Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Evaluation of injectable progesterone on the reproductive efficiency of goats in the off-season

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Goats are seasonally polyestrous; therefore, the production of kids is restricted to only one time of the year, but with estrous cycle manipulation, this production can be distributed throughout the year. The present study aimed to estimate the reproductive efficiency of Boer female goats, after administration of injectable P4, combined or not with the male effect, during the off-season in the southern region of Paraná, evaluating the pregnancy rate, twin births, number of kids born per group and concentration of births. The study was conducted in a beef goat farm, using 73 goats randomly assigned to three experimental groups. In the G1 group (n=22), females were stimulated by the male effect; in the G2 (n=25), animals received 75 mg of injectable P4, intramuscularly, at the beginning of the experiment. In G3 (n=25), goats received 75 mg of injectable P4, intramuscularly, at the beginning of the experiment, and were subjected to the male effect. For the male effect, goats were separated from the females in a pen with a distance of 300 meters, without physical, visual, or olfactory contact for 60 days. Then, they were placed together with the goats of the G1 and G3 groups. Females of the G2 group remained in contact with the goat (visual and olfactory) before starting the experiment. These goats were in a breeding season of 60 days. To check the effect of treatment on the pregnancy rate, the percentage of concentrated births at the end of January, and the percentage of twin births, data were compared in an Excel contingency table, and tested by the Chi-square test, with 5% probability. For the average kid/group, data were tested by analysis of variance, followed by Tukey's test, with a 5% probability in SigmaPlot. The pregnancy rate in G1 was 77.27%, in G2 of the 80% and in G3 of the 76%. The twin births in G1 were 41.20%, in G2 of 55% and G3 of 52.63%. The number of kids born in G1 was 24, in G2 of the 30 and G3 of the 33. There was no significant difference in these parameters between the groups studied ($p>0.05$). There was a significant difference ($p<0.05$) in the percentage of births at the end of January, in which G1 (74.47[a]%) and G3 (52.63[a]%) had a significantly higher percentage than G2 (45[b]%), concentrating on the births of kids. In this way, we can conclude that the use of injectable P4 showed a similar pregnancy rate to the other groups, despite not synchronizing the births.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Oviductal cell explants from bovine females at different stages of the estrous cycle

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The *in vitro* culture of oviductal explants was developed with the objective of evaluating the characteristics and functions of oviductal cells. However, despite all the advances related to the culture of these cells, the correlation between the influence of hormonal domains and the ability to form explants has not yet been evaluated. The aim of this study was to evaluate the ability of isthmus cells from bovine females at different stages of the estrous cycle to form oviductal explants. Oviducts were collected immediately after slaughter and were classified into three experimental groups, according to the ovarian characteristics of the reproductive tracts from which they were retrieved. Thus, oviducts from reproductive tracts containing ovaries with follicles larger than 8 mm (FG Group), with follicles smaller than 8 mm (FP Group), and with CL (CL Group) were selected. In the lab, isthmus cells from each experimental group were recovered and microscopically classified from 1 to 5 (according to the number of cells obtained pre-culture; 1=very few amount of cells; 5=very high number of cells) and cells were cultured at 39°C and 5% CO2 for 24 hours. Then, post-culture, the number of explants formed in each experimental group was evaluated. Three uterine tubes were collected from each experimental group in each replicate and 5 replicates of this design were performed (n=45). Data were submitted to analysis of variance and Tukey's test. To compare the number of formed explants between groups, the Kruskal-Wallis test and the Dunn test were performed. A significance level of 5% was considered (Software R 3.6.1 - R core Team, 2019) and results are presented as mean (± Standard Deviation). The FG group had a higher (P=0.05) visual score for the number of cells (3.4 ± 0.9) than the CL group (2.2 ± 0.4). The number of formed explants differed (P=0.004) among all groups, being higher in the FG group (364.0 ± 139.1) than in the other groups. The CL group had a lower (8.0 ± 11.0) number of formed explants. Estrogen has mitogenic effects because it stimulates the expression of genes related to cell proliferation and may play a key role in the action of growth factors. This may explain, at least in part, the greater number of explants formed from oviductal cells under estrogenic influence (FP and FG). On the other hand, progesterone (P4) is responsible for reducing the capacity of intercellular connections. In this sense, it may be suggested that, intercellular junctions and connections were compromised in oviductal cells explants of CL group during the culture period, a stage of fundamental importance for the formation of the explants It was concluded that, in vitro, isthmus cells under estrogenic influence are more efficient in providing greater formation of oviductal explants, although further studies are still needed for functional and structural characterization of these structures.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Ovarian response and oocyte recovery in superovulated goats with low nutritional condition undergoing different periods of diet supplementation

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In ruminants, it is known that improving oocyte quality is dependent on nutritional balance, which has a direct effect on the donor´s response. The purpose of the study was to evaluate the effect of different diet supplementation (DS) flushing periods on ovarian response and oocyte recovery in goats with low body condition scores (BCS). Thirty-six crossbred adult does with low subcutaneous fat deposit thickness (6.0±0.2mm) and low BCS (2.3±0.1;mean±SD) were selected, with BCS ranging from 1-5 measured at quartile intervals. Does were segregated into three groups (n=12): 0D,7D,14D, with 0 (without DS), 7, and 14 days of DS prior to oocyte recovery, respectively. Goats with homogeneous body weights (35.8±0.7 kg;P>0.05) were fed a diet to meet the energy requirement for breeding according to the NRC. Estrus was synchronized by the insertion of an intra-vaginal P4 device for 12 days, 14 days prior to oocyte recovery, followed by a 0.075mg i.m. dose of D- cloprostenol (PGF2α analogue) 60h before device removal (DR). Ovarian superstimulation was performed using 180 mg pFSH i.m. at 12h intervals from the 10th to the 12th day, followed by a single dose of 0.025mg GnRH i.m. 14h later withdrawal of P4. Oocyte recovery was performed 36h after DR. After slaughter, ovaries were washed, weighed, and the number and diameter of visible follicles were determined and the presence of hemorrhagic bodies were verified to determine ovulation rate (Hemorrhagic bodies/Total follicles+hemorrhagic bodies x 100). Ovaries and oviducts were then used to recover in vivo-matured cumulus-oocyte complexes (COCs) by follicular aspiration and flushing, respectively. Collected COCs were denuded by repeated pipetting in 0.1% hyaluronidase in TCM-Hank's medium. Denuded oocytes were observed under a stereomicroscope to determine the maturation status by the presence of the first polar body (metaphase II). Data were analyzed by GLM ANOVA using groups (14D,7D,0D) as the main effect, tested for ovary weight, number of visible follicles (small,<6 mm;large,>6 mm), follicular density (number of visible follicles+hemorrhagic bodies/weight per ovary), and retrieved oocytes. Ovulation and maturation rates were compared by the Kruskal-Wallis and the Chi-square tests. Increasing the nutritional flushing period to 14 days was effective (P<0.05) in increasing the ovulation rate (30.4%) compared to the 0D (22.5%). However, dietary treatments did not influence the in vivo maturation rate (28.4%,75/262) of the recovered oocytes. Additionally, no statistical differences were found for ovarian weight (3.0±0.1g), number of small (5.7±0.6 per goat) and large follicles (11.7±1.0 per goat), follicular density (7.8±0.5/g), and oocyte recovery (21.8±1.3 per goat) between the flushing lengths. Considering these results, a period of 14 days of flushing with DS promotes positive effects on the ovulation rate in low BCS adult goats.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

NPPC-mediated GV redistribution during pre-*in vitro* maturation alters cumulus-oocyte communication, maturation kinetics and gene expression in cattle

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Pre-IVM has been used to extend cumulus-oocyte communication and to synchronize nuclear and cytoplasmic maturations, improving oocyte competence. We tested the efficiency of a pre-IVM protocol with natriuretic peptide type C (NPPC) on COC communication maintenance and maturation rate in cattle, when compared with regular maturation medium (TCM199 and gonadotrophins, termed Cm group) or modified medium (Mm group, TCM199, 0.4% BSA, 10 ng/mL IGF-1, 100 ng/mL AREG, 10^{-2}UI/mL rhFSH, 5 µg/mL estradiol, 150 ng/mL progesterone). COCs recovered from 2-8 mm ovarian follicles were (1) directed submitted to Cm or Mm medium during 19h, or (2) pre- in vitro matured for 9h (base medium with 0.4% BSA, 100 nM NPPC, 10^{-4}UI/mL rhFSH, 500 ng/mL oestradiol, 50 ng/mL progesterone, 50 ng/mL androstenedione) followed by 19h in Mm (Pm- Mm group). Maturation rates (first polar body extrusion) were calculated after 19h of maturation. COCs at the end of pre-IVM (Pm-0h group) were compared with immature COCs (COC-0h control group). TZPs were stained with phalloidin at 0h and 9h, and quantified. Expression of TZP-retraction related genes, *ERK2* and *PRKACA*, were determined in cumulus cells at 0h and 9h by RT-qPCR. As a result, Pm-Mm group had lower maturation rate (68.37±8.35%, $p<0.01$) when compared with Cm (73.19±11.97%) and Mm (79.13±8.64%). TZP density was lower ($p=0.017$) in Pm-Mm (3.0/10 µm) at 9h IVM compared with COC-0h (3.4/10 µm). *ERK2* and *PRKACA*were upregulated 1.7 and 1.8-fold in Pm-0h compared with COC-0h, and 1.39 and 1.54-fold in Mm or 1.28 and 1.29-fold in Pm- Mm when compared with their corresponding 9h control ($p<0.01$). Next, we aimed to evaluate meiotic arrest in Pm-0h vs COC-0h adopting a novel classification system based on chromatin condensation and nuclear envelope characteristics (Hoechst 33342 and nuclear lamina immunostaining). NPPC supply during 9h promoted ($p<0.05$) a GV stage redistribution, from a GV2 predominance in COC-0h (21.86% GV1; 55.06% GV2; 23.08% GV3) to increased GV1 and GV3/GVBD categories in Pm-0h (34.53% GV1; 16.55% GV2; 29.5% GV3; 19.42% GVBD). Labeling nascent RNA assay during the last 4h of pre-IVM showed that ~15.12%(13/86) of this novel GV1 population were transcriptionally active. Since we also observed a population with higher GVBD rate in Pm-0h, we examined maturation rates after 9h. Pm-Mm showed 24.20 ± 12.69% ($p<0.05$) oocytes already matured at 9h IVM, while no mature oocytes were observed in Cm and Mm. These data show that pre-IVM with NPPC during the first 9h modulates differently each GV stage of bovine COCs, speeding-up some GV3 and “returning” a population back to GV1 stage, leading to different maturation kinetics and TZP maintenance throughout IVM. This pre-IVM system with 100 nM NPPC could be helpful if applied to GV1-enriched COC populations.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Influence of glucose and oxygen tension on the cell differentiation of *in vitro* produced bovine embryos

