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Caloric restriction during gestation in mice decreases ovarian reserve in the offspring

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The aim of this study was to evaluate the effect of caloric restriction during gestation on ovarian follicular count in the offspring in mice. For this, we used 14 female mice and 7 male mice of the C57BL/6 lineage maintained with standard diet and water ad libitum under controlled light and temperature conditions. The mice mated at the ratio of one male to two females at the same period in separate cages. Ten days after confirmation of copulation the females were divided into control group (n= 7) and caloric restriction (CR) group (n= 7), which received a diet consisting of 50% of what was consumed by the control group in the day before. Mice were subjected to this restricted diet for 6 days. After delivering and weaning (21 days of age) males and females were separated according to the initial group and received ad libitum diet until 3 months of age. The mice were evaluated every 14 days from weaning to euthanasia, when the ovaries were collected. For histological evaluation the ovarian samples were submitted to serial cut in a microtome, stained with hematoxylin-eosin. Images of ovarian sections captured with a digital camera coupled to a composite light microscope using the 40X objective. Oocytes were classified as primordial, primary, secondary and tertiary. The statistical analyzes carried out used the software GraphPad Prism 6, assuming a level of significance of 5%. The weight gain was increased in the female offspring of the CR group after weaning ($P < 0.0001$). We observed more primordial (1064 ± 128 vs 2740 ± 231; $P<0.0001$) and transition (1042 ± 131 vs 2914 ± 210; $P<0.0001$) follicles in mice subjected to CR during gestation. Primary, secondary, tertiary and total follicles were not different between groups ($P>0.05$). These results suggest that there were less follicles in the reserve of CR mice, but the rate of activation was not different, as we do not see more follicles in the primary stage. This indicates that differences in the ovarian reserve can be occurring during fetal ovarian development. Therefore, CR during gestation negatively affects weight gain and the size of the ovarian reserve of the offspring in mice.

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In vivo evaluation of the effect of sperm on gene expression in bovine oviductal epithelial cells

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The interaction between sperm and bovine oviduct epithelial cell (BOEC) provide a favorable environment to maintain the viability of sperm with a higher potential for fertilization. Studies related to sperm binding to the oviduct, have showed that this binding influences the transcription of genes from these cells, modifying the oviduct environment. The aim of this study was to find out the effect of sperm interaction with oviduct cells 18 h after artificial insemination (AI) on relative expression of genes *FUT 6*, *NQO1*, *CST6*, *B3GNT3*, *CKB*, *RARRES2*, *MIF* and *FOS*. All genes were selected from a RNAseq assessment (data not showed). Nelore heifers were synchronized with an estradiol and P4 based protocol. Heifers identified on estrus were divided into 2 groups: heifers inseminated (n=9) with a total of $8x10^6$ *cryopreserved* semen pool from 6 Nelore bulls (group IA+); heifers inseminated with saline solution (group AI-). Eighteen hours after AI, all heifers were slaughtered, and BOEC from isthmus appraised for any variation in transcripts level of selected genes by real time PCR. For each assay, five different biological replicates were performed. Data were analyzed by analysis of variance (ANOVA) and differences between means were compared by Tukey's test ($P<0.05$). Only the *FOS* gene showed increased on relative abundance of mRNA in the IA+ group ($P=0.03$). All other genes assessed presented similar expression between groups. The gene *FOS* is related to cell proliferation and differentiation, which transcription occurs fast and in a transitory way. Based on these results, it was concluded that interaction of sperm and BOEC from isthmus, modulates the expression of the *FOS* gene. Finnancial support: EMBRAPA, CAPES, FAPDF.

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Effectiveness of *in vitro* maturation strategies to reduce the lipid accumulation in buffalo embryos

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In vitro embryo production (IVEP) is a procedure that can promote genetic improvement in a short time frame. However, the success rates obtained with this biotechnology in buffaloes are still inconsistent, which can be associated with the high concentration of lipids in the cytoplasm of oocytes and embryos. Considering the potentially positive impacts of strategies to replace/reduce the supplementation of fetal bovine serum (FBS) during IVEP, the objective of this study was to evaluate the effects of reducing the concentration of FBS and/or use of 5 mM L-carnitine (LC) during *in vitro* maturation on the development and lipid accumulation of buffalo embryos. In the first experiment, we aimed to determine the lowest concentration of FBS in the IVM medium able to maintain the embryo development rate obtained by the control group (10% FBS). Buffalo oocytes were placed in IVM in bovine serum albumin (BSA) medium supplemented with 0%, 2.5%, 5% or 10% FBS for 22 h, and then fertilized in Talp-IVF medium for 24 h, and *in vitro* cultured in modified SOF medium supplemented with 1.5% FBS at 38.5 °C and 5% CO_2 atmosphere in air for 7 days. Blastocyst rates were evaluated and the data analyzed using the analysis of variance (ANOVA) and Tukey test. After defining the lowest effective concentration of FBS as 5% [27/79; 34.18%^{a}, similar to 10% - 52/105; 34.67%^{a} and superior to 0% (11/104 - 10.58%^{b}) and 2,5% (16/83 - 19.28%^{b}) groups], we performed a second experiment in which the 0%, 5% and 10% FBS groups were also evaluated regarding the addition of 5 mM of L-carnitine in the IVM medium. The blastocysts produced in this experiment were submitted to lipid quantification tests, involving staining followed by observation by optical (OilRed O) and confocal (BODIPY 493/503) microscopy. The lipid quantification data were evaluated by the nonparametric Kruskal-Wallis test. All the statistical analyses were performed with the SPSS version 22.0.0.0 software, except for the lipid data, which were evaluated with GraphPad Prism 7 version 7.03. No difference was observed between the 5% (60/184 - 32.61%^{a}) and 10% FBS (82/227 - 36.12%^{a}) groups in blastocyst rate, which were superior to 0% (34/270 - 12.59%^{c}) and groups supplemented with L-carnitine (5% FBS-LC: 32/144 - 22.22%^{b} and 10% FBS-LC: 38/153 - 24.84%^{b}). There was no difference regarding embryo lipid accumulation. The results indicate that it is possible to reduce the FBS concentration to 5% in IVM media for buffalo embryo production and the supplementation of the maturation medium with L-carnitine at a concentration of 5 mM did not cause an increase in the embryo production of this species. Furthermore, alterations in the lipid accumulation during the IVEP were not found, with or without the presence of FBS and addition of L-carnitine during the IVM, indicating the need for further research, mainly involving the *in vitro* culture step of buffalo embryos.

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Influence of circulating concentrations of estradiol and progesterone on endometrial area and pituitary responsiveness to GnRH

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The aim was to evaluate the influence of high/low circulating P4 and E2 on endometrial area (EA) and on LH/FSH release after GnRH challenge. In a previous experiment, we developed a proestrus-like hormonal milieu using exogenous hormones (Motta et al. Anim Reprod, 15: 1031, 2018). For the new experiment, 43 nonlactating multiparous Holstein cows were submitted to the following protocol: D-7: 2 mg estradiol benzoate (EB) and 1g P4 implant; D0: implant removal, 0.526 mg PGF and two 2g P4 implants; D1: 0.526 mg PGF; D4 and D5: aspiration (OPU) of all follicles ≥5 mm. On D5 cows were randomized into 4 groups (n=10 or 11 per group): HighE2&LowP4, HighE2&HighP4, LowE2&HighP4, and LowE2&LowP4. LowP4 cows had one implant removed after OPU on D5 and 18 h later the second implant was removed. HighP4 cows had both implants maintained. HighE2 groups received a total dose of 0.8 mg EB divided into 8 treatments given 6 h apart in increasing doses (0.04, 0.04, 0.08, 0.08, 0.12, 0.12, 0.16, and 0.16 mg), starting after OPU on D5. Blood samples for P4 were taken on D5, just before the first implant removal and 18, 30 and 48 h later. The EA was evaluated by ultrasound after OPU on D5 and 12, 24 and 48 h later. The endometrial thickness from uterine horns was measured and then converted to EA (πr^2). On D7, after the last EA evaluation, all cows were treated with 8.4 µg buserelin (GnRH) and blood was collected just before GnRH and at 0.5, 1, 2, 3, 4, 5, and 6 h later for LH and FSH. Data were analyzed by PROC MIXED in SAS and the area under the curve (AUC) by the package MESS in R program ($P \leq 0.05$; tendency = $0.05 < P < 0.1$). Results are presented below in the following order: HighE2&LowP4, HighE2&HighP4, LowE2&HighP4, and LowE2&LowP4. Circulating P4 did not differ within groups with Low or with High P4 concentration. Therefore, data from groups were combined. At time 0, High and Low P4 groups had similar circulating P4 (1.9±0.2 vs 1.8±1.2 ng/mL), but at time 18 (1.2±0.1 vs 2.2±0.2 ng/mL), 30 (0.03±0.01 vs 2.1±0.2 ng/mL) and 48 (0.01±0.0 vs 2.4±0.2 ng/mL), Low P4 cows had lower circulating P4. The EA was similar among groups at time 0 (159.5±12.5 mm^2) and 12 (166.2±10.9 mm^2) but it differed at time 24 (224.4±11.4^a; 152.2±13.5^c; 162.1±11.6^{bc}; 178.6±10.2^b mm^2) and 48 (293.3±22.0^a; 151.5±11.3^c; 163.7±8.5^{bc}; 189.9±16.1^b mm^2). The FSH peak (2.4±0.4^a; 1.6±0.1^b; 0.8±0.1^c; 1.1±0.1^{bc} ng/mL) and AUC (7.3±0.9^a; 5.9±0.4^a; 3.0±0.4^b; 3.9±0.4^b ng^2) differed among groups and the time of peak tended to differ (1.8±0.2^a; 2.2±0.1^{ab}; 2.3±0.1^{ab}; 2.4±0.2^b h). The LH peak (12.2±1.6^a; 8.1±1.4^b; 2.6±0.43^c; 7.3±1.4^b ng/mL), AUC (36.9±5.1^a; 19.9±2.4^b; 8.3±0.9^c; 19.4±2.7^b ng^2) and time of peak (1.8±0.2^c; 2.0±0.0^{bc}; 2.5±0.2^a; 2.3±0.2^{ab} h) differed among groups. In conclusion, EA only changed under high circulating E2 and low P4. Further, the greatest LH and FSH surges after GnRH challenge were obtained when circulating P4 was low and E2 was high. Acknowledgements: FAPESP, CAPES, CNPq, and GlobalGen

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Pulses of Prostaglandin F2α metabolite during late embryonic development in Bos indicus cattle

