocell 1, based on trehalose, fructose, amino acids, soy lecithin, 7% glycerol, and 10% egg yolk; 3. Criocell 2, same base as Criocell 1, but with 5% egg yolk. Testicles of an adult Red Deer from the Brasília Zoo (DF, Brazil) were transported at 5°C to the Embrapa Cerrados Laboratory. The material was stored for an additional two days at 5°C to simulate prolonged transport. Subsequently, spermatozoa were collected by incising the tail of the epididymis, and sperm motility, vigor (scale 0–5), and concentration were evaluated. The spermatozoa were diluted in the three cryopreservation solutions and frozen in 0.5 ml straw with 20x106 cells using an automated freezing curve (TK-3000 SE machine). Straws were thawed in water bath at 37°C for 30 seconds and subjective motility, membrane integrity (Propidium Iodide and Carboxyfluorescein Diacetate), acrosome integrity (fluorescein isothiocyanate-conjugated peanut agglutinin) and DNA integrity (Tunel) were assessed to evaluate the impact of cryopreservation. The data obtained of thawing of 3 straws from each treatment were evaluated by Kruskal-Wallis test and Dunn test at a significance level of 5% ($p<0.05$). Initial fresh sperm motility was 40% with a vigor score of 2. After dilution in the different media, total motility improved; Botubov reached 50% motility, Criocell 1 reached 55% and Criocell 2 reached 60%. In all treatments, vigor increased from 2 to 3. There was no significant difference ($P>0.05$) in post-thaw sperm motility among the three cryoprotectant solutions (45.00±5.00%, 31.66±2.88% and 45.00±5.00% for Botubov, Criocell 1, and Criocell 2, respectively). Membrane integrity was also similar across treatments (29.16±2.46%, 24.5±5.26% and 27±4.33%). However, treatments with Botubov and Criocell 2 provided better protection of the acrosome (40.17±2.98% and 32.42±1.88%, respectively; $P<0.05$) compared to Criocell 1 (25.67±3.18%). None of the treatments were damaging to DNA, with over 97% of spermatozoa showing intact DNA. These results demonstrate that storing testicles at 5°C reduces sperm metabolism and maintains viability for up to two days, which is sufficient for long distance transportation to laboratories. Additionally, Botubov and Criocell 2 (with reduced egg yolk content at 5%) better protected epididymal spermatozoa from this species during cryopreservation. Considering the specific characteristics of each species, we inferred that epididymal spermatozoa from Red Deer adapt better to Botubov and Criocell 2 during the cryopreservation process.

Acknowledgments: Brasília Zoo, Embrapa Cerrados.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Effects of Lippia sidoides Essential Oil on Cumulus Cells Viability and on Activity of Antioxidant Enzymes in Vitrified Bovine Ovarian Tissue

Sueline Cavalcante Chaves[1], Leopoldo Rugieri Carvalho Vaz da Silva[1], Andreza de Aguiar Silva[1], Erica Costa Marcelino[1], Francisco das Chagas Costa[1], Venância Antonia Nunes Azevedo[1], Regislane Pinto Ribeiro[1], Laryssa Gondim Barrozo[1], Solano Dantas Martins[2], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará, [2]Universidade Federal do Delta do Parnaíba

