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Injectable long-acting P4 supplementation stimulates IFNt-signaling, but not pregnancy success after transfer of multiple embryos in beef cattle

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In cattle, the P4 supplementation at early diestrus stimulates conceptus growth increasing IFNt-signaling, however also anticipates luteolysis occurrence. These paradoxically effects are associated with inconsistency on pregnancy rates. Using a multiple embryo transfer model, we aimed measure the luteolytic and embryotropic effects triggered by the P4 supplementation in beef cattle. This model allows to eliminate pregnancy failures related to the unique embryo transfer (Therio.73,250-260,2010). The hypothesis were (1) the embryo presence inhibits the early luteolysis induced by P4 supplementation and (2) P4 supplementation stimulates embryos retention. Experiment was conducted in two replicates; estrous cycles of non-lactating multiparous cows were synchronized by an estradiol/P4-based protocol followed by estrus detection twice a day (day 0 = estrus; D0). Cows detected in estrus (n=70) were split to receive an IM administration of 150 mg of long-acting P4 (iP4, Sincrogest, Ourofino Saúde Animal) or vehicle (no-iP4) on D4 and transcervical transference of none or 5 embryos produced in vitro on D7 on a 2x2 factorial arrangement. The CL development and function was evaluated daily by B-mode and Color Doppler ultrasonography from D3 to 21. Criteria for determining the day of luteolysis was when the CL area was <2.0 cm2 and colored blood flow signals covered ≤ 25% of total CL area. Pregnancy was confirmed by ultrasonography on D30. Abundance of transcripts for the interferon-stimulated gene ISG15 was measured by qPCR in peripheral blood mononuclear cells (PBMCs) isolated on D14, 16, 18 and 20. Transfer of multiple embryos (5 embryos: 16.60 ± 0.47 days vs. None embryo: 17.91 ± 0.26 days; $P < 0.05$), but not iP4 ($P > 0.10$), reduced luteal lifespan of animals. Regarding the embryotropic effect of iP4, there was no difference in the proportion of cows presenting functional CL on D20 between cows underwent to embryo transfer treated with iP4 (72.2% [13/18]) or not (77.8% [14/18]). Similarly, there was no difference on conception rate between iP4 treated cows (44.4% [8/18]) compared with no-iP4 treated cows (55.6% [10/18]). Despite of similar conception rate, the pregnant cows treated with iP4 presented greater ($P=0.05$) abundance of ISG15 mRNA on D18 (2.69-folds) and D20 (2.05-folds), than no-iP4 treated cows. In conclusion, our results demonstrated that luteolysis occurrence was not anticipated by iP4 supplementation, but it was by multiple embryo transfer. The iP4 supplementation at early diestrus stimulates the abundance of ISG15 mRNA in cows that underwent multiple embryo transfer, although not increase the embryo retention.
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Spatial differences in energy substrates across the bovine uterus

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The bovine endometrium is a dynamic tissue that undergoes spatio-temporal functional changes directed by the ovarian hormones, estradiol and progesterone. The arrangement of vessels that irrigate the uterus allows a greater input of ovarian steroids to the cranial portion of the uterine horn ipsilateral to the CL. In cattle, the morula-stage embryo enters in the apical uterine horn on days 4–6 post-mating and then develops into a blastocyst. Development of the preimplantation embryo/conceptus depends on uterine secretions to supply nutrients and growth factors. The uterine luminal fluid (ULF) is composed of molecules synthesized and secreted by endometrial cells as well as selectively transported from blood. Transport of molecules to the uterine lumen is a spatially programmed process, but knowledge of the biochemical composition of the ULF along the uterine horns is lacking. The aim was measure the abundance of energy substrates in spatially defined regions of the uterine environment. Estrous cycles of multiparous, non-lactating Nelore cows (n=7) were synchronized and uterine horns ipsi- and contralateral to the CL were isolated and divided in anterior, middle and posterior thirds, starting from the uterotubaric junction 7 days after estrus. Each uterine third was washed individually with D-PBS to obtain regional ULFs. Subsequently, intercaruncular endometrial samples were collected from each uterine third in the mesometrial side. Glucose and pyruvate were quantified in the ULF using fluorometric assays and concentrations were adjusted to units of endometrial area. Relative abundance of transcripts for Glucose transporters (SLC2A1 and SLC2A4) and Pyruvate transporter (SLC16A7) were measured on endometrial samples by qPCR. Protein quantification of SLC2A1 on endometrium was performed by Western Blot. Metabolite concentration on ULF and relative abundance of transcripts and protein was analyzed by split-plot ANOVA (SAS 9.3) and included the effects of horn (ipsi vs. contra) and third (anterior vs. middle vs. posterior) and their interaction. Concentration of pyruvate was similar across uterine horns and thirds. In contrast, concentration of glucose was 48% greater ($P<0.05$) in the anterior ULF of the ipsi- compared to the contralateral horn and was similar between the remaining thirds of both horns. Abundance of transcripts for SLC2A1, SLC2A4 and SLC16A7 was not affected by horn or third, neither the protein abundance of SLC2A1. The regional pattern of the glucose composition across the bovine uterus was not consistent with transcripts and protein abundances of main transporters in the endometrium, indicating a complex-regulation of the energy substrates-transport to the uterine lumen. In conclusion, glucose, but not pyruvate ULF abundance in the bovine uterine environment is associated with physical proximity to the CL-containing ovary.
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Presence of a pre-hatching embryo influences the metabolite composition of the uterine environment in beef cattle

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The oviductal and uterine environments play critical roles in the success of early embryonic development. *In vitro*, the bovine pre-implantation embryo secretes bioactive molecules (embryotropins) from early developmental stages. We reported that the endometrial transcriptome is altered by the presence of an embryo 7 days after estrus. However, potential effects of embryotropins in the uterine environment *in vivo* are unknown. We hypothesize that exposure to an embryo changes the biochemical composition of the uterine fluid in the cranial region of the pregnant uterine horn. The present study aimed to assess a spatially defined region of the uterine environment for responses to a day 7 embryo *in vivo*. Uterine luminal fluid (ULF) and endometrium were collected from the cranial region of the uterine horn ipsilateral to the CL 7 days after estrus from sham-inseminated (Con; n=8) or artificially inseminated and confirmed pregnant (Preg; n=10) cows. We performed absolute quantification of 205 metabolites in the ULF, including amino acids and biogenic amines, acylcarnitines, phophatidylcolines, lysophophatidylcolines, sphingolipids, hexoses and eicosanoids using electrospray ionization and tandem mass spectrometry. Metabolite concentrations were normalized by the endometrial area. Relative abundance of endometrial transcripts was determined by Real-Time PCR. Statistical analysis was carried out by one-way ANOVA with FDR correction for multiple comparisons. Of the 205 metabolites quantified, 166 were detected in 50% or more samples. Twenty two (13%; $P<0.05$) metabolites showed different concentration between Con and Preg ULF samples. Concentration of two metabolites (12-S-HETE and 15-S-HETE), associated with the Lipoxygenases pathway, were significantly greater (2.5 and 2.8-folds, respectively) in the Preg group. While the remaining 20 metabolites were less abundant in the Preg ULF, including Glycine (0.7-fold) and Sarcosine (0.6-fold). A Quantitative Enrichment Analysis revealed that Arachidonic Acid Metabolism and Glycine, Serine and Threonine Metabolism pathways were positively enriched and decreased in the Preg group, respectively. Relative abundances of transcripts for Lipoxygenases (*ALOX5*, *ALOX5AP*, *ALOX15B* and *ALOX12*) were evaluated by qPCR in endometrial samples and were similar between groups. However, a downregulation of *SLC6A9* (a Glycine transporter; 0.76-fold; $P=0.01$) transcripts was found in the Preg endometrial tissue, suggesting an endometrial origin of regulation that was consistent with the lower Glycine concentration in the ULF. Although the endometrial or embryonic side of modulatory biochemical processes can only be speculated, it is clear that regulation is complex. We conclude that uterine environment changes in response to embryo presence and believe that differences in ULF metabolite composition are important for both uterine receptivity and embryo development. Acknowledgments: FAPESP, CNPq e CAPES.

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Changes in the profile of PGFM following an oxytocin challenge during the first 60 days of pregnancy in dairy cows

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It has been shown that pregnant (P) cows on d18 have a higher concentration of prostaglandin F2α (PGF) and its metabolite 13,14-dihydro-15-keto-PGF2α (PGFM) in the uterine lumen than non-pregnant (NP) after challenge with oxytocin (Parkinson et al., J. Reprod. Fert. 90, 337-345, 1990). However, little is known about PGF release throughout other stages of pregnancy. The aim of this study was to evaluate and characterize the profile of PGFM, before and during a challenge with oxytocin, throughout the first 2 months of pregnancy in lactating Holstein cows (n = 121) inseminated by FTAI (GnRH–7d–PGF–1d–PGF–3h–GnRH–16h–AI). On d11 (n = 23), 18 (n = 23), and 25 (n = 12) after AI, and on d 32 (n = 13), 39 (n = 13), 46 (n = 12), 53 (n = 13), and 60 (n = 12) of pregnancy, cows were challenged with 50 IU of oxytocin, i.m. Blood was collected before (0 min), 30, 60, 90, and 120 min after challenge for plasma concentration of PGFM (pg/mL) by ELISA. Ultrasound evaluations were performed for pregnancy diagnosis on d32, 39, and 60 post-AI. In addition, cows on d11 and 18 after AI had whole blood collected for concentrations of interferon-stimulated genes (ISGs) using RT-PCR, as a marker of an elongating embryo. Samples from cows on d11 were used to establish a confidence interval, to identify a cut off value of maximum expression of ISGs for NP cows. The analysis of PGFM used data only from NP cows on d18, based on ISGs, or cows diagnosed pregnant on d32, using ultrasound. Data were analyzed by PROC MIXED of SAS 9.2 comparing P vs. NP on d11 and 18 in one analysis and comparing the effect of day of pregnancy from d11 to 60 in a second analysis. On d11, there was no difference between P and NP with low PGFM before and after oxytocin challenge and no effect of oxytocin on PGFM. On d18, NP tended to have greater basal PGFM than P (16.3 vs. 9.5; $P = 0.08$) and had 3-fold greater PGFM after oxytocin (72.9 vs. 24.4; $P < 0.05$). Comparing only P cows from d11 to 60, the basal PGFM concentrations increased ($P < 0.0001$) as pregnancy progressed with d11 and 18 of P, lower than d25 and later days of pregnancy. After oxytocin, PGFM increased throughout gestation, and there was an interaction between gestation day and time after challenge ($P < 0.001$). Pregnant cows on d18 had little increase in PGFM following oxytocin but it tended ($P = 0.06$) to be greater compared to the negligible PGFM after oxytocin in P cows on d11. The oxytocin-induced PGFM in P cows on d25 (48.5 ± 9.1) was greater than P cows on d18 (22.0 ± 3.3; $P = 0.006$), especially 60 min after challenge ($P = 0.01$) when values were 2.8-fold higher on d25 However, there was no difference between d25 and 32 (68.9 ± 12.2). The oxytocin-induced PGFM had maximum values 60 min after challenge in P cows for d25 to 60 (d25 = 48.3 ± 8.6; d32 = 72.1 ± 12.0; d39 = 89.4 ± 18.7; d46 = 93.3 ± 14.5; d53 = 136.5 ± 19.3 and d60 = 106.1 ± 29.4). Basal and oxytocin-induced PGFM was greater on d53 (26.9 ± 4.6; 122.4 ± 18.4) and d60 (28.0 ± 4.2; 106.4 ± 19.5) of P than on d25 (22.6 ± 3.7; 48.5 ± 9.1), with intermediate values on d32 (30.5 ± 4.8; 68.9 ± 12.1) and d39 (25.8 ± 3.2, 80.7 ± 16.5). Thus, consistent with previous reports, the CL of early pregnancy is protected due to suppression of PGF secretion probably due to actions of interferon-tau. However, during the second month of pregnancy, PGF secretion is not suppressed since basal PGFM and oxytocin-induced PGFM secretion are greatly elevated (equal or greater than in d18 NP cows) indicating alternative mechanisms for protection of the CL during the second month of pregnancy.
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Sex steroids drive the remodeling of oviductal extracellular matrix and regulate embryo receptivity in cattle

