A215 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Histone post-translational modifications H3K9me3 and H3K27me3 roles in the kinetics of the first cleavages: causes or consequences?

Jessica Ispada[1,2], Otávio Luiz Ramos Santos[1], Aldcejam Martins Fonseca Junior[1], Camila Bruna de Lima[1,2], Kelly Annes[1], Erika Cristina dos Santos[1], James L Chitwood[3], Pablo Juan Ross[3], Marcella Pecora Milazzotto[3]

[1]UFABC - Universidade Federal do ABC, Santo André; [2]USP - Universidade de São Paulo, São Paulo; [3]UC - University of California Davis, Davis.

The timing of the first cleavages is a tool used for selection of human embryos. The same approach has been adapted for cattle and studies identified that the kinetics during the first cleavages generates blastocysts with different gene expression patterns and DNA methylation levels. These differences might be associated with epigenetic changes. Based on that, we investigated the dynamics of histone H3 lysine 9 trimethylation (H3K9me3) and lysine 27 trimethylation (H3K27me3) and transcripts of factors involved in the control of these epigenetic marks in fast and slow developing embryos. For this, bovine embryos were produced in vitro and, at 40 hours post insemination (hpi), were classified as Fast (4 or more cells) or Slow (2-3 cells) and collected at 40hpi (FCL and SCL), 96hpi (FGA and SGA) or 168hpi (FBL and SBL). For H3K9me3 and H3K27me3, embryos (35-62 nuclei at 40hpi, 67-97 at 96hpi and 402-467 at 168hpi from 7-15 embryos from 3 replicates and 21-33 nuclei at 40hpi, 57-83 at 96hpi and 147-286 at 168hpi from 5-9 embryos from 3 replicates, respectively) were immunostained with specific antibodies. The fluorescence intensity of each nucleus was quantified using ImageJ and analyzed by Student's t-test or one-way ANOVA. For transcript quantitation, RNAseq data was accessed from a previous report using the same kinetics classification model (Milazzotto et al, 83(4):324-36 2016). In this study, H3K9me3 increased in fast and slow embryos in the transition from 96 to 168hpi (SGA 8,8±0,3 AU vs FGA 6,7±0,5 AU; FBL 17,8±0,3 AU vs SBL 14,1±0,2 AU). Also, higher levels of H3K9me3 were present at 40hpi in fast embryos (FCL 15,7±0,8 AU vs SCL 9,9±0,9 AU), whereas slow embryos presented its higher intensity during the genome activation stage (P<0.0001). Gene expression analysis of proteins involved with H3K9me3 revealed overexpression of KDM3A and KDM7A at 40hpi and SETDB1, SETDB2, SUV39H1, KDM1A, KDM1B, KDM3A and KDM3C at 168hpi in slow embryos. No differences in H3K27me3 fluorescence was observed at 40hpi (FCL 4,8 ±0,8 AU vs SCL 4,0±0,8 AU; P=0,89). At 96hpi, fast embryos presented higher H3K27me3 intensity than slow embryos (FGA 7,8±0,5 AU vs SGA 5,4±0,4 AU) and, in both kinetics groups, the level increased from 96hpi to blastocyst stage, with higher levels detected in SBL than in FBL (FBL 11,2±0,6 AU vs SBL 21,4±1,0 AU; P <0.0001). At 40hpi, slow embryos presented higher expression of KDM7A and KDM3A and less of EZH1 transcripts and at blastocyst stage, higher expression of KDM3A and EZH2 and lower expression of KDM6B were observed in slow embryos. It is possible to conclude that the kinetics of the first cleavages is related with histone post-translational modifications in embryos and, apparently, the difference of H3K9me3 levels between fast and slow embryos appears since the first cleavages, probably being inherited from gametes, whereas H3K27me3 became different between groups only at the genome activation stage.

A216 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Using pregnancy-associated glycoproteins to provide early pregnancy diagnosis in cattle

Fabio Girardi Frigoni[1], Mariana Dulce Delle Vedove Ortolan Sayeg[1],
Luiz Fernando Rodrigues Féres[2], Fernando José Delai Pardo[3], Mariana Pallú Viziack[1],
Carlos Alberto Rodrigues[4], Pietro Sampaio Baruselli[1]

[1]FMVZ/USP - Universidade de São Paulo - Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP;
[2]Fazendas do Basa - Fazendas do Basa, Leopoldina, MG; [3]Idexx - Idexx, Itaim Bibi, São Paulo, SP; [4]Agrindus - Fazenda Santa Rita, Descalvado, SP, Brasil.