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The first cell differentiation event that occurs in the embryo determines the inner cell mass (ICM) and the trophectoderm (TE). In the mouse, glucose (GLC) is essential for this process and oxygen tension (O2) also interferes with TE formation. The roles of GLC and O2 in this event in bovine embryos are unknown. We hypothed that glucose is required and that higher O2 tension is prejudicial for TE differentiation in the bovine embryo. The objective of this study was to evaluate the effect of presence or absence of GLC in different O2 levels on the formation of the TE in IVP bovine embryos. Embryos were cultured in serum-free KSOM medium and randomly submitted to treatments on day 4 (D4), according to a 2x2 factorial model, in which GLC (present [GLC+] or absent [GLC-]) and O2 (low [5%O2] or high [20%O2]) were the independent variables. Considering five IVP replicates, cleavage, and blastocyst rates were obtained at D4 and D8, respectively. Embryos at D8 were subjected to autofluorescence analysis to quantitate NADH and FAD+. The blastocysts had their zona pellucida removed, followed by paraformaldehyde 3.8% fixation for subsequent immunofluorescence targeting GATA3. Images were obtained using a laser scanning confocal microscope. Total, TE (GATA3-positive), and ICM (GATA3-negative) cell counts were obtained. Embryos were also harvested for q-RT-PCR in four replicates to analyze gene expression of GATA3, YAP1, SOX2, CDX2, TFAP2C and OCT4. YWHAZ and H2A were used as reference genes. Rates, measurements, and cell counts were analyzed by ANOVA using SAS 9.4 software. The model included each independent variable and its interaction and comparisons between specific conditions was performed using the slice function. Q-RT-PCR was analyzed using PROC MIXED of SAS with pre-planned comparisons of specific conditions. A level of significance of 5% or less was considered statistically significant. There was an effect of O2 (p=0.018, n=5 replicates) on cleavage rates, although no differences were observed in blastocyst rates. NADH was higher in GLC- compared to GLC+ (p=0.014, n=74 total embryos) and no differences in FAD+ were observed. Total cell count data were not different between variables (n=23 total embryos). There was an effect of O2 in the ICM cell count, as the 5%O2 (n=12 embryos) group presented more cells than 20%O2 (p=0.032, n= 11). No effect of GLC, O2, or their interaction was observed on TE cell count or the TE/total cell ratio. CDX2 (0.007) and TFAP2C (0.038) were increased in GLC- 20%O2 compared to GLC+ 20%O2. SOX2 was decreased in GLC+ 20%O2 compared to GLC+ 5%O2 (p=0.027) or compared to GLC- 20%02 (p=0.005). GATA3, YAP1, and OCT4 did not present differences among conditions (n=4 replicates). In conclusion, TE differentiation occurred in the absence of glucose in bovine embryos and, interestingly, this condition increased the expression of TE-related genes CDX2 and TFAP2C in a higher O2 tension.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Assessing the influence of Hippo signaling pathway in bovine early embryo development by targeting the *LATS2* gene

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The first cell differentiation event during embryo development is the segregation of the trophectoderm (TE) and the inner cell mass (ICM). In the mouse, this process involves the Hippo signaling pathway in response to stimuli such as cell polarization and cell contact. LATS2 kinase is one of the effectors of the Hippo pathway, directly regulating the localization of co-transcription factor YAP1 and eventually the expression of TE-related genes. Evidence suggests that this event may not be similarly regulated in bovine embryos. We thus hypothed that the Hippo signaling pathway does not participate in the segregation of the TE in bovine embryos. The objective of this study was to delete *LATS2* in developing bovine embryos by gene targeting using CRISPR/Cas9. IVP embryos were divided into three groups: uninjected Control (CTRL), microinjection control with only Cas9 (CAS9) or Cas9 plus two different guide RNAs targeting *LATS2* (LATS2 gRNA). Embryos at 10 hours post fertilization (hpi) were injected with 70 ng/µl TrueCut™ Cas9 Protein v2 (ThermoFisher) and 12.5 ng/µl of each gRNA, when applicable. Cleavage, blastocyst (blastocyst/total), and development (blastocysts/cleaved) rates were recorded at 90hpi and 186hpi respectively, in 7 replicates. Embryos at 186hpi were fixed for subsequent immunofluorescence targeting YAP1, visualized with a laser scanning confocal microscope. After imaging, embryos were recovered for genotyping analysis by PCR. Also, embryos at 186hpi were individually harvested for RNA extraction and subsequent q-RT-PCR for absolute quantification of *LATS2, CDX2, WBP1* and, *SOX2*. Data were analyzed by ANOVA followed by Tukey's comparison of means considering a 5% level of significance. Cleavage and blastocyst rates were unchanged among all groups. However, the development rate was reduced in LATS2 gRNA (16.05 ± 6.78) compared to CTRL (39.42 ± 6.78). Genotyping revealed that one of the imaged LATS2 gRNA embryos (1/3) presented the expected 172 base pair deletion. Sequencing revealed that another LATS2 gRNA embryo (1/3) had indels at one of the targeted sites. Confocal imaging revealed that edited embryos did not display blastocoel and nuclear YAP1. Five out of nine LATS2 gRNA embryos did not present *LATS2* expression and were used for further gene expression analysis. *CDX2, WBP1* and *SOX2* were reduced in the LATS2 gRNA group compared to other groups (n=5 CTRL, n=4 CAS9). In conclusion, gene editing of *LATS2* impaired trophectoderm differentiation and reduced gene expression of differentiation-related genes in bovine embryos, suggesting participation of the Hippo signaling pathway in the first lineage segregation.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Evidences of ineffectiveness of insulin-like growth factor 1 (IGF-1) on bovine *in vitro* embryo production: systematic review and meta-analysis

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Unsatisfactory results on in vitro maturation (IVM) affect embryonic development, survival, and implantation. During IVM several components are added to improve oocyte competence and blastocyst quality. Many studies have investigated the effects of insulin-like growth factor 1 (IGF-1) during IVM; however, the results are contradictory. To clarify the efficacy of IGF-1 during IVM, here was performed a meta-analysis of all previous studies regarding bovine oocyte competence and further in vitro embryo development. A specific question was structured according to PICO: "Does IGF-1 improve in vitro embryo production in cattle during IVM? Consulted were: PubMed/MEDLINE, Embase, and Scopus. For selected studies, risk of bias was analyzed using SYRCLE® by two independent investigators. Data analysis was performed using R software and the "META" package for meta-analysis. The metaprop function was used to evaluate the proportion of data and estimate random effects for each analysis. The forest function was used to construct data representation in a forest plot. A total of 12 of 43 papers were included in the statistical analysis. Meta-analysis did not provide effects of IGF-1 on blastocyst yield (heterogeneity: I^2= 25%, p = 0.25 - 95% CI = 0.33 [0.31;0.35]; n= 1525). Similarly, no effects were observed on apoptosis rate (heterogeneity: I^2 = 91%, p = < 0.01 - 95% CI = 0.18 [0.07; 0.41]; n= 820) and cleavage rate (heterogeneity: I^2= 95%, p = < 0.01 - 95% CI = 0.64 [0.49; 0.76]; n= 1928). In conclusion, it was obtained that IGF-1 did not improve embryo development or increase blastocyst yield. Moreover, the high heterogeneity of apoptosis and cleavage rates was found to perpetuate controversy about the efficacy of IGF-1. Considering all the results together, a new strategy to modulate and increase the bioavailability of IGF-1 during IVM in cattle can be hypothesized.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

A single Day-7 *in vivo* or *in vitro* derived bovine embryo modifies the endometrial transcriptome, and the small extracellular vesicles (sEVs) secreted by them alter the miRNA profile in luteal cells