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The extracellular matrix (ECM) is a group of molecules that offers structural and biochemical support to cells and interacts with them to regulate their functioning. Also, growth factors (GF) can be stored in the ECM and locally released during the ECM remodeling process. Here, we hypothesize that the abundance of ECM components and remodelers is different in the oviduct of cows treated to ovulate larger or smaller follicles. It was employing an animal model that, using synchronization protocols, produces two groups: cows that ovulate small follicles (SF-SCL; n= 20) or large follicles (LF-LCL; n=21) and therefore have differences in their luteal development and E2 and P4 plasmatic concentrations. In preliminary studios, these animals had significant differences in their fertility. Ampulla and isthmus samples were collected after slaughter in day four (Day 0 = ovulation induction by GnRH) and immediately frozen or fixed in 4% buffered formalin. After RNA extraction, transcriptome (n = 3 / group) were evaluated by RNA sequencing. Human MMP Antibody Array (Abcam: ab134004) was used to detect and quantify several ECM remodelers proteins in parallel. While the paraffin embedded samples were used to localize and quantify the immunostaining for type I Collagen. Digital analysis was performed using Image J and the immunostaining signal was analyzed separately for each structural layer (i.e., tunica mucosa, t. muscularis, and t. serosa). Data analyses were performed using the GLIMMIX procedure of SAS. The model included the fixed effects of group, region, and their interaction. The transcriptome analysis revealed clusters with overrepresented ontology terms and activation of pathways associated with ECM organization, and remodeling in the LF-LCL group, especially in the isthmus region. Molecules up-regulated in LF-LCL cows could be further classified as ECM components (Collagens), ECM remodelers (ADAMs and MMPs), and ECM-related growth factors. Protein-intensities for MMP3, MMP8, MMP9, MMP13, and TIMP4 were 65.84, 43.50, 44.30, 76.08, and 65.23% greater for the LF-LCL compared to the SF-SCL group, respectively ($P < 0.05$). Additionally, the protein expression of MMP1 and TIMP2 tended to differ between groups, and was greater in the LF-LCL group (71.48 and 63.39% greater intensity in comparison to SF-SCL; $P < 0.10$). When type I collagen content was quantified in the t. mucosa, even though no region effect was observed, it was detected a stronger signal in SF-SCL in comparison to LF-LCL samples on both regions (LF-LCL ampulla: $14.48 \pm 5.21\%$ and isthmus: $17.05 \pm 2.52\%$; SF-SCL ampulla: $27.77 \pm 1.70\%$ and isthmus: $29.55 \pm 3.58\%$; $P < 0.01$). We concluded that ECM remodeling process takes place in the oviduct of high fertility cows (LF-LCL) when the embryo is being transported from the oviductal lumen into the uterine environment. This remodeling process is more intense and probably occurs earlier in these cows, when compared with low fertility cows (SF-SCL).

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Pregnancy-associated plasma protein-A (PAPP-A) added during in *vitro* maturation: effects on meiosis progression, oocyte DNA fragmentation and gene expression in bovine cumulus-oocyte complexes

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The insulin-like growth factor (IGF) is a stimulatory factor for oocyte maturation and embryonic development *in vivo* or *in vitro*. The bioavailability of free IGF is restricted by IGF-binding proteins (IGFBPs) and increased by pregnancy-associated plasma protein-A (PAPP-A), which cleaves the bond between IGF and IGFBPs. Thus, we added PAPP-A to the *in vitro* maturation (IVM) of *cumulus*-oocyte complexes (COCs) and evaluated effects on oocyte meiosis progression, DNA fragmentation and transcriptional profile of cumulus cells and oocytes. For this, COCs from a local abattoir were submitted to IVM for 24h with TCM199 medium supplemented with PAPP-A (P1:1ng/mL, P10: 10ng/mL e P100: 100ng/mL) or not (Control). After IVM, oocytes were submitted to evaluation of DNA fragmentation (TUNEL assay) and meiosis progression (stained with Hoechst 33342; n=5 replicates with approximately 88 oocyte/group). Further, after the same IVM treatments groups, oocytes (n=4 replicates with 20 oocytes/group) were separated from their respective cumulus cells (n=6 replicates with *cumulus* cells from 20 COCs/group) and each cell type was analyzed for the transcriptional profile of 96 genes (3 reference genes and 93 target genes) by RT-qPCR using Taqman® assays in the HD-Biomark System®. The mRNA abundance of target genes was normalized with the geometric mean of reference genes (ACTB, GAPDH and PPIA), and the effects of PAPP-A supplementation on meiosis progression (%), apoptosis rate (%) and mRNA abundance (fold-change) were tested by ANOVA, using JMP software (SAS Institute Cary, NC). Means were compared with Tukey-kramer or Wilcoxon tests and differences were considered significant when $P \leq 0.05$. The addition of PAPP-A did not alter the percentage of oocytes in metaphase II (Control: 57.8±12.7, P1: 49.9±10.4, P10: 45.1±12.9, P100: 54.8±10.3) or oocyte DNA fragmentation (Control: 42.2±2.7, P1: 50.1±10.4, P10: 55.0±12.9, P100: 45.2±10.3). Regarding the mRNA abundance of transcripts in cumulus cells, FOXO3 was lower in P100 group (Control: 0.005±0.0002; P1: 0.004±0.0001; P10: 0.004±0.0004; P100: 0.003±0.0006). In oocyte, the mRNA abundance of DNMT1 was lower in P100 group when compared with P10 group (P10: 1.035±0.065 and P100: 0.841±0.026); H1FOO was higher in P100 group when compared with P1 group (P1: 2.211±0.078 and P100: 3.031±0.229), TXNRD1 was higher in P100 group when compared with control group (Control: 0.099±0.005 and P100: 0.140±0.006) and CPT1B was higher in P100 group when compared with P1 and P10 groups (P1: 0.004±0.0004; P10: 0.005±0.001 and P100: 0.009±0.002). In conclusion, the addition of PAPP-A, particularly at 100ng/mL, could modify the oocyte competence and maybe results in further developmental changes in blastocysts due to the down-regulation of FOXO3 (involved on premature primordial follicle activation) and DNMT1 (related in methylation pattern) and up regulation of TXNRD1, H1FOO, CPT1B (involved in oxidative stress processes, control of gene expression during oogenesis and fatty acid beta-oxidation) in matured bovine COCs.

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Pregnancy-associated plasma protein-A (PAPP-A) increases free IGF-1 on *in vitro* maturation medium and modulates important genes related to embryonic development in bovine *in vitro*-produced blastocysts

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The insulin-like growth factor (IGF) acts as an important modulator for oocyte maturation and early embryonic development and its bioavailability is modulated by action of pregnancy-associated serum protein A, which breaks down the binding of IGF-1 to IGF binding proteins (IGFBPs). So, the aim was to verify the effect of PAPP-A on the modulation of free IGF-1 during bovine oocyte in vitro maturation (IVM) and its impacts on embryo yield and transcriptional profile of blastocysts. For this, ovaries were obtained from a local abattoir and COCs (5 replicates; 20 COCs/replicate per group) were in vitro matured in humidified atmosphere with 5% CO2 in 90 ul drops, into serum free medium on the absence (control group) or presence of PAPP-A (100 ng/mL; P100 group). After 24 h IVM, the medium was collected to measure levels of free IGF-1 by in house ELISA and the matured oocytes were submitted to IVF (5% CO2) with semen from Nelore bull and thereafter, presumptive zygotes were cultured in controlled atmosphere of 5% CO2, 5% O2 and 95% N2 until blastocyst stage. Medium was exchanged (45 ul – 50%) on the third and fifth day of culture. On day 3 and 7, the cleavage and blastocyst rates were verified, respectively. On day 7, blastocysts were collected to analyze the embryonic gene expression (n=4 pools for control group and n=5 pools for P100 group; 3 blastocysts/pool). The transcriptional patterns of 91 genes were analyzed by RT-qPCR using Taqman® assays in the HD-Biomark System®. The in vitro performance (cleavage and blastocyst rates) was calculated as percentage and transformed to arcsine. The mRNA abundance of target genes was normalized by geometric mean of four reference genes (ACTB, GAPDH, PPIA and SDHA) and data were transformed to fold change. The levels of free IGF-1 was calculated and transformed to fold change. The statistical analysis was performed with t-test, using JMP software (SAS Institute Cary, NC) and differences were considered significant when $P \leq 0.05$. In summary, the addition of PAPP-A (100 ng/mL) increases 1.27-fold change the levels of free IGF-1 ($P=0.03$) on IVM medium. There was no difference in embryo yield. The rates of cleavage and blastocysts were, respectively, 83% and 12% for control group and 79% and 17% for P100 group. The mRNA abundance of genes related to cellular stress (ATF4, GPX4 and H1F1A) and lipid metabolism (FASN and SREBF1) was lower in embryos of P100 group. On the other hand, genes related to cellular proliferation/differentiation (MAPK1) and pluripotency (POU5F1) were up-regulated in embryos of P100 group. In conclusion, the addition of PAPP-A during oocyte IVM increases the bioavailability of free IGF-1 and modulates the expression of important, genes related to cellular stress ,lipid metabolism, embryo development and pluripotency genes in in vitro-produced bovine embryos.
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Follicular viability and tissue damages are influenced by time and temperature of ovarian transportation

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Despite the great prospect of preantral follicles, their application in studies or in assisted reproductive techniques comes up against the fact that these ovaries are generally at some distance from the manipulation centers. Thus, the effects of ovarian transportation should be evaluated in order to minimize the injuries to the follicular population. Thus, the aim of this study was to establish the best time interval and the ideal temperature of the holding medium to transport the bovine fetuses' ovaries from the slaughterhouse to the laboratory. The preantral follicles morphology and tissue viability by confocal microscopy were the evaluated parameters. For this, the work was divided into two experiments. In experiment 1, ovaries of bovine fetuses were collected in test tubes and stored in thermal bottles with temperatures of 4°C, 22°C, and 33°C to test in which temperature range would maintain the follicular morphology and tissue viability. Experiment 2 was carried out, evaluating the parameters by the time the ovaries were manipulated after their collection., and each group was manipulated with intervals of 4-, 10- and 16 hrs. The control group was immediately fragmented and fixed at the slaughterhouse (H0). Experiment 1 demonstrated that the highest proportion ($P < 0.05$) of viable preantral follicles was observed at 4°C. Furthermore, the group of 33 °C showed the highest fluorescence intensity relative to tissue degeneration emitted by propidium iodide. The proportion of normal primordial follicles was lower ($P < 0.05$) in all groups after in vitro culture compared to control and D0. Experiment 2 demonstrated that the follicles morphology and tissue viability were better during the 4 hs-interval compared to the 10 and 16-hour intervals. The group transported for 4 hrs presented the lower fluorescence emission relative to apoptotic cell index ($P < 0.05$) compared to the control (H0) and to the other treatments. In conclusion, the follicular morphology and tissue viability were influenced by time and temperature of ovarian transportation. Also, the results showed that the best temperature for the transport of bovine fetuses ovaries is 4 °C within the time interval of 4 hours.