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The period of late embryonic/early fetal mortality (LEM) in cattle occurs simultaneously with initiation of active placentation. The objective of this study was to evaluate differences in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion and pulsatility throughout late embryonic development period in cattle that maintained pregnancy as $PGF_{2\alpha}$ may play a role in the initiation of placental attachment. Pregnancies were established in Brahman females using industry standard estrous synchronization protocols with insemination occurring on day 0 (n = 25). Pregnancy diagnosis was performed on days 28, 40 and 60 via ultrasound. A subset of pregnant heifers (n = 4) and cows (n = 4) were fitted with coccygeal vein cannulas inserted~65 cm into the caudal vena cava for indirect sampling of utero/ovarian drainage. Blood samples were collected every 15 min for 6 h on day 29, day 31, day 34, day 37, and day 39. All animals maintained pregnancy until day 40 when catheters were removed; however, two animals were diagnosed as non-pregnant at the final day 60 pregnancy diagnosis (LEM). Serum concentrations of $PGF_{2\alpha}$ metabolite (PGFM) were measured with a validated ELISA (Interassay CV = 8.76%, Intraassay CV = 6.32%). Data were analyzed using repeated measures in SAS 9.4 and pulses were identified using AutoDecon Pulse2 accounting for unequally spaced sampling days. Average basal PGFM concentrations differed by day ($P<0.01$) with greatest concentration on day 31 (110.36 ± 5.86 pg/mL) and minimal concentrations on day 39 (9.45 ± 7.52 pg/mL) in animals that maintained pregnancy. Heifers had increased ($P = 0.01$) basal PGFM at day 31 (156.86 ± 16.31 vs 78.81 ± 8.89.61 pg/mL) but decreased ($P<0.01$) PGFM at day 34 (26.93 ± 8.49 vs 54.65 ± 6.03 pg/mL) compared to cows. LEM cow had elevated PGFM at day 31, 34, and 37 but not at 39 compared to cows which maintained pregnancy and exhibited twice as many PGFM pulses (8 vs 3) throughout the collection period; however, the LEM heifer exhibited comparable concentrations and pulses to animals that maintained pregnancy on all days. Number of PGFM pulses were increased ($P<0.02$) at day 31 (1.9 ± 0.26 pulses) compared to day 34 (0.17 ± 0.23 pulses), 37 (1.00 ± 0.33 pulses) and 39 (0.33 ± 0.33 pulses) in successful pregnancies. There was no difference in peak amplitude by day ($P = 0.68$) or parity ($P = 0.76$). These data indicate that concentrations and number of pulses of PGFM are increased during the initiation of active placentation during pregnancy; however, more data is needed to determine differences in PGFM in cows undergoing late embryonic/early fetal mortality. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from the USDA National Institute of Food and Agriculture.

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Profile of type I and II interferon receptor transcripts in peripheral blood mono and polymorphonuclear cells during early gestation in Nelore heifers

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We aimed with this study to analyze the abundance levels of type I and II interferon-tau (IFNT) receptors (IFNAR I and II) in peripheral blood mono (PBMC) and polymorphonuclear (PMN) cells in pregnant Nelore heifers. Twenty-nine heifers (18-20 months) had their estrous cycle synchronized and were subjected to fixed-time artificial insemination (FTAI) on D0. Pregnancy diagnosis was performed by transrectal ultrasonography on D25 and D28 through the detection of the embryonic vesicle and heartbeat. On days 0, 10, 14, 16, 18 and 20, 25 mL of blood were collected in heparinized tubes by puncture of the jugular vein for the isolation of PBMCs and PMNs cells. The isolation was performed by Ficoll® Paque Plus gradient (GE Healthcare, Chicago, USA), in an adapted method (Jiemtaweeboon S et al. 2011. ReprodBiol and Endoc., 9:79-89). Samples from 8 pregnant and 9 non-pregnant heifers were submitted to RNA extraction using the Direct-Zol RNA Miniprep Kit (Zymo Research, Irvine, USA) according to the manufacturer's instructions. The expression of the target genes (*IFNAR I* and *II*) was normalized in relation to the reference genes (*GAPDH* and *PPIA* for PBMCs; and *GAPDH* and *ACTB* for PMNs). For statistical analysis, the transcript abundance levels were evaluated by analysis of variance (ANOVA) with repeated measures of time, considering the random effect of heifer and the fixed effects of group (pregnant or non-pregnant), day and interaction of group by day using the PROC MIXED SAS software (SAS Institute). For PMNs, no significant (P>0.1) differences were detected in the *IFNAR I* expression, while for *IFNAR II*, only a time effect (P= 0.01) was observed, indicating an increase on transcript abundance from D0 to D16, with a progressive decrease on D20 in pregnant heifers. For PBMCs, only a time effect (P= 0.02) was observed for *IFNAR I* expression, characterized by an increase on the transcript abundance between D10 and D16, followed by progressive reduction on D18 and D20. Although an interaction of group by time was not significant (P=0.11), a subsequent analysis indicated that *IFNAR I* abundance on PBMC in the pregnant heifers progressively increased from D0 to D16 and followed a progressive decrease from D16 to D20; whereas, no difference (P>0.05) was detected along the evaluated days in the non-pregnant heifers. Also, the *IFNAR I* abundance on D20 was greater (P=0.04) in the pregnant than non-pregnant heifers. No significant (P>0.1) effects were detected in the *IFNAR II* expression. In conclusion, for PMN, only *IFNAR II* transcript abundance varied during early pregnancy but its expression is independent of the pregnancy status; whereas, for PBMC the pregnancy status may affect the temporal expression of *IFNAR I* at D20, which could be involved in the IFNT signaling mechanisms to guarantee the success of maternal recognition of gestation. Acknowledgments: FAPESP (2015/10606-9; 2017/13472-9; 2018/25393-9).

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Role of L-carnitine in *in vitro* maturation medium on oocyte nuclear maturation in domestic cats

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Oxidative stress may have detrimental effects on the oocyte, fertilization process, and subsequent embryo development. L-carnitine is an endogenous substance that, in addition to its significant role in lipid metabolism, has protective effects on the cell through its antioxidant actions. In this sense, data from the literature show that the supplementation of this antioxidant in the range of 0.3 – 0.6 mg/mL during *in vitro* maturation (IVM) exerts a beneficial effect on the progression of meiosis (Zare et al., Int J Reprod Biomed, 15(12):779-786, 2017). The objective of this study was to evaluate the effect of 0.5 mg/mL of L-carnitine during IVM in the resumption of meiosis in domestic cats oocytes. A total of 20 ovaries from queens of reproductive age were collected during elective ovarian hysterectomy at the Veterinary Hospital of Universidade Federal Fluminense, Niterói-RJ, and transported at 4 °C in 0.9% saline. Five replicates were performed. The cumulus-oocyte complexes (COCs) were recovered by slicing and washing each ovary with 1.5 mL PBS, in a 60 mm Petri dish. Subsequently, grade I and II COCs (Wood and Wildt, J Reprod Fertil, 110:355-360, 1997) were washed with TCM199 supplemented with HEPES and $NaHCO_3$ and allocated into one of two groups. Each group was transferred to a well of a 4-well dish with 500 µL of maturation medium (MM) containing TCM199 supplemented with HEPES and $NaHCO_3$, 4 mg/mL BSA, 0.5 µg/mL FSH, 1 µg/mL 17β-estradiol, 0.2 mM pyruvate and 50 µg/mL of antimycotic and antibiotic solution, with or without the addition of 0.5 mg/mL L-carnitine. The COCs were incubated for 24 h at 5% CO_2, 38,5 °C and humidified atmosphere. Afterwards, they were denuded using 100-500 IU/mL hyaluronidase and vortexed for 6 min. The oocytes were washed in PBS and 1% BSA and fixed in 200 µL 4% paraformaldehyde and 1% BSA, where they were kept for at least 24 h. After washing with PBS and 1% BSA, the oocytes were transferred to a slide and stained with 10 µL of 1 µg/µL Hoechst (diluted in TCM199). Then, they were evaluated under fluorescence microscopy, being classified as matured (MII), germinal vesicle breakdown (GVB), germinal vesicle (GV) and degenerated (DEG). For the statistical analysis, the chi-square test ($p<0.05$) was used. Data are presented as mean ± SEM. There was no difference between the parameters of the groups with and without the presence of L-carnitine ($p>0.05$), which were, respectively: MII 51.4 ± 5.6% and 38.2 ± 8.8%, GVB 29.7 ± 7,7% and 26.5 ± 7.4%, GV 2.7 ± 1.2% and 8.8 ± 7.8% and DEG 16.2 ± 4.0% and 26.5 ± 8.2%. In conclusion, although there was no significant difference, the group with the presence of L-carnitine in MM reached a nuclear maturation rate 13.2% higher than the group without the addition of the antioxidant.

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Expression of interferon stimulated gene 15 in the vaginal mucosa cells as a pregnancy diagnostic methodology during early pregnancy in cattle

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The aims of the study were 1) to evaluate the using of vaginal cytology as an alternative to determine the expression of interferon stimulated gene 15 *(ISG15)* during early pregnancy, and 2) to compare this method with the isolation of peripheral polimorphonuclear blood cells (PMNs) to determine *ISG15* expression *on day 20 after timed-artificial insemination (TAI) in beef heifers.* Nelore heifers (n=31) weighting 422 ± 47.8 kg were submitted to an estradiol/progesterone based protocol to synchronize ovulation for TAI on day 0. On days 16, 18 and 20, samples of vaginal cells were collected using a cytological brush (Cytobrush; Viamed Ltd, West Yorkshire, UK), which was placed in the fornix surrounding the external cervix os to recover the superficial cells from vagina. On day 20, blood samples (30ml) were also collected from jugular vein for PMNs isolation. The pregnancy diagnosis was performed on day 30 by detection of an embryo with heartbeat, and animals were classified on Pregnant (P; n=16) and Non-Pregnant (NP; n=15) groups. The *ISG15* expression was evaluated by RT-qPCR in the vaginal cell samples on days 16, 18 and 20 in randomly selected animals (n=7/group). On day 20, the relative *ISG15* expression between P and NP groups was compared between the vaginal cells and PMNs (n=13 P and 12 NP/cell type). Two reference genes (*GAPDH* and *ACTB*) selected from five tested genes using Normfinder program were used for normalization of relative expression of *ISG15* in both cell types. For the comparison between the two methods, the relative *ISG15* expression on day 20 in each P heifer was divided by the averaged expression in the NP group. The results were analyzed by ANOVA and PROC MIXED procedure (SAS), considering the main effects of group (G), time (T) and its interaction (TG). No significant effects (P>0.1) of G, T and TG were observed for the *ISG15* expression in vaginal cells. However, when evaluated on day 20 with a large number of animals, a greater abundance of *ISG15* was observed in the P group for the vaginal cells (relative expression to reference genes: 0.22±0.09 vs. 0.05±0.01; P=0.08) and PMNs (2.77±0.42 vs. 1.12±0.36; P<0.008). When compared the relative *ISG15* expression in the P group to NP group in the two cell types, no difference (P>0.1) was found between the PMNs (fold change: 2.47) and vaginal cells (fold change: 4.18). In conclusion, the use of *ISG15* expression in vaginal cells sampled by cytological brush does not show to be an efficient predictor of pregnancy status in cattle, as it presented a high variation among the P samples on day 20.