The objective of this study was to evaluate the viability of PAG (pregnancy-associated glycoprotein) ELISA kit to provide early pregnancy diagnosis. Two experiments were done. Experiment 1: 150 bovine females (78 Gir and 72 crossbreeds) were synchronized with 1.9g progesterone (P4) device (CIDR® Zoetis, Brazil) and 2mg of estradiol Benzoate (BE) (RicBE®, Tecnopec, Brazil) on Day 0. On Day 8, the P4 device was removed, and all animals received 0.5mg Sodic Cloprostenol (Ciosin®, MSD, Brazil), 1mg of estradiol cypionate (CE; ECP®, Zoetis, Brazil) and 300 IU of eCG (Folligon®, MSD). The embryo transfer (ET) was done 9 days after the P4 device removal. Blood samples were collected from all animals on day 19 (n=150) and on day 21 post-ET (n=125), corresponding to 26 and 28 days of gestation. Ultrasound (US) examinations were performed on day 19 post-ET, using a 7.5 MHz linear array transducer. Observation of allantoic fluid or a visible fetus yielded a positive gestation diagnosis. At US, 46.7% (70/150) cows were pregnant and 53.3% (80/150) were not pregnant. Results were classified as positive diagnosis (vp), false positive diagnosis (fp), negative diagnosis (vn), and false negative diagnosis (fn). Sensitivity [100 vp/(vp+fn)], specificity [100 × vn/(vn+fp)], positive predictive value (PPV) [100 × vp/(vp+fp)], negative predictive value (NPV) [100 × vn/(fn+vn)} and accuracy [(vp+vn)/(vp+vn+fp+fn)] were calculated for each PAG test (26 and 28). Statistical were done using PROC FREQ of SAS 9.4 version. Nemar's test compared sensibility and specificity of PAG 26 and PAG 28. US results were used as a gold standard. The PAG26 test yielded 98.7% of sensitivity, 92.0% of specificity, 92.5% of PPV, 98.6% of NVP and 95.3% of accuracy. The PAG28 test reached 100% of sensitivity, 89.6% of specificity, 89.2% of PPV, 100% of NVP and 94.4% of accuracy. No statistical differences were found in sensibility and specificity (P=1.0) between PAG 26 and PAG 28. Experiment 2: 195 Holstein heifers were synchronized with 3mg norgestomet auricular implant (Crestar® MSD, Brazil) and 2mg BE (Sincrodiol®, OuroFino, Brazil) on Day 0. On Day 8, the implant was removed, and 0.5mg i.m Sodic Cloprostenol (Ciosin®, MSD, Brazil), 1mg CE (Sincroecp®, OuroFino, Brazil) and 300IU eCG (Folligon®, MSD) were administrated. FTAI was performed on Day 10 as well 0.1mg Gonadorelin (Fertagyl®, MSD, Brazil) was administrated. Blood samples were collected on day 26 post-FTAI (n=195) and US examinations were done. The PAG ELISA 26 post-FTAI reached 94.2% of sensitivity, 96.0% of specificity, 92.9% of PPV, 96.8% of NVP and 95.4% of accuracy. In conclusion, no differences were found in the efficiency of pregnancy diagnoses comparing PAG 26 and PAG 28 in the ET program. Furthermore, the PAG 26 presents elevated efficiency for early pregnancy diagnosis in the AI program. Therefore, the use of PAG ELISA on 26 days of gestation can be an alternative to early pregnancy diagnose in the AI and ET programs.

A217 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Toxicity of dimethylsulphoxide in cryopreservation of bovine ovarian tissue

Camila Bizarro-Silva[1,2], Marcela Bortoletto Cerezetti[2], Camila Bortoliero Costa[2], Rafael Luiz Stolf[2], Maysa Lopes Orsi[2], Sofia Botsaris Delchiaro[2], Anne Yaguinuma de Lima[2], Higor Souza de Camargo[2], Marcelo Marcondes Seneda[2]

[1]PUCPR - Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Toledo, PR, Brasil; [2]UEL - Universidade Estadual de Londrina, Londrina, PR, Brasil.