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Effect of increased circulating insulin with propylene glycol on ovarian dynamics in Holstein cows

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Based on the assumption that cows with high feed intake have lower circulating steroid concentrations due to increased catabolism by the liver, it is necessary to identify new strategies to alleviate these problems as alternatives to hormonal supplementation. Several studies have suggested that increasing circulating insulin would stimulate steroidogenesis in ruminants. Therefore, we tested the hypothesis that augmented circulating insulin during pre and post follicle deviation periods would increase follicle and corpus luteum (CL) development and function. Estrous cycles of 16 non-lactating and non-pregnant Holstein cows were synchronized using an intravaginal progesterone (P4) device of 1.9 g (CIDR; Zoetis, SP, Brazil) and i.m. treatment with 100 µg gonadorelin diacetate (GnRH ; Cystorelin, Merial, Canada) on day -12 of the protocol. On day -5, 25 mg were administered i.m. of dinoprost tromethamine (PFG2α; Lutalyse, Zoetis). On day -3, the P4 device was removed and cows received 25 mg of PGF2α i.m. On days 10 and 11 ± 1 of the estrous cycle all follicles > 5 mm were aspirated to synchronize the emergence of a new follicle wave. The second day of aspiration was considered D1 of the experiment. On D1, treatments were initiated and cows were divided into two groups: water (control; C) or propylene glycol (P) provided orally in four daily doses of 300 mL every 6 h for 3 consecutive days (D1 to D3, pre follicular deviation period), and another 3 consecutive days (D5 to D7; after follicular deviation period). The experimental design was a Latin square in a 2x2 factorial arrangement. Thus, four groups of 16 cows each were formed: 1) CC = water pre and post follicle deviation; 2) CP = water pre and propylene glycol post follicle deviation, respectively; 3) PC = propylene glycol pre and water post follicle deviation, respectively; and 4) PP = propylene pre and post follicle deviation. Blood samples were taken 0 (immediately before), 15, 30, 60 and 120 min after propylene glycol for circulating insulin and glucose, and daily for P4 measurement. Ovarian ultrasound examinations were performed daily for evaluation of follicular and luteal dynamics until ovulation, as well as during the following 9 d of CL development post ovulation. Statistical analysis was performed by the MIXED procedures of SAS. Plasma insulin concentrations (µIU/mL) were greater for groups receiving P compared to controls (0, 15, 30, 60 and 120 min: 17.5 ± 1.4, 26.3 ± 1.4, 31.2 ± 1.4, 21.8 ± 1.4, 16.9 ± 1.5 vs. 12.1 ± 1.5, 11.6 ± 1.5, 11.2 ± 1.5, 10.8 ± 1.5, 11.1 ± 1.5; P ≤ 0.05). Glucose concentrations were also greater in the groups receiving P. Despite increased circulating insulin and glucose due to treatments with propylene glycol, there was no difference (P > 0.05) among groups for rate of pre deviation follicle growth (1.5 ± 0.14, 1.3 ± 0.15, 1.5 ± 0.14, 1.4 ± 0.15 mm/d), or post deviation follicle growth (1.2 ± 0.13, 1.4 ± 0.14, 1.4 ± 0.13, 1.4 ± 0.13 mm/d), ovulatory follicle diameter (15.5 ± 0.56, 16.1 ± 0.55, 15.6 ± 0.52, 15.6 ± 0.54 mm), CL volume, or circulating concentrations of P4 for the groups CC, CP, PC and PP, respectively. Thus, increasing circulating insulin using treatment with propylene glycol four times per day, either before or after the expected time of deviation, did not alter growth rate of the dominant follicle, luteal volume, or circulating P4.
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17-beta estradiol action on the synthesis of endometrial PGF2alpha in cows

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17β-E2 stimulates expression of the endometrial ER and OXTR receptors. The activation of OXTR induces the PGF2α cascade synthesis. The hypothesis was that the signaling enzymes and PGF2α synthesis are regulated by 17β-E2. The objective of this study was to determine the effects of 17β-E2 on expression of key transcripts and proteins in PGF2α synthesis. Cyclic and non-lactating Nelore cows were synchronized by application of 3mg BE (Ourofino, Cravinhos, Brazil) and insertion of P4 device (1g; Ourofino, Cravinhos, Brazil). After 8 days, the P4 device was removed and PGF2α (0,5mg; Ourofino, Cravinhos, Brazil) was applied, followed by 4 days of estrus observation (D0). On D15 cows were randomly assigned into three groups: Control (C, untreated), Placebo (P; 6mL of ethanol 50%, IV) and Estradiol (E; 3mg 17β-E2 plus 6mL of ethanol 50%, IV).Uterine biopsies were collected at the times 0h (C; N=10), 4h (E4h; N=11 and P4h; N=10) or 7h (E7h; N=10 and P7h; N=11) for PCR, WB e ICQ assay. Plasma concentration of PGFM concentrations were measured at D15. Group E presented decrease in CL area (cm2) and blood flow (%) and P4 concentration ($P<0.05$), compared to group P. Continuous dependent variables were analyzed by factorial 2 X 2 (PROC MIXED; SAS 9.3 program). PCR and WB were analyzed separately at times 4 and 7 hours. The Kruskal-Wallis test was performed for IHC. Compared to group P, functional and structural luteolysis of group E was anticipated 2 and 3 days, respectively. Group E presented greater concentration of PGFM at 4h, 6h, and 7h (38%, 119% and 150%; $P<0.05$), compared to the group P. At 4 hours, the abundance of the genes ESR1(fold change; FC=0.3), ESR2(FC=0.1), PRKCα(FC=0.5), PRKCβ(FC=0.3), PLA2G4(FC=0.2), AKR1B1(FC=0.2), and AKR1C4(FC.=0.3) was lower in the E4h, while OXTR was greater in the same samples compared to the P4h (FC=4.7; $P<0.05$). The gene expression of PTGS2 was not different between groups E4h and P4h ($P>0.05$). At 7 hours, E7h also showed lower abundance of ESR1(FC=0.8), PRKCα (FC=0.5), PRKCβ (FC=0.5), AKR1B1 (FC=0,2), and AKR1C4 (FC=0,2; $P<0.05$) and there was a tendency for lower ESR2 expression, compared to P7h (FC=0.3; P=0.08). However, there was no difference in the abundance of OXTR, PLA2G4, and PTGS2 between E7h and P7h ($P>0.05$). The abundance of the enzyme PKCα was decreased in both, E4h and E7h, relative to the samples P4h and P7h, respectively. E4h showed greater PGR immunostaining in the glandular epithelium (GE; $P<0.05$) and there was a tendency for greater PKCγ immunostaining in the luminal epithelium, compared to the P4h (P=0.08) e for reduced ERα immunostaining in the GE of the E4h compared to the E7h (P=0.1). 17β-E2 stimulates the plasmatic concentration PGFM and trancription of OXTR, but inhibits the transcription of molecules of the PGF2α-synthesis cascade. The increased concentrations of PGFM have led to speculate that the enzymes of the PGF2α synthesis cascade were activated by 17β-E2.

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Cyclic GMP modulators (NPPC and Sildenafil) effects during *in vitro* maturation on cytoplasmic lipid content and transcripts abundance in bovine cumulus-oocyte complexes and embryos

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Recent studies have indicated the influence of C-type natriuretic peptide (NPPC) on meiosis resumption in mammalian oocytes. NPPC is synthetized by granulosa cells and binds to the natriuretic peptide receptor 2 (NPR2) present mostly in cumulus cells. NPR2 activation induces the synthesis of cyclic guanosine monophosphate (cGMP), which is transferred via gap junctions from cumulus cells to the oocyte, where the cGMP inhibitory action over PDE3A maintains high concentration of cAMP in the oocyte, sustaining meiosis arrest. The concentration of cGMP is controlled by the balance between its synthesis and degradation, which is carried out by the PDEs themselves. Sildenafil is specific inhibitor of PDE5 (inhibiting cGMP hydrolysis), utilized during IVM to delay spontaneous meiosis resumption. The signaling pathway activated by cGMP/PKG may trigger a lipolytic action through phosphorylation of perilipin and lipases. The objective of this study was to evaluate the effects of natriuretic peptide type C (NPPC) and/or sildenafil during in vitro maturation (IVM) on lipid content (LC) by Nile Red staining and gene expression in cumulus-oocyte complexes (COCs) and embryos by Fluidigm Biomark™ HD system and on embryo production. In experiment I, cumulus-oocyte complexes (COCs) were cultured in maturation medium with a NPPC (100 nM) associated or not with a Sildenafil (10 μM PDE5 inhibitor) for 24h and after this period, the samples were collected to lipid content (oocytes) and gene expression (COCs). In experiment II, COCs were cultured in the same conditions as experiment I and the COCs were submitted to IVF and IVC. The developmental rates, embryo LC and gene expression were evaluated on D7. Statistical analyses were performed using the JMP Software, the effects of the treatments on lipid content, gene expression and embryonic development were analyzed by one-way ANOVA followed by Tukey parametric post hoc testing or non-parametric Kruskal-Wallis test. Differences with probabilities of $P < 0.05$ were considered significant. NPPC during IVM decreased the LC in oocytes compared with control group (16.10 and 20.60 FI, respectively, $P < 0.05$), but genes involved in lipid metabolism and glucose transport were not affected in COCs ($P > 0.05$). NPPC increased one gene involved in cumulus expansion (PTX3) compared with control group ($P < 0.05$). Embryo development rates and their LCs were not affected in d7 ($P > 0.05$). Analysis of embryos transcripts showed that when COCs were matured with NPPC, transcript abundance was not different from the control group ($P > 0.05$). Only FOXO3 was increased relative to sildenafil or NPPC+sildenafil ($P > 0.05$). Sildenafil treatment during IVM increased HSF1 and PAF1 ($P < 0.05$) and decreased REST transcripts abundance relative to controls ($P < 0.05$). When both NPPC and sildenafil were used during IVM, only REST was lower than control ($P < 0.05$). PAF1 and HSPA1A were reduced relative to sildenafil alone and FOXO3 relative to NPPC alone ($P < 0.05$). This study reports, for the first time, the effect of NPPC during IVM on cytoplasmatic LC in bovine oocytes and effects on genes involved in cumulus cell expansion. However, the reduce LC in oocytes and the an increase in the expression of cumulus expansion related genes had not affected embryo production, still, these embryos recover the LC after IVC. We acknowledge São Paulo Research Foundation (FAPESP) for funding (Grant 2012/50533-2, 2013/05083-1 and 2015/20379-0).