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Early or late blastocoel expansion on cytoplasmic lipid content, cell number and apoptosis of *in vitro* produced blastocysts

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In vitro embryo production (IVP) is one of the reproductive biotechnologies most applied in the field nowadays, aiming to rapidly increase the number of animals produced and the genetic gain achieved. Although well established, it still has limiting factors for the appropriate embryonic development when compared to in vivo. One of these factors is the increased lipid amount in IVP embryos when compared to in vivo embryos. This altered lipid content of the IVP embryos has been associated with a reduced survival after cryopreservation, hindering a broad commercial application of this biotechnology. The number of blastomers and apoptotic cells are other factors directly related to embryo viability. In this study, the lipid content, number of blastomers and percentage of apoptotic cells were evaluated in bovine expanded blastocysts at day 7 (D7) and 8 (D8) of embryonic culture. Bovine ovaries were recovered from a commercial slaughterhouse and transported to the laboratory, in physiological saline at 35°C. Cumulus oocyte complexes (COCs) were aspirated from follicles of 2 to 8 mm in diameter and only those with uniform cytoplasm and multilayered cumulus cells were selected. The COCs were in vitro matured and fertilized at 38.5°C and 5% CO2 in air. After fertilization, the presumed zygotes were cultivated at 38.5°C and 5%CO2, 5%O2 and 90% N2. The cleavage was evaluated at day 4 and the blastocysts were collected at D7 and D8, and submitted to lipid quantification (by Sudan Black B), nuclear fragmentation and total cell number (by TUNEL). For statistical analysis, the data were submitted to the t-test using PROC GLIMMIX of SAS. The lipid content was higher ($P<0.05$) in blastocyst derived from D8 when compared to D7 blastocysts (5.4± 0.3 vs 7.9 ± 0.2 respectively). Blastocysts derived from D7 presented more ($P<0.05$) blastomeres than the D8 blastocysts (105.9 ± 6.2 blt vs 82.0 ± 6.1 blt, respectively). On the other hand, blastocysts derived from D7 presented less ($P<0.05$) apoptotic cells when compared to the D8 blastocysts (6.1 ± 1.1 blt vs 11.3 ± 1.4 blt, respectively). The late blastocoel expansion of the embryos at D8 was important for increased cytoplasmic lipid accumulation, reduction in the number of blastomeres and increase in the number of apoptotic cells. Therefore, we conclude that the early blastocoel expansion, on D7, favors embryonic quality and can be used as a morphological predictor of competence.

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Effects of *in vitro* growth and prematuration on mRNA levels for *GDF9*, *CCNB1*, *H1FOO*, *cMOS*, *PARN* and *eIF4E* in oocytes from small bovine antral follicles

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During the meiotic maturation of bovine oocytes, the mRNAs are translated between the metaphases and will aid in the later developmental processes. Thus, the objective of this study was to evaluate mRNA levels for *GDF9*, *cyclin B1*, *H1FOO* (oocyte specific binding histone), *cMOS kinase*, *PARN* (poly (A) ribonuclease) and *eIF4E* (cap binding protein) in oocytes derived from small follicles antral (1.0-3.0 mm in diameter) before and after growth, prematuration and *in vitro* maturation (IVM). For this, cow ovaries (n = 40) were obtained from a local slaughterhouse and transported to the laboratory in saline solution. In the laboratory, cumulus-oocyte complexes (COCs) were aspirated from small antral follicles, classified and those that had cumulus compact cells and oocytes with no signs of cytoplasmic degeneration were intended for *in vitro* culture. The COCs were cultured individually for 48 h in TCM-199 supplemented with 4% PVP, 1 µg / ml estradiol, 4 mM hypoxanthine, 0.2 mM pyruvic acid, 2.2 mg / ml sodium bicarbonate, 0 mg / mL LH, 0.5 mg / mL FSH, 5% FBS and 100 IU / mL penicillin and 50 µg / mL streptomycin. The *in vitro* pre-maturation medium was TCM-199 supplemented with 0.2 mM pyruvic acid, 5.0 mg / mL LH, 0.5 mg / mL FSH, 0.4% BSA, 10 µM cilostamide, 100 IU / mL penicillin and 50 µg / mL streptomycin. COCs were cultured for 20 hours. For IVM (22h), the same pre-maturation medium was used, but without cilostamide. Morphology, oocyte diameters, meiotic progression were analyzed and four groups containing 10 oocytes were stored at -80 °C for RNA extraction and subsequent evaluation of mRNA expression before the pre-maturation and maturation period. The percentages of Germinal Vesicle (GV) in the different treatments were evaluated by the Mann Whitney test to analyze the developmental stage of the oocyte. The mRNA levels were quantified at the end of 18 days of culture by the real-time PCR technique. The gene expression data were analyzed by the Kruskal-Wallis test, followed by Dunn's nonparametric multiple comparison test. Differences were considered significant when $P < 0.05$ or $P > 0.05$ when there was no difference. After the growth and pre-maturation of the COCs of the small follicles, only 17.0% of the oocytes had a resumption of meiosis. However, after IVM, meiosis resumption rate was 80%. These pre-mature oocytes showed increased mRNA levels for *GDF9*, *PARN* and *eIF4E* when compared to those before culture. However, there was no significant increase ($p < 0.05$) in mRNA levels for *cMOS*, *CCNB1* and *H1FOO* after pre-maturation. In conclusion, prematuration increased the expression levels of *GDF9*, *PARN* and *eIF4E* genes. Furthermore, the increased expression levels of these genes in oocytes may be a good indicator of oocyte viability during *in vitro* culture. However, further studies are needed to confirm the effect of premature oocyte development.

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Endometrial expression of oxytocin receptor, and interferon-stimulated gene 15, and circulating PGFM after oxytocin challenge differ between AI and IVP derived pregnancies on days 18 and 32

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The aim was evaluate oxytocin-induced prostaglandin F metabolite (PGFM) in pregnant cows from artificial insemination (AI) or *in vitro* produced (IVP) embryos on d18 and d32, and its association with factors that impact the success of pregnancy, such as circulating progesterone (P4), conceptus length on d18 and embryo size on d32. Moreover, aimed to quantify and localize oxytocin receptors (OXTR) and interferon-stimulated gene 15 (ISG15) expression in uterine endometrium. Non-lactating *Bos indicus* (Nelore) cows (n=142) were submitted to a synchronization protocol, and randomly assigned to one of the following groups: non-inseminated (NI), AI on d0 (48 h after implant removal), or received an IVP embryo on d6.5. Then, NI, AI and IVP cows were slaughtered on d18 or d32, according to AI or IVP groups. One d before slaughter (d17 and d31) cows were challenged with 50 IU oxytocin, i.m., and blood samples were collected before (0 min), 60 and 120 min after oxytocin for circulating PGFM. Samples for P4 were collected on d6.5 and on the d of oxytocin. After slaughter, uterus was collected and dissected for conceptus, embryo and OXTR analysis. Statystical analysis were performed using PROC MIXED of SAS 9.4. There was no difference (P>0.05) between AI vs IVP for conceptus length on d18 (44.6±4.3 vs 53.3±5.9 cm), or P4 on d6.5 and d17. However, embryo size on d32 (1.8±0.2 vs 1.3±0.1 cm) was bigger, and P4 on d31 (8.5±0.9 vs 6.6±0.5 ng/mL; P=0.07) tended to be higher in AI than IVP. For basal circulating PGFM on d31, AI and IVP were similar (47.3±6.4 vs 39.8±4.8 pg/mL), and both were respectively 3 and 2.5-fold higher than NI (15.7±3.6 pg/mL), and 2.6 and 2.2-fold higher than IVP on d17 (17.9±3.9 pg/mL). AI cows on d17 had intermediate basal circulating PGFM (33.3±6.6 pg/mL) which whereas greater than IVP on d17, but similar to NI and both groups on d31. The highest increase in PGFM after challenge was detected at 60 min for all groups. The NI cows had higher PGFM after oxytocin challenge than IVP (7.6- vs 0.2-fold increase). The AI group on this same d had an intermediate response, which did not differ from other groups. Cows from IVP on d17 had lower oxytocin-induced PGFM than AI on the same d, although on d31 both groups had higher PGF release than IVP d17. In addition, OXTR were significantly highly suppressed on pregnant cows on d18, especially in IVP cows, but highly expressed in NI cows and in both groups on d32, being AI higher than IVP. The ISG15 had lower expression in NI and d32 groups, whereas was highly expressed in d18 pregnant cows for both groups. In conclusion, the PGF production pathways are induced after the first month of pregnancy in uterus, suggesting alternative mechanisms for CL maintenance than PGF suppression. Moreover, the signaling differences between IVP and AI pregnancies affected molecular and endocrine environment, influencing PGF release during these two critical time points. Acknowledgements: FAPESP, CNPq, CAPES, FEALQ.

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In vitro production of bovine thermotolerant embryos

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Considering the climatic changes and the elevation of temperature, the reproductive indices in dairy herds of countries with tropical climate are critical. High metabolic rate and the high uterine temperature are related to embryonic death. This experiment was designed to develop a heat treatment protocol for bovine embryos in order to induce thermotolerance in Girolando embryos. Subsequently, those embryos will be characterized (HSP protein expression) and tested as an alternative to conventional embryos during the summer season. For this experiment, oocytes were collected from F1 (½ Gir and ½ Holstein) donors by ultrasound guided follicular aspiration (OPU, ovum pick-up) (CEUA EGL 3956180316). Oocytes were in vitro fertilized with Holstein bull semen for production of ¾ Holstein embryos. A mild heat treatment protocol was designed and tested at 96, 120 or 144h. p.i.. The embryos were submitted to the heat treatment at a temperature of 38.5 to 40.5 °C for 6 hours. A homemade incubator was settled with a water filled plastic box placed at a heat stage. Temperature increase or decrease was controlled manually by opening or closing the plastic box. Treated embryos were moved to cryotubes with 200ul medium and 200ul of mineral oil and remained for 40 min in the incubator for gas equilibration with a loose cover. After that, cryotube was tightly closed and sealed with parafilm and transferred to heat treatment chamber. Heat treatment was carried out as six 1h cycles. At each cycle, every 7.5 min a 0.5° C increase was induced up to 40.5°C, and then every 7.5 min a 0.5°C decrease was induced until 38.5°C. Control remained in the incubator at 38.5 °C. Two replicates were performed, and blastocyst rates at d7 were evaluated as well as the number of cells and apoptosis rate of the blastocysts. No differences were observed in the blastocyst rates (C = 28.57, TT96hpi = 35.14, TT120hpi = 23.81, TT144hpi = 19.77, p> 0.05, Fisher's exact test, n = 298 cleaved embryos) or on the apoptosis index (C = 6.47 ± 3.93, TT96hpi = 7.41 ± 4.05, TT120hpi = 7.07 ± 5.21, TT144hpi = 4.54 ± 2.71, p> 0.05, ANOVA and Dunnett, n = 50 blastocysts). The mean number of cells did not differ in any treatment compared to the control group (C = 70.53 ± 20.03, TT96hpi = 67.46 ± 13.65, TT120hpi = 57.80 ± 8.74, TT144hpi = 78, 00 ± 14.87, p> 0.05, ANOVA and Dunnett, n = 50 blastocysts). The results allow us to conclude that heat treatment developed can be used at any of the tested moments without being harmful to embryos. The 144h.p.i. can be preferred due to the proximity to embryo transfer (168 h.p.i.), so that the post-transfer effects are prolonged. Acknowledgements: Fapemig and CNPq.