The objective of this study was to use histology for evaluating the toxicity of three different concentrations (1M, 1.5M, and 3M) of dimethylsulfoxide (DMSO) before and during vitrification of bovine ovarian tissue. The ovarian cortex of *Bos taurus indicus* (n=10) was fragmented (6 mm^3, N=7 per animal) and randomly distributed between the control (non-vitrified) and three groups with different concentrations of DMSO: i) 1M; ii) 1.5M or iii) 3M. Before the cryopreservation, a test was performed to assess the toxicity of DMSO to ovarian tissue without vitrification. To achieve this, the fragments were exposed for 20 min at 20°C in 1.8 mL of essential minimal medium (MEM) containing DMSO at the same three concentrations and then fixed in Bouin for 24h for subsequent histologic evaluation. For vitrification, fragments were placed in cryotubes containing 1.8 mL of MEM plus one of three concentrations of DMSO at 4°C for 15 min. The fragments were then exposed to the same concentrations of DMSO supplemented with 0.25 M sucrose and 10% fetal bovine serum for the period of 15 min at 4°C. Subsequently, the ovarian tissues were dried and placed in contact with the metal cube surface partially immersed in liquid nitrogen for vitrification during 30 seconds. Once vitrified, the sample was stored at -196°C for 7 days. After warming, fragments were then fixed in Bouin and processed for classical histology with Schiff Periodic Acid and Hematoxylin staining. The analyzes were performed considering morphological aspects (integrity and follicular degeneration) before and after cryopreservation. The data were submitted to ANOVA and Tukey's test, considering P≤0.05. The control contained largely intact follicles and a small portion of degenerate follicles, according to physiological patterns. In the histological analysis during the toxicity test all concentrations of DMSO presented a higher level of degenerated follicles, comparing to the control (1M: 28.3% (210/741); 1.5M: 49.8% (252/506); 3M: 65.7% (349/531) and control: 18.7% (80/428); P<0.05). The concentration of 3M DMSO during the toxicity test had an injurious effect the integrity on follicles (34.4% (182/531); P<0.05) when compared to the other concentrations of DMSO. After vitrification/warming, the fragments in 1M, 1.5M, and 3M DMSO showed similar percentages of intact follicles (1M: 62.0% (345/557); 1.5M: 55.2% (266/482) e 3M: 33.8% (127/375; P>0.05). However, 1M of DMSO resulted in percentages of degenerate follicles statistically equivalent to control (38.0% (212/557) and 18.7%, respectively; P<0.05; 1.5M: 44.8% (216/482) and 3M: 66.1% (248/375). In addition, 3M of DMSO was more related to follicle degeneration (66.1%; P<0.05) in comparison to other groups. Therefore, it is suggested that the concentration of 1M DMSO showed to be less toxic to the preantral follicles of bovines after vitrification and warming of the ovarian tissue.

A218 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Oxidative stress during the cryopreservation of ovarian bovine tissue with dimethylsulfoxide

Camila Bizarro-Silva[1,2], Andressa Guidugli Lindquist[2], Suellen Miguez González[2], Larissa Zamparone Bergamo[2], Denis Vinicius Bonato[2], Ana Carolina Rossaneis[2], Waldiceu Aparecido Verri Junior[2], Marcelo Marcondes Seneda[2]

[1]PUCPR - Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Toledo, PR, Brasil; [2]UEL - Universidade Estadual de Londrina, Londrina, PR, Brasil.

The objective of this study was to evaluate the oxidative stress caused to bovine ovarian tissue after vitrification with three different concentrations (1M, 1.5M and 3M) of dimethyl sulfoxide (DMSO). The ovaries from *Bos taurus indicus* cows (n = 10) were collected from a local abattoir, fragmented (6 mm^3; n = 4 per animal) and being placed in a control treatment (placed directly on the liquid nitrogen) and three vitrified groups: i) 1M of DMSO; ii) 1.5M of DMSO and iii) 3M of DMSO. For vitrification, the fragments were placed in 2.0 mL cryotubes and submitted to equilibration solution and transferred to the vitrification solution. Thus, the ovarian fragments were exposed to 1.8 mL of Minimum Essential Medium (MEM) containing one of the following DMSO concentrations (1M, 1.5M and 3M) for 15 min at 4°C. After the equilibration, the fragments were transferred to 1.8 mL in MEM with the same concentrations of DMSO, supplemented with 0.25 M sucrose and 10% fetal bovine serum for 15 min at 4°C. Follicles from all fragments (n = 40) were analyzed for oxidative stress to evaluate the presence of reactive oxygen species and antioxidant capacity after vitrification. Samples of cryopreserved ovarian tissue (n=30) were heated and the cryoprotectant removed for the execution of the kinetic-colorimetric assays for determination of superoxide anion production in homogenates of tissue (10 mg /ml in 1.15% KCl) using the modified nitroblue tetrazolium (NBT) assay. The reduction of NBT was measured at 600 nm (Multiskan GO, Thermo Scientific) and tissue weight was used for normalization of the data. The ability of the sample to resist oxidative damage was determined by the neutralization of free radicals (ABTS) by cation sequestration of 2,2 V-azinobis (3-ethylbenzothiazoline-6-sulfonate). For the statistical analysis of the oxidative stress assays, the data were submitted to ANOVA and Tukey's test, considering a value of P≤0.05. It was observed that the vitrified samples in 1M and 1.5M DMSO presented similar superoxide anion levels when compared to the control (12.05 in 1M; 15.05 in 1.5M, 26.20 in 3M of DMSO and 16.21 OD/mg protein in the non-vitrified control, P> 0.05). For the antioxidant capacity of the fragments submitted to vitrification with 1M DMSO presented similar results with the control (151.01 in 1M, 184.35 in 1.5M; 246.98 in 3M of DMSO and 168.77 equivalent of the Trolox/mg protein in the non-vitrified control, P> 0.05). Conversely, vitrified ovarian tissue samples analyzed for the identification of reactive oxygen species presented reduction in superoxide anion levels and antioxidant capacity at 1M and 1.5M DMSO concentration when compared to 3M (P <0.05). It is therefore suggested the use of 1M DMSO for vitrification of ovarian tissue because it presents lower levels of reactive oxygen species and a higher antioxidant capacity.