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Pregnancy success depends on adequate communication among the embryo, endometrium, and corpus luteum (CL). SEVs are part of these interactions, carrying miRNAs. However, the sEVs role in this communication still deserves investigation. Herein, we investigated the global endometrial transcriptome in response to the embryo's presence and origin (in vivo or in vitro); the miRNA contents of sEVs retrieved from media conditioned (CM) by endometrial explants (EE) cultured with a single in vivo or in vitro blastocyst, and the miRNA profile of luteal explants (CLE) exposed to sEVs retrieved from CM. For this, EE obtained from the uterus of previously synchronized cows were cultured in the presence or absence of a single Day-7 in vivo (EE-AI) or in vitro (EE-IVF) blastocyst for 6 hours. Total RNA extraction from EE was done using miRNeasy Mini Kit (QIAGEN). The RNA libraries preparation was performed using Illumina TruSeq Stranded mRNA Sample Prep kit. The sequencing was performed in one lane of Hiseq 2500 V4 (2x100pb). The CM-EE, CM-EE-AI, and CM-EE-IVF were collected to isolate sEVs. SEVs size and concentration were determined using nanoparticle tracking analysis (Nanosight NS300, Malvern Panalytical). The CLE were obtained from abattoir-ovaries with the CL in stage 2 (apex of corpus luteum red or brown) and exposed to sEVs for 6 hours. Relative levels of 382 bovine miRNAs were determined by RT-qPCR in sEVs from CM and CLE. The size and concentration of sEVs and expression of miRNAs were compared using ANOVA. The means were compared by Tukey's test ($p \leq 0.05$). We found, in the EEs, 45 differentially expressed genes (DEGs) associated with embryo presence and 211 associated with embryo origin (P.adjust ≤ 0.1). These DEGs associated with embryo presence and origin modulate the immune response to viruses, metabolism, and hippo signaling pathways (P-value ≤ 0.1). SEVs concentration (p=0.8101) and size (p=0.8076) were similar among the groups in CM. Nevertheless, miR-145, miR-196b, miR-199b, miR-382, and miR-1388-3p were differentially expressed in sEVs secreted by EE, EE-AI, and EE-IVF in CM. Those miRNAs are predicted to modulate Hippo, Wnt, and MAPK signaling pathways. Moreover, miR-24, miR-124b, miR-135b, miR-149-3p, miR-193a, miR-216b, miR-342, miR-381, miR-409b, and miR-502a were differentially expressed in CLE treated with sEVs from CM by EE, EE-AI, and EE-IVF. The predicted pathways modulated by those miRNAs are involved in cell proliferation, differentiation, survival, and growth. These results demonstrate that the embryo presence and origin alter endometrial genes profile, the miRNAs content of sEVs secreted by endometrial and embryonic cells, and CLE miRNA profiles treated with those sEVs. Therefore, we propose that the sEVs-mediated embryo-endometrium-corpus luteum interactions can regulate CL viability to ensure pregnancy success.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Nano.Follicle: preliminary insights of a closed 3D system for production of bovine *in vitro* embryo

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Despite the success of IVP, current conditions in human reproductive medicine and animal reproduction are far from ideal. The low production may be related to the usual microenvironment of embryo culture: the two-dimensional (2D) approach. Although the three-dimensional (3D) system has gained attention in recent years, little progress has been made. Moreover, cell behavior, signaling, and extracellular matrix (ECM) components are key factors to improve the follicular environment. Here, we propose a closed system for in vitro maturation of oocytes (IVM-3D); Nano.Follicle, which provides a more suitable microenvironment for bovine oocyte competence. To understand the effects of this new IVM-3D system, we performed bovine in vitro embryo production (IVEP) and evaluated cleavage rate, blastocyst yield, and initial embryo development. For this purpose, ovaries were collected from a local abattoir and cumulus oocyte complexes (COCs) were obtained (n= 30 COCs/group with six replicates). COCs were matured for 24 hours using the 2D system or the Nano.Follicle approach with the same IVM base medium. After maturation, we subjected the matured COCs to IVF for 18 hours, and the presumptive zygotes were cultured for seven days. Homoscedasticity of the data was assessed with Shapiro-Wilk, and the effect was tested with unpaired T test. Differences were considered significant when $P<0.05$. Preliminary results show that the Nano.Follicle system can ensure cleavage rate (76.73%; p= 0.3856) and blastocyst yield (27.63%; p= 0.9292) compared to the 2D system (85.12%; 24.82%) Moreover, the initial embryonic development was not affected by the Nano.Follicle system. In conclusion, we present a new nanobiotechnology system that enables in vitro development of bovine embryos. In addition, we propose Nano.Follicle as a new approach for a mimetic follicle microenvironment for future applications in reproductive science.

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Impact of paternal age on induced sperm capacitation and *in vitro* embryo development: a mouse model

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Aging is the accumulation of unrepaired cellular damage and compensation which compromises and promotes anatomical and physiological changes. Few studies focus on male reproductive efficiency throughout life and the prognosis and diagnosis of male infertility is still a challenge. Studies on the capacitation status have been related to fertility. We hypothesize that spermatozoon from senile mice shows a lower response to *in vitro* induction of capacitation with consequences on embryo production. The aim was to evaluate the response to the capacitation induction and relate it with blastocyst and embryo development rates. Experimental groups were composed of 4 (control, n=5), 19 (n=5), 24 (n=7) months-old males, and 10-15 weeks-old females (n=24). Females were superovulated and allocated with the males, and mating was verified by the vaginal plug. After 12 hours of hCG, females were euthanized, and the presumptive zygotes collected (D0.5) and transferred to KSOM droplets at 38.5°C, 5% CO_2, 5% O_2, and 90% N_2, under high humidity. Cleavage rate was observed at D1.5 and blastocyst stages and rates, and embryo development at D4.5. Males were euthanized, and semen was collected from the epididymis tail and vas deferens. Semen was submitted to *in vitro* induction of sperm capacitation for 1 hour in HTF medium and evaluated by the chlortetracycline assay (CTC). About 100 cells were counted using the filter 355nm/465nm under epifluorescence, considering CTC1 as non-capacitated, CTC2 as capacitated, and CTC3 as acrosome reacted. Data were analyzed using SAS System for Windows 9.3. For embryo development, T-TEST was performed, and for CTC ANOVA analysis (PROC GLM). A level of 5% significance was considered. There was no difference in cleavage rate when comparing 19 and 24 groups with the control, nor between the groups 19 vs. 24. On D4.5, the blastocyst formation rate differed between 24 vs. 19 (p=0.03). Embryo development rate differed between 24 vs. 4 (p=0.03), but not among the other groups. Regarding embryo kinetics, it was observed that the 19-group presented a delay in blastocyst development, being most at early blastocyst (p=0.003), compared to the control. The same observation could be made for the blastocyst stage (p=0.01). However, at the expanded blastocyst (Bx) stage, both groups 19 (p=0.03) and 24 (p=0.04) presented lower rates of Bx when compared to the control. For the hatched blastocyst, there was a difference between 24 vs. 4 (p=0.001) and 24 vs. 19 (p=<.0001), but not between 19 vs. 4. In the response to *in vitro* induction of sperm capacitation, the senile group (19 and 24) presented fewer cells with CTC2 status when compared to the young (p=<.0001). The 19-group had more cells with reacted sperm (CTC3) compared to the control (p=0.01). There was no difference in the CTC1 status between groups (p=0.08). In conclusion, the senile group showed a lower response to *in vitro* induction of capacitation, possibly negatively influencing embryo production.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Zinc oxide nanoparticles supplementation on *in vitro* maturation of bovine oocytes and its effects on the embryo quality, lipid accumulation, and preimplantation development

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One of the biggest challenges in bovine genetic improvement programs that use *in vitro* embryo production techniques is improving embryonic quality and survival after cryopreservation. Among the associated factors are lipid accumulation and excessive production of reactive oxygen species (ROS) associated with *in vitro* culture conditions, which do not provide all the necessary nutrients for perfect embryo development, as occurs *in vivo*. Supplementation of IVM medium with zinc oxide nanoparticles (ZnO-NPs) has been shown to have a beneficial effect in reducing free radicals (Isaac, et al., Biochemical and Biophysical Research Communications, 494: 656-662, 2017) and increasing the rate of development and re-expansion of vitrified bovine blastocysts (Abdel-Halim BR.; Moslhy, WA.; Helmy, N.A.; Asian Pac. J. Reprod. 7:161-6, 2018). This study evaluated the effects of adding three concentrations (0, 1.0, and 1.5 µg/mL) of ZnO-NPs in IVM medium on the development, lipid accumulation, and total cell number of bovine embryos. ZnO-NPs were characterized by morphology, and stability using dynamic light scattering (DLS), Scanning Electron Microscopy (SEM), and zeta potential analyses. Oocytes obtained from slaughterhouse ovaries were *in vitro* matured (TCM199 with 10% FCS, hormones, and sodium pyruvate) and exposed to ZnO-NPs in the following concentrations: 0µg/mL (control), 1,0 µg/mL (treat.1) and 1,5 µg/mL (treat.2). Then, were *in vitro* fertilized (Talp-FIV with 0.6% BSA) and the presumptive zygotes were partially denuded and cultured in SOF medium supplemented with 1.5% FCS. On D7, the blastocysts were fixed in paraformaldehyde and subsequently exposed to Hoechst 33342 and Bodipy 493/503 dyes for one hour for nuclear evaluation and lipid accumulation, respectively, in epifluorescence microscopy. Images of each embryo (approximately 30 embryos per group) were captured on an EVOS 5000 inverted microscope, and data on lipid accumulation were obtained by relative fluorescence values of images analyzed with the ImageJ program (version 1.53e). Five replicates were performed, totaling approximately 100 oocytes per group. Statistical analyses were performed in GraphPad Prism 9 software, and proportions were analyzed by Chi-Square Test ($\chi 2$). The addition of ZnO-NPs did not interfere ($P > 0.05$) in the cleavage rates (0µg/mL: 81.5%; 1.0 µg/mL: 73.5%, 1.5µg/mL: 79.5%), blastocyst developmental (D7) (0µg/mL: 36.9%; 1.0 µg/mL: 37.5%, 1.5µg/mL: 37%) and the accumulation lipid profile of blastocysts on D7. However, mean total cells were higher ($P< 0.05$) in the group treated with 1.0µg/mL ZnO-NPs (n=112.23) when compared to the control group (n=89.9). We conclude that, at the concentrations tested, ZnO-NPs do not affect embryo development and that adding 1.0 µg/mL ZnO-NPs to IVM can improve the quality of bovine embryos produced *in vitro*.