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Use of lipolysis supplement during IVM reduces lipid content of bovine oocytes but do not affect blastocyst cryosurvival

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In vitro produced embryos have high lipid content and this characteristic is related to cryotolerance. Since lipid accumulation first occurs during in vitro maturation (IVM), our hypothesis was that the use of lipolysis regulators during IVM would reduce lipid content of the oocytes and consequently of the embryos and improve cryosurvival. We evaluated the effect of a lipolysis supplement (L-carnitine, linoleic acid and forskolin) during IVM on total lipid content of oocytes and in vitro produced blastocysts and their influence on cleavage, blastocyst and survival rates after vitrification. CCOs were obtained from slaughterhouse ovaries, in 6 replicates, and randomly distributed in two groups: Control (C, TCM 199 + 10% SFB) and Lipolysis supplement (L, TCM 199 + 10% SFB and lipolysis supplement (2,5 mM L-carnitine, 150 µM linoleic acid and 15 µM forskolin) for 24 h IVM. After IVM, part of matured oocytes was denuded with Tryple Express (Gibco, Grand Island, NY) solution and fixed for Oil Red O staining and most followed IVF and IVC for 7 days. All steps were performed at 38.5ºC, 5% CO2 and maximum humidity. Samples of denuded oocytes and day 7 blastocysts from C and L groups were fixed in 4% paraformaldehyde and stained with Oil Red solution for 30 min. Lipid content was estimated by stained cytoplasm area fraction (µm²) and staining levels (pixels) using ImageJ® software. Bl and Bx grade 1 were vitrified in vitrification solution 1 (HSOF + 7.5% ethylene glycol [EG] + 7.5% dimethyl sulfoxide [DMSO]) for 3 minutes and solution 2 (HSOF + 15 % ET + 15% DMSO) for up to 30 seconds. Subsequently, the embryos were transferred to vitrification forks and submerged in liquid nitrogen. The lipolysis supplement reduced lipid content in L oocytes compared to Control group considering staining levels (C- 49.90 ± 1.59, L - 45.00 ± 1.86, P <0.046, T test, n = 192,104-88 per group) and area fraction (C - 127.25 ± 4.06, L – 114.77 ± 4.76, P<0.046, T test, n = 192,104-88 per group). In blastocysts, there was no difference between groups (C - 62.38 ± 2.68, L- 66.78 ± 2.61, P = 0.27, T Test, n = 40, 25 -15 per group). There was no difference in developmental rates or survival rates after vitrification. The mean rate of cleavage (C - 74.88 ± 5.52, L - 78.21 ± 5.76, P = 0.45, T Test, n = 152, 74-78 per group) and blastocyst (C - 42.24 ± 5.75, L - 31.46 ± 3.10, P = 0.09, T Test), as well as the reexpansion rate (C - 64.36 ± 8.58a, L - 54.78 ± 7.73a, P = 0.39, Test T, n = 196, 108-88 per group) and hatching (C - 23.42 ± 5.66a, L - 24.51 ± 6.87a, P = 0.93, Test T, n = 72, 36 per group) of vitrified blastocysts 48 hours after warming did not differ between groups. We conclude lipolysis supplement use during IVM was efficient in reducing the total lipid content of oocytes but levels were similar to control in blastocysts. No effect was detected in developmental rates or survival after vitrification. Acknowledgments: FAPERJ, EMBRAPA, Pesagro-Rio.

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Monitoring lipids behavior in bovine preimplantation embryos

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In addition to their important role as structural units and in cell signaling pathways, lipids are a potential energy source for the embryo. However, an inverse relationship between embryonic quality and lipid content has been established. In general, factors that contribute to compromised viability such as high oxygen tension and non-optimized media culture supplementation also result in an increase in lipid content. It is therefore imperative to overcome the barriers in lipid analysis and understand how the culture system modulates lipid content to enable embryo formation. In this context, this study proposes to comprehensively monitor the impact of the interaction between glucose supplementation and oxygen tension in the lipid profile of individual bovine embryos. Using a factorial experimental design (2x3), embryos were produced *in vitro* by standard protocols and cultured in 20% or 5% O_2, and also in distinct glucose concentrations (0, 2 and 5mM). Blastocysts (n=10/group) were collected on day 7 and submitted to the Bligh&Dyer lipid extraction protocol. Then, using a sensitive profiling method based on mass spectrometry (Multiple Monitoring Reactions), we monitored 178 lipids from triacylglycerol (TAG), free fatty acids (FFA) and cholesteryl ester subclasses. Absolute intensity of each lipid was gathered in a matrix, normalized and submitted to multivariate analysis and univariate statistics. Results show that the augmentation of glucose in the culture media only impacts the lipid profile if the embryos are being cultured in a non-optimized oxygen tension (higher in 2mM and 5mM compared to 0mM in 20% O_2; $p<0.05$). This was confirmed by a non-supervised multivariate analysis (Principal Component Analysis), where the model was able to separate the groups cultured in different oxygen tensions (PC1+ PC2 = 67.5%), but not those produced with distinct glucose supplementation. Therefore, we investigated the general behavior of these lipids in both oxygen tensions and observed that although embryos cultured in 20% O_2 presented generally higher relative amounts of lipids ($p<0.000$), this was not a widespread effect. TAG and Cholesteryl esters were augmented in the embryos cultured in 5% O_2 ($p<0.000$), while only FFA were significantly increased in embryos cultured at 20% O_2 ($p<0.000$). Moreover, important fatty acids such as palmitic and stearic acids were found to have relative amounts around 2 times higher in the groups cultured in 20% O_2. With this results it is possible to point out that (i) glucose supplementation alone is not responsible for an increase in lipid relative amounts; (ii) the higher oxygen tension drives lipid metabolism to the production of free fatty acids that will likely be oxidized to produce energy and (iii) in 5% O_2, lipid metabolism is orientated towards the production of TAGs and Cholesteryl esters, probably representing a protection against mitochondrial damage and a way to ensure correct membrane formation and energy stock.

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Presence of CL and/or intravaginal progesterone insert affect ovulation and subsequent CL development after gonadorelin treatment

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The aim of this study was to determine the effect of presence of corpus luteum (CL) and/or an intravaginal progesterone (P4) insert on ovulatory response and subsequent CL development, after administration of 100 µg gonadorelin acetate (GnRH). Non-lactating Holstein cows were submitted to a presynchronization protocol: D-17: 2 mg estradiol benzoate and a 2 g P4 insert previously used for 7 d; D-9: 0.530 mg cloprostenol sodium (PGF) and 1 mg estradiol cypionate; D-7: 100 µg GnRH. Only cows that ovulated were used (n = 90, Age = 5.0 ± 2.3 years; BCS = 3.3 ± 0.1; 4 replicates). On D -1.5 cows were randomly assigned to a 2x2 factorial design (CL x P4 insert). Groups designed to have no CL at GnRH challenge received 0.530 mg PGF on D -1.5 and a second dose 24 h later. On D0 all cows were treated with 100 µg GnRH. Simultaneously, cows from P4 insert groups received a 2 g P4 device, that was maintained for 14 d. Dominant follicle (DF) size, ovulatory response, and development of the subsequent CL were assessed by ultrasonography on D0, D2, D7, and D14, respectively. For a subset of cows (n = 35), CL development was evaluated daily from D5 to D14. Statistical analyses were performed by MIXED and GLIMMIX of SAS 9.4 (means ± SEM; $P \leq 0.05$). Only cows with DF ≥ 10 mm on D0 were considered in the analysis. There was no difference on DF diameter on D0 among treatments or between ovulated and non-ovulated cows. There was an effect of presence of CL on the ovulatory response ($P<0.001$), but there was no effect of P4 insert or interaction between these factors: with CL = 58.1% [52.4% (11/21) and 63.6% (14/22) with and without P4 insert, respectively]; without CL = 95.5% [90.9% (20/22) and 100% (22/22) with and without P4 implant, respectively]. The presence of CL at GnRH negatively affected the volume of the new CL on D7. Cows with CL on D0 had smaller subsequent CL than cows without CL (2.9 ± 0.3 vs. 4.2 ± 0.2 cm^3; $P = 0.001$). Moreover, there was an interaction for CL volume on D14. Cows without CL on D0 that did not have a P4 insert had greater CL than cows with a P4 insert at GnRH (6.3 ± 0.4 vs. 3.5 ± 0.6 cm^3; $P = 0.001$). From D10 to D14, all cows from the group without CL and without P4 insert on D0 maintained the CL, whereas the other groups presented a significant decrease in mean CL volume. The results confirm that CL presence at GnRH administration exerts a suppressive effect on ovulatory response of a 7-day old follicle and on subsequent CL development. Although the insertion of an intravaginal P4 device has not affected ovulation, surprisingly, it negatively influenced CL development/maintenance by D14, even in cows that did not have a CL at the time of GnRH challenge. Thus, it is very likely that P4 supplementation at the time of ovulation induction causes a suppressive effect on the subsequent CL lifespan by anticipating luteolysis. Acknowledgments: FAPESP, CNPq, and CAPES.

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Metabolic gene expression and lipid accumulation in bovine embryos produced *in vitro* from semen of Nellore bulls selected for residual feed intake

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Feed costs account for more than half of the total cost of cattle production, therefore improving feed efficiency (FE) is desirable to improve the economy in the livestock sector. FE can be measured by residual feed intake (RFI), which is defined as the difference between the actual and the predicted dry matter intakes based on the body size and performance of each animal. Animals selected for lower RFI (high efficiency) present lower feed consumption with no effect on growth rates, however, small changes in body composition towards greater lean and less fat have already been reported in low RFI animals. Considering the differences in the metabolism of selected animals for FE, this experiment aimed to investigate the expression of genes related to metabolism and intracytoplasmic lipid accumulation in embryos produced *in vitro* from semen of Nellore bulls classified for high (less efficient; n=3) and low (more efficient; n=3) RFI. The semen was cryopreserved when the animals were 24 months old and was used to inseminate *in vitro*-matured oocytes recovered from ovaries obtained in a slaughterhouse. Putative zygotes were culture until day 7 when blastocysts (n=44) were stained with the lipophilic dye Sudan Black B for determination of the intracytoplasmic lipid content and expanded blastocysts (3 pools per treatment, each containing 3 blastocysts of each bull) were collected to assess the abundance of 88 transcripts by RT-qPCR using a microfluidic platform (BioMark HD System™, Fluidigm®). The ΔCt values were calculated relatively to the geometric mean of five most stable reference genes and fold changes were calculated as $2^{-\Delta Ct}$. Data were analyzed by t test ($P<0.05$). Blastocysts rates on day 7 (18.2%; averaged) and intracytoplasmic lipid content (131.3 arbitrary units of pixels; averaged) were unaffected by FE ($P>0.05$). Transcript levels of *CD36* and *ACAT1* genes (lipid metabolism) were up-regulated ($P<0.05$), whereas several other transcripts were down-regulated ($P<0.05$) in low RFI group, including genes related to lipid metabolism (*HMGSC1* and *PPARGC1*), embryo development and quality (*NANOG* and *IFNT2*), epigenetic regulation (*H3F3A*) and stress response (*HSF1*). These genes were uploaded to the R package clusterProfiler for analysis of gene ontology (GO) and the program identified 23 enriched GO biological processes. We also identified 4 KEGG pathways related to *ACAT1* and *HMGCS1* genes. The results suggest that there is a genetic association between paternal dietary efficiency and the expression of genes related to metabolism of descending embryos, and the increase in metabolism is associated with lower RFI (ie, higher efficiency). However, the up-regulation of transcripts related to lipid metabolism did not reflect in an increase on embryonic lipid content. In conclusion, the gene expression and metabolic pathways of *in vitro*-produced embryos are affected by paternal RFI. Financial support: FAPESP (#2015/06733-5 and #2012/50533-2) and CNPq (#307416/2015-1).