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Molecular and endocrine factors involved in future dominant follicle dynamics during the induction of luteolysis in *Bos indicus* cows

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The growth profiles of the future dominant (DF) and subordinate (FS) follicles and the gene expression of the granulosa cells during luteolysis induction in Bos indicus cows were evaluated. Forty non-suckled multiparous Nelore (*Bos indicus*) cows scoring 3.0±0.2 body contition score (1-thin to 5-obese) and between 5 and 7 years-old were synchronized with a progesterone and estradiol based protocol. After synchronization, cows with a CL were evaluated by ultrasonography every 12 hours, beginning at eight days post ovulation. Cows identified with a follicle of at least 6.0 mm in diameter in the second wave were split into two groups (BD– before deviation and AD- after deviation. In the BD group cows received 500µg of cloprostenol when the DF reached a mean diameter of 7.0mm (6.5 to 7.5mm). In the AD group, cows received 500µg of cloprostenol when the DF follicle reached a mean diameter of 8.0 mm (7.5 to 8.5 mm). Cows in both groups were submitted to the aspiration of the DF at 96 and 72 hours after was given cloprostenol. Follicular aspirations were performed to quantify IGF1r, LHr and PAPPA transcripts in the granulosa cells. Statistical analyses were performed using the Statistical Analysis System for Windows (Statistical Analysis Software 9.3, SAS Institute Inc., Cary, NC, USA). The GLIMMIX procedure was used to determine significant differences between groups for the variables related to follicular dynamics. Relative gene expression analysis was performed by the REST software Relative gene expression using the Pair-Wise Fixed Reallocation Randomization Test. The relative expression values are expressed as the mean ± SEM. Transcripts abundance were compared between BD and AD groups. The diameter of the DF at the moment of prostaglandin administration (BD=6.76 ±0.13 mm and AD=8.17±0.13 mm; P=0.001) and growth rate of the SF (BD=-0.04±0.09 mm/day and AD=0.07±0.14 mm/day; P=0.05) were greater in the AD group. There was greater abundance of LHr transcripts in BD cows (P=0.04). The remaining variables tested were similar between the experimental groups (P>0.05). In conclusion, the induction of luteolysis before follicular divergence does not interfere with dominant follicle dynamics. However, it causes granulosa cell LHr down regulation.
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Endometrial cytological and microbiological evaluation of postpartum dairy cows from properties in the Caparaó, ES

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Dairy farms have experienced a contrast between increasing milk production and reducing the fertility of high-yielding cows. Among the factors involved in the impairment of fertility, uterine conditions, such as different types of endometrites, stand out. Thus, the aim of this study was to diagnose and classify the occurrence of uterine infections through endometrial cytological and microbiological examinations of dairy cows. Fifty-five cows (20 primiparous and 35 multiparous) lactating cows belonging to breeders from the Caparaó, ES region were used. Subclinical endometritis was diagnosed by endometrial cytology at 30 days postpartum when more than 3% of neutrophils were identified from 200 cell. However, animals in estrogenic phase, 18% neutrophils was considered (Salasel et al., Theriogenology, 74: 271-1278, 2010). Clinical endometritis was identified by the presence of cloudy, catarrhal or purulent uterine secretions. The culture, isolation and identification of microorganisms from the endometrial microbiological examination were performed according to a technique described by Koneman et al. (Microbiological Diagnosis, 5th ed. 2001, p.720-785). Results were submitted to descriptive statistics. In the microbiological examination, 52.7% (29/55) of the samples presented positive growth in plates containing blood agar media in aerobiosis. Anaerobic growth was observed in 47.2% (26/55), and in 27.2% (15/55) of the samples there was no microbiological growth. All positive samples were evaluated by catalase test, obtaining 100% of catalase positive samples. Thirteen (23.6%) animals were diagnosed with clinical endometritis (9 multiparous and 4 primiparous) by observation of mucopurulent, purulent, catarrhal or sanguineo-purulent secretion. According to endometrial cytology, 49 animals (89.1%) had no uterine infection, while 6 animals (10.9%) presented subclinical uterine infection. It was observed that only 46.1% of clinical uterine infection, diagnosed by secretion observations, also demonstrated a diagnosis of uterine infection in the cytology, suggesting that it may have be originated from cervicitis or vaginitis. Gram staining showed predominance of positive cocci (09/29) in aerobiosis and anaerobiosis (07/26). Despite several treatments described for endometritis, the early diagnosis of this pathology is essential for its success. Thus, vaginoscopy, cytology and endometrium microbiological examinations are important alternatives for diagnosing these conditions.

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In vitro evaluation of progesterone production of luteal cells on pregnant domestic cats

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Feline species represents an important experimental model for wild felids with physiological similarities (Pope, C.E., Theriogenology, 163-174, 2000). Several factors are involved in steroidogenesis process of luteal cells and in domestic cats this knowledge is unrecognized. Our objective was performed standardization of luteal cell culture in vitro of domestical cats and measurement of P4 production in culture medium during pregnancy. Nine pairs of ovaries were obtained through OSH tecnic performed in three gestational phases: initial, intermediate and final. The ovaries were isolated and maintained in phosphate-saline buffer solution (DMPBS-FLUSH, Saint Louis, USA) containing 1% antibiotic and antimycotic solution (10,000 IU penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml; Sigma Aldrich, Saint Louis, USA) and amikacin (4µL/40mL). After ovary scarification, then were submitted to collagenase type 1 action (1mg / ml; C0130, Sigma Aldrich, Saint Louis, USA) plus DMEN high glucose (cod.41965-039, Sigma Aldrich, Saint Louis, USA) to obtain the luteal cells. The resulting contents were filtered (70um) and centrifuged serially (3 times) for 10 minutes at 20oC. The final pellet was resuspended and cell viability and concentration determined (10x10 5 cells/ ml for plating, 500 ul / well). The culture medium was collected in 2 stages: M1 (36 hours after beginning of cell culture) and M2 (60 hours after after beginning of cell culture) and stored in cryotubes (1mL) at - 80 ° C. The P4 assays were accomplished at PROVET laboratory by radioimmunoassay (RIE). The results were analyzed by ANOVA, two-way and average were compared in pairs by Student-Newman-Keuls method, with significance when $p \leq 0.05$. In initial phase of pregnancy there was a higher production of P4 at M1 when compared to M2 ($p = 0.043$); however, in intermediate and final phases there was no difference between analyzed moments ($p = 0.71$ and 0.27, respectively) . At M1, the lowest production of P4 occurred in intermediate phase of gestation, (0.74 ± 0.25 ng/m), and at M2 there was no difference between gestational phases. According to results, P4 secretion in culture medium was higher in initial gestation after 36 hours of cellular culture (M1), which was expected, since P4 exercises an important role on endometrium, modulating the regulation of important genes for uterine receptivity and concept growth (Ayad, A. et al., Theriogenology, 1503-1511, 2007). In intermediate and final stages of pregnancy, P4 concentrations differs from those observed in serum profile, suggesting a possible interference of placental progesterone, a fact emphasized by Siemieniuch et al. (Reprod Biol Endocrinol., 89, 2012). According to results obtained a standardized method of cellular culture for feline species was established although P4 mensurements in culture medium during pregnancy did not follow seric pattern known.

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Evaluation of ultrasound parameters in the diagnosis of the functionality of corpus luteum after ovulation induction in prepubertal Nellore heifers

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Due to the common occurrence of premature regression (PR) of the corpus luteum (CL) in heifers after first ovulation (OV), the aim of the present study was to determine differences in luteal dynamics in relation to the functionality of these ovarian structures. Fifty-seven prepubertal Nellore heifers (BW 289.61±32.28 kg, BCS 5.66±0.65 and 17.47±0.81 months old) were divided into two treatment groups for OV induction: GP4+GnRH and GGnRH. In the first, an intravaginal progesterone (P4) device (CIDR®, Zoetis, São Paulo, Brazil) of 3rd use was used for 10 days, and 48 hours after its removal 0.02 mg of buserelin acetate (GnRH hormone analogue) (Sincroforte®, Ouro Fino, São Paulo, Brazil) was applied intramuscularly, and in the second group only GnRH was used. Formed CLs were monitored by B-mode and color Doppler ultrasonography every two days until their functional regression (determined by the decrease in Doppler vascular signal and serum P4 concentrations below 1 ng/mL). For each day of evaluation was determined the area and percentage (%) of vascularization of CL and serum concentration of P4. The CLs with luteal phase greater than 16 days were classified as normal duration (ND), and those with duration less than 16 days as prematurely regressed. The characteristics were compared between treatments, CLs functions, evaluation days and their interactions, using the MIXED procedure of SAS program ($p \leq 0.05$). Six animals (6/57 = 10.5%) did not respond to treatment, due to the absence of CL within 96 hours after GnRH administration. Based on the results found for the serum P4 concentration, another category of CLs was established in the study, the nonfunctional (NF), which were those that presented at all days of evaluation values less than 1 ng/mL. In GP4+GnRH was observed 80.7% (21/26) of CL with ND and 19.2% (5/26) with PR; and in GGnRH, 4.0% (1/25) of the CLs were of ND, 44.0% (11/25) RP and 52.0% (13/25) NF. The area and % of vascularization of CL presented interaction between the treatments and the CLs functions ($P<0.0001$ and $p=0.05$ respectively). The NF CLs presented the lowest area (31.9 ± 11.4^{c}mm2) in comparison to the other categories (GP4+GnRH: ND - 112.1 ± 8.2^{a} and PR - 78.4 ± 13.8^{b}; GGnRH: ND - 154.4 ± 22.9^{a} and PR - 58.3 ± 10.7^{b}), and the ND of the highest % of vascularization (GP4+GnRH: ND - 20.8 ± 1.2^{a} and PR - 15.7 ± 2.1^{b}; GGnRH: ND - 18.1 ± 3.3^{ab}, PR - $13{,}6\pm2.3^{b}$ and NF - 10.9 ± 2.6^{b}). The concentration of P4 presented interaction with the treatments, CLs functions and evaluation days ($p=0.03$), with higher values for ND CLs in both treatments, intermediate values for prematurely regressed and values always lower than 1 ng/mL for NF. In conclusion, formed CL after OV induction of prepubertal heifers presented differences in the functionality, being able to be differentiated by ultrasound parameters area and % of vascularization of CL and serum concentration of P4. Acknowledgment: FAPESP (2015/13079-0), Ouro Fino and Centro APTA Bovinos de Corte (Instituto de Zootecnia/Sertãozinho – SP).

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Changes in the expression of epigenetics related genes during the embryonic genome activation in cattle

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Genome activation is an important event during early embryonic development. The transition from maternal to embryonic genome is gradual and in bovines its peak is evident in embryos at the 8-6 cells stage. During this period the depletion of maternal transcripts by degradation or translation and the replacement by new transcripts of embryonic origin occurs. Epigenetic control is an important component of transcriptional regulation and can be modulated by non-coding RNAs, mainly involved with post-transcriptional mechanisms such as gene silencing and modulation, as well as epigenetic changes related to histone modification and DNA methylation. Considering that epigenetic changes are essential to control gene transcription, the aim of this study was to evaluate genes related to these alterations before and after the major genome activation in bovine embryos. For this, IVF embryos were produced and collected with 4 and 8-16 cells for the analysis of genes related to epigenetic alterations. Samples were pooled into 5 polls of 10 embryos each. Total RNA was extracted by combining the QIAzol reagent (Qiagem) with the miRNeasy kit. Reverse transcription was performed with the high-capacity cDNA reverse transcription kit (Applied Biosystems) and the analysis of gene expression through the Biomark HD System (Fluidigm). Relative levels were analyzed by Student's T-Test. Six differentially expressed genes related to epigenetic changes were identified. Four genes (*DNMT1*, *DNMT3A*, *DNMT3B* and *H1FOO*) are increased in 4-cell embryos and two genes (*H2AFZ* and *HDAC2*) in embryos of 8-16 cells stage. The 4-cell embryos showed higher levels of three DNA methyltransferases (DNMTs), enzymes involved in the maintenance of gene methylation patterns, and could be related to maintenance of the inactive embryonic genome. For this same reason, higher expression of *H1FOO* in 4-cell embryos, a key histone H1-encoding gene, was expected based on chromatin remodeling occurring during early embryonic development. The genes *H2AFZ* and *HDAC2*, higher in the group of embryos of 8 to 16 cells, are related to histone coding. The *HDAC2* is associated with embryonic development and cell differentiation and the *H2AFZ* controls gene expression through regulation and chromatin silencing. As we know, embryonic genome activation is a critical period of early embryonic development, and in vitro culture may alter the pattern of gene expression. Thus, is important to understand the pattern of expression of epigenetic modifiers during critical embryonic developmental periods in order to improve the production of in vitro bovine embryos.