Ovarian tissue vitrification is a strategy for fertility preservation; however, the use of traditional cryoprotectants can induce oxidative stress and cellular toxicity, limiting its effectiveness. Therefore, natural antioxidants, such as the essential oil of Lippia sidoides, have been studied as alternatives to reduce these adverse effects. This study aimed to analyze the effects of L. sidoides essential oil on bovine cumulus cells (CCs) and its antioxidant potential in the vitrification of bovine ovarian tissue. The CCs, isolated from bovine cumulus-oocyte complexes (COCs), were cultured in α-MEM at 38.5°C with 5% CO_2 until reaching 70-80% confluence, in three replicates from a pool of four animals, with analyses performed separately for each treatment. To test cytotoxicity, CCs were cultured in 96-well plates with 0 (control), 4, 40, and 400 µL/mL of the essential oil. After 48 hours, cell viability was assessed using calcein-AM and ethidium homodimer-1. For vitrification, ovarian tissues from four animals were exposed to a solution containing α-MEM, 10% DMSO, 0.25 mol/L sucrose, and the same oil concentrations. During warming, samples were immersed in α-MEM with 10% FBS and decreasing concentrations of sucrose. The tissue fragments were macerated and centrifuged for spectrophotometric assays, with total protein concentration determined for normalization of the results. Thiol levels were measured with DTNB, superoxide dismutase (SOD) activity by inhibition of adrenaline auto-oxidation, catalase (CAT) by H_2O_2 consumption, and glutathione peroxidase (GPX) by NADPH oxidation. Normality was tested using the Shapiro-Wilk test; ANOVA/Tukey and Kruskal-Wallis tests were used for statistical analyses ($p < 0.05$). CCs treated with 4 µL/mL of the essential oil showed a significant increase in calcein-AM fluorescence compared to the control ($p < 0.05$), indicating preserved viability. ethidium homodimer-1 fluorescence was reduced in CCs exposed to 40 µL/mL, suggesting lower cell mortality. After vitrification, SOD activity was significantly increased in ovarian tissue vitrified with 4 µL/mL of the oil compared to the non-vitrified control ($p < 0.05$), with no differences in CAT, GPX, or thiol levels. Despite these promising results, functional reproductive outcomes, such as oocyte developmental competence or fertilization rates, were not evaluated, and further studies are recommended. The findings indicate that L. sidoides essential oil may be an innovative additive for cryopreservation protocols, contributing to advances in fertility preservation.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Impact of live BVD virus vaccines on the serological diagnosis of P80

Lopes César Mugabe[1], Rafaella Lull dos Santos[1], Fabricio Dias Torres[2], Fabiane Pereira de Moraes[3], Cesar Andrei Marques da Silveira[1], Breno Weissheimer[1], Rogério Ferreira[4], Bernardo Garziera Gasperin[1], André Gustavo Cabrera Dalto[1], Monique Tomazele Rovani[1]

[1]Universidade Federal do Rio Grande do Sul, [2]Axys Análises, [3]Universidade Federal de Pelotas, [4]Fundação Universidade do Estado de Santa Catarina

Serological diagnosis of Bovine Viral Diarrhea (BVD) uses ELISA to detect antibodies against the viral non-structural protein NS3 (P80). Inactivated vaccines do not induce anti-P80 antibodies, but replicating live vaccines may interfere with the tests. This study evaluated whether commercially available replicating Bovine Viral Diarrhea Virus (BVDV) vaccines affect the detection of P80. Angus and Brangus cattle (12-36 months old), seronegative for P80, were allocated into four groups: replicating vaccines A (n=41), B (n=37), C (n=36), and Control (saline solution; n=39). Samples were collected on days 0, 3, 6, 9, 30, 60, and 90 post-treatments for analysis of total antibodies and P80 (IDEXX BVDV Total Ac, IDEXX BVDV P80 Ac and IDEXX, Brazil). In a subgroup (n=27), viral detection was also performed using antigen detection (IDEXX BVDV Ag/Serum Plus) and RT qPCR (Virotype BVDV 2.0; Indical Bioscience, Germany) in nasal swabs and whole blood on days 0, 3, 6, and 9. Additionally, a group of animals (n=13) (A, n=4; B, n=4; C, n=3 and control group, n=2) was sampled and tested 240 days after treatment for P80 analysis. Data were analyzed using mixed models for repeated measures, with groups as fixed effects and animals as random effects. Post-hoc comparisons were performed using the Tukey's test (JMP Pro 18). Vaccination stimulated total antibody production, evidenced by the progressive increase in the sample/positive (S/P) ratio in the vaccinated groups, which was significantly higher than in the control group (P<0.0001). Vaccinated animals showed progressive seroconversion sample/negative (S/N) ratio (%) values for P80 on D30 (A:52.9±1.8; B:44.5±2.0; C:44.8±2.1), on D60 (A:28.7±1.6; B:16.8±1.7; C:18.7±1.8), and D90 (A:22.1±1.7; B:13.6±1.8; C:12.6±2.0); and differed from the control group (D30: 99.1 ± 2.0; D60: 87.2±1.7; D90: 90.0±1.9) (P<0.0001). The control group did not present positive animals in any of the evaluations, while the vaccinated groups demonstrated a progressive increase in the percentage and frequency of positive animals. Vaccine group A presented an increase from 0% (D0; 0/41) to 90.5% (D90; 19/21) of positive animals, while vaccine group B presented 0 (0/37), 30.6 (11/36), 89.2 (33/37) and 90% (18/20) on days 0, 30, 60 and 90, respectively, and group C presented a percentage of positive animals ranging from 0% (D0; 0/36) to 93.3% (D90;14/15). At 240 days after treatments (S/N (%)), vaccinated groups remained positive (A: 10.30±0.67; B:19.97±7.31; C:21.92±11.52), while the Control remained negative (102.82±4.08). No viral excretion was detected in whole blood and nasal swab samples. The results confirm that replicating live vaccines interfere with the interpretation of ELISA tests for P80 for at least up to 90 days post-treatment. Therefore, vaccination history must be considered to ensure accurate herd health monitoring. The authors thank INCT Reprodução Animal, FAPERGS, CNPq and CAPES for their financial support.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Mfn2 deletion in oocytes leads to increased mitochondrial DNA replication and decreased autophagy and mitophagy, disturbing mitochondrial inheritance