A219 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Metanalyses of RNA-seq data reveal crucial role of lncRNAs during bovine early embryo development

Ricardo Perecin Nociti[1,2,3], Rafael Vilar Sampaio[2], Carlos A O Biagi Júnior[4], Wilson A Silva Jr[4], Vera Fernanda Martins Hossepian de Lima[1], Pablo Juan Ross[3], Flavio Vieira Meirelles[2]

[1]FCAV-UNESP - Sao Paulo State University, Jaboticabal, São Paulo, SP, Brasil; [2]FZEA-USP - University of Sao Paulo, Pirassununga, SP, Brasil; [3]UC-DAVIS - University of California Davis, Davis, CA; [4]FMRP-USP - University of Sao Paulo, Ribeirão Preto, SP, Brasil.

Long non-coding RNAs (lncRNAs) are a relative new class of non-coding RNAs involved with gene regulation in a stage and tissue specific way. However, little is known about their role during the transcriptional events during bovine embryo development. The bovine genome (Bos_taurus.ARS-UCD1.2) has 1480 annotated lncRNAs and 23515 putative lncRNAs (NONCODE). We hypothesized that RNA-seq public data from the NCBI GEO (Gene Expression Omnibus) could be combined to explore the lncRNAs transcriptional changes through embryo development. Furthermore, we used 9 experiments (GSE52415, GSE61717, GSE25082, GSE74675, GSE44023, GSE48147, GSE56513, GSE85563, SRR7757966), a total of 115 samples from gametes (sperm and oocytes) up to day 19 (D19) of development and all development stages were used, and each stage was contrasted with the previous and with the following stage. We used SRA Toolkit to download data and to convert, from SRA format to fastq format; fastQC for quality check; Trim Galore for read trimming; multiQC for report compiling. For read alignment and gene count we used STAR. We used R for all statistical analysis. For differential gene expression we used DESEQ2 using p adjusted2, for enrichment and co-expression analyses we used the clVALID and ClusterProfiler packages respectively. Our results showed that 6457 transcripts were differently expressed among the contrasts evaluated. The blastocyst stage showed the greatest differences among the stages analyzed. We identified 62 transcripts with the highest expression level during maternal to zygote transition stage, that are enriching the DNA replication, modulation, alkylation, methylation, and demethylation pathways. We also found 33 transcripts that had lower expression levels only during the blastocyst stage, enriching the lamelipodium pathway. A total of 189 transcripts had the highest mean levels of expression during the embryo-maternal recognition period enriching the pathways of lipid metabolism, cholesterol efflux, organization to external stimuli, and cell migration. We also identified 11 lncRNAs clusters, in which we detected 1284 lncRNAs broadly expressed. On the remaining cluster we found 121, 7, 15, 40 and 6 lncRNAs that exhibited the highest expression level during GV to 4 Cells stages; at embryonic genome activation period; at sperm and from Blastocyst to D19; from the embryonic genome activation until D19 and only at blastocysts stage, respectively. We conclude that public bovine embryo sequencing data can be used and combined, been a great information source of bovine embryo development. Furthermore, our analysis suggests a crucial role of lncRNAs during maternal to zygote transition and blastocyst formation and development.

A220 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Embryonic Culture Supplementation (ECS) – a new culture system based on oviduct and uterus fluid composition improves bovine embryo production and quality

Erika Cristina dos Santos[1], Aldcejam Martins da Fonseca Junior[1], Camila Bruna de Lima[1], Jessica Ispada[1], Kelly Annes[1], João Vitor Alcantara da Silva[2], Marcella Pecora Milazzotto[1]

[1]UFABC - Universidade Federal do ABC, Santo André, SP, Brasil); [2]UMC - Universidade Mogi das Cruzes, Mogi das Cruzes, SP, Brasil.