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Cumulus cell gene expression as a biomarker of blastocyst´s development in dairy cows involuntarily culled

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Intensive genetic selection and management in Holstein breed has resulted in modern dairy cow with very high milk yields. Thus, a high metabolic activity to maintain the level of production, which increases the metabolism of steroids, affecting reproductive physiology and reduce oocyte competence, even more in dairy cows involuntarily culled. Consequently, selecting oocyte with the highest development potential is of great importance in assisted reproductive technology. The aim of our study was to identify the cumulus cell (CC) gene expression associated with oocyte the high development potential to blastocyst in involuntarily culled dairy cows (referred as dairy cow). COCs from crossbred cattle were used as control. The donors were identified at the moment of ovary collection and grouped in dairy cows (Holstein–Friesian breed) or crossbred cattle (mostly Angus). CC biopsy from cumulus oocyte complex (COCs) was performed in 184 COCs from dairy and 188 COCs from crossbred cattle, divided in 7 replicates per group. Eight genes were analyzed in CCs biopsies (LUM, KRT18, KRT8, AGPAT9, CLIC3, BMPR1B, GATM and SLC38A3. In vitro maturation (IVM) and fertilization (IVF) as well embryo culture (IVC) was performed individually. IVM was performed in TCM199 for 22 h; oocytes were fertilized using frozen–thawed semen during 18 h and both at 39˚C under 5% CO_2 atmosphere. IVC was performed using SOFaa culture medium at 39˚C under 5% CO_2, 5% O_2 and 90% N_2 during 7 days. At day 7, embryos were classified; after scoring the developmental fate of embryos derived from the COCs that were biopsied, two groups were created: G1 (n=10); CCs from COCs that developed to blastocysts and G2 (n=10); CCs from COCs that did not develop to blastocysts stage. Also, the expression level of 12 genes (OCT4, SOX2, NANOG, CDX2, GATA6, TP1, BCL2L1, BAX, CASP3, LPIN1, LPIN2 and ELOVL5) were evaluated in individual blastocysts derived from dairy cows COCs (n=10) and controls (n=10). Statistical analysis were conducted using chi square test for embryo development and Wilcoxon non-parametric test for gene expression analysis using the software InfoStat (Buenos Aires, Argentina). Less than 10 % (7.4 ± 2.1%; mean±SEM) of COCs derived from dairy cows were able to develop to blastocyst stage (In control: 20.7 ± 2.7%;). Also, 68.4 % of blastocysts (day 7) from crossbred COCs were at expanded or hatching stage while only 20 % of blastocysts from dairy cows were at these stages (p=0.004). Two genes, LPIN1 and CASP3, were differentially expressed ($P<0.05$); LPIN1 was downregulated while CASP3 was upregulated in blastocysts derived from dairy cows COCs. The gene expression analysis of cumulus cells showed that more competent oocytes (G1) had a higher expression of six genes: LUM, KRT18, KRT8, CLIC3, BMPR1B and SLC38A3 ($P<0.05$). A positive correlation of gene expression in cumulus cell with embryonic developmental ability ($r=0.7$, $P<0.05$), can be used as biomarkers of blastocyst´s development in involuntarily culled dairy cows.

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The effect of using estradiol cipionate on antecipated luteolysis control in *Bos indicus* (Nelore) cows suplemented with a long acting injectable progesterone at the beginning of diestrus

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In cattle, lower estradiol concentration in the preovulatory period have been associated with premature luteolysis. The objective of this study was to evaluate the effect of estradiol cypionate (ECP) on proestrus associated with a treatment of a long acting injectable P4 (iP4) at beginning of the diestrus on the expression of genes that control the release of PGF2α in the endometrium [progesterone receptor PGR], Oxytocin receptor (OXTR), estradiol receptor 1 (ESR1)] in Nelore cows. The ovulation of 80 cyclic cows was synchronized with a conventional E2 plus P4 protocol, which started on day -10 (D-10). After the P4 device removal (Sincrogest®, OuroFino Saúde Animal) in the D-2, animals received 0 or 1 mg of ECP (SincroCP®, OuroFino Saúde Animal). After 48 h following P4 device removal, all animals received 1 µg of buserelin acetate [(GnRH), Sincroforte®, OuroFino Saúde Animal). Therefore, the groups were defined as: GnRH + ECP (n = 12), GnRH + P4 (GnRH + ECP), GnRH + ECP (n = 12) and GnRH + P4 (N = 13) and GnRH + ECP + P4 (n = 13). Endometrial tissue samples were collected transcervically on D1, 3, 5, 7, 13 and 16 after ovulation using cytobrush. Color Doppler ultrasonographic evaluations were performed from day D3 to 25 to evaluate the development and regression of CL. Data analysis was performed with PROC GLIMMIX (SAS, 9.4), considering statistical difference P ≤ 0,10. Structural luteolysis occurred earlier (16.9 ± 0.3 vs. 18.4 ± 0.4; P = 0.10) in animals receiving iP4 compared to groups not receiving iP4. The GnRH + ECP + P4 group presented a greater (P <0.05) rate (53.9%) of early luteolysis (≤ D16) compared to GnRH group (0%). Regardless of use of ECP, animals that received iP4 and presented early luteolysis demonstrated: lower CL area at D6, 7, 15 and 16, lower blood flow at CL at D14, 15 and 16 after ovulation (P = 0.01), greater P4 concentration in D4, 5 and 6 and lower concentration in D14, 15 and 16 (P <0.0001) compared to those not having early luteolysis. In general, there was a greater abundance of transcripts for PGR in D1, which decreased over time (P <0.0001). Cows receiving ECP presented lower ESR1 expression over time compared to animals that did not receive ECP (P = 0.03). Cows that received iP4 and presented early luteolysis there was greater OXTR expression in D16 compared to animals that did not have early luteolysis (P <0.0001). In conclusion, the early luteolysis induced by iP4 is associated with an increased transcript for the endometrial OXTR gene. It is speculated that this molecular event is associated with early release of PGF2α pulses that will lead to CL regression. Furthermore, the increase in P4 concentration in the initial diestrus followng iP4 use may have damaged the development of the CL, which may have influenced the early luteolysis of these animals. However, estradiol (through of the ECP use) was not able to reverse this luteolysis process. Furthermore, iP4 treatment had no effect on the modulation of the PGR and ESR1 receptors in the initial diestrus, so the modulation of these receptors at this time had no influence on early luteolysis response. Acknowledgments: FAPESP, CNPq, CAPES, Ourofino Animal Health.

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Eight-cell embryo developmental rates after been expose to gaseous sublethal stress

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High gaseous pressure (HGP) has been reported as a sublethal stressor able to induce embryo stress response, providing cell protection to subsequent stress, such as cryopreservation, although the involved cellular mechanisms are little understood. This principle was investigated in this report aiming to evaluate the effect of 16 MPa high gaseous pressure (HGP) during 2 (P1 group) and 4 hours (P2 group) on the development and viability of 8-cells stage murine embryos. From 60 superovulated 6 weeks old Mus musculus domesticus females, it was recovered 1303 8-cells stage embryos that were aleatory segregated in control (C group) and experimental groups (P1 and P2). HGP was applied on embryos of experimental groups while control embryos were immediately after recovery cultured in vitro in mKSOM media + 0.4% BSA during HGP treatments at 37ºC under atmosphere of 5% CO_2, 5% O_2, 90% N_2 with satured humidity. After, embryos from experimental groups were also cultured at the same in vitro conditions. Blastocysts rates were observed after 48h in vitro culture and compared by Chi-square test ($P < 0.05$). No differences were observed in blastocysts rates between control and experimental groups: (C) 94.2% (419/445); (P1) 95.4% (395/414), (P2) 94.1% (418/444). We concluded that murine 8-cells embryos could be exposed to16 MPa HGP during 2 and 4 h without loss of in vitro embryo viability to reach blastocyst stage. This approach will be studied as subletlhal stressor in order to induce embryo cell response aiming to improve survival rates in a subsequent stress such as cryopreservation.

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Study of the relationship of transcription and metilation profiles of genes related to lipid metabolism, cell differentiation and cell stress/death in bovine blastocysts with different kinetics of development

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Blastocysts with distinct kinetics in early cleavages present differences in global methylation DNA, which potentially lead to the activation/suppression of genes from various biological pathways, such as lipid metabolism, cell death/stress and cell differentiation (Ispada, et al. Anim. Reprod.13(3): 708, 2016). In this work, genes previously identified as differentially methylated were investigated aiming to stablish the relation between these two parameters and its impact on essential pathways for embryo viability. For this, bovine embryos were in vitro produced by conventional protocols, using sexed semen (female). After 40 hours of insemination the embryos were classified as Fast-cleavage (FBL - 4 or more cells) or Slow-cleavage (SBL - 2 or 3 cells), remaining in culture until the blastocyst stage (12 blastocysts per group obtained in 4 replicates). These embryos were analyzed through BioMark™HD for the characterization of gene transcripts related with lipid metabolism (9), stress (10) and cell differentiation (4). For statistical analysis, PPIA gene was used as endogenous control for ΔCt calculation, later submitted to Student's t-test. All results will be presented in SBL in relation to FBL. Upregulation of ACSL3, ELOVL6, PPARA and FADS was observed in the transcripts of genes related to lipid metabolism and previously identified as hypomethylated genes, whereas PPARG and PTGS2 showed no statistical difference. SCD and FASN genes, although hypermethylated, showed upregulation or non-difference, respectively. ACSL6, which did not show difference in DNA methylation, showed upregulation. Gene transcripts related with cell survival/death that were hypomethylated, BAX, PA2G4, HSPA1A and NOS2 were dowregulated and BID was upregulated, whereas NFE2L2 had no statistical difference. Furthermore, the CASP9 transcripts were observed as downregulated and TXNRD1 and FOXO3 showed no difference, although these genes were hypermethylated, and DDIT3 was upregulated, although it did not had DNA methylation difference. Regarding the control of cell differentiation, it was observed that of the genes POU5F1 and SALL4 (hypomethylated) only the POU5F1 presented statistical difference, being dowregulated. However, the NANOG gene that showed no DNA methylation difference was upregulated. Herewith, we can conclude that differentially methylated pathways identified previously between blastocysts with distinct development kinetics during the first cleavages are also altered in transcripts and could lead to metabolic differences with impact in embryo viability. In addition, the lack of correlation between DNA methylation status and some transcripts may be a result of other epigenetic mechanisms influence.
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Genome wide association test with scrotal hernias in pigs