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miRNAs identified in corpus luteum of IVF recipient cows are absent in SCNT recipient animals on day 19 of pregnancy

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Corpus luteum (CL) is responsible by P4 production and it is necessary for establishing and maintenance of pregnancy in cattle. Normal luteal function is regulated by miRNAs, that are small non-coding RNA molecules involved in post-transcriptional regulation of target genes. However, the role of miRNAs and its potentially regulated pathways are poorly known in the CL of cows carrying conceptus derived from different biotechnologies (IVF or SCNT). Therefore, our hypothesis is that miRNAs are exclusively expressed in the CL of cows carrying IVF or SCNT conceptus on day 19 pregnancy. For this, COCs recovered from ovaries collected at local abattoir were used to produce SCNT blastocyst and, IVF embryos were made from oocytes collected by OPU. Nellore cows had the estrus synchronized and received one embryo (IFV or SCNT), on day 7 after expected estrus. CL were collected on day 19 of pregnancy, in animals carrying IVF (n=3) or SCNT (n=3) conceptus. The CL function was evaluated by P4 concentration in blood serum collected from the jugular vein on days 9, 14 and 19 of pregnancy. Mature miRNAs were reverse transcribed using MiScript HiSpec Buffer. The relative levels of 384 miRNAs were evaluated. The data were normalized by the geometric mean of miR-99b, RNU43 snoRNA and Hm/Ms/Rt U1 snRNA. miRNAs were considered exclusively expressed when the expression was detected in all CL samples of one group and not detected (not expressed) in all samples of the other group. On days 9, 14 and 19, serum P4 concentrations were similar between IVF and SCNT groups (Bridi, et al., Animal Reproduction, 15:480, 2018). A total of 288 mature miRNAs were identified in CL samples from both groups, with one exclusive miRNA in the SCNT-CL and three exclusive miRNAs in the IVF-CL. The identification of bovine genes modulated by each miRNA was performed using TARGETSCAN software. After gene identification, the code Ensembl Transcript ID was used to determine enriched pathways regulated by these miRNAs using DAVID Bioinformatics Resources 6.8, NIAID/NIH. The miRNA bta-miR-129-3p, uniquely detected in the SCNT CL group, modulated signaling pathways that include MAPK (14 genes), oxytocin (9 genes), GnRH (7 genes) and estrogen (6 genes). Moreover, bta-miR-141, bta-miR-302a and bta-miR-875, which were uniquely detected in the IVF CL, regulate PI3K-Akt (40 genes), MAPK (33 genes), Hippo (25 genes) and oxytocin (18 genes) pathways. The results show that, on day 19, the expression of different miRNAs in the CL of recipient cows can be modulate by conceptus of different origins (IVF or SCNT). Furthermore, the exclusive miRNAs of both groups regulate oxytocin signaling pathway in the CL, that have an important biological role in the maintenance of luteal function and, consequently in the establishing of pregnancy. Funding: FAPESP grants 2014/22887-0; 2016/50433-9; 2017/19681-9, 2017/50438-3 and 2018-13155-6. Acknowledgments: WTA.

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Profile of new early pregnancy markers identified by transcriptomic analysis in peripheral blood immune cells in beef heifers

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We aimed with this study in pregnant (P) and non-pregnant (NP) heifers: 1) to discover new pregnancy markers (PM) by RNA sequencing (RNAseq) in peripheral blood mononuclear cells (PBMC) on day 18 post-AI; and 2) to assess the mRNA profile of new PM in PBMC and peripheral blood polymorphonuclear cells (PMN) at early pregnancy. Nelore heifers (N=21) were AI in fixed-time (D0). On D10, 14, 16, 18 and 20, blood was collected for isolation of PBMC and PMN, and P4 concentration assay and color Doppler ultrasonography was performed to evaluate the corpus luteum (CL). Pregnancy diagnosis was done on D28 and heifers were *classified* in P (N=9) and NP (N=12). Heifers (N=6/group) with different (P<0.05) plasma P4 concentration, CL area and blood perfusion on D18 were selected and RNAseq was done on PBMC samples. RNAseq analysis indicated 220 differentially expressed genes (200 up regulated in P). Twenty genes found on RNAseq of PBMC with the highest fold-changes or no overlapping between P and NP, were assessed by qPCR from D10 to 20 (N=6/group). Reference genes were used for expression normalization (*GAPDH* and *PPIA*). Data were analyzed by ANOVA using the PROC MIXED procedure (SAS) and considering the main effects of group (G), time (T) and their interaction (GT). For PBMC, G and T effects (P<0.1) and GT interaction were observed for *IFI6*, *RSAD2*, *IFI44*, *IFITM2*, *TNFSF13B* and *LGALS3BP*, reflecting a greater (P<0.1) expression in the P group on D18 and D20 for *IFI6*, *RSAD2*, *IFI44* and *IFITM2*, and on D16 and D18 for *TNFSF13B*. For *CLEC3B*, *OAS2* and *LOC100139209*, a T effect (P<0.05) and GT interaction (P<0.1) were detected, reflecting a greater (P<0.05) expression in the P group on D20 for *OAS2* and *CLEC3B*. For *DMKN*, a GT interaction (P<0.05) reflects an increase on D16 in the P group. For *A2M*, *BPI*, *ANG*, *PLSCR2*, and *DRAM1*, only a T effect (P<0.05) was observed, reflecting a progressive increase from D10 to D20. For *LIG1*, a greater (P<0.1) expression was observed in the NP group from D10 to D20. For PMN, a T effect and GT interaction (P<0.1) were observed for *IFI44*, *RSAD2*, *OAS2* and *LGALS3BP*, reflecting a greater expression in the P group on D18 and 20 for *RSAD2* and *LGALS3BP*, and on D20 for *IFI44* and *OAS2*. An interaction (P<0.05) was also detected for *IFI6*, *C1R*, *RHOT1* and *LIG1*, indicating an increase in the P group on D16, D18 and D20, respectively, for *RHOT1*, *C1R* and *IFI6*, and a decrease in *LIG1* expression in NP group on D20. However, no effect (P>0.1) was observed for *SIGLEC1*, *SORD*, *C1R* and *RHOT1* in *PBMC* and for *IFITM2* in PMN. In conclusion, 9 genes presented increased expression on PBMC of P in at least one-time point from D16 to D20; but only 4 of these genes retained the expression increased on PMN (*IFI6*, *IFI44*, *RSAD2* and *LGALS3BP*). Thus, results indicate potential genes to be used as novel pregnancy predictors in immune cells in cattle at early gestation. Acknowledgments: FAPESP (2015/10606-9; 2017/13994-5).

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Influence of apical domain formation on the segregation of cell lineages in early development bovine embryos

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The first event of cellular differentiation in mammals consists in the segregation between the inner cell mass (ICM) and the trophectoderm (TE). Biological processes that comprise this event are not yet clear during bovine embryo development and studies in mouse suggest that cellular contractility and formation of an apical domain plays a role in this event. In this study, we tested the hypotheses that blocking cellular contractility would block apical domain and inhibit TE formation or that direct inhibition of apical domain formation would inhibit TE formation in bovine embryos. First, we evaluated the presence of an apical domain during bovine embryo development by immunofluorescence of apical domain proteins PARD6B (Novus Biologicals, Littleton, CO USA) and EZR (Abcam, Cambridge, MA, USA) in IVP embryos. We observed that EZR is present since 8-cell stage while PARD6B becomes apically localized at the blastocyst stage. To test the effect of cellular contractility on TE formation we treated IVP embryos with blebbistatin (Bb), a myosin light chain kinase inhibitor. We assessed embryos at 90 hours post-insemination (90hpi) and those at 8-cell stage or further ahead in development were submitted to the following treatments: control, 25μM Bb (+)- and 25μM Bb (-)- (Cayman Chemical, Ann Arbor, USA). Embryos were kept in treatments until 186hpi when development rates (blastocysts/treated embryos) were assessed and embryos fixed with paraformaldehyde (Merck KGaA, Darmstadt, Germany). Developmental rates were analyzed by ANOVA followed by Tukey's adjustment for comparison of means after 5 replicates. Unexpectedly, no statistical difference ($p<0.05$) was observed considering developmental rates among all three groups: control 47.24±7.30% (45/96), Bb (+)- 60.24±7.30% (58/96) and Bb (-)- 49.50±7.30% (46/96). Immunofluorescence revealed that EZR was practically abolished in Bb (+)- treated embryos while present in the other groups. YAP (Abcam), a HIPPO-pathway related protein, was nearly undetected in Bb (+)- treated embryos while visible in other treatments. Also, CDX2 (Abcam), a commonly used marker for TE cells, was not observed in Bb (+)- embryos. To confirm these results, we used the same experimental design and statistical analysis to test if inhibition of apical domain establishment blocks TE formation. Embryos were submitted to following treatments: Control, vehicle (DMSO, Merck) and 7.5μM U73122 (Cayman Chemical), a phospholipase C inhibitor. No statistical difference was observed considering developmental rates among all three groups: control 41.8±3.27% (51/122), vehicle 35.29±3.27% (44/124) and U73122 35.27±3.27% (45/128). Combined, these results led us to conclude that inhibition of contractility or inhibition of the apical domain do not block formation of the TE in bovine embryos, suggesting that different biological processes are involved in ICM/TE segregation in bovine embryos. Funded by FAPESP grants 2017/09576-3, 2017/25574-0, 2018/08285-8.

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Effect of cysteamine during *in vitro* maturation of bovine oocytes on embryo development

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In in vitro embryo production (IVP), oxidative modifications via increased reactive oxygen species (ROS) represent a major culture induced stress. Anti-oxidant systems such as glutathione (GSH) can attenuate deleterious effects of oxidative stress decreasing ROS thus protecting the zygote and early embryo. Previous studies suggest that addition of cysteamine to in vitro maturation (IVM) medium can increase intracellular GSH synthesis, improving pronucleus formation, cleavage rates and embryo development. The aim of the present work was to investigate the effects of cysteamine during IVM with conventional FSH stimulation or utilizing the IVM phase of the follicular system (FS), recently proposed by Ovarian Molecular Physiology Laboratory research group. The FS base medium consisted of TCM199 (with Earle's salts, bovine serum albumin, amikacin, pyruvate) supplemented with rhFSH, amphiregulin, insulin-like growth factor 1, estradiol and progesterone (Soares et al., Reproduction, Fertility and Development, 29:2217-2224, 2017). Five replicates were performed to compare four experimental groups: FSH (basic medium supplemented with rhFSH 10-1 UI/mL); FSH+C (FSH medium supplemented with cysteamine 1 mM/mL); FS and FS+C (FS medium supplemented with cysteamine 1 mM/mL). Ovaries were obtained from a slaughterhouse and COCs recovered by aspiration were submitted to IVM for 24h, followed by in vitro fertilization for 18h and in vitro culture (IVC) for seven days. Blastocyst rate was calculated in relation to total oocytes subjected to IVM and blastocyst cell numbers were assessed by Hoechst 33342 staining. Rates of expanded and hatched blastocysts were calculated in relation to total blastocysts. Data were arcsine transformed and compared with Tukey (parametric data) or Wilcoxon (non-parametric data) tests. Differences were considered significant when $P \leq 0.05$. Addition of cysteamine did not alter blastocyst rate ($P > 0.05$; FSH 24.67±5.37; FSH+C 33.50±4.95; FS 25.96±4.92; FS+C 22.95±5.56), expanded and hatched blastocysts rates ($P > 0.05$; FSH 94.44±3.51; FSH+C 83.57±5.61; FS 85.83±4.86; FS+C 83.36±7.65), nor the total number of embryonic cells ($P > 0.05$; FSH 118.40±7.41; FSH+C 123.39±7.36; SF 124.97±9.75; SF+C 124.37±9.63). In conclusion, addition of cysteamine to the IVM medium did not improve embryo production. Supported by FAPESP 2017/07588-4.