The systems of *in vitro* production (IVP) of bovine embryos aim to mimic the female reproductive tract in order to generate blastocysts more similar to those produced *in vivo*. However, the amount and availability of nutrients in a dynamic system, such as the *in vivo,* may be greater than that required for a static *in vitro* system. In fact, in rats, the embryo development in systems with reduced amounts of nutrients increased blastocyst quality and rates. To verify that, we developed a sequential culture media – *Embryonic Culture Supplementation (ECS)* – based on the salt-based composition of SOF (Synthetic Oviduct Fluid) and supplemented with the amount of energy substrates and amino acids present in the composition of bovine oviduct (*ECS1-100*) and uterus (*ECS2-100*) fluids. We also supplemented *ECS* with half of the concentration found in these biological fluids (*ECS1-50* and *ECS2-50*) to verify if the reduction of such substrates could be beneficial to embryo development and quality. All media used in this experiment were supplemented with 8mg/mL of Bovine Serum Albumin (BSA) and antibiotics. Embryos were produced *in vitro* by using conventional protocols and after fertilization they were transferred to one of the following groups: <u>*Control*</u> (C) - SOFaa: supplemented with 2% essential amino acids, 1% nonessential amino acids, 1.5 mM glucose), <u>*ECS100*</u> – zygotes cultured in ECS1-100 or <u>*ECS50*</u> – zygotes cultured in ECS1-50. At Day 4 (D4), cleavage rates were assessed and embryos were transferred to their correspondent group (Control, ECS2-100 and ECS2-50) where they remained until blastocyst (D7). Blastocysts were assessed by means of rates, energy metabolism (mitochondrial activity, NADH, FAD and ATP production) and oxidative stress (generation of ROS). All data were analyzed by ANOVA and Student's T-test (n=128 blastocyst/16 replicates). Embryo production was positively affected by the reduction of energy substrates and amino acids in culture (Cleavage rates – C = 75.9 ± 2.1; ECS50 = 81.6 ± 1.4; ECS100 = 76.8 ± 3.3, p= 0.03/ Blastocyst rates – C = 34.0 ± 1.4; ECS50 = 41.1 ± 1.8; ECS100 = 37.3 ± 1.9, p= 0.03). Blastocysts from ECS 50 presented reduction of ROS comparing to control group (p= 0.03) and increase of mitochondrial activity (p= 0.01) and NADH production (p= 0.01) when comparing with ECS 100. Mitochondrial activity was also higher in control group (p= 0.04) comparing with ECS 100. No differences were found among groups related to FAD and ATP production. Based on these results, we can conclude that ECS is not only able to support embryo development to blastocyst, but the reduction in energy substrates and amino acids concentration (ECS 50) seems to be beneficial for embryo production and blastocyst quality. Acknowledgment: FAPESP 2016/00350-0 and FAPESP 2017/18384-0.

A221 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Applied use of interferon-tau stimulated genes expression to detect pregnancy status in Nelore cattle submitted to timed-AI

Gabriela Dalmaso de Melo, Barbara Piffero Mello, Catia Aparecida Ferreira, Cecilia Constantino Rocha, Amanda Guimarães da Silva, Leonardo Marin Ferreira Pinto, Carlos Alberto Souto Godoy Filho, Leonardo Amaral, Igor Garcia Motta, Gilmar Arantes Ataide Junior, Ed Hoffmann Madureira, Guilherme Pugliesi

USP - University of São Paulo, Pirassununga, SP.

This study aimed to evaluate the use of interferon tau stimulated genes (ISGs) expression in peripheral blood polymorphonuclear cells (PMN) associated with color Doppler ultrasonography (Doppler-US) to detect pregnancy 20 days after timed-AI (TAI) in beef cattle. Nelore cows (n=144) and heifers (n=103) were submitted to TAI (D0). On D20, PMNs were isolated from jugular blood by Ficoll gradient (GE Healthcare), and the RNA was extracted. Expression of target genes (ISG15 and OAS1) was quantified by qPCR and normalized to reference genes (GAPDH and ACTB). Pregnancy diagnoses were performed on D20 by luteolysis detection with Doppler-US, and on D30 and D70 by detection of embryo and fetus with heartbeat, respectively. Animals were classified as: pregnant (P; fetus on D70), non-P (NP; no active CL on D20), early embryo loss (EEL; active CL on D20 but NP on D30) and late embryo loss (LEL; embryo on D30 but NP on D70). ISG expression was analyzed by ANOVA using PROC MIXED of SAS considering the effects of group, category and their interaction. ROC curves were created and the area under the curve (AUC), accuracy (Ac), specificity (Sp) and sensitivity (Se) were calculated for pregnancy predictions on D20 (ISGs and Doppler-US) compared to the standard diagnosis method on D30. Expression of ISG15 and OAS1 were greater (P<0.01) in the P (2.0 ± 0.2 and 2.4 ± 0.2, respectively) compared to the NP (0.5 ± 0.1 for both) and EEL (0.9 ± 0.2 and 0.8 ± 0.2, respectively) groups but did not differ from the LEL group (1.6 ± 0.8 and 1.2 ± 0.5, respectively). A greater (P<0.01) ISG15 expression was also observed in heifers than cows (fold change: 1.55). ROC analysis indicated that ISG15 and OAS1 were significant (P<0.01) predictors of pregnancy in heifers (AUC= 0.81 and 0.86, respectively) and cows (AUC= 0.77 and 0.82, respectively). The optimal cutoff value for ISG15 was 4-fold increased in heifers than cows; whereas for OAS1, the same cutoff value was determined for both categories. Doppler-US presented a higher Ac (89% for heifers and 93% for cows) compared to ISG15 (80% for heifers and 72% for cows) and OAS1 (81% for heifers and 75% for cows). Doppler-US, ISG15 and OAS1 methods resulted, respectively, in Sp of 76%, 82% and 65% in heifers, and 83%, 70% and 75% in cows, and Se of 100%, 78% and 74% in heifers, and 100%, 78% and 75% in cows. When both genes were evaluated only in females with an active CL on D20, the Ac was improved for OAS1 in heifers (91%) and cows (82%) due to reduction of false positive results. In conclusion, heifers have greater ISG15 expression than cows, which resulted in different cutoff values for both categories. ISG15 and OAS1 expression were increased in P than NP and EEL animals, suggesting they are good predictors of pregnancy. However, ISG expression (mainly OAS1) can only be used to reduce false positive results of EEL females, as both genes presented lower Se and Ac compared to Doppler-US method.