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Hernia is an abnormal protrusion of an organ or tissue through a defect or natural opening in the covering skin or muscle. In the swine industry, it is considered the most common congenital defect involved with high morbidity and mortality in the herds. The scrotal hernias are found in high frequencies ranging from 1.7 to 6.7%, and they have been linked to several boar lines and breeds, with moderated to high heritability (0.2- 0.86). However, even avoiding the use of those boar lines, hernias have not being completely eliminated from herds. Therefore, new approaches are needed to elucidate the genetic mechanisms involved with this condition to better select animals. Therefore, the objective of this study was to conduct a genome-wide association test (GWAS) in a cross-bred swine population (Landrace, Large-White and Pietrain) for the identification of genetic markers associated with the appearance of scrotal hernias. Animals were all from the same commercial swine herd located in the Northwest region of the RS. Piglets had similar age and were kept with the sow until 28 days of age and then weaned; castration was conducted during the first week of age. After weaning, piglets were moved to a group-housing with 100 piglets per pen with mixed sex. The phenotype classification was based on visual appearance of scrotal hernias. Each affected pig was matched to a healthy control from the same pen. In the total, 68 animals were genotyped using the Porcine SNP60 Beadchip, out of those, 41 animals had the presence of hernias and 27 were healthy animals. Markers and animals were submitted to a quality control process to remove individuals with difference in their genetic background and SNPs with a Minor Allele Frequency< 1% or if they failed in more than 10% of the samples. After after quality control, 50,797 SNPs from 18 healthy animals and 35 piglets with scrotal hernia were tested using an allelic X^2 test. From this test, we have identified two markers (MARC0114274, $P = 1.6 \times 10^{-7}$ and CASI0004285, $P = 1.6 \times 10^{-5}$) located on SSCX at 50,001,848 bp and 55,903,957 bp, respectively, and one with unknown location (MARC0063079, $P < 1.6 \times 10^{-5}$) associated with appearance of scrotal hernias in this population. In this study, we were able to refine the region linked with the appearance of hernias previously identified by Grindflek, E., BMC Genet. V. 7, P.1-12, 2006. We have identified that the segregation of these predisposing alleles for hernias in this population is via maternal inheritance. Therefore new approaches will be needed to eliminate those predisposing alleles to hernias out of this population, by removing the carriers females and replacing the boars being used.
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Hematological profile and reproductive tract lactic acid bacteria during the estrous cycle in beef cattle

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The interactive role of the immune system and the microbiota in regulating the physiological outcome of specific organs has been increasingly explored across different species and body systems. Considering the marked fluctuations of ovarian steroids throughout the estrous cycle, the aim of the this work was to assess effect of the phase of the estrous cycle on the response of systemic blood variables and on the isolation of lactic acid bacteria (LAB). Thirteen cyclic cows received on Day -3 (D-3) an injection of 25 mg of dinoprost. Observation of estrus behavior was performed twice daily, beginning on D-2. A cross over design was setup, where in the first round cows were randomly selected to be collected either on D-3 (diestrus; n=5) or on D0 (estrus; n=8). Subsequently, cows collected on D-3 and D0 on the first round were collected on D0 (n=5) and D-3 (n=8), respectively, on the second round. The interval from sampling to estrus (SEI) was calculated in hours. Uterine sampling was performed by washing the uterine horn ipsilateral to the dominant follicle with 60 ml of saline. Vaginal secretion was obtained from the vaginal fornix with a Metricheck-like apparatus. For the assessment of LAB, uterine washing and vaginal secretion were seeded in Man Rogosa Sharpe agar and broth and incubated in anaerobic chamber at 37ºC for 48 hours. Blood samples were collected by jugular venipuncture on D-3 and D0 to determine hematological profile. Total leukocyte count was performed on Neubauer chamber, and differential leucocyte count was obtained by Quick Panoptic-stained blood smear. Packed cell volume (PCV) and total plasma protein (TPP) were determined by microhematocrit method and refractometry, respectively. Microsoft Excel was used to compare group means by Student´s T-Test and run regression analyses. Fibrinogen, PCV, eosinophil, neutrophil and lymphocyte counts did not vary between D-3 and D0 ($P>0.05$). TPP concentration and leukocyte count were observed at greater values, whereas monocytes decreased on D0 ($P<0.05$). Furthermore, both TPP ($r=0,66$; $r2=0,44$; $P<0.01$) and leukocytes ($r=0,63$; $r2=0,4$; $P<0.01$) were positively, whereas monocytes ($r=-0,58$; $r2=0,33$; $P<0.05$) was negatively correlated with SEI. No LAB growth was detected on any uterine or vaginal samples, except for a single animal, whose sample collection was the only one carried out 24 hours post-estrus. It is suggested that the major endocrine events taking place around estrus interfere with the population of circulating leukocytes as well as total plasma protein. Such changes may impact the regulation of local reproductive tract immune cell population and the regulation of the colloid osmotic pressure, which in turn may influence the uterine influx of plasma-derived substances. We thank the State Foundation for Agricultural Research (FEPAGRO; Uruguaiana, RS) for providing the animals.

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In Vitro viability of murine 2-cells embryos after high gaseous pressure exposure

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Successfully genome activation is the first critical checkpoint in embryo development and it is an essential step for correct cell metabolism and differentiation. In mice it occurs at 2-cells stage and the further embryo development can be used as quality indicator for in vitro culture conditions. Many studies were made to evaluate high pressure effects on embryonic developmental capacity and its transcriptional response. Sublethal stress caused by high pressure activates different genes as embryo cell response that confers protection to a subsequent challenge such as cryopreservation (Pribenszky, Biol Reprod, 83, 690, 2010; Rodrigues, Reprod Fertil Dev, 25, 282, 2012). The present report aimed to evaluate the effect of 28 MPa high gaseous pressure (HGP) during 2 hours on the development and viability of 2-cells stage murine embryos to reach hatched blastocyst stage. A total of 148 2-cells embryos recovered from 10 six weeks old superovulated Mus musculus domesticus females were aleatory segregated into control (C) and experimental (P) groups. HGP was applied on embryos of P group while control embryos were immediately after recovery cultured in vitro in mKSOM media + 0.4% BSA for 120 h at 37ºC under atmosphere of 5% CO_2, 5% O_2, 90% N_2 and satured humidity. After HGP treatment, embryos from P group were also cultured at the same in vitro conditions. Blastocysts hacthing rates were observed after 120 h in vitro culture and compared by Chi-square test ($P < 0.05$). No differences were observed in hatching rates between control and experimental groups: (C) 74.2% (43/60); (P) 74.0% (65/88). Therefore, we concluded that 28 MPa HGP can be used as sublethal stressor on 2-cells embryos without compromising in vitro embryo development, viability and genome activation overcoming. Transcriptional response analysis will be conducted to identify if modifications at molecular level could affect later organism development.

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Maternal breed influence in Holstein-Gyr reciprocal F1 embryos

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Since several reproductive particularities exist between Bos taurus and Bos indicus, a common question is whether maternal contribution affects embryo developmental capacity. We compared reciprocal embryo development, in order to detect the most efficient maternal breed for F1 production in tropical conditions, and understand possible origins for any disparities. Crossbred embryos were produced by fertilization of Gyr oocytes with Holstein semen (HGyr) or by fertilization of Holstein oocytes with Gyr semen (GHol). Blastocysts were transferred to recipients and post-transfer development was assessed until birth. Spermatozoids bearing X-chromosome were used in all replicates of the experiment for IVF, and only viable oocytes (grade I, II and III). Mean number of viable oocytes and blastocysts were compared between groups using Mann Whitney Test. Rates of viable oocytes (percentage of viable oocytes in regards to total oocytes), cleavage, blastocyts, and pregnancy 30 and 60 days, calving, and embryonic loss were compared between groups using Fisher's Exact Test. Gestation length was compared between groups using T Test. We performed 88 OPU sessions in Gyr and 90 OPU sessions in Holstein donors, and retrieved 1040 Gyr oocytes and 609 Holstein oocytes. Oocyte quality, assessed by mean viable oocyte number (Gyr=8.94±0.69, Holstein=4.20±0.33; P<0.0001) and viable oocyte rate (Gyr=74.73%; Holstein=62.07%; P<0.0001), were decreased in Holstein oocytes. Embryo cleavage (HGyr, 562/694 – 80.97%; GHol, 255/343 – 74.34%; p=0.015) was decreased in GHol group. Overall, we observed a reduction of 2.39 fold in the total number of F1 blastocysts obtained in GHol group in this experiment (GHol 106 vs HGyr 254), and comparison of mean blastocyst number per OPU revealed decreased numbers for GHol (GHol 1.36±0.15, HGyr 3.34±0.35; P<0.0001). Blastocyst rate (per cleaved embryos) was similar between groups (GHol 41.56, HGyr 45.19; p=0.36). Post implantation development revealed similar pregnancy rates 30 days after IVF (GHol 38.57, HGyr 47.92; p=0.24) and 60 days after IVF (GHol 31.43, HGyr 44.44; p=0.08), but decreased calving rates for GHol group (GHol 22.86, HGyr 40.97; p=0.01). Embryonic loss was increased for GH group (GHol 40.74, HGyr 14.49; p=0.01). Gestation length of F1 embryos was affected by reciprocal cross (GHol 281.23±0.71, HGyr 286.72±0.96; P<0.0001). We conclude despite similar genetic background, maternal/paternal breed has deep influence on embryo development in bovine. We acknowledge CNPq (Grant 309271/2009-6), Embrapa (Grant 01.13.06.001.05.01.003), Faperj (Grant 111.466/2014) and Fapemig (Grant PPM 00167/15) for financial support.

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Influence of endometrites on ovarian structures and oocyte quality of cows

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The objective of this study was to diagnose clinical endometritis (CE) and subclinical (SE) by means of macroscopic, cytological and histological evaluations of bovine uterus, its influence on ovarian structures and alterations, number of oocytes recovered and oocyte quality. A total of 171 reproductive tracts of females of different ages, from unknown races and / or crosses, slaughtered in a slaughterhouse were collected. ES were diagnosed by endometrial cytology and identified more than 3% of neutrophils from 200 cells. Females in the estrogenic phase, with follicle between (12,5 and 15,5 mm) in diameter was considered the percentage more than 18% of neutrophils. The CE were identified by the presence of uterine secretion. The diagnosis of CE and ES was confirmed in the histopathology by the observation of neutrophil infiltrates in the endometrium. The ovaries were evaluated macroscopically for ovarian structure and alterations, being: dominant follicle (DF), corpus luteum (CL), ovarian cysts (OC) and oophorites (OP). The oocyte quality was performed according to the number of cumulus cell layers and cytoplasmic aspect, being: grade I (GI), grade II (GII), grade III (GIII) and grade IV (GIV). The results were submitted to descriptive statistics, parametric ANOVA and Tukey's test. Non-parametric data were analyzed by the Kruskall Wallis test and Dunn test at 5% significance. CE were present in 8,2% (n = 14) of the animals, while SE in 4,1% (n = 7), both confirmed by histopathological evaluation. DF were observed in 32,7% of the animals (n = 56), of these, 7,1% (n = 4) presented CE and 5,3% (n = 3) SE. CL were found in 42,1% (n = 72) of the animals, of these 11,1% (n = 8) were diagnosed with CE, and 5,5% (n = 4) with SE. CF and OF were found in 1,2% (n = 2) of the animals with CE. In the oocyte retrieval analysis, animals with CE, ES and healthy presented mean recovery of 10,3 ± 7,1, 15,0 ± 9,6 and 10,6 ± 8,3 oocytes / animal, respectively. In the assessment of oocyte quality, healthy animals presented GI oocytes in 12,4% (n = 188), GII in 19,8% (n = 300), GIII in 42,4% (n = 643) and GIV in 25,3% (n = 384). Animals with CE showed 11,7% (n = 17) of GI oocytes, 13,1% (n = 19) GII, 33,1% (n = 48) GIII and 42,1% (n = 61) GIV. In those diagnosed with SE, GI oocytes were obtained in 15,2% (n = 16) of the animals, GII in 9,5% (n = 10), GIII in 29,5% (n = 31) and GIV in 47,7% (n = 48). In the comparison of the quality degree between the groups, no difference was observed between the rates of GI, GII, GIII and GIV oocytes. The presence of endometrites did not influence ovarian structures and alterations and number of oocytes recovered per animal. The endometrites altered the oocyte quality within each group, however, it did not affect the oocyte quality when evaluated between the groups.