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Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Oleic acid supplementation affects prostaglandin synthesis in bovine trophoblast cells cultured *in vitro*

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In cattle early embryonic mortality, caused by failures in maternal recognition of pregnancy (MRP), is one of the major causes of reproductive failures. Oleic acid (OA) determines modifications in the synthesis of prostaglandins that may favor MRP. The objective was to determine the effects of supplementation with OA (Sigma, O1383) in the *in vitro* culture of bovine trophoblastic cells (CT-1) on the synthesis of prostaglandin E2 (PGE2) and prostaglandin F2α(PGF2α). The CT-1 were cultured in plates with culture medium supplemented with 10% fetal bovine serum (FBS), incubated at 38.5°C in a humidified atmosphere with 5% CO2. After 5 days of cultivation, they received the same medium free of FBS for 24 hours. Then, the wells received 4 mL of medium without FBS containing AO at concentrations of 0, 50, 100, 200, 500 or 1000 µM; for 48 or 72 hours. For each concentration and time, a well was constituted in each repetition, with a total of 5 repetitions. 300 µL of medium was collected from each well 48 or 72 hours after administering the treatments. PGE2 and PGF2α concentrations were determined by enzyme- linked immunosorbent assay. Statistical analysis was performed using PROC MIXED from SAS. The concentration of PGF2α at 48 hours was higher (P < 0.0001) in treatments with 50 µM, 100 µM, 200 µM and 500 µM of OA (88.97 ± 7.11; 95.31 ± 7.11; 114.88 ± 7.11 and 107.83 ± 7.11 ng/ml; respectively) compared to the control group (61.52 ± 7.11 ng/ml), the 1000 µM OA treatment (58.44 ± 7.11 ng/ml) did not differ from the control group. The concentration of PGF2α at 72 hours was higher (P < 0.0001) in treatments with 50 µM, 100 µM, 200 µM and 500 µM of OA (64.08 ± 2.89; 76.45 ± 2.89; 70.31 ± 2.89 and 59.31 ± 2.89 ng/ml; respectively) compared to the control group (49.40 ± 2.89 ng/ml), the 1000 µM OA treatment (39.41 ± 2.89 ng/ml) was lower than in the control group. CT-1 supplemented with 200 µM, 500 µM and 1000 µM of OA showed an increase (P = 0.0151) in PGE2 synthesis within 48 hours (20.61 ± 3.39; 20.11 ± 3.39 and 19.11 ± 3.39 ng/ml, respectively) compared to the control group (4.80 ± 2.93 ng/ml). At 72 hours, there was a tendency (P = 0.0872), towards an increase in PGE2 synthesis, when CT-1 supplemented with 50 µM, 200 µM and 1000 µM of OA (18.60 ± 3.60; 20.20 ± 3.60 and 19.00 ± 3.60 ng/ml; respectively) were compared to the control group (5.40 ± 3.60 ng/ml). In groups treated with OA for 48 hours, the PGE2/PGF2α ratio was lower (P = 0.0460) when CT-1 was supplemented with 200 µM and 500 µM of OA (0.62 ± 0.06 and 0.56 ± 0.06 ng/ml) compared to the control group (0.82 ± 0.06 ng/ml). At 72 hours, there was a tendency to decrease the PGE2/PGF2α ratio (P = 0.0834) only when the TC-1 were supplemented with 500 µM of OA (0.62 ± 0.10 ng/ml) compared to the control group (0.93 ± 0.09 ng/ml). In conclusion, OA supplementation at a concentration of 1000 µM decreases PGF2α synthesis with 72 hours and increases PGE2 synthesis with 48 hours in the *in vitro* culture of CT-1.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Response of bovine luminal endometrial epithelial cells to stimulus provided by conditioned medium from cultured bovine trophoblast spheroids

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A major gap in the current knowledge on the field of pre-implantation biology in cattle is the nature of one-to-one endometrium and embryo biochemical interactions that result in positive or negative pregnancy outcomes. Understanding such interactions is critical for the development of strategies to favor pregnancy maintenance. However, models to study the embryo-endometrium interactions in vitro are limited. The objective was to validate an in vitro model to determine the response of bovine uterine epithelial cells (BUEC) to a bovine trophoblast cell line (CT-1). The hypothesis was that the conditioned medium of CT-1 regulates transcription of IFN-stimulated genes (ISGs) in BUEC. The CT-1 were cultured for 10 days with DMEM + GlutaMAX supplemented with 10% of fetal bovine serum (FBS), 1% of non- essential amino acids, 1% of antibiotic-antimycotic and 0.001% of beta-mercaptoethanol, in a humidified incubator at 38.5 °C with 5% CO2. Within 10 days, confluent CT-1 clumps were placed onto petri dishes and cultured for another 7 days until they became spheroids. Five hundred spheroids were washed in DPBS and incubated in 10 mL FBS-free medium for 24 h. After incubation spheroids were removed and the conditioned medium was stored at -20°C until use. Four-passage cryopreserved BUEC were thaw and propagated in the same previous environmental conditions with DMEM/F12 supplemented with 10% of FBS, 3% penicillin-streptomycin, and 2% amphotericin B. When reached confluence BUEC were plated in 96-well cell culture plates, submitted to 4 h starvation and treated with CT-1- conditioned medium. Conditioned medium was used in the following dilutions 1:1, 1:3, 1:10 and 1:30. A negative control (FBS-free medium) and positive control (IFN-t 10ng/mL) were also used as treatment. All treatments were incubated for 24 h. After treatment BUEC were harvested for PCR of target ISGs transcript. Statistical analyzes were performed using the PROC MIXED from the SAS software, considering it as an experimental unit. We evaluated the effect of treatment on the gene expression of ISGs. There was a linear increase in the expression of ISG15, RSAD2 and OAS1Y in the cells submitted to the conditioned medium. The 1:1 dilution yielded the greatest ISGs expression of all treatments (P<0.0001). Positive control had a 10 times increased expression of ISGs transcripts when compared with negative control (P<0.0001). The level of expression of ISGs in the positive control was similar to the numerical value of 1:3 conditioned medium treatment. We concluded that BUEC responds to CT-1 conditioned medium treatment by upregulating the expression of ISGs, suggesting that one of the main components released by CT-1 cells in culture is IFN-t. This system may be used to study embryo-endometrium signaling in vitro.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Different nutritional plans in the pre puberty period do not alter the corpus luteum function in ewe-lambs post puberty

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The aim of this study was to investigate the influence of 3 nutritional plains in pre-puberty of ewe-lambs on the gene expression of acute insulin regulatory protein steroidogenesis (StAR) and 3β-hydroxysteroid dehydrogenase (HSD3B) in the corpus luteum (CLs) and plasma progesterone after puberty. 24 lambs (7/8 Dorper) between 6-7 months of age, 15-25 kg of live weight, were used. Groups were randomly assigned to one of 3 nutritional plans (n=8): G-Sub (70%-80% of National Research Council [NRC] requirements); G-Control (100-110% [NRC]; and G-Super (140% [NRC]). Lambs in the G-C and G-Sub groups were pastured on Panicum maximum cv. Tanzania and *ad libitum* access to water and mineral salt, with only the G-C group supplemented with commercial feed at 1.5% of live weight (BW). The G-Super lambs were housed and fed a total diet with a roughage:concentrate ratio of 20:80, containing 16% crude protein (CP) and 72% total digestible nutrients (TDN), up to a weight gain of 200 g/day according to NRC. Upon reaching a body weight of 35-40 kg (breeding weight in this breed), 120 and 180 days after the start of the different feeding schedules, the lambs were synchronized by inserting a slow-release vaginal progesterone device (Easy-Breed CIDR®, Pfizer, Brazil) for 12 days. On the day of implant removal (D12), 0.075 mg cloprostenol (Veteglan®, HertapeCalier, Brazil) and 300 IU eCG (Novormon®, MSD, Brazil) were administered intramuscularly. Ewe-lambs were slaughtered eight days after CIDR removal, and jugular blood was collected for subsequent progesterone measurement by radioimmunoassay. The ovaries were removed, 100% ovulation was observed, and fragments of CLs (approximately 40 mg) were preserved in liquid nitrogen (-196 ºC) and then stored in a freezer at -80 ºC. These fragments were subjected to the total extraction protocol of TrizolTM (InvitrogenTM, ThermoFisher Scientific Inc., Carlsbad, California, USA). Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied ByosystemsTM¬, ThermoFisher Scientific, Vilnius, Lithuania, USA) according to the manufacturer's protocol. qPCR in StepOnePlusTM (Applied Biosystems®, Foster City, California, USA) was performed for quantitative analysis of the relative gene expression of StAR and HSD3B. The delta delta CT method was used for gene expression. Statistical analysis (ANOVA) showed no difference in gene expression of HSD3B1 (p=0.1085) and StAR (p=0.6516) between nutritional plans. In addition, plasma concentrations of progesterone in the G-sub, G-C and G-super groups were 2.72 ± 0.44, 3.36 ± 0.81, 2.71 ± 0.35 ng/ml, respectively (p=0.3039). We conclude that prepubertal undernutrition does not affect progesterone production following ovulation induced by a hormone protocol in ewe-lambs.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Gene targeting of NANOG changes gene expression of bovine embryos