Anim. Reprod., v.16, n.3, p.675, Jul./Sept. 2019

675

Can we can predict estrus in Gir (*Bos indicus*) heifers using behavior monitor device system?

**Mariana Dulce Delle Vedove Ortolan Sayeg[1], Luiz Fernando Rodrigues Féres[3],
Luísa Helena Bartocci Liboni[4], Jessica Cavanelas Fernandes[1], Leonardo Salgado Maia[2],
Diego Marcondes Guerra[2], Fabio Girardi Frigoni[1,2], Augusto Rodrigues Felisbino Neto[1],
Laís Ângelo de Abreu[1], Marcos Henrique Alcantara Colli[1], Pietro Sampaio Baruselli[1]**

[1]USP - Universidade de São Paulo, Butantã, São Paulo, SP; [2]CRV Lagoa - CRV Lagoa, Sertãozinho, SP; [3]Faz Basa - Fazendas do Basa, Leopoldina, MG; [4]IFSP - Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Jardim Canaa, Sertãozinho, SP, Brasil.

Heat detection is one of the greatest challenges in dairy farms all over Brazil. Technologies like Ovalert device can be used in reproductive management to identify which cows are in heat, increasing service and pregnancy rates and improving the financial performance of the farm. The goal of this study was to evaluate the efficiency of heat detection by the electronic device Ovalert (CRV Lagoa, Brazil) in Gir (*Bos indicus*) heifers based on data of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. For that, 54 Gir heifers received the Ovalert collar at least 8 days before the beginning of the experiment, then they were synchronized with 1,9g progesterone device (Cidr ® Zoetis, Brazil) and 2mg of Estradiol Benzoate (RicBE®, Tecnopec, Brazil) on day 0 of the protocol. On day 8, the progesterone device was removed, all animals received an Estrotect® device, 0,5mg i.m Sodic Cloprostenol (Ciosin®, MSD, São Paulo, Brazil), 1mg of estradiol cypionate (ECP®, Zoetis, Brazil) and 300 IU of eCG (Folligon®, MSD). Estrotect was used as a control to determinate true estrus and fake estrus on the Ovalert system and they were evaluated twice a day to determinate the presence of the estrus. The heat in Ovalert system was determined by the warning generated by the increase in animal activity. Ultrasound exam was made on day 18 to determine the presence of corpus luteum (ovulation rate). Statistical analyses were performed using WEKA Software. The ovulation rate was 79.6% (43/54). Cows were considered in estrus when Estrotect was positive. Cows with Estrotect positive and Ovalert negative was considered false negative. All cows had positive Estrotect in this experiment (54/54) and 94.4% (51/54) was also positive in Ovalert, so the index of false negative was 5.5% (3/54). In this study, no false positive were found (0% for false positive index). The sensibility, that is the capacity of Ovalert determinate the estrus in the animals that are really in estrus (confirmed by estrotect) was 94.4%. The specificity, that is the capacity of the system to determinate not heat in cows that are not in heat, was unable to determinate because all cows were in heat. Positive predictive value (the probability that the cow with a positive result in Ovalert is actually in heat and the accuracy (probability of the test providing correct results) are also 94.4%. The negative predictive value was 0 because of the absence of false negative animals. In conclusion, indices reveal high sensitivity of the test and high accuracy. In this way, despite the lack of negative individuals, the indexes reveal that the heat can be predicted using a behavior monitor device system in Gir (*Bos taurus indicus*) heifers.