Mirela Brochado Souza Cáceres[1], Lindomar de Oliveira Alves[1], Angelica Camargo dos Santos[1], Marcos Roberto Chiaratti[1]

[1]Univerdade Federal de São Carlos

Deletion of mitofusin 2 (MFN2) is associated with impaired mitochondrial dynamics and mitochondrial DNA (mtDNA) instability. MFN2 is necessary for mitochondrial fusion and, in turn, integrity of the mitochondrial network. In heteroplasmic models containing a mixture of mtDNA from different mouse lineages, this scenario offers a unique opportunity to investigate how loss of MFN2 influences mitochondrial dynamics and mtDNA inheritance. This work aimed to investigate the effect of Mfn2 deletion on mitochondrial dynamics in heteroplasmic oocytes, focusing on autophagy, mitophagy and mtDNA replication. Eight-week-old females (0%, 10-40% and 50-80% of heteroplasmic; defined by real-time PCR using 3 replicates) were hormone-primed with eCG and, after 44 hours, the ovaries were enzymatically digested for oocyte isolation. Oocytes were then cultured for 16 hours in EdU-containing medium under low O2 tension and, subsequently, fixed and stored for immunofluorescence. The EdU was revealed with the Click-iT™ EdU Cell Proliferation Kit (Life Scientific, C10337), while target proteins were identified through immunocytochemistry using the following primary antibodies: TFAM (Sigma, SAB1401383), COX IV (Cell Signaling, 11967S), LC3B (Sigma, L7543) and P62 (Cell Signaling 5114S). Following labelling with secondary antibodies anti-mouse-Alexa 488 (life technologies, A11001) and anti-Rabbit-Alexa 594 (life Technologies, A11012), samples were visualized with the aid of a Airyscan 1 confocal microscope (Zeiss). Image quantification was performed using Zen 10.1 software, with statistical analysis in Graph Prism 9 ($P<0.05$). As a result, analysis of oocytes from females with a range of heteroplasmies indicated that the heteroplasmic condition leads to increased autophagy and mitophagy—evidenced by elevated levels of LC3B (~80%) and P62 (~200%) in oocytes with >50% heteroplasmy—despite not affecting mtDNA replication. In parallel, deletion of Mfn2 enhances mtDNA replication, as shown by increased mt-EdU/TFAM incorporation (~30% in Mfn2−/− vs. ~10% in wild-type), while also impairing autophagy and mitophagy, with LC3B and P62 levels reduced to ~30% and ~40%, respectively. Given the instability of the heteroplasmic condition in the germline, these data point to a possible role of auto/mitophagy in removing a less fit mtDNA towards reestablishment of homoplasmy. In addition, the effects of Mfn2 knockout on oocytes might explain why Mfn2 is required for purifying selection in heteroplasmic mice, highlighting the important role of mitochondrial dynamics to mtDNA inheritance.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Microfluidic Device as an Alternative for Bovine Oocyte Pre-Maturation