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Anisomycin inhibits ERK1/2 MAPK activities in activated bovine oocytes

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Pioneering studies by our research group have demonstrated the advantages of using Anisomycin, a protein synthesis inhibitor, in the activation of bovine oocytes in embryos generated by intracytoplasmic sperm injection (ICSI), somatic cell nuclear transfer (SCNT) and parthenogenesis. However, the precise mechanism by which anisomycin releases the oocyte's meiotic arrest and allows the activation of oocytes and further embryonic development is unknown. Therefore, the objective of the present study was to evaluate the effect of the activation of bovine oocytes by anisomycin on the inhibition of the MAPK activities. For this, oocytes were activated using ionomycin (ION), ionomycin plus anisomycin (ION/ANY) and ionomycin plus cycloheximide (ION/CHX; activation control). Also in vitro fertilized oocytes (IVF) at 6 hours and MII-oocytes were included as controls. The evaluations were conducted at 1, 4 and 15 hours post activation (hpa) and 3, 6 and 17 hours post fertilization (hpf, in the case of IVF 2 extra hours to allow the penetration of the spermatozoon), respectively. The status of phosphorylation of ERK1/2 were measured by immunoblotting using GAPDH as loading control. Differences between treatments were analyzed using ANOVA and to identify the differences between groups, Tukey's post-test was performed with a level of significance of $p<0.05$. The preliminary results of two biological repetitions showed no differences in the status of phosphorylation of ERK1/2 at 1 hpa-3 hpf. However, assessment at 4 hpa-6 hpf showed a low level ($p<0.05$) of phosphorylation of ERK1/2 in oocytes activated by ION/ANY (0.03±0.003), compared to MII-oocytes (2.4±0.9) and oocytes activated by ION (2.2±0.5). It was also observed a decreased, although not significant, in the status of phosphorylation of ERK1/2 in oocytes activated by ION/ANY (0.03±0.003), compared to oocytes activated by ION/CHX (0.36±0.2). Oocytes activated by ION/CHX did not show differences in relation to MII-oocytes and oocytes activated by ION, meanwhile, the level of phosphorylation of ERK1/2 in IVF-oocytes (3.3±1.4) was greater ($p<0.05$) than the other treatments. The analysis at 15 hpa-17 hpf showed a low level ($p<0.05$) of phosphorylation of ERK1/2 in oocytes activated by ION/ANY (0.03±0.01) and ION/CHX (0.03±0.02), in relation to MII-oocytes (1.6±0.5), oocytes activated by ION (1.3±0.1) and IVF-oocytes (1.5±0.7). In conclusion, anisomycin showed a similar pattern of phosphorylation than cycloheximide, one of the most common exogenous oocyte activation treatments, since both compounds showed to inhibit the ERK1/2 MAPK activity by dephosphorylation at 4 hpa-6 hpf and 15 hpa-17 hpf. Further studies are necessary to determine the global effect of anisomicyn on the MAPKs. Acknowledgment: The provision of ovaries by our local Slaughterhouse (Frigorífico Temuco) and funding support from FONDECYT 1181453 CONICYT-Chile are gratefully acknowledged.

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Formulation of a conjugated polymer-drug system for the *in vitro* antibacterial evaluation in the treatment of subclinical endometritis in cows

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The objective was to obtain a chitosan-enrofloxacin conjugate system for the treatment of subclinical endometritis (ES) in cows. Chitosan, a biopolymer known for its mucoadhesive, anti-inflammatory and antibacterial properties, which could induce a synergistic effect with enrofloxacin, a veterinary fluoroquinolone known for its good absorption and high bioavailability. The conjugate was achieved through a "crosslinking" reaction, the process of joining two or more molecules through a covalent bond, chitosan and enrofloxacin. The presence of the carboxylic acid in the enrofloxacin and the amino group in the chitosan facilitated the conjugate reaction. The activation of these molecules occurred through the use of the reagents EDC (N- (3-Dimethylaminopropyl) -N'-ethylcarbodiimide)) and NHS (N-hydroxysuccinimide), allowing the formation of an amide bond between the polymer and the drug. A controlled release system is expected after the break of the amide bond thanks to the proteases produced by the bacteria present in the aforementioned disease. To obtain the enrofloxacin-conjugated value, fluorescence quantification was performed by a multipurpose spectral scan plate reader Varioskan Flash (Thermo scientific), using the parameters described by Lihua *et al* 2018. The results shown that a conjugate with reproducible drug values was obtained, 23.1 µg ± 1.95 (n = 3) of enrofloxacin per mL of formulation. Size, polydispersity index (PDI) and zeta potential were measured using the Zetasizer nanoZS® (Malvern Instruments, UK). The size of the conjugated system "Chitosan-enrofloxacin" (459.4 nm), will allow us to use it as a component for a nanoparticle formulation, and thus be able to encapsulate the enrofloxacin bounded effectively. The value obtained from 0.8 of PDI shows us that there are different sizes of particle population. For this reason, it is recommended to work on decreasing this indicator to obtain a more stable conjugate. The zeta potential of the conjugate was positively charged, thus giving good mucoadhesive properties to the formulation by interacting with negatively charged cells present in the endometrial mucosa. Currently, the implementation of the bacterial susceptibility test *in vitro* to the polymer-drug conjugate system is being developed to three bacterial strains (*Trueperella pyogenes, Bacteroides fragilis* and *Fusobacterium necrophorum*) identified in ES, using the method developed by Patel J. *et al* 2015 described as microdilution in MH broth. The *in vitro* antibacterial study will verify if enrofloxacin can be released from chitosan after incubation with bacteria; and if the conjugation method using the carboxyl group of enrofloxacin will not alter its antibacterial activity.

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Morfofunctional and endocrine aspects related to cloprostenol-induced luteolysis in equine females

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The objective of this study was to evaluate variables related to morphological, functional and endocrinological alterations related to cloprostenol sodium induced luteolysis in equine females. Twenty-five females aged between 60 and 182 months, between days 7 and 10 of the estrous cycle were used. After selection, (0h) the animals' blood was collected using vacuum collection tubes without anticoagulant. On the same day, ultrasound evaluation of the ovaries was performed using B-mode and color Doppler technology (Mindray-M5™). They were recorded from each ovary that had the corpus luteum (CL), a sequence of 252 frames in B mode and 150 frames in Doppler mode. Immediately after the evaluations, 0.250mg of Cloprostenol sodium (Clocio™-Bimeda, Monte Mor-Brazil) was applied IM. Blood samples and the same ultrasonographic evaluations were done 12, 24, 36 and 48 hours after luteolytic application. Mode B images were used to measure the perimeter and area of the corpus luteum (CL). The color Doppler images were used to determine the vascularization score on a scale of 1 to 4 (according Siqueira et al., J. Dairy Sci., 96:6461-72, 2013). Serum obtained from the blood samples were used for the measurement of progesterone (P4) via Electrochemiluminescence (ECL) using Cobas E411 equipment and commercial Elecsys ™ kits Progesterone III (Roche - Germany). The B mode CL measurements and P4 concentrations were accessed by Anova and compared between the days using Tukey's test. Vascularization scores between the different days were compared by the Kruskal Wallis test. Significant probabilities less than 5% were considered. The intra-assay coefficient of P4 dosages was 1.67%. The mean P4 concentrations were 6.64 ± 5.18^{a}; 2.59 ± 1.97^{b}; 1.18 ± 0.99^{b}; $0.63+0.49^{c}$ e 0.34 ± 0.30^{c} ng/mL for times 0, 12, 24, 36 and 48 hours ($P<0.05$). The mean CL circumferences were 7.83 ± 1.49^{a}; 7.53 ± 1.32^{a}; 7.29 ± 1.53^{ab}; $7.07+1.51^{b}$ and 5.63 ± 1.63^{c} cm and area 3.99 ± 1.53^{a}; 3.85 ± 1.34^{a}; 3.72 ± 1.51^{ab}; 3.36 ± 1.35^{b} and 2.81 ± 0.66^{c} cm^2 for the times 0, 12, 24, 36 and 48 hours ($P<0.05$). The mean values of CL vascularization score were 3.88^{a}; 3.38^{ab}; 2.38^{b}; 2.19^{b} and 1.25^{c} for 0, 12, 24, 36 and 48 hours, respectively ($P<0.05$). The efficiency of luteolysis was 100%. The reduction of P4 concentration was observed at 12 hours, and reduction of vascularization at 24 hours that is, at the 1st and 2nd evaluation after the application of the product. On the other hand, the morphological regression of CL occurred only in the 3 evaluation, at 36 hours, 24 and 12 hours later than endocrinological functional and regression, respectively. It is concluded that the product used is efficient in causing luteolysis in equine females. Functional regression and reduction of P4 concentrations precede morphological changes in CL. Supported by: Bimeda, Biotran, Unifenas, Capes and CNPq.

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Lipid content in maturation media alters the lipid profile of oocytes and blastocysts

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Lipids are an important energy source for oocytes and embryos during in-vitro production, since they are stored as lipid droplets in cytoplasm and can be used according to the energy demand. However, high amounts of lipid droplets may increase apoptosis rates in blastomeres and impact embryonic survival after cryopreservation. Based on that, the aim of this study was to evaluate how the increase or reduction in lipid supplementation during in vitro maturation could affect bovine oocytes and blastocysts development. Bovine CCOs were in vitro matured in maturation media supplemented with 10% FBS (CO group), 10% of delipidated FBS (-lip group) or 10% FBS plus lipid extracted from FBS (+lip group). After maturation, oocytes were collected for analysis or fertilized and cultured until Day 7. Cleavage (Day 3) and blastocyst rates (Day 7) were assessed and the blastocysts were collected for analysis. Lipid profile for both oocytes and blastocysts were determined by the quantification of lipid droplets (Sudan Black B staining) and the characterization of lipid content by MRM-MS (multiple reaction monitoring-mass spectrometry). The results from embryo rates and lipid staining were analyzed by ANOVA followed by Tukey test (5 Prism GraphPad Inc.) and MRM-MS by principal components analysis (PCA). There was no difference in cleavage rates (CO:72±5%; -lip: 74±5%; +lip: 73±4%; P= 0.98), while there was a higher conversion of blastocysts in +lip group (CO: 30±3%; -lip: 30±3%; +lip: 42±3%; P=0.02). In addition, although the quantification of lipid droplets were similar for oocytes (CO: 11.0±1.7AU; -lip: 8.0±0.9AU; +lip: 9.3±1.2AU; P<0.30), blastocyst from CO group presented a higher amount of lipids than the other groups (CO: 7.6±0.8AU; -lip: 4.6±0.8AU; +lip: 4.9±0.5AU; P=0.0107). The PCA analysis of MRM data revealed that, for both oocytes and blastocysts, the lipid profiles were similar between +lip and -lip groups, which differed from CO. Among these lipids, +lip and -lip had an increase in triacylglycerols (TAG) and cholesterol (oocyte - TAG CO: 0.9±0.01AU; -lip: 1.9±0.1AU; +lip: 1.6±0.05AU; P<0.0001; Cholesterol CO: 3.8±0.1AU; -lip: 6.7±0.3AU; +lip: 6.8±0.4AU; P=0.0004/ blastocyst - TAG CO: 8.3±0.3AU; -lip: 22.4±0.8AU; +lip: 17.5±0.4AU; P<0.0001; Cholesterol CO: 4.0±0.1AU; -lip: 8.1±0.3AU; +lip: 7.3±0.3AU; P<0.0001) when compared to CO group. Also, blastocysts from -lip group had lower relative amounts of fatty acids than +lip group (CO: 11.7±1.5AU; -lip: 9.0±0.7AU; +lip: 15.2±0.9AU; P=0.0003). Different lipid supplementation during in vitro maturation impact the lipid composition of oocytes, leading to changes in blastocyst production and metabolism. Surprisingly, maturation in the presence of higher lipid levels seems to contribute positively to the blastocysts development rates. Acknowledgement: FAPESP (2016/23272-4).