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Does lipopolysaccharide affect the rate of cleavage and embryonic development *in vitro*?

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Cows with postpartum uterine infection reduce the growth of dominant follicles and the production of estradiol, which results in postpartum delay in ovulation (Sheldon, Biology Reproduction, 81, 1025, 2009). Gram-negative bacteria, such as Escherichia coli, contain on its outer membrane lipopolysaccharide (LPS) that produces an acute inflammatory response at systemic level, which causes an increase in body temperature and serum levels of pro-inflammatory cytokines (Carroll , Innate Immunity, 15, 81, 2009). Thus, the objective of this work was to evaluate the effect of the supplementation of MIV medium with LPS (0.1, 1.0 and 5.0 µg / mL) in the cleavage rate and initial embryo development in vitro in cattle. In vitro embryo production was performed in a 5% CO2 incubator at 39°C using commercial media (Progest - Biotechnology in animal reproduction, Botucatu, SP). Oocytes (COCs) were obtained from slaughterhouse, washed and selected by morphology. COCs of grade I, II and III were randomly distributed into four groups (n = 50 COCs / group) according to the addition of LPS in the IVM medium (G1: 0 µg / mL, G2: 0.1 µg / mL, G3: 1.0 µg / mL and G4: 5.0 µg / mL LPS, SIGMA-ALDRICH®, St. Louis, MO, USA). IVM occurred for 22 hours. IVF was performed with a concentration of 1x106 sperm/mL during 20 hours. After this period, the probable zygotes were cultured in CIV medium covered with mineral oil for 7 days. At day 3, the cleavage rate (cleaved / inseminated) was assessed and on the same day, and also at day 5, 70% of the culture medium was renewed. On day 7 the embryonic development rate (blastocysts / inseminated) was evaluated. Thus, 4 replicates were performed with approximately 150 oocytes / group. The effect of LPS on the cleavage and embryo development rate was analyzed by repeated measurements of ANOVA after data transformation and the Tukey post-hoc test. There was no difference for cleavage rate, G1: 21.5 ± 3.3 (58.9%); G2: 22.5 ± 8.1 (55.6%); G3: 23.7 ± 5.3 (60.1%); G4: 30.2 ± 10.6 (72.9%) (P> 0.05); or embryonic development: G1: 6.7 ± 2.1 (13.7%); G2: 8.0 ± 3.5 (15%); G3: 9.3 ± 5.1 (17.7%); G4: 10.7 ± 8.1 (19.3%) (P> 0.05). These data suggest that exposure of oocytes to LPS does not affect the initial embryonic development in vitro in cattle.

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Modulation of cholesterol biosynthesis pathway in bovine embryos produced *in vitro*

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Embryos with high amounts of lipids have their impaired development, with increased embryo death and lower survival after cryopreservation. A component which is present in both cytoplasmic lipid droplets as the cell membranes is cholesterol, which has been suggested by recent studies as a major lipid to be investigated, having demonstrated differences in the amounts of related genes transcribed this pathway in embryos cultured in the presence and absence of serum, as well as embryos with different kinetics as compared to those produced in vivo. Therefore, the aim of this study is to test if different concentrations of a inhibitor (I) of cholesterol biosynthesis affects the blastocysts rate and the lipid content of IVP bovine embryos. In such a purpose, slaughterhouse cows oocytes (n=250 oocytes/group; 3 replicates) where submitted to IVM, IVF (5% of CO_2, 38.5°C and saturated humidity) and in the IVC (5% of CO_2, 5% of O_2, 38.5°C and saturated humidity) were divided in six treatment groups (inhibitor dose): I (20µM), 100x I, 10x I, 0,1x I, 0,01x I, and control (C). After 40hpi the cleavage and additional rate was evaluated, in the embryos droplets, the inhibitor doses corresponding to each treatment. Expanded blastocysts (n=15 blastocysts/group) were collected and submitted to lipid quantification by Sudan Black B prepared following previously stablished. The ImageJ software was used to convert the images in gray scales and determinate, in gray intensity by area, the mean of lipid content by embryo. The test t Student was used for the comparison between kinetics and ANOVA to the else analysis involving comparison between the three treatments. The cleavage rate was the same to all groups, once the inhibitor wasn't added yet ($P>0.05$). In the 100x I treatment, no embryo survived after the addition of this inhibitor dose. There was no difference between the else groups ($P>0.05$). Embryos form the treatments 0,01x I, 0,1x I and C did not presented differences in the lipid content (0,01x I: 11,67 AU±1.47; 0,1x I: 11.73 AU±1.32; C: 11.23 AU±1.14. $P>0.05$), just as the treatments 10x I e I (10x I: 15.51 AU±1.36; I: 15,16 AU±1.29. $P=0.82$). But the embryos from the treatments 10x I (15.51 AU±1.36) and I (15,16 AU±1.29) presented higher ($P< 0.05$) lipid content when compared to the group C. Concluding, i) the most concentrate dose of the inhibitor blocked the embryo development, demonstrating the importance of the cholesterol biosynthesis in the early development; ii) the dose of 10x I, 0,1x I, 0,01x I and I did not affected in the blastocyst rate; iii) 10x I and I embryos presented higher lipids amount, possibly, by a deviation in the substrate from the cholesterol biosynthesis pathway to the triglycerides pathway and PL reflecting in a higher amount of lipid cytoplasmic droplets when compared to C.

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TIMP1 levels in follicular cells and extracellular vesicles pattern in follicular fluid from ovarian follicles of different stages of the bovine estrous cycle

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During the bovine estrous cycle ovarian follicles are exposed to different physiological profiles, which can influence oocyte quality and atresia contents. Extracellular vesicles (EVs) are nanovesicles carrying bioactive molecules (mRNA, miRNA and proteins) with size between 50-150 nm found in follicular fluid (FF). It has been recently shown that transcript levels of metallopeptidase inhibitor 1 (TIMP1) are increased in follicular cells (FCs) in atresia. Our hypothesis is that *TIMP1* levels are associated with the pattern of EVs originated from follicles at different stages of the estrous cycle. To test this hypothesis, slaughterhouse ovaries were collected in pairs and classified according to the appearance of the corpus luteum in stage 1 (hemorrhagic corpus luteum, characteristic of the post ovulatory period) and stage 3 (developed corpus luteum, orange, characteristic of the diestrus period). Next, we evaluated the transcript levels of *TIMP1* in FCs of the different stages, as well as the EVs pattern, and the IVP rates from the oocytes of each stage. In order to do that, follicles between 3-6 mm exposed to different moments of the luteal phase were punctured for collection of FCs, FF, and cumulus oocyte complexes (COCs). FCs were placed at -80 ° C for subsequent mRNA extraction and evaluation of *TIMP1* levels by RT-PCR. The EVs were isolated from FF by two ultracentrifugations at 100,000xg for 70 minutes and were analyzed for concentration and particle size. The COCs were selected and kept in an incubator at 38.5 ° C, 5% CO_2. After 26 hours of maturation the oocytes were parthenogenetically activated and cultured in SOFaa at 38.5 ° C, 5% CO_2. Data analysis were performed using Student's T-test with significance level of 5%. The results showed an increase in *TIMP1* levels in stage 3 ($p = 0.051$) compared to stage 1. There was no difference between size (113.8 ± 9.40; 123.62 ± 7.62 nm) and concentration ($5.2x10^{11} \pm 2.06x10^{11}$; $6.52x10^{11} \pm 1.28x10^{11}$ particles/mL) of the EVs at the analyzed stages. As for IVP, there was no difference between cleavage rates at day 3 (68.89 ± 15.54; 67.28 ± 10.55%) and blastocysts rates at day 7 (30.09 ± 9.71; 33.80 ± 13.32%) for stage 1 and 3 respectively. Our results demonstrate that *TIMP1* levels are increased in FCs of stage 3 follicles of the estrous cycle. However, it was not possible to observe differences in size and concentration of EVs found in FF as well as in IVP, probably due to the exclusion of poorer quality oocytes prior to IVP. In this way, we believe that although we cannot find differences in the EVs size and concentration, the vesicles contents in the different stages of the estrous cycle can influence the PIVE results. These results will help to understand the molecular events involved in follicular maturation and oocyte quality in cattle. Funding: FAPESP (2014/22887-0; 2015/21674-5; 2015/21829-9; 2017/02037-0).

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The ketone body acid β–hydroxybutyric does not affect H3K9ac levels in bovine oocytes maturated *in vitro*

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Recently the ketone body acid β–hydroxybutyric (BOHB) was demonstrated to be a potent inhibitor of histones deacetylases (HDACs) both in vitro and in vivo. It makes this molecule a potential candidate to connect the animal nutrition with the regulation of gene expression via chromatin modifications. The modern high-producing dairy cows present reduced fertility, which might be caused by altered levels of metabolites (e.g. non-esterified fatty acids and ketone bodies) originated from metabolic disorders affecting the maturing oocytes. The process of oocyte maturation is characterized by changes in chromosome morphology and dynamic changes on histone modifications. HDACs are chromatin-remodeling proteins that participate in this process catalyzing histone deacetylation, which is essential for normal chromosome condensation and segregation. To gain mechanistic insights about the epigenetic effects of BOHB on maturing oocytes, we decided to investigate whether exposure of immature cumulus-oocyte complexes (COCs) with BOHB levels normally found circulating in blood of ketotic cows and paralleled in follicular fluid affect the oocyte in vitro maturation and histone acetylation. We supplemented the in vitro maturation (IVM – TCM199 +10 % FCS + 50 mg/mL hCG + 1 mg/mL FSH) medium and treated the COCs with 2 mM BOHB during the IVM (~ 21-23h). We denuded the oocytes and observed the maturation rate based on the presence of the first polar body. We carried out 10 independent replicates, culturing a total of 994 oocytes in the control group (0 mM BOHB) and 1013 oocytes in the treated group (2 mM BOHB). We did not observe difference between the groups, with the control group presenting maturation rate of 74.27 ± 2.22% and the treated group 69.74 ± 2.01%, (P=0.15; Student's t test). Since BOHB is a HDAC inhibitor, we decided to investigate whether exposure of COCs to BOHB affect histone acetylation levels as well the relative nuclear area in oocytes, an indirect measure of chromatin condensation. After confocal measurement of 61 oocytes from each group, we did not observed difference on the H3K9ac levels in the control oocytes (52.76 ± 2.23; N=61) compared with the treated with BOHB (56.95 ± 2.74; N=61; P=0.24; Student's t test). Regarding the relative nuclear area, there is no difference in the diameter of the metaphase plate, with the control group presenting 0.06058 ± 0.003816 (arbitrary units), and the treated group 0.06039 ± 0.003852, (P= 0.97; Student's t test). Our data suggest that BOHB levels normally found in cows with ketosis does not compromise the in vitro maturation neither the H3K9ac levels in bovine oocytes.
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Prostaglandin E2 in ovulation of prepubertal female mice

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The objective of this study was to evaluate the effect of prostaglandin E2 (PGE2) on ovulation of prepubertal mice. In Experiment 1, 96 prepubertal female BALB/C mice were given 5 IU of eCG intraperitoneal (i.p.) on Day 0. On Day 2, females were randomly distributed into 3 Groups to receive: 1) 0.5 mL of PBS (n=31), 2) 5µg of GnRH (Gonadotropin Releasing Hormone; Gonaxal®, Biogénesis-Bagó, Curitiba, Brasil; n=32), and 3) 25 µg of PGE2 (Prostaglanin E2; Sigma-Aldrich, MO, USA; n=33). On Day 3, mice were killed and the oviducts were collected for cumulus oocyte complex (COC) detection and counting according to Bogle et al. Reproduction, v. 142, p. 277-283, 2011. Number of oocytes was analyzed by one-way analyses of variance and Tukey's test was used to determine differences among treatments. The proportion of ovulated mice was compared among groups by chi-square test or Fischer, when appropriate. The proportion of mice that ovulated was higher (P<0.001) in the GnRH group (93.7%, 30/32) than PBS (9.7%, 3/31) and PG E2 (3%, 1/33) groups. Similarly, the mean number of oocytes observed per treatment group was higher (P<0.001) in the GnRH-treated group (10.2 ± 1.4) compared with PGE2 (0.1 ± 0.1) and PBS (0.1 ± 0.1) groups. The proportion of mice that ovulated was similar in the PBS and PGE2 groups. In the Experiment 2, 50 prepubertal female BALB/c mice were randomly distributed into PBS (n=18), GnRH (n=16) and PGE2 (n=16) groups. The mice were treated similarly to the experiment 1, except mice from PGE2 group that received 250 µg of PGE2 i.p. There were no difference in the proportion of mice that ovulated in females treated with GnRH (93.7%, 15/16) and PGE2 (93.7%, 15/16), and both were higher (P<0.001) than PBS group (0%, 0/18). Similarly, the mean number of oocytes observed per treatment group was higher (P<0.001) in the GnRH-treated group (8.9 ± 1.3) and PGE2 (6.4 ± 0.8) compared with PBS (0.0 ± 0.0) groups. The results demonstrated that the PGE2 dose of 250 µg successfully induces ovulation in prepubertal female mice.