Manuela Brauner Freitas[1], Luca Angi Souza[1], Schaienni Fontoura saldanha[1], Helena Fabiana Reis de Almeida Saraiva[1], Franciele Flores Vit[1], Felipe Perecin[1], Flávio Vieira Meirelles[1], Juliano Coelho Da Silveira[1]

[1]University of São Paulo

The heterogeneity of nuclear maturation stages resulting from ovarian follicle aspiration contributes to asynchronous maturation during IVP. This condition is not corrected during IVM. Therefore, a promising alternative is *in vitro* pre-maturation (Pre-IVM) with the addition of C-type natriuretic peptide (CNP) to synchronize meiotic resumption, potentially improving IVP outcomes. This study evaluated oocyte development in Pre-IVM medium by comparing a dynamic system with continuous flow in a microfluidic device (MD) to conventional static culture in plates (P). The hypothesis is that the MD could improve oocyte synchrony and allow real-time collection and diagnosis. The MD consists of a PDMS plaque with 1 mm × 1 mm wells, and oocytes were cultured in pairs (approximately 14 oocytes in total) under a 1 µL/min flow rate. Immature oocytes were collected from slaughterhouse-derived ovaries and divided into five groups: Im (immature; fixed immediately after selection), D9h (device, 9 h in Pre-IVM), P9h (plate, 9 h in Pre-IVM), D18h (device, 9 h in Pre-IVM + 9 h in IVM), and P18h (plate, 9 h in Pre-IVM + 9 h in IVM). Groups D9h and P9h were incubated for 9 h in Pre-IVM medium in the device and plate, respectively. Groups D18h and P18h underwent 9 h of Pre-IVM followed by 9 h in IVM medium containing AREG. Three experimental replicates were performed. Media were prepared according to SOARES et al. (Reproduction, Fertility and Development, vol. 29, no. 11; 2017; 2217–2224). All groups were analyzed for meiotic stage using immunofluorescence (IF) of the germinal vesicle (GV) with anti-Lamin, transzonal projections (TZPs) with ActinGreen 488, and chromatin with Hoechst. Gene expression analysis was performed using RT-PCR on cumulus cells (CCs), targeting EGFR, ERK2, CPEB1, MAPKAPK2, and PRKACA, normalized to ACTB and PPIA. TZP and gene expression data were analyzed using Student's t-test (considering differences significant when $p<0.05$), while GV distribution was compared using proportions. For immature oocytes, GV stages were: GV1 (18%), GV2 (32%), GV3 (50%), and GVBD (0%). In D9h and P9h groups, the stages were: GV1 (7% and 3%), GV2 (14% and 9%), GV3 (53% and 59%), and GVBD (26% and 29%). In D18h and P18h: GV (15% and 16%), GVBD (30% and 42%), M1 (30% and 29%), and M2 (25% and 13%), respectively. No differences were observed between groups for TZPs or mRNA expression of EGFR, ERK2, CPEB1, MAPKAPK2, and PRKACA. However, at 18 h, BMP15 expression increased in CCs from COCs exposed to microfluidics ($p<0.048$). These findings suggest that the microfluidic device may be a viable alternative for Pre-IVM and IVM, showing performance comparable to conventional static culture. Additionally, it may be used as a tool for real-time culture medium collection as a diagnostic strategy. This modulation may promote synchronization among bovine oocytes, contributing to improved IVP success rates.