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The increase of lipid levels in oocytes at germinal vesicle stage and its association with mitophagy in obese mice

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The subfertility of obese females and the increase of lipid content in their oocyte has been deeply studied. However, the possible relationship between oocyte lipid accumulation and removal of damaged mitochondria by mitophagy is unknown. This study aimed to evaluate the association between the increase in lipid accumulation with the reduction of mitophagy in obese mice oocytes. For that, six-week-old females were submitted to control or high fatty acid diet for 12 weeks, originating control and obese groups, respectively. Afterwards, mice were superstimulated with intraperitoneal administration of 5 U.I of eCG to collect immature oocytes at the germinal vesicle (GV) stage. Lipid storages were determined in 36 (control) and 35 (obese) oocytes retrieved from 7 females per group using fixed oocytes. The lipid content was estimated based on lipid area/oocyte area using Bodipy 493/503 and confocal microscopy. Moreover, NAD(P)H levels were assessed in 92 oocytes from 4 control mice and 71 oocytes from 3 obese mice by autofluorescence (using DAPI filter in epifluorescence microscopy). To evaluate mitophagy, 30 oocytes from 4 control females and 18 oocytes from 3 obese females were submitted to immunofluorescence to determine COX IV and LC3B. Additionally, 14 out of 30 (control group) and 13 out of 18 (obese group) oocytes were used to perform co-localization analyses of COX IV and LC3B. This was performed using images that were captured every 0.2 μm (z-stack axis). These images were subjected to 3D reconstruction and Mender´s coefficiency using JACoP plugin in FIJI software. The data were submitted to one-way ANOVA, considering a randomized block design using body weight as blocking variable. We detected an increase ($p<0.05$) in lipid content in oocytes from obese group (0.0169±0.0082) when compared to control oocytes (0.0104±0.0080). An increase ($p<0.05$) in NAD(P)H levels was also identified in oocytes from obese group (3.66 ±1.44) compared to control (3.28±1.73). Regarding mitophagy, we observed an increase ($p<0.05$) of LC3B intensity in obese group when compared to control (16.37±9.08 and 7.97±2.38, respectively), but no difference in COX IV levels (28.6±9.90 and 29.98±8.99, for control and obese, respectively). Likewise, it was observed a decrease ($p<0.05$) in COX IV-LC3B co-localization in oocytes from obese (0.307±0.107) in comparison to control group (0.463±0.148), suggesting a reduction of mitophagy associated with obesity. In conclusion, we were able to show the negative effects of obesity in lipid accumulation and in oxidative status in oocytes, and also a potential association of this condition with disrupted mitophagy. Supported by FAPESP (grants 2017/19825-0 and 2018/13155-6).

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Post implantation measurements of female PIVE embryos can show risk of pregnancy loss in cows

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The aim of this study was to investigate relationships between pregnancy losses and ultrasound size measurements of Girolando female bovine embryos. The study was performed at the Campo Experimental de Santa Mônica – Embrapa Gado de Leite, Valença (RJ), between January and April 2018 (CEUA/EGL – 3956180316). Girolando recipients (n=92) aged 3 to 6 years with body condition score 4 were treated with hormones for estrous synchronization and received fixed-time embryo transfer (FTET) at D7 post-ovulation. Girolando ¾ grade 1 blastocysts (according to International Embryo Technology Society - IETS standards) *in vitro* produced using sexed sorted semen were used. Positive pregnancy diagnosis was performed 24 days after ET (considered as the D31 of gestation) if the visualization of embryonic vesicle in a B-mode ultrasound examination was performed. A Mindray DP2200 with linear transducer at a 7.5 MHz frequency was used. Non-pregnant animals (n = 57) were excluded from subsequent analyzes. We compared the measurements of Embryonic Vesicle Diameter (EVD), Crown Rump Length (CRL) and Biparietal Diameter (BPD) of embryos that completed gestational development (Control group) (n=30) and of embryos whose gestation was lost up to 90 days (Pregnancy Loss group) (n=5). The 35 pregnant animals were followed up by ultrasonography every 6 days up to D90 or until the fetal heart beat ceased. Among the five gestational losses, only one occurred between D43 and D49. The other four occurred between 60 and 90 days of pregnancy. Measurements of EVD and CRL were performed at D37, D43, D49 and D55 of gestation, while those of BPD, at D43, D49 and D55. The results were analyzed by ANOVA repeated measurements. Significance level of 5% was adopted. The results showed higher EVD in the Control group (37.51 ± 3.56 mm) compared to the Pregnancy Loss group (32.92 ± 2.84 mm) at D55 and no difference between the groups was detected at D37 (14.81 ± 2.09 mm vs. 12.30 ± 1.65 mm); D43 (21.22 ± 2.32 mm vs. 21.48 ± 3.46 mm); and D49 (29.36 ± 2.35 mm vs. 27.79 ± 2.92 mm). The CRL measurements showed higher sizes in the Control group at D37 (16.17 ± 1.65 mm vs. 13.14 ± 1.73 mm) and D55 (46.13 ± 2.74 mm vs. 41.88 ± 5.47 mm). No difference was detected at D43 (23.11 ± 1.72 mm vs. 22.20 ± 2.46 mm) and at D49 (31.94 ± 1.78 mm vs. 30.97 ± 0.74 mm). The BPD measurements did not show any differences between the groups at evaluated moments. These findings indicate that decreased embryo/fetal growth during the first two months of pregnancy may suggest pregnancy loss. We suggest the measurement of fetuses and vesicles at D55 and routine assessment of pregnancies with EVD less than 34 mm and fetuses smaller than 43 mm in this period. Acknowledgements to Coordenação de Pessoal de Nível Superior – CAPES (Financial Code 001) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (CVZ APQ 00972/16).

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Influences of *in vitro* mimicking of estrus cycle phases on gene expression profiles of bovine oviduct epithelial cells obtained from pre-ovulatory or mid-luteal phase

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Estradiol (E2) and progesterone (P4) play key roles on morphological and functional changes of the bovine oviduct epithelial cells (BOEC) *in vivo*. However, few *in vitro* culture systems have been described to reproduce the functional changes on BOEC. Therefore, the aims of the present study were to mimic the estrus cycle phases on a 3D *in vitro* culture system of BOEC derived from pre-ovulatory or mid-luteal phases and verify the expression of genes encoding steroids receptors and proteins related to fertilization. For that, bovine oviducts were collected at a slaughterhouse (n=4 cows for each phase). BOECs from ampulla were collected separately from each animal and cultured in trans-well inserts (3D system) that allows the cell polarization. BOECs from pre-ovulatory or mid-luteal phases were culture for 14 days within four treatments, i) LUT: mimic of the luteal phase [P4 (100 ng/mL) and E2 (75 pg/mL) for 14 days]; ii) LUT_FOL: mimic of luteal phase followed by a follicular phase [luteal phase for 11 days, one transition day (low P4 and low E2), and E2 (300 pg/mL) and P4 (10 ng/mL) for two days]; iii) LUT_FOL_OverE2: mimic of luteal phase followed by a follicular phase with over high E2 levels [luteal phase for 11 days, one transition day, and E2 (600 pg/mL) and P4 (10 ng/mL) for two days], or iv) CONTROL: vehicle of E2/P4 dilution (0.5% ethanol). The relative mRNA abundance of genes related to fertilization (*OVGP1, HSPA5, FUCA1*, and *FUCA2*) and steroid receptors (*ESR1, ESR2*, and *PGR*) were detected by RT-qPCR. The results were normalized with the geometric mean of the two best reference genes (*18S* and *RLP15*). ANOVA followed by the Tukey-Kramer test was used to assess effects of treatments on BOEC culture using $P < 0.05$ as significance level. The effect of treatments was analyzed separately in BOEC from cows at pre-ovulatory or mid-luteal phases. In BOEC from cows at pre-ovulatory, a higher abundance of *OVGP1* was detected in LUT_FOL and LUT_FOL_OverE2 compared with CONTROL and LUT groups, whereas in BOEC from cows at mid-luteal phase, LUT group presented lower *OVGP1* levels compared with CONTROL. In BOEC collected at pre-ovulatory, the *ESR1* abundance was lower in LUT, LUT_FOL, and LUT_FOL_OverE2 compared with CONTROL, whereas in BOEC collected at mid-luteal phase there was no treatment effect on *ESR1* abundance. *FUCA1* and *FUCA2* showed higher abundance in LUT, LUT_FOL, and LUT_FOL_OverE2 compared with CONTROL group in BOECs from both pre-ovulatory and mid-luteal phases. No effect was observed on *ESR2, HSPA5,* and *PGR.* In conclusion, E2 and P4 modulate the gene expression of *OVGP1*, *FUCA1*, *FUCA2*, and *ESR1* during polarized culture system of BOEC. Moreover, this modulation is driven differentially by BOECs from pre-ovulatory and mid-luteal phases, thus it is an important bias to be considered during primary cell culture. To elucidate this, the global gene expression is ongoing in the same samples. Supported by FAPESP (#16/25685-4, 17/13481-8, 18/06674-7).

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Conceptus-derived products in circulation during early pregnancy in cattle receiving parthenogenetics vs normal embryos

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Establishment of pregnancy in cattle involves regulated interactions between maternal and paternal genetics to obtain proper conceptus development. In rodents, parthenogenetic embryos are known to have well-developed embryo proper but poor placenta proliferation, but limited information is available in cattle. We hypothesized that recipients receiving parthenotes will have decreased interferon-stimulated genes (ISG) expression and decreased circulating pregnancy-associated glycoprotein (PAG) concentration. This experiment aimed to determine differences in pregnancy establishment and conceptus-derived products in parthenogenetic embryos (PA) compared with biparental embryos (CON). Parthenote embryos were produced in vitro using a validated chemical activation method and control biparental embryos produced in vitro using industry standard techniques. Cows (n=30) were synchronized and embryos transferred 7 days after estrus onset (day 0). Experiment was divided in three replicates of embryo transfer, with cows on the PA group receiving 2-3 embryos per round, and CON cows receiving 1 embryo. Blood samples were collected on days 7, 15 and daily from days 21 to 40 for peripheral blood leukocytes and serum. Transrectal ultrasonography was performed daily to monitor conceptus development. Trizol (Invitrogen, Carlsbad, USA) was used to extract RNA from buffy coats, cDNA was synthesized and RT-PCR performed to determine relative expression of ISG15, MX2, OAS1 and PPIA (house-keeping). An in-house ELISA was used to measure serum PAG concentration. Dependent variables (ISG expression levels and PAG) were analyzed using PROC GLM (SAS 9.4) to test difference among groups. As expected, the PA group had decreased pregnancy rate at day 30 (13%, 2/15) compared to CON (33%, 3/9) and pregnancy was maintained up to day 40 of gestation in both groups. Fold change on day 22 over the baseline was decreased in PA for both OAS1 (7.0 vs 2.4, P=0.02) and ISG15 (13.2 vs 4.2, P=0.07) compared to CON embryos, but no difference was observed for MX2. Circulating PAGs increased from days 24 forward in both groups, but the PA group had reduced concentrations (0.81±0.44 vs 5.23±0.44; P=0.01) at day 32 of gestation. These results indicate that parthenogenetic embryos can establish pregnancy in cattle; however, there are significant decreases in conceptus-derived products in circulation. Overall, these findings suggest that this is a suitable model to investigate parental versus maternal contributions to placental development in cattle. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from USDA NIFA.