A223 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Development and validation of a low-cost ELISA methodology for bovine insulin quantification

Guilherme de Paula Nogueira, Marco Antonio Maioli, Ana Flavia Tereza Paiva, Waneska Stéfani Spinelli Frizzarini, Devani Mariano Pinheiro

FMVA-UNESP - Faculdade de Medicina Veterinária - UNESP, Araçatuba, SP, Brasil.

The reproductive processes are dependent on numerous variables, including the animal's metabolic status. Monitoring parameters, such as the quantification of metabolic hormones can be decisive in research to improve reproductive efficiency. The aim of this work was to develop and validate a low cost and specific ELISA for insulin quantification in cattle plasma. First, bovine insulin was processed for zinc removal according to Sodoyez et al. (J. Biol. Chem, 250: 4268-77, 1975) and subsequently conjugated to biotin according to Maioli et al. (Vet. Bras., 37: 1545-53, 2017). Anti-insulin antibody production was performed in guinea pigs by intradermally injection of zinc-free bovine insulin emulsified in Freund's adjuvant, complete on the first immunization and incomplete thereafter (every 28 days). The antiserum was obtained by cardiac puncture at 42, 63 and 100 days of treatment. For the assays, the antibody dilution and biotinylated hormone concentration were determined in bidimensional tests, using microwell plates coated with Goat anti-Guinea-Pig IgG (0.25 μg/well), blocked with 1% BSA, as in Kekow et al. (Diabetes, 37: 321-26, 1988), using tetramethylbenzidine as a chromogen. Insulin was quantified in samples from 5 lactating Holstein cows submitted to the glucose tolerance test (Adamiak et al., Biol. Reprod., 73: 918-26, 2005) for biological validation and the results were expressed in μUI/ml. Assay precision was determined by the coefficients of variation (CV) in assay from high (CA) and low (CB) insulin concentrations controls, and the limits of detection (LOD) and quantification (LOQ) were determined according to Shrivastava et al. (Chron, Young Sci., 2: 21-25, 2011). For determination of these parameters, 689 samples were quantified into 20 assays. Basal insulin concentration of the tested animals was close to 20 μIU/mL. Five minutes after intravenous glucose infusion there was a peak in the hormone concentration (136 ± 11.71 μUI/mL), which decreased around 120 min, reaching the basal values. Mean insulin concentration for CA and CB was 121.75 and 26.02 μUI/mL, respectively, with intra-assay CV being 7.1% (CA) and 7.59% (CB) and the inter-assay CVs were 13.92% (CA) and 15.09% (CB). The LOD was 1.57 μUI/mL and the LOQ was 4.76 μUI / mL. In conclusions, the methodology was considered fully developed and can be applied for quantification of bovine insulin with a calculated cost of U\$1.00 per sample.

A224 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Relative gene expression of esr1 and esr2 in the uterine horns of bitches along diestrus

Vanessa da Silva Alves Gossler[1], Francislaine Anelize Garcia Santos[2], Aline de Oliveira Santos[1], Ana Paula Mattoso Miskulin Cardoso[3], Lilian Francisco Arantes de Souza[1], Natalia Caroline de Oliveira Marçal[1], Lauren Chrys Soato Marin Schaffer[1], Paula de Carvalho Papa[2], Ines Cristina Giometti[1]

[1]Unoeste - Universidade do Oeste Paulista, Presidente Prudente, SP; [2]USP - Universidade de São Paulo, São Paulo, SP; [3]Unesp - Universidade Estadual Paulista "Júlio de Mesquita Filho", Presidente Prudente, SP, Brasil.

The diestrus stage in the bitch is characterized by the increase of Progesterone (P4) in the first half of diestrus and by fluctuations of Estradiol (E2) in the second half of diestrus. E2 has higher concentrations in the middle of the diesturs around day 40, declining later (Papa and Hoffmann, Reproduction in Domestic Animals, 46: 750-756, 2011). The effects mediated by E2 depend on the binding of E2 to the estrogen receptors. In general, ERα (whose gene is ESR1) promotes cell proliferation and ERβ (ESR2) appears to play an antiproliferation role (Vivar et al., J Biol Chem, 285: 22059-22066, 2010). The objective of this study was to determine the gene expression of ESR1 and ESR2 in the uterus of bitches during diestrus (days 10, 20, 30, 40 and 50 post-ovulation). For this, the uterine horns of 25 clinically healthy bitches of different ages and with no defined racial pattern were used. The uterus was obtained from dogs that underwent ovariohysterectomy (OSH) at 10, 20, 30, 40 and 50 days after ovulation, thus constituting the five groups studied in this experiment (n = 5 / group). The uterine horns were harvested and stored in TRIzol® (Thermo Fisher Scientific, California, USA) in the freezer at -80 ° C and thereafter the relative abundance of mRNA of ESR1 and ESR2 were evaluated by RT-qPCR, using GAPDH as reference gene. The data were evaluated using the Kruskal-Wallis test followed by Student Newman (p <0.05). ESR2 presented higher relative gene expression (p <0.05) at 10, 20 and 30 days after ovulation when compared to 40 and 50 days. There was no significant statistical difference for the relative gene expression of ESR1 in the studied groups. It is concluded that only the gene expression of ESR2 undergoes alteration during diestrus, decreasing after day 30 post-ovulation, and may have been a consequence of the incremental increase of E2. Support: FAPESP (process number: 2014/00739-9) and CAPES - Financing Code 001.