Support: FAPESP 21/06645-0.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Noninvasive miRNA delivery to oocyte cytoplasm using engineered extracellular vesicles via transzonal projections

Camila Azzolin de Souza[1], Gislaine dos Santos[2], Maria Alice de Almeida[1], Mariani Fiorenza[1], Matheus Andrade Chaves[1], Cibele Maria Prado[1], Flávio Vieira Meirelles[1], Felipe Perecin[1], Juliano Coelho Da Silveira[1]

[1]Universidade de São Paulo, [2]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

Microinjection is a conventional technique for delivering exogenous molecules into oocytes and zygotes; however, it can be harmful to the embryos due to the invasive intervention. An alternative involves harnessing extracellular vesicles (EVs), naturally occurring nanocarriers present in follicular fluid, facilitate cell-to-cell communication and thus represent promising candidates for noninvasive delivery systems. This study hypothed that engineered EVs can deliver exogenous miRNA to oocytes during IVM by exploiting transzonal projections (TZPs) from cumulus cells (CC). Follicular fluid-derived EVs (ffEVs) were isolated and engineered using EXO-FECTTM to load a bovine miRNA mimic, miR-20a, which is associated with oocyte competence through regulation of the PI3K/AKT signaling pathway. To evaluate the delivery efficiency a nematode miRNA, prd-miR-39, which there is no record in the bovine genome, was also loaded. Three independent experiments were conducted. The first assessed whether exogenous miRNA could enter into the oocyte. COCs were allocated to four groups: control, ffEVs, ffEVs + control mimic, and ffEVs + miR-20a. The supplementation occurred for either 2 and 12h. The second experiment evaluated the roles of cumulus cells and ffEVs in mediating miRNA transfer. Oocytes were matured with or without CC in four groups: control, ffEVs, ffEVs + miR-20a, and free miR-20a, for 12h. The third experiment investigated the importance of TZPs in EV-mediated delivery. COCs were supplemented with prd-miR-39-loaded ffEVs during either the first or the last 12h of IVM (24h IVM) when TZPs are present or retracted, respectively. Groups included: control, ffEVs, ffEVs + prd-miR-39, and free prd-miR-39. Following IVM, CC and oocytes were collected separately and ZP were removed using Tyrode's solution. The levels of miR-20a and prd-miR-39 were quantified via RT-qPCR (5 replicates/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's test, with significance set at $p < 0.05$. Results indicated a significant increase in miR-20a levels in CC after 2 h incubation, although oocyte uptake was not yet evident. After 12h, miR-20a levels significantly elevated in both CC and oocytes supplemented with miR-20a-loaded ffEVs. Moreover, delivery efficiency was higher with engineered ffEVs than with free miRNA, particularly in the presence of CC. Notably, prd-miR-39 levels in oocytes were significantly increased only following supplementation during the first 12h of IVM, underscoring the critical role of TZPs in facilitating EV-mediated miRNA transfer. This study demonstrates that engineered EVs can noninvasively deliver exogenous miRNA to the oocyte cytoplasm via cumulus cell TZPs. These findings establish a novel platform for manipulating oocyte molecular content, with broad implications for genetic modulation or manipulation of oocytes from low-quality follicular environments.

Funding: FAPESP 21/06645-0, 2022/09461-0, CAPES 001.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

NPR2 and EGFr levels in extracellular vesicles and cumulus cells: a potential novel regulatory mechanism involved in bovine oocyte maturation

Luca Angi Souza[1], Juliano Rodrigues Sangalli[1], Tiago Braga da Silva[1], Helena Fabiana Reis de Almeida Saraiva[1], Felipe Perecin[1], Lawrence Charles Smith[1,2], Flávio Vieira Meirelles[1], Juliano Coelho Da Silveira[1]