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The modulation of the cholesterol biosynthesis pathway impacts metabolism, viability and cryotolerance of *in vitro* produced embryos

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Phospholipids and cholesterol are the main constituents of membranes and their distribution pattern and abundance may affect the membrane fluidity, permeability and thermal phase behavior, characteristics that are fundamental in cryopreservation. In this study, we modulate the cholesterol biosynthesis pathway (CBP) throughout the *in vitro* embryo development and characterize its impact on embryo viability. Oocytes were in vitro matured, fertilized and, at the timing of *in vitro* culture (SOFaa supplemented with 8% FBS at low oxygen tension) zygotes were divided in 3 groups: C - control, I – inhibition of CBP by simvastatin (20 μM) and S – stimulation of CBP by IGF-1 (100ng/mL). Blastocysts were collected at day 7 and submitted to lipid quantification [Sudan Black B (n=10-20 per group)], nuclear fragmentation and total cell number [TUNEL (n=50 per group)], apoptosis [Caspase-3 (n=50 per group)], cryotolerance [vitrification (n=100 per group)] and lipid profile [NMR (n=3 per group]. Lipid content of the culture media was also evaluated by NMR. Cleavage and blastocyst rates, cytoplasmic lipid droplet content, TUNEL and Caspase were analyzed by ANOVA and Tukey post test. NMR data were submitted to partial least square discriminant analysis (PLS-DA) by using MetaboAnalyst2.0 and survival, development and hatching rates post warming were compared by Chi square Test. Inhibition of CBP had deleterious effects on embryo development and quality since cleavage and blastocyst rates were lower in I group and percentage of nuclear fragmentation and total lipid content were increased when compared to C and S ($P<0.05$). On the other hand, embryos derived from S group presented less apoptotic cells, evidenced by lower nuclear fragmentation and caspase-3 activity when compared to C and I. Modulation of CBP had influence on the overall lipid metabolism, since all lipid classes presented higher relative abundance in blastocysts from C group, except for ômega-3 that were increased in S and I groups and cholesterol that decreased on I group, as expected ($P<0.05$). The lipidome analysis of the spent culture media revealed that the relative abundance of fatty acids and cholesterol were lower and omega-3 and triglycerides were higher on S group ($P<0.05$) when compared to C and I. These changes in cellular viability as well as lipid metabolism led to a diminished survival rate after cryotopreservation for I group ($P<0.05$). Data from pregnancy rates after embryo transfer are still being collected. These data allow us to conclude that the CBP is crucial for the maintenance of different cellular functions. Inhibition of this pathway results in diminished embryo quality and survival after cryopreservation, while the stimuli of CBP improve cellular functions, however without impact cryopreservation (FAPESP: 2018/01965-3, 2016/05986-0, 2017/18384-0).

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Small extracellular vesicles from follicular fluid modulate EIF4E in cumulus cells during bovine oocyte *in vitro* maturation

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Small extracellular vesicles (EVs) are particles secreted by cells that carry bioactive molecules. Small EVs are found in follicular fluid and can be related to oocyte maturation. The objective of this study was to quantify RNA in zona pellucida (ZP, Exp.1) and to evaluate transcripts related to RNA transport pathway in cumulus cells (Exp.2) after small EVs supplementation during bovine oocyte *in vitro* maturation (IVM). For Exp.1 we collected slaughterhouse ovaries and aspirated small follicles (3-6 mm) to obtain follicular fluid and cumulus-oocyte complex (COCs). Follicular fluid was centrifuged twice (120,000 xg, 70 min) to pellet small EVs, which were diluted in maturation medium. COCs were matured in 100 µL drops of maturation medium with or without small EVs. After 0 (immature), 2 and 4 h of IVM, 10 COCs per group were labeled with SYTO RNA Select (ThermoFisher; 0.05nM), a selective dye to probe RNA molecules, during 30 min at 37°C and denuded oocytes were fixed in PFA 4% during 15 min. A total of 21 slices for each oocyte were imaged, with an interval of 1 µm each, using a confocal microscope (Zeiss LSM 700) at 40x/1.2 objective. RNA molecules localized in ZP were counted using ImageJ. For Exp.2 slaughterhouse ovaries were collected in pairs and classified in early or late estrus cycle stage according to corpus luteum morphology. COCs were *in vitro* maturated with small EVs from follicular fluid of different estrus cycle stage (n=20 COCs/group) or without EVs (control). After 24 h of IVM cumulus cells were collected and RNA extraction (miRNeasy Mini Kit; QIAGEN), reverse transcription (High Capacity; ThermoFisher) and quantitative RT-PCR (Power SYBR Green; Applied Biosystems) were performed. We analyzed eight transcripts related to RNA transport pathway, which were normalized by geometric mean of two reference genes (*PPIA* and *YWAHZ).* Six replicates were realized for each experiment. Statistical analysis was performed using SAS by ANOVA following Tukey's test at a significance level of 5%. Results from Exp.1 showed that COCs matured with small EVs increased RNA mean at 2 and 4 h of IVM compared to immature COCs ($p<0.05$). Also, COCs matured without small EVs increased RNA mean at 4 h of IVM compared to immature COCs ($p<0.05$). Results from Exp.2 demonstrated that small EVs from late estrous cycle increased *EIF4E* in cumulus cells, a translation initiator factor, comparing to control group. In conclusion, results showed that RNA molecules increase in the ZP during IVM overtime independent of EVs supplementation. However, small EVs from late estrous cycle increase *EIF4E* levels in cumulus cells, suggesting a role for EVs modulating protein translation. Based on our findings, small EVs did not increase the quantity of RNA in ZP but could modulate RNA transcripts in cumulus cells. Further experiments are necessary to determine the role of this mechanism in oocyte quality (Funding: FAPESP grant 2014/22887-0; 2017/02037-0; 2018/14869-2).

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Morphometry of reproductive system in Dorper Sheep submitted to 3 feeding plans in pre-puberty

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Nutritional status is the main factor that influences the animal's ability to reproduce. The onset of puberty and the maintenance of reproductive function are physiologically linked to nutrition and body condition. Therefore, the aim of the present study was to evaluate the influence of three feeding planes on the morphometry of reproductive system in pre pubertal Dorper sheep. 24 lambs (7/8 Dorper), aged between 6 and 7 months, were randomly assigned to 1 of 3 groups: G1 (70-80% of the requirement of the National Research Council [NRC]), G2 (100-110% [NRC]) and G3 (140% [NRC]). The ewes of G1 (n=8) and G2 (n=8) were maintained on pasture of Panicum maximum cv. Tanzania with access to water and mineral salt ad libitum, and only those in the G2 group received 1.5% of the live weight of commercial 2x daily feed. G3 ewes (n = 8) were confined during the experimental period, receiving a total diet in the following proportions: concentrate of 20:80, 16% CP and 72% NDT, aiming at daily gain of 200g/day according to NRC, being the mineral salt ad libitum. Initially the sheep received 3.5% of the live weight of the total diet (hay + ration), and this percentage increased until reaching an average of 4.5 to 5% of the live weight. Upon reaching body weight of 35 kg, sheep were synchronized by insertion of a vaginal progesterone delivery device (CIDR®, Pfizer, Brazil) for 12 days. On the day of implant withdrawal, 0.075 mg of cloprostenol (Veteglan®, HertapeCalier, Brazil) and 300 IU of equine chorionic gonadotrophin (eCG, Novormon®, MSD Saúde Animal, Brazil) were administered intramuscularly. Eight days after CIDR removal, all animals were slaughtered and the reproductive tract removed for weighing and morphometry. The number of antral follicles was measured. The data were analyzed by ANOVA using the MIXED procedure (SAS, version 9.4). The number of cervix rings were analyzed using non-parametric Kruskal-Wallis test. The number of cervical rings was greater ($p < 0.05$) in G1 (7.66 ± 0.21) compared to G2 (6.57 ± 0.29) and G3 (5.8 ± 0.20). G2 had a greater number of rings than G3. The width of the uterine horn was greater in G1 (2.63 ± 0.15 cm) than in G3 (1.86 ± 0.16 cm) independent of the side of the horn (ipsilateral or contralateral). The length vs width of the ovary of G1 (1.38 ± 0.05 cm) was greater than G3 (1.17 ± 0.04 cm). The corpus luteum weight did not differ between G1 (0.46 ± 0.1 g), G2 (0.42 ± 0.09 g) and G3 (0.6 ± 0.10 g). The number of antral follicles (12.35 ± 1.91 / 15.12 ± 1.78 / 13.22 ± 1.71) did not differ between groups. Based on the results obtained we can conclude that the food restriction does not negatively affect the size and weight of the reproductive organs of Dorper ewe lambs.

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Effect of protein source during *in vitro* maturation on the development and sex of *in vitro* produced bovine embryos

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Several studies have shown that the use of different protein sources (PS) such as FBS and BSA during the IVC of bovine embryos affect quality and embryonic development (Nedambale et al., Theriogenology, 62:437-449, 2004). The most affected parameters are, blastocyst rates, total cells number, percentage of apoptotic cells, metabolism, cryotolerance and gene expression. Considering that most of the studies focus on the effect of PS during IVC, the present study aimed to evaluate whether changes in PS during IVM would affect blastocyst rate and sex of bovine embryos. In order to do this, two experiments were carried out. In the first one, COCs obtained from slaughterhouse ovaries were distributed in 2 groups: IVM-FBS (COCs matured in the presence of 10% FBS); and, IVM-BSA group (COCs matured with 0,4% BSA), after this, both groups were fertilized and cultured in the presence of FBS up to day 8 of development. The second experiment was similar to the first one (IVM-FBS and IVM-BSA groups), differing only in fertilization and culture until D8 that were performed in the presence of BSA instead. In both experiments the embryo rate, the development kinetics and the sex of the embryos were evaluated. Sexing was performed in 30 blastocysts / group by PCR. Data of the blastocyst rate and embryo sex were analyzed using chi-square test ($p < 0.05$). The results showed that when IVC was performed in the presence of FBS, the PS during IVM affected ($p < 0.05$) the blastocyst rate in D8 (IVM-FBS = 64.8% n = 105; IVM-BSA = 50.5% n = 105). When embryos were cultured in the presence of BSA, no effect ($p > 0.05$) of PS during IVM was observed at the blastocyst rate at D8 (IVM-FBS = 41.1% n = 350, IVM-BSA = 37.3 % n = 346). The PS used during IVM did not affect the sex of the embryos ($p > 0.05$), regardless of whether the culture medium was supplemented with FBS, IVM FBS (Male 53.33%, Female 46.66% n = 30) and IVM BSA (Male 56.66%, Female 43.33% n = 30) or with BSA, IVM FBS (Male 46.66%, Female 53.33% n = 30) or IVM BSA (Male 56.66%, Female 43.33% n = 30). It can be concluded that the PS during IVM does not influence the sex of the embryos and only affects the embryonic development if the culture is carried out in the presence of FBS.