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The second event of cell differentiation in mammals consists of the separation between the epiblast (EPI) and the primitive endoderm (PE). This occurs shortly after the segregation of the inner cell mass (MCI) and the trophectoderm (TE). It is known in mice that NANOG drives EPI differentiation, although it leads to FGFsecretion that acts on neighbor cells, allowing GATA6 and subsequent SOX17 expression in the PE. Overall, we aim to test the hypothesis that NANOG is necessary for the specification of EPI in bovine embryos. The specific objective of this study was to assess expression of genes related to this second cell differentiation event after gene editing of NANOG using CRISPR/Cas9. The experimental group consisted of IVP-derived embryos microinjected at 16 hours post fertilization with 80 ng/μl of two different guide RNAs (gRNA) targeting NANOG homeobox domain and 70 ng/μl of TrueCut™ Cas9 Protein v2 (ThermoFisher), while the control group was not microinjected. Embryos were cultured until 216 hours post fertilization (hpi) and harvested individually for genotyping by PCR or gene expression analysis of *NANOG, GATA6* and *SOX17* by absolute q-RT-PCR, using 11 injected and 5 control embryos. Cleavage (at 90hpi), blastocyst (number of blastocysts/number of zygotes) and development (blastocysts/cleaved embryos) rates were recorded in five replicates. Data was analyzed by ANOVA followed by Tukey's comparison of means. The level of significance was considered 5% or less. We observed a significant decrease in cleavage (44.40 ± 2.93%) and blastocyst (7.95 ± 2.92%) rates in the injected group compared to the control group (73.87 ± 2.93% and 19.63 ± 2.92%, respectively); however, no significant changes were observed in development rates (17.71 ± 4.84% vs. 26.88 ± 4.84%). Genotyping by PCR and agarose gel electrophoresis revealed that a minority (1/6 tested) of injected embryos presented expected gene deletion caused by editing from both gRNA. Q-RT-PCR analysis revealed that *NANOG* expression was significantly reduced, but not extinguished in injected embryos. Interestingly, expression of GATA6 was not different between groups, while expression of another PE marker, *SOX17*, was significantly reduced in injected embryos. In conclusion, targeting of *NANOG* in bovine embryos using CRISPR allowed blastocyst formation while reducing *SOX17* gene expression.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Bovine oocyte and cumulus cells can exhibit distinct response to heat shock during in vitro maturation

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Heat shock (HS) increases the generation of reactive oxygen species (ROS) in bovine oocytes and reduces developmental competence (Ascari et al., Dom. Anim. Endocrinol. 60:50-60, 2017). However, its effect on the surrounding cumulus cells has not been fully elucidated although those cells play an important role on oocyte competence. Zinc oxide nanoparticles (ZnO-NP) can modulate the generation of ROS in mammalian cells (Saptarshi et al., Nanomedicine, 10:2075-2092, 2015). This study evaluated the effect of ZnO-NP and HS during in vitro maturation of cumulus cells-oocyte complexes (COCs) on the generation of ROS and mitochondrial activity in oocytes and their surrounding cumulus cells. Immature COCs were randomly distributed in four in vitro maturation (IVM) groups: Control (IVM under 38.5°C for 24h), ZnO-NP (IVM at 38.5°C + 1 µg/mL ZnO-NP for 24h), HS (IVM under 41.5°C for 24h) and HS+ZnO-NP (IVM under 41.5°C + 1 µg/mL ZnO-NP for 24h). In the first trial (seven replicates), in vitro matured oocytes were in vitro fertilized, and the presumptive zygotes were cultured for eight days. Blastocyst data was analyzed by the Logistic procedure of SAS 9.0. In the second trial (two replicates), cumulus cells were removed from oocytes after IVM. The denuded oocytes and respective cumulus cells were stained with CellROX green, MitoTracker Red CMXRos and Hoechst 33342 reagents (Thermo Fischer). Images were captured using epifluorescence microscope and fluorescence intensity calculated by ImageJ software. Data was analyzed by the Mixed procedure of SAS. Values are shown as mean±SEM. The lowest ($P<0.05$) blastocyst rates were found in HS (22.4±8.7%) and HS+ZnO-NP (18.4±3.2%) groups and the highest ($P<0.05$) was found in ZnO-NP (52.2±7.3%) group when compared to control (42.1±9.2%). Oocytes of ZnO-NP (22.7±1.1 arbitrary units [a.u.]), HS (23.4±1.3 a.u.) and HS+ZnO (22.6±0.9 a.u.) groups displayed more ROS ($P<0.05$) than oocytes of control group (18.3±0.9 a.u.). Mitochondrial activity increased ($P<0.05$) only in the HS+ZnO-NP group (35.4±2.6 a.u.) and no differences ($P>0.05$) were found between control (21.7±2.5 a.u.), ZnO-NP (27.9±2.8 a.u.) and HS (28.0±2.9 a.u) groups. In contrast to heat-shocked oocytes, heat-shocked cumulus cells (HS group) displayed lower ($P<0.05$) generation of ROS (6.9±0.2 a.u.) and mitochondrial activity (3.7±0.1 a.u.) than cumulus cells of control (10.2±1.1 and 6.8±0.8 a.u., respectively) and ZnO-NP (10.3±0.7 and 6.3 ±0.4 a.u., respectively) groups. The generation of ROS (8.9±1.4 a.u.) and mitochondrial activity (6.7±1.1 a.u.) in cumulus cells of HS+ZnO-NP group were similar ($P>0.05$) to control group. In conclusion, bovine oocytes and the surrounding cumulus cells can exhibit a distinct response to HS during IVM. The lower ROS generation and mitochondrial activity induced by HS on cumulus cells can be modulated by ZnO-NP supplementation in the IVM medium.

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Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Potential of melatonin on the *in vitro* maturation of Nellore bovine oocytes

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Melatonin exerts anti-apoptotic and antioxidant effects, attenuating the formation of reactive oxygen species. The hypothesis is that the addition of melatonin in the *in vitro* maturation medium (IVM) of oocytes increases the number of blastocysts produced *in vitro*. The objective was to evaluate the concentrations of 0.01 nM, 10 nM and 10000 nM of melatonin in the IVM medium of oocytes on the rate of cleavage and production of blastocysts 7 (D7) and 9 (D9) after *in vitro* fertilization. In ovaries of slaughtered Nellore females, ovarian follicles with 2 to 8 mm in diameter were aspirated, using a 10 mL syringe coupled to an 18G needle. Oocytes with homogeneous cytoplasm and multiple layers of *cumulus cells* were cultured in base medium for IVM plus 10% fetal bovine serum (FBS) supplemented or not with different doses of melatonin (Sigma Aldrich, M5250): control (without melatonin), 0.01 nM (M 0.01), 10 nM (M 10) and 10000 nM (M 10000) melatonin for 24 hours. 468 oocytes were obtained, in 7 repetitions performed on different days, which were divided into 4 groups with 117 oocytes/group. After IVM, the oocytes were co-cultured with 1 x 10^6 spermatozoa/mL, for 18 hours, using the semen of a single Nellore bull (D0). Subsequently, the possible embryos were transferred to synthetic oviduct fluid medium (SOF) added with 2.5% FBS, where they remained in *in vitro* culture (IVC) for 9 days. All cultivation was carried out in Petri dishes, in drops with 100 µL of medium submerged in mineral oil (Botupharma®); in an incubator at 38.5°C and in a humid atmosphere with 5% CO2. The cleavage rate on D3 and the number of blastocysts at different stages (total blastocysts, initial blastocyst, blastocyst, expanded blastocyst and hatched blastocyst) on D7 and D9 were evaluated. Data were submitted to analysis of variance (ANOVA) followed by application of contrasts for comparison between groups (control group *vs*. all treatment control group *vs*. M 10000 group and M 0.01 group *vs*. 10000 group), by the SAS software. The addition of melatonin did not affect the cleavage rate (P = 0.2081). On D7, there was a trend towards a greater number of total (P = 0.0892) and hatched blastocysts (P = 0.0686) in the M 10000 group compared to the M 0.01 group. On D9, there was a tendency for blastocyst production (P = 0.0804) in the M 10000 group to be higher when compared to the control. On D9, the M 10000 group had a higher (P = 0.0216) production of total blastocysts when compared to the M 0.01 group. The M 10000 group showed a tendency (P = 0.0741) for a higher production of hatched blastocysts, when compared to the M 0.01 group on D9. It is concluded that supplementation with melatonin in the IVM medium did not increase the number of blastocysts produced, however comparing the tested doses, the production of blastocysts was higher in the dose of 10000 nM than in the dose of 0.01 nM.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Conjugated linoleic acid supplementation alters prostaglandin synthesis and transcript abundance in bovine trophoblast cells cultured *in vitro*