[1]Universidade de São Paulo, [2]Université de Montreal

During IVM, oocytes frequently resume meiosis prematurely (around 6 h after COCs are placed in the medium), leading to incomplete cytoplasmic maturation, reduced developmental competence, and lower blastocyst rates. To address this issue, protocols combining C-type natriuretic peptide (CNP) to maintain meiotic arrest and amphiregulin (AREG) to later trigger resumption via epidermal growth factor receptor (EGFR) activation have been proposed. However, approximately 20% of COCs resume meiosis prematurely after 9 h of CNP-based pre-IVM, which may be due to low levels of natriuretic peptide receptor 2 (NPR2), the key receptor mediating CNP's arresting effect. AREG is then added to activate EGFR and support meiotic progression. Our project aims to elucidate the mechanisms by which cumulus cells acquire NPR2 and EGFR to refine pre-IVM/IVM protocols and enhance oocyte competence. In unpublished preliminary data, we detected NPR2 and EGFR proteins in small extracellular vesicles (sEVs) from bovine follicular fluid. To investigate further, we pooled follicular fluid and cumulus cells from approximately 100 small follicles (3-6 mm) and 10 large follicles (8-14 mm) from ovaries without corpus luteum, obtaining four pools per group. After serial centrifugation and -exclusion chromatography, sEVs were isolated from both small (S-EVs) and large (L-EVs) follicles. Western blot analysis for NPR2 and EGFR was performed on S-EVs, L-EVs, small-follicle cumulus cells (S-CCs), and large-follicle cumulus cells (L-CCs). Protein normalization was performed with Qubit (for sEVs and CCs) and additionally by β-actin (for CC lysates), and statistical analysis used Student's t-test ($\alpha = 0.05$). Both NPR2 and EGFR were detected in EVs regardingly the follicle . NPR2 levels in S-EVs (450.3 ± 214.1) versus L-EVs (1,079.0 ± 786.2; $P = 0.47$) and in S-CCs (0.18 ± 0.05) versus L-CCs (0.08 ± 0.03; $P = 0.13$) showed no statistically significant differences. In contrast, EGFR was significantly more abundant in S-EVs (4,889.8 ± 1,832.6) than in L-EVs (157.5 ± 82.5; $P = 0.04$), and also higher in S-CCs (3.91 ± 0.52) than in L-CCs (0.53 ± 0.16; $P < 0.01$). These results suggest that sEVs carry EGFR and could be involved in the acquisition by cumulus cells, potentially influencing the regulation of meiotic resumption, while the role of sEVs in NPR2 transfer remains inconclusive. These findings may contribute to refining IVM and Pre-IVM systems by enhancing the oocyte's responsiveness to CNP and AREG through increased receptor availability mediated by EV uptake. This could improve oocyte competence for fertilization and subsequent embryonic development in IVP protocols.

Financial support: CAPES - Finance Code 001, and FAPESP 21/06645-0; 21/09886-8; 24/03052-6.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Prospecting for automated extraction of mathematical variables representative of the morphology of the *in vitro* produced equine embryo

Malu Maria Felix Manzieri[1], Vinicius Casado Moraes[1], Ester de Oliveira Ballarini[2], Marcos Antonio Gonçalves[2], Perla Fleury[2], José Celso Rocha[1], Marcelo Fábio Gouveia Nogueira[1]

[1]Universidade Estadual Paulista, [2]*In Vitro* Equinos

Embryo quality has traditionally been assessed based on morphology. With the recent development of artificial intelligence (AInt)-based programs for embryo analysis, numerical vectors must be extracted from digital embryo images to serve as inputs for training an AInt model specific to equine blastocysts. Thus, this work focuses on developing an automated digital image processing method to obtain mathematical variables that represent the morphology of *in vitro*-produced equine blastocysts (via ICSI). Initially, algorithms and prompts previously developed for the processing of digital images of bovine (IVP) and human (IVF) embryos were applied using the MatLab® platform. In a pilot experiment, 51 digital images of blastocysts were provided by *In Vitro* Equinos Ltda. The images were captured using an EP50 camera (Olympus) coupled to an inverted microscope (IX73 Olympus, 20x magnification) and saved in .JPG format (1,600 x 1,200 pixels). Preliminary data aiming to obtain predictive significant variables, as fully automatic segmentation of the zona pellucida (ZP), trophectoderm (TE) and inner cell mass (ICM), proved to be inefficient in most of the raw images. Subsequently, 10 images were adjusted for resolution (640 x 480 pixels), aspect ratio (0.75) and saturation (grayscale). This improved significantly (around 90%) the segmentation efficiency of each embryonic structure by the tested software. Additionally, new methodologies for morphological assessment optimization were applied, which the Watershed Transform presented more accurate results (>90%) in isolating the ZP, TE and ICM by identifying regions with similar gray intensities and assigning random colors to enhance analytical processing. Therefore, the initial results suggest the feasibility of the computational approach for morphological evaluation of equine embryos. Next steps include expanding the image database, refining the numerical variable extraction methods, and integrating artificial or convolution neural networks (i.e., AInt) for automated classification. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Processes Numbers #2024/23599-0, 2024/03739-1, 23/16156-1 and 2021/11747-6.