A225 Support biotechnologies: Cryopreservation and cryobiology, imaging diagnosis, molecular biology and "omics"

Modulation of mitochondrial acetyl-coa production impacts histone acetylation levels of *in vitro* produced bovine blastocysts

Aldcejam Martins da Fonseca Junior[1], Erika Cristina dos Santos[1], Jessica Ispada[1], Camila Bruna de Lima[1], João Vitor Alcantara da Silva[2], Kelly Annes[1], Marcella Pecora Milazzotto[1]

[1]UFABC - Universidade Federal do ABC, Santo André, SP; [2]UMC - Universidade de Mogi das Cruzes, Mogi das Cruzes, SP, Brasil.

Glycolysis is a metabolic pathway that converts glucose into pyruvate that can be driven to the tricarboxylic acid cycle (TCA) by an intermediate step in which it is converted to acetyl-CoA. The TCA cycle is responsible for the production of citrate that can be exported from the mitochondria to the cytosol and also generate acetyl-CoA. Acetyl-CoA is an important regulatory factor for histone acetylation. Lysine 9 of histone H3 acetylation (H3K9ac) is one of the main epigenetic mechanisms responsible for the genome reprogramming after fertilization. We investigated the possible interaction between acetyl-CoA biosynthesis and the epigenetic control H3K9ac. Bovine embryos were *in vitro* cultured in the presence of the following modulators: dichloroacetate (DCA) – inhibitor of pyruvate dehydrogenase (PDH) phosphorylation, carrying more pyruvate to the mitochondria, increasing acetyl-CoA generation or iodoacetate (IA) - inhibitor of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), diminishing the pyruvate synthesis which leads to a decrease in pyruvate/acetyl-CoA generation. Embryos were produced by conventional protocols and cultured in a SOF based culture media. At D4 of culture, corresponding to the major genome activation turn, embryos were transferred to new drops of media supplemented with the modulators: Control group – no supplementation; DCA1, DCA2, and DCA5 – supplementation with 1mM, 2mM or 5mM of DCA respectively; IA1, IA2, and IA5 – supplementation with 1uM, 2uM or 5uM of IA, respectively. Embryos were cultured until day 7 when collected and fixed for immunostaining of H3K9ac. Images were acquired by software (LAS, v.3, Leica, German) and the quantification of fluorescence intensity was measured by the mean value of pixels of each nucleus (ImageJ v.1.8.0 NIH, EUA). A minimum of 88 nuclei were analyzed per group. All values were submitted to ANOVA with Tukey or Kruskal-Wallis post-test with $P<0.05$ for fluorescent data as well as for embryo production. Blastocyst rates did not differ among groups for DCA (Control: 32.5%±7.9; DCA1: 37.7%±6.8; DCA2: 32.3%±8.0; DCA5: 21.6%±4.0; $P = 0.461$) and IA (Control: 24.65%±4.8; IA1: 40.03%±5.0; IA2: 43.13%±9.3; IA5: 24.80%±8.3; $P = 0.186$. DCA supplementation resulted in increased H3K9ac mark in all doses when compared to control (Control: 22.90±0,4AU; DCA1: 32.6±0.5AU; DCA2: 41.5±0.7AU; DCA5: 35.6±0.9AU; $P <0.0001$). IA1 and IA2 had a diminished H3K9ac mark but, interestingly IA5 did not present differences when compared to control (Control: 43.8±0.6AU; IA1: 34.7±0.5AU; IA2: 30.1±0.6AU; $P <0.0001$/ IA5: 41.2±1.4AU; $P =0.118$). In conclusion, the addition of modulators of acetyl-CoA generation allows blastocyst development in similar rates that the control. In addition, these changes in embryo metabolism directly influence the acetylation profiles for H3K9 in blastocysts, which can further affect cellular differentiation and potentiality, impacting the development.