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Early embryonic mortality, caused by failures in maternal recognition of pregnancy (MRP) in the first three weeks after fertilization, represents the major cause of reproductive inefficiency in beef cattle females. Modifications in prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) concentrations may benefit MRP. The addition of conjugated linoleic acid (CLA) in endometrial and fetal cell culture affected prostaglandin synthesis, however such effect on bovine trophoblastic cells (CT-1) is unknown. We aimed to determine the effects of CLA (mixture of cis- and trans-9, 11- and -10,12-octadecadienoic acid Sigma- Aldrich, USA, O 5507) on PGE2 and PGF2α synthesis and the expression of transcripts involved with MRP in bovine trophoblast. The CT-1 were cultured for 24 days, incubated at 38.5°C in a humidified atmosphere with 5% CO2 in culture bottles. After this step, the CT-1 were transferred to culture plates containing medium added with 10% fetal bovine serum (FBS), where they were cultivated for 5 days. Next, the CT-1 received culture medium free of FBS for 24 hours. The CT-1 were supplemented with different concentrations of CLA (0, 10, 20, 50 or 100 µM) in an SFB-free medium for 24, 48 and 72 hours. After collection from the culture medium, the CT-1 were lysed with 1 mL Trizol for 5 minutes and stored at -80ºC. The transcript abundance was determined by qRT-PCR and PGE2 and PGF2α were quantified by ELISA. Statistical analysis was performed using PROC MIXED from SAS (summer 9.2, SAS Institute Inc., Cary, NC, USA) considering the well the experimental unit. In all groups treated with CLA the concentrations of PGE2 (P = 0.0285) and PGF2α (P = 0.0001) were reduced with 24 and 72 hours of culture compared to control. In addition, CLA at all doses tested increased the PGE2 /PGF2α ratio (P = 0.0001) with 24, 48, and 72 hours and determined a quadratic effect on the relative expression of PTGER4 (P = 0.0026), PTGES2 (P = 0.0273), and MMP9 (P = 0.0256) transcripts. The relative abundance of PTGER4 was reduced in CT-1 cultured with 100 µM CLA when compared to the control group and supplemented with 10 µM CLA (0.0470 ± 0.0054 control vs. 0.0532 ± 0.0054 10 µM -CLA *vs*. 0.0223 ± 0.0061 100 µM -CLA). It is concluded that treatment with CLA decreases the synthesis of PGE2 and PGF2α and increases the PGE2:PGF2α ratio in a dose-dependent manner. The CLA supplementation determines a quadratic effect on the expression of transcripts related to prostaglandin metabolism (PTGES2, PTGER4) and on extracellular matrix remodeling (MMP9).

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Can follicular fluid used at the beginning of culture improve the quality of embryos produced *in vitro*?

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The Follicular Fluid (FF), formed by blood plasma compounds and metabolites secreted by granulosa and theca cells, exhibits an essential role in oocyte quality and sperm capacitation, on top of stimulating fertilization and embryo development. With the objective of accessing the effects of Bovine Follicular Fluid (BFF) on *in vitro* early embryo development, bovine ovaries were collected from a local abattoir and their follicles with a diameter between 8 and 10 mm were aspirated. The FF obtained was deposited in 15 mL tubes, centrifuged in 600g for 25 minutes, filtered through a 0,22µm membrane and stored in a -22ºC freezer. The *cumulus oophorus* complexes (COCs) were matured and fertilized using the laboratory's standard protocols, then randomly assigned between a BFS Group (*in vitro* culture supplemented with 10% bovine fetal serum) and a BFF Group (*in vitro* culture supplemented with 10% bovine follicular fluid) during the first 72 hours of *in vitro* culture. Following this period, the embryos from both groups were transferred to new droplets of culture medium with 10% bovine fetal serum. They remained in this new medium until the 8th day of culture, upon which they were washed with PBS, stored in RNA later^{tm} and then used for gene expression analysis. The cleavage rates (2nd day), blastocyst formation rates (7th day), embryo development kinetics (7th and 8th day) and gene expression data were submitted to ANOVA (Tukey's post-test, $P<0.05$). There was no difference ($P>0.05$) in cleavage rates (80.66±13.01 *vs* 77.00±11.93), blastocyst formation (39.66±5.00 *vs* 45.60±10.52) and in embryo development kinetics between the BFS and BFF Groups. The analysis of the gene expression of genes related to cell protection against oxidative and thermal stresses revealed no difference for SOD ($P=0.489$) and HSP70 ($P=0.427$) in between the different experimental treatments. However, the BFF Group displayed a higher expression ($P=0.001$) of OCT-4 (2.74 ±0.6) when compared to the BFS Group (1.1±0.5), with OCT-4 being an important regulator of transcription during cell differentiation, correlated with good embryo quality suggesting that the addition of BFF improves the quality of *in vitro* produced embryos.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Oviductal magnetic spheroid is effective to study the estrous cycle stage in the oviduct

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For long time, the oviductal cells, specially the epithelial, have been inefficiently cultured under two-dimensional (2D) conditions. Successfully, 3D systems have been promoted cellular functionality and polarity maintenance. The magnetic 3D culture system (Greiner Bio-One CELLSTAR®) has been applied to form the oviductal magnetic spheroids (OMS). As known, estradiol (E2) increases, while the progesterone (P4) decreases, oviductal cell ciliation and secretory activity. Therefore, we hypothed that OMS is hormone responsive. Hence, we aimed to evaluate the OMS's response before luteal (L), pre-ovulatory (Pre-Ov), and post-ovulatory (Post-Ov) treatments simulation. In an attempt to reduce interference, bovine oviduct epithelial cells (BOEC) and stromal cells (BOSC) were collected at two estrous cycle stages: follicular (high E2 milieu, n=6 animals) and luteal (high P4 milieu, n=5 cows). The ampullary cells were separately cultured (2D). At 80% confluence, cells in solution were magnetized by centrifugation with the nanoshuttle™-PL (NS), and seeded in 96-well plate at 7:3 proportion (BOEC:BOSC), 10,000 cells/well. A magnetic force induced cell aggregation, forming the OMS within three days. The next 7 days, all the OMS were treated to simulate luteal phase (P4, 100 ng/mL), followed by 3-days Pre-Ov (E2, 300 pg/mL), and finally 4-days Post-Ov (no hormone). Acetylated-alpha tubulin (acTUB, cell ciliation marker) and OVGP1 (specific secreted oviductal protein) were analyzed per immunoassay in all OMS. Comparisons were analyzed between L (D7), Pre-Ov (D10), and Post-Ov (D14) in cells collected at follicular or luteal stages. The raw intensity density was normalized by the spheroid area using ImageJ (version 1.53t). Data were analyzed in the GraphPad Software (version 8), using ANOVA or t-test, with P 0.05 as significance level. In general, great variation was observed between individuals. No difference was observed in the acTUB levels. The OVGP1 levels were similar in OMS submitted to L treatment ($P>0.05$) when comparing the stage of cell collection. But when OMS were submitted to Pre-Ov and Post-Ov treatments, higher OVGP1 levels were observed in cells collected in luteal (P 0.01) than the follicular stage. Comparing the treatments in OMS from cells collected at the luteal stage, a progressive increase was observed from L to Pre-Ov and from Pre-Ov to Post-Ov in OVGP1 levels ($P=0.004$), with no difference in follicular stage ($P=0.1113$). The OMS is responsive to hormone treatment in a more similar pattern to the oviductal tissue if cells are collected at the luteal and follicular stage of the estrous cycle.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Bovine polymorphonuclear cells are able to endocytosis labeled extracellular vesicles within minutes