Animal Reproduction

THEMATIC SECTION: 38TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

SUPPORTING BIOTECHNOLOGIES: CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSTIC IMAGING, MOLECULAR BIOLOGY AND "OMICS"

Thymol improves the viability of bovine cumulus cell, and antioxidant defense in bovine ovarian tissue during vitrification

Andreza de Aguiar Silva[1], Francisco das Chagas Costa[1], Sueline Cavalcante Chaves[1], Venância Antonia Nunes Azevedo[1], Erica Costa Marcelino[1], Leopoldo Rugieri Carvalho Vaz da Silva[1], Gabrielle de Oliveira Ximenes[1], Solano Dantas Martins[2], Valdevane Rocha Araújo[2], José Roberto Viana Silva[1]

[1]Universidade Federal do Ceará, [2]Universidade Federal do Delta do Parnaíba

This study aimed to evaluate the cytotoxic effects of thymol on bovine cumulus cells, and to investigate its antioxidant potential during the bovine ovarian tissue vitrification. For this, cumulus cells (CCs) were isolated from bovine COCs (grade 1 and 2) and cultured in α-MEM at 38,5 °C in a humidified atmosphere containing 5% CO2, until reaching 70-80% confluence. To assess the cytotoxicity, CCs were seeded in 96-well plates and treated with thymol at different concentrations (2.5, 25.0 and 250.0 µg/mL). Untreated cells served as negative control. After 48 h of incubation, cell viability was assessed using 4mM of calcein-AM and 2mM ethidium homodimer. For the vitrification procedure, ovarian tissues samples were exposed to a vitrification solution composed of α-MEM, 10% dimethyl sulfoxide (DMSO), 0.25 mol/L of sucrose and thymol at different concentrations (2.5, 25 and 250 µg/mL). For warming the samples were sequentially immersed in wells composed of α-MEM, 10% FBS, and decreasing concentrations of sucrose (0.5M; 0.25M; 0M) for 5 minutes each. Following warming, fragments were macerated and centrifuged for spectrophotometric assays. The total protein test was performed to obtain the total protein concentration in each sample. Thiol level was determined using 5.5' -dithiobis(2-nitrobenzoic acid). Superoxide dismutase (SOD) activity was measured by adrenaline auto-oxidation inhibition. Catalase (CAT) was evaluated by hydrogen peroxide (H2O2) consumption. Glutathione peroxidase (GPX) activity was measured by oxidation of NAPH. Data normality was assessed with the Shapiro-Wilk test. Analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to compare CAT activity in vitrified tissues among treatments. Protein (SOD, GPX), thiol levels and the percentage of viable CCs were compared using the non-parametric Kruskal-Walli's test. Statistical significance was set at $p < 0.05$. The cytotoxicity assesses showed that, calcein-AM fluorescence intensity in CCs cultured with 2.5, 25.0 and 250.0 µg/mL of thymol did not differ significantly from the control group ($p>0.05$). However, ethidium fluorescence intensity was significantly reduced in CCs treated in medium supplemented with 25 µg/mL of thymol, when compared to control ($p <0.05$). After vitrification, SOD activity was significantly increased in ovarian tissue exposed to 25 µg/mL of thymol compared to non-vitrified tissue ($p<0.05$). No significant difference in thymol addition on CAT and GPX activity was observed in vitrified tissues ($p>0.05$). Thiol content significantly reduces in vitrified tissue with 2.5 µg/mL of thymol in comparison to non-vitrified tissue ($p<0.05$), while other thymol concentrations did not alter thiol levels. In conclusion, thymol at 25.0 µg/mL demonstrated cytoprotective effects in CCs and enhanced antioxidant defense in vitrified ovarian tissue by increasing SOD activity and preserving thiol levels at higher concentrations.