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Polymorphonuclear cells (PMN) are phagocytes from the innate immune response that can respond to stimuli within seconds. PMNs were considered prejudicial to physiological processes like pregnancy for a long time. However, with advanced studies, the beneficial functions of these cells are now known. PMN are responsible for various functions in the organism, including intercellular communication in different systems and cells. Intercellular communication can be mediated by several factors, such as small extracellular vesicles (sEVs). However, information about PMNs interaction mediated by sEVs is unclear. Therefore, this study aims to determine the capability and time of endocytosis of sEVs by PMNs. For that, Bovine purified PMN were isolated by density gradient (1.077 g/mL) centrifugation and diluted in IDMD media supplemented with 1% BSA. Cells for time-lapse imaging were seeded in a 35 mm culture plate with a coverslip bottom in a density of $1x10^4$cells in 200 µL and stained with DNA dye. Cells for flow cytometry analysis were seeded at a density of $1x10^6$/mL. Follicular fluid-derived sEVs, isolated by ultracentrifugation at 120000xg twice and stained with PKH26, a lipophilic membrane dye, were added to the culture in a concentration of $1x10^{10}$particles/mL. Timelapse was set to register the co-culture every minute (min) for 60 min. Images were taken with an epifluorescence microscope (Leica Thunder 3D Imager DMi8). In the end, a video was made to establish when sEVs started to be endocytosed. Flow cytometry was performed at 10, 15, 30, and 60 min. DNA dye was used to discriminate cells from noise and to measure the number of events positive and median fluorescence intensity (MFI) for PKH26 within the cells. The flow cytometry machine used was CytoFlex (Beckman and Coulter). For imaging results, it was possible to detect PKH26-labeled sEVs in the perinuclear area of PMNs after 9 min of co-incubation. After 30 min, it is possible to detect a few more sEVs in the perinuclear area, which did not increase at the end of the 60 min. Flow cytometry results demonstrated similar findings. Positive events to PKH26 were detected at 10 min within the cellular population and were stable until 60 min. MFI was also higher at different times than in the cells that did not receive stained sEVs. Polymorphonuclear cells endocytosed labeled EVs within minutes in vitro, this rapid endocytosis could be due to the phagocytosis characteristics and half-life of PMNs. This mechanism could play a role in the fast immune response related to pregnancy recognition. In the future, it will be essential to determine the endocytosis process based on dose and time response as well as different sources of sEVs.

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Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of hydroxychloroquine on *in vitro* maturation of bovine oocytes

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The aim of this work was to evaluate the cytotoxicity of hydroxychloroquine (HCQ; Sigma- Aldrich®; St. Louis, MO, USA) on in vitro maturation and further embryonic developmental potential in a bovine IVEP system. Cumulus-oocyte complexes (COCs) originating from slaughterhouses were selected and matured in vitro (IVM) in TCM 199 medium supplemented with 10% FBS, 0.5 µg/mL of FSH, 5 µg/mL of LH, and 11 µg/mL of pyruvate and 10 µg/mL of gentamicin in a 5% CO2 incubator, in air, at 38.5 oC for 22 hours. IVM was performed in the presence of 6, 12 and 24 µM of HCQ diluted in maturation medium (Control group without HCQ) and in the first experiment, part of oocytes were evaluated for nuclear maturation by Hoechst 3342 staining (2 µg/mL for 10 min) and the other part of oocytes were parthenogenetic activated (5 µM of Ionomycin for 5 minutes followed by 2mM of 6-DMAP for 3h) and cultivated in SOF medium supplemented with 2.5% FBS and 6 mg/mL BSA for 7 days in a 5% CO2 incubator, in air, at 38.5 oC. Cleavage and blastocyst rates were evaluated on the 3rd and 7th days of culture. In the second experiment, COCs were treated with 24 µM of HCQ and the accumulation of acidic vesicles, indicative of autophagy, was investigated both in cumulus cells and in oocytes shortly after the end of the IVM by acridine orange staining (1µg/mL for 30 minutes) followed by fluorescence intensity analysis (20X objective). The data obtained were calculated as mean and standard deviation and analyzed by ANOVA and Tukey's post-test (GraphPad Software®, La Jolla, CA). Results showed that none of HCQ dosages have affected (p>0.05) nuclear maturation of oocytes. With regards to the further development of oocytes, while cleavage was not affected (p>0.05), it was observed a gradual reduction (p <0.05) in blastocyst formation according to the increase in HCQ concentration in IMV (6 µM = 43.0%; 12 µM = 37.7% and 24 µM = 30.0%) compared to the control group (48.8%). In the second experiment, it was observed that treatment with 24 µM of HCQ in IVM led to an increase of the fluorescent signal indicative of acidic vesicles accumulation in cumulus cells (2.8 times; p>0.001) and in oocytes (1.7 times; p<0.001) compared to Control indicating a possible blockage of the autophagy flux during IVM. Thus, we concluded that although HCQ in IMV did not affect nuclear maturation of oocytes, the embryonic developmental capacity of these gametes was impaired in a dose-dependent manner by the treatment with HCQ in IVM. The increase of acidic vesicles accumulation in both oocyte and cumulus cells after HCQ treatment indicated a possible disturbance of the autophagic flux which might be related to negative effects observed in embryo development. Further studies must be done to address this hypothesis.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of hydroxychloroquine on the development and autophagy of *in vitro*- produced parthenogenetic bovine embryos

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The purpose this study was to evaluate the effect of hydroxychloroquine (HCQ) on the developmental potential, autophagy, and apoptosis of pre-implantation bovine embryos. For this, cumulus-oocyte complexes (CCOs) from slaughterhouse were matured *in vitro* in TCM-199 supplemented with 10% SFB, 0.5 µg/mL FSH, 5 µg/mL LH, 11 µg/mL pyruvate, and 10 µg/mL gentamicin for 26 hours. The COCs were denuded by pipetting with hyaluronidase (0.3 mg/mL) then mature oocytes were parthenogenetic activated with ionomycin (5µM for 5 minutes) followed by incubation in 6-DMAP (2mM for 3h). Parthenotes were cultivated in SOF medium with 2.5% SFB and 6 mg/mL BSA for 7 days in with 5% CO2 in air. In the first experiment, parthenogenetic bovine embryos were cultured *in vitro* for 7 days with HCQ in a dose-dependent manner (1µM, 6µM, 12µM, and 24µM) and cleavage (D3) and blastocyst (D7) rates were evaluated. In the second experiment, we chose the intermediate concentration that showed effects on development at the first experiment (12µM), then embryos were cultivated with HCQ and embryo development, autophagy (acridine orange staining), and apoptosis (TUNEL test) were evaluated on D3, D5 and D7. Data were analyzed by ANOVA (first experiment) and t test (second experiment) adopting a significance level of 5% (Prism 6 software). In the first experiment, cleavage was reduced only at the highest concentration of HCQ (24µM) compared to the control (45.2 vs. 69.6%, respectively; $p<0.05$). Blastocyst rate was not affected by the treatment with 1µM of HCQ ($p>0.05$) but significantly reduced with the increase in HCQ concentration (6µM = 28.7%; 12µM = 11.2%; 24µM = 0.0% vs. 43.1% in Control; $p<0.05$). In the second experiment, it was confirmed that IVC with 12µM of HCQ had no effect either on cleavage in relation to the control (72.5% vs. 76.0%, respectively; $p>0.05$) or morula formation (76.8 vs. 73.8%, respectively; $p>0.05$), but reduced the blastocyst rate compared to the control (12.7 vs. 37.3%, respectively; $p<0.05$). Acridine orange staining of HCQ-treated embryos showed a gradual accumulation of acidic vesicles (indicative of autophagy blockage) on D5 and D7 ($p<0.05$), but not on D3 ($p>0.05$). Apoptosis was not detected on days 3 and 5, but a higher apoptotic index was observed in HCQ-treated blastocysts on D7 compared to the control group ($p<0.05$). In conclusion, we showed HCQ in IVC negatively affected development starting at 6µM, particularly with regards to the blastocyst formation in both quantitative and qualitative terms (causing more apoptosis). This negative effect was associated with an accumulation of acidic vesicles at the morula and blastocyst stages, indicating a possible blockage of autophagic flux. To our knowledge, this is the first study evaluating the effects of HCQ on preimplantation embryo development in mammals. We believe that our results can contribute to the study of autophagy in embryology and provide new information and discussion about HCQ toxicity in the reproductive field considering the effects observed even at 6µM, which represents just a two-times higher concentration found in serum of humans taking the recommended dosage of HCQ.

Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Exploring intraovarianism during fetal development of Zebu breeds: insights into gonadal development

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Intraovarianism refers to mechanisms within an ovary that influence follicle and luteal dynamics, represented by greater follicle activity in the right ovary (RO) than left ovary (LO) during the follicular waves into the interovulatory interval. This phenomenon is known in cows, heifers, and newborn dairy calves. However, the nature of intraovarianism is not known, having only evidence that the difference in the number of follicles between the ovaries or vascularization may be involved. This study aimed to research the intraovarianism phenomenon in fetal development and evaluate its presence in zebuine fetuses of the Nelore, Guzerat and Gir breeds (n=40) between 120 and 270 days of gestation obtained from slaughterhouses. The fetuses were measured for their weight (W, Kg), crown-rump length (CRL, cm), and head width (HW, cm) for fetal age estimation and grouping into six age categories (a: 120-150 day b: 140-170, c: 150-180, d: 180-210, e: 210-240, f: 240-270) for comparison of gonadal development. The right and left ovaries were collected, dissected, and subjected to biometrics analysis: weight, length, width, and depth. The data were checked for normality and homogeneity of variances and analyzed by two- way ANOVA to study the effect of gestational age and ovary on dependent variables. Statistical analysis and graphing were performed using the Prism GraphPad version 7.0 software for MacOS. Preliminary results showed no differences were observed between RO and LO on weight ($p= 0.9232$), length ($p= 0.6082$), width ($p= 0.6176$), and depth ($p= 0.9863$), indicating the absence of intraovarianism during the fetal development in zebu breeds. The gestational age significantly affected gonadal development, with notable ovarian growth from 240 days of gestation in all measured variables ($p<0.01$). The main phase of fetal gonadal development occurs from 240 days of gestation for all variables (weight: 411.1% increase in the mean of the last gestational age category compared to the mean of the other length: 62.9% increase; width: 77.4% increase; and depth: 77.1% increase). Future work includes the evaluation of intraovarianism in fetal development in taurine breeds, comparing gonadal development with zebuine data. Furthermore, histological and gene expression analyses will be performed to compare ovarian vasculature and follicular population between RO and LO and investigate genes involved in gonadal development and growth.

Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Extracellular vesicles from blood serum and uterine fluid are modified by endometritis and negative energy balance in dairy cows

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Dairy cows usually present a negative energy balance (NEB) and uterine diseases during the post-calving period, which has a significant economic impact due to the reduction in animals available to the next reproductive season. Extracellular vesicles (EVs) are mediators of intercellular communication and can modulate several reproductive processes. The aims of this study were: 1) to investigate if EVs present in the serum of animals 7 days post-calving can predict animals with endometritis at 30 days post-calving and; 2) evaluate if NEB intensity modifies EVs miRNA contents present in the uterine fluid of dairy cows at 60 days post-calving. First, blood samples were collected from individual dairy cows at weeks 1 and 2 post-calving. Serum samples were subjected to metabolite analysis to determine the NEB intensity (Low NEB: NEFAs 0.3 to 0.8 mmol/L and BHB 0.55 to 1.1 mmol/L; High NEB: NEFAs ≥ 0.9 mmol/L and BHB ≥ 1.2 mmol/L). At 30 and 60 days post-calving animals were evaluated and a total of six animals presented uterine alteration out of 13 animals total. Healthy animals (n=7, being 3 Low and 4 High NEB) had the estrous cycle synchronized and on Day 5 after artificial insemination (AI) the collection of uterine fluid (UF) was performed. EVs isolated were analyzed for particle and concentration by nanoparticle tracking analysis. Total RNA from uterine fluid EVs from dairy cows with Low and High NEB was isolated and analyzed for the relative expression levels of 383 miRNAs. Differences between treatments were assessed by Student's t-test. The EVs mean particle concentration at week 1 post-calving decreased in the endometritis compared to the control group (Control: 343 x10^8 ± 107.6x10^8 particles/mL; Endometritis: 135.3 x10^8 ± 16.3x10^8 particles/mL) and the mean showed no difference (Control: 156.8 ± 15.8 nm; Endometritis: 160.9 ± 7.0 nm). No differences were identified in EVs mean particle concentration isolated from uterine fluid (Low NEB: 57.3x10^8 ± 18.4x10^8 particles/mL; High NEB: 25.7x10^8 ± 6.5x10^8 particles/mL) or mean (Low NEB: 189.52 ± 5.6 nm; High NEB: 192.7 ±4.2 nm) between the groups. EVs isolated from uterine fluid 60 days post-calving presented 316 miRNAs in common between the two groups and one (miR-369-3p) exclusive to the Low NEB group. A total of 28 miRNAs were differently expressed being 27 upregulated in Low NEB and 1 upregulated in High NEB. The miR-369-3p, which is exclusive in Low NEB, is related with decrease inflammatory response, which may suggest that animals with low NEB have a more favorable uterine environment to receive an embryo at 60 post-calving days. In conclusion, the EVs evaluation in serum can help in the early diagnosis of endometritis as well as the understanding of the NEB intensity modulation of the uterine environment at 60 post-calving days in dairy cows, which can help decide the best time for the uterus to receive an embryo.

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Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Effects of an early weaning system on growth and reproductive characteristics of Nelore heifers during rearing

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We aimed to evaluate the effects of early weaning (150 days vs. 240 days) of Nellore calves during two consecutive generations on the second-generation heifers" body growth and reproductive characteristics. For this study, 34 heifers born in 2021 were subjected to the effects of either early weaning (n=16) or conventional weaning (n=18) during their fetal programming (weaning of the previous generation when they were in the uterus) and were also subjected to the same weaning regime in 2022. Thus, the effects of early weaning during the gestational period and calf-rearing period were analyzed. From 90 days of age until weaning (150 days or 240 days), a *creep-feeding* was provided. From weaning to 16 months, the heifers received a protein-energy supplement (10g/kg of body weight; BW). Calves were weighed at birth, 5 and 8 months. At 14-16 months, heifers were subjected to a puberty induction protocol (150mg of intramuscular long-acting progesterone) and timed- insemination (TAI) protocol. From 12 to 18 months, all heifers were evaluated every 28±3 days for BW, body condition score (BCS; scale 1 to 9), average daily weight gain (ADG), rump fat thickness (RFT), reproductive tract score (RTS; 1-5), ovary score (1-5), dominant follicle (DF) size and blood perfusion on the day of TAI, and puberty and pregnancy rates. Data were analyzed using the MIXED or FREQ procedures of SAS. For BW, a significant (P<0.0001) interaction between treatment and time indicated that conventional-weaned heifers were heavier from 8 to 13 months and 15 to 16 months compared to early-weaned heifers; whereas no difference between groups was observed at 5, 14, and 18 months. For ADG, an interaction of treatment and time (P<0.0001), indicated that conventional-weaned heifers gained more weight between 5 and 8 months; whereas, early-weaned heifers had a higher daily weight gain between 12 and 14 months. For BCS, there was only a time effect (P<0.0001), indicating an increase over time. For RFT, an interaction between treatment and time indicated that conventional-weaned heifers tended (P<0.07) to have greater rump thickness compared to early-weaned heifers at 13 months. The weaning strategy did not affect RTS and ovary score, but a time effect (P<0.0001) reflected an increase in these endpoints over time. The DF diameter two days before TAI (overall mean: 10.5±0.4 mm) and at TAI (overall mean: 12.7±0.5 mm), blood perfusion of the DF at TAI (overall mean: 21.5±2.4%), and puberty (overall: 38.2%) and pregnancy rates (overall: 55.9%) did not differ (P>0.1) between weaning groups. In conclusion, precocious heifers born in the early weaning system and receiving a quality of nutrition can overcome the reduced BW gain from 5 to 8 months of age due to lack of suckling compared to heifers in the conventional weaning system, and present comparable body and reproductive development and performance in their first breeding season.

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Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Co-culture of bovine oviduct spheroids enhances the embryo development in vitro at Day 7 under high oxygen tension

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Bovine IVP is an established biotechnology, but it still does not completely mimic the oviductal environment. So, the environment in which embryos are exposed during IVP can affect their quality and several factors such as co-culture and oxygen tension can influence them. Therefore, this study investigated the role of the co-culture with bovine oviduct epithelial spheroids (bOES) during IVC under high and low tensions of oxygen. Oviducts in periovulatory stage were obtained in a local slaughterhouse and oviduct cells were cultured and on day three of cell culture, the bOES presenting 100-200 µm were selected for further co-culture. In parallel, ovaries were aspirated, and Grade 1 and 2 COC were selected and submitted to IVM (50-80 COC/well) for 22-24 h, in an incubator of 5% CO2, at 38.8 °C. Frozen spermatozoa (1.106 sptz/mL) were selected by Percoll and used for IVF (50-80 COC/well), for approximately 22 h in an incubator of 5% CO2, at 38.8 °C. The IVC was performed in four different systems, with/out bOES and each one under two different oxygen tensions [5% O2 (LO) vs 20% O2 (HO)]. In both bOES groups, there was an equal amount (1:1, maximum of 25:25) of presumptive zygotes and bOES in the IVC droplets (25 µL) of modified SOF media, at 38.8 °C for eight days. The cleavage rate was evaluated on day two, the blastocyst rate on D7-8, and hatching rate at D8 (nb of hatched/blastocysts). Three replicates were performed. Data were evaluated by two-way ANOVA. No statistical difference (P>0.05) was observed for cleavage rate among groups, being 77% (193/250) in HO, 82% (204/250) in HO-bOES, 65% (228/351) in LO, and 81% (287/353) in LO-bOES. Blastocyst rate at D7 from the initial number of COC and from cleaved ones, respectively, were higher (p=0.005; p=0.004) in HO-bOES [26% (64/250) and 31% (64/204)] than HO [11% (28/250) and 14% (28/193)] and similar (P>0.05) to LO [28% (100/351) and 44% (100/228)], and LO-bOES [26% (92/353) and 32% (92/287)]. For blastocyst rate at D8 from the initial number of COC had no difference (P>0.05) among groups, being 22% (56/250) in HO, 34% (84/250) in HO-bOES, 36% (125/351) in LO, 32% (112/353) in LO-bOES. However, the blastocyst rate at D8 from cleavage was observed a lower rate (P=0.011) of HO being 29% (56/193) than 41% (84/204) for HO-bOES and this one was similar (P>0.05) to 55% (125/228) for LO and 39% (112/287) for LO-bOES. Of note, the hatching rate was similar (P>0.05) among groups, being 21% (12/56) for HO, 25% (21/84) for HO-bOES, 38% (47/125) for LO, and 29% (33/112) for LO-bOES. In conclusion, co-culture with bOES was able to enhance the in vitro development of blastocysts in D7 when high oxygen tension is applied for IVC.

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