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Redox potential profile of the reproductive tract environment at diestrus and estrus in beef cattle

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Drastic fluctuations of ovarian steroid hormones trigger a number of molecular and cellular mechanisms that drive relevant reproductive tract functional outcomes. The aim of the present work was to assess the redox potential of the uterine and vaginal compartments under the regulation of diestrus and estrus endocrine environment. Thirteen cyclic cows were assigned in a cross over design, where in the first round cows were randomly selected to have vaginal and uterine samples collected either on D-3 (diestrus; n=5) or on D0 (estrus; n=8). Observation of estrus behavior was performed twice daily, beginning on D-2. Cows received on D-3 an injection of 25 mg of dinoprost. Subsequently, cows collected on D-3 and on D0 on the first round were collected on D0 (n=5) and on D-3 (n=8), respectively, on the second round. Uterine sampling was performed by washing the uterine horn ipsilateral to the dominant follicle with 60 ml of saline, and vaginal secretion was obtained by scooping up the vaginal fornix with a Metricheck-like apparatus. Reactive oxygen species (ROS) were assessed by spectrofluorimetric method, using the 2',7'-dihydrodichlorofluorescein diacetate (DCHF-DA) probe. Antioxidant capacity was determined by using "ferric reducing antioxidant potential" (FRAP) assay. Microsoft Excel was used to compare group means by Student´s T-Test and run regression analyses. Uterine FRAP and ROS content did not differ between D-3 and D0 ($P>0.05$). Vaginal secretion from D-3 was not processed for redox potential. Uterine washings from D-3, obtained within 108 and 36 hours pre-estrus, showed a linear increase of ROS ($r=-0,84$; $r2=0,71$; $P<0.05$) and FRAP ($r=-0,76$; $r2=0,57$; $P<0.05$) content as the sampling to estrus interval (SEI) decreased. On D0, for washings obtained within 36 hours prior and 24 hours post-estrus, longer SEI were positively correlated with higher ROS ($r=0,67$; $r2=0,45$; $P<0.05$) and FRAP ($r=0,68$; $r2=0,47$; $P<0.05$) counts. On vaginal secretions, ROS ($r=-0,69$; $r2=0,47$; $P<0.05$) and FRAP ($r=-0,7$; $r2=0,48$; $P<0.05$) were negatively correlated with SEI. At estrus, the lowest FRAP and ROS counts in uterine washing and the highest counts in vaginal secretion were expressed by the only cow that was sampled 24 hours after estrus detection. It is suggested that the uterine and vaginal redox environments are regulated by the endocrine events that take place around estrus. We speculate that estradiol concentrations play a major role in the proposed regulation. As ROS and FRAP concentrations were highly positively correlated with each other, it is possible that antioxidant agents are stimulated as the oxidant activity rises; however, further investigation is warranted to determine what the precise mechanism is. It is hypothesized that major endocrine changes happening around estrus trigger equally relevant changes in the uterine and vaginal environment. We thank the State Foundation for Agricultural Research (FEPAGRO; Uruguaiana, RS) for providing the animals.

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Requirement of follicular estradiol at the time of luteolysis in Nelore cows supplemented with progesterone at early diestrus

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In beef cows, long-acting injectable progesterone (iP4) supplementation in the early diestrus has paradoxical effects, as both it increases conception growth and fertility as it induces early luteolysis in a part of treated animals. Estradiol (E2) from the ovarian follicles during the luteal phase plays a central role in the induction of PGF2α release and lysis of the corpus luteum (CL). We tested the hypothesis that follicular E2 is necessary for the occurrence of luteolysis in cows treated with iP4 at the beginning of diestrus. Cyclic Nellore cows (n = 35) were synchronized and three days after ovulation (day 3; D3), the animals were randomly assigned to receive 300 mg (im) of iP4 (Sincrogest®, Ourofino Saúde Animal) and also, to be submitted or not to daily follicular aspiration (FA) from D9 to 18, thereby composing 4 groups: -iP4-FA (n=6), +iP4-FA (n=8), -iP4+FA (n=6), e +iP4+FA (n=7). Ovarian ultrasonography was performed from D6 to subsequent ovulation using B-mode for evaluation of the largest follicle in D6, development and regression of CL, and Doppler color for evaluation of CL blood perfusion area (blood perfusion x CL total area). The day of structural luteolysis was considered to be the day when there was a 25% reduction in the largest CL area measured between D8 and 10, and 50% of the luteal blood perfusion in D8. The data were analyzed by PROC MIXED of SAS (9.3 version) for the main effects of treatment with iP4, FA, day, and their interactions. The groups treated with iP4 had a smaller follicular diameter in D6 (9.6 ± 0.4 vs. 11.8 ± 0.5 mm; $P = 0.03$) and lower mean value for CL area between D8 and 10 (2.9 ± 0.3 vs. 2.4 ± 0.2 cm2; $P = 0.02$) compared to the placebo groups. Cows from the +iP4+FA group presented earlier luteolysis compared to the +iP4-FA group (D16.4 ± 0.7 vs. 18.2 ± 0.6 d; $P = 0.05$). In the moments prior to luteolysis (D12, 13 and 14), cows from the +iP4+FA group with luteolysis ≤ D16 had CL with a lower total and blood perfusion areas compared to cows of the same group with luteolysis > D16 (2.1 ± 0.2 versus 2.67 ± 0.2, $P = 0.08$; 1 ± 0.2 vs. 1.35 ± 0.2 cm2; $P = 0.07$, respectively). Luteolysis occurred in the presence of smaller follicles in the groups submitted to FA (2.8 ± 0.6 vs. 10.75 ± 0.7 mm; $P < 0.0001$) compared to groups in which there was no FA. Cows submitted to FA had a longer cycle compared to non-aspirated cows (24.9 ± 0.8 vs. 22.1 ± 0.9d; $P < 0.01$). In summary, iP4 supplementation 3 days post ovulation affected the development of the dominant follicle of the first wave and CL. In addition, a deficiency in size and blood perfusion of CL from animals that entered luteolysis up to D16 in the +iP4+FA group was noted. It was concluded that despite extending the duration of the cycle, follicular aspiration was not able to postpone the timing of luteolysis in cows supplemented with iP4. Thus, we reject the hypothesis that follicular E2 is necessary in this process.

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Variation of seroprevalence of bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), leptospirosis and neosporosis in dairy herds from different regions of Rio Grande do Sul

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Reproductive disorders, such as embryonic death, abortion, repeat breeder cows lead to decreased reproductive efficiency and consequent milk production. Frequently, the etiology of reproductive losses is related to the occurrence of infectious diseases, such as leptospirosis, infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) and neosporosis, causing great economic losses. Strategies for diseases prevention may vary from region to region according to disease prevalence as well as their respective risk factors. The objective of the study was to estimate seroprevalence among individuals for leptospirosis, IBR, BVD and neosporosis in dairy cattle in different meso regions of the state of Rio Grande do Sul. Simple random sampling was performed considering an expected seroprevalence of 50% for IBR, BVD and leptospirosis and 15% for neosporosis. The 95% confidence level and 5% sample error were the other parameters used in the calculation of the sample size. Blood samples were collected in different mesoregions: MR1 (north-west and northeast, n=459), MR2 (north-west, n= 259), MR3 (south-west and southeast, n=373) for laboratory diagnosis by enzyme-linked immunosorbent assay (ELISA) Seroprevalence for IBR was was 61% (MR1), 54.8% (MR2) and 59.7% (MR3), with no difference (P> 0.05) among mesoregions. For BVD no difference (P> 0.05) was found between the seroprevalences according to the mesoregions (45.5% MR1, 30.1% MR2 and 39.9% MR3). For the neosporosis, higher serum prevalence (34.6%; P <0.05) was observed in the north-west mesoregion (MR2) compared to other mesoregions (24.5% MR1 and 21.7% MR3). In the case of leptospirosis, the highest serum prevalence was 27.5% in the southeast-southwest mesoregion (MR3), which differed (P <0.05) from other mesoregions (15.2% MR2, 17.8% MR1). The results indicate that IBR and BVD were homogeneously distributed according to the mesoregions studied. However, mesoregions with higher sero-prevalence were identified for leptospirosis and neosporosis, indicating a spatial variation in the health problems of these diseases.

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Effect of *in vitro* co-culture of buffalo embryos with bovine cumulus cells on the potential for early embryonic development

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In vitro embryo production (IVEP) in buffaloes is a promising technique for the multiplication of genetic material from maternal origin, however, embryo production rates are still lower than those observed in cattle, probably due to the lower quality of buffalo oocytes (fragility of the zona pellucida and the cumulus cells). Thus, cell co-cultures are widely used during IVC of buffalo embryos, especially with cumulus cells from the IVM stage. The present study aimed to evaluate the possible benefits provided by the co-culture of buffalos embryos with bovine cumulus cells newly obtained in the IVM, believing in the contribution of these cells to the production of growth factors that, in turn, stimulate the initial embryonic development. After IVM for 22 h in TCM199 medium supplemented with 10% FBS, hormones, sodium pyruvate and antioxidants, buffalo oocytes were fertilized in Talp-IVF medium supplemented with 0.6% BSA for 24 h. Then the development culture was performed in modified SOF medium supplemented with 2.5% FBS and 6 mg/mL BSA, and the structures maintained at 38.5 °C and 5% CO_2 atmosphere in air during 7 days, when the blastocyst production rate was evaluated. Three replicates of IVEP were performed, totalling approximately 100 oocytes per group. Analyses were performed in the program GraphPad Prism 7, and the proportions of blastocysts were evaluated by Fisher's exact test. Although there was a higher average blastocyst production in the group co-cultured with bovine cells, there was no difference (P>0.05) between the groups (co-culture with buffalo cells - 17/106 - 20.0% , Co-culture with bovine cells - 22/110-16.0%). Attanasio et al. (Theriogenology, v.74, p.1504-1508, 2010) performed a study in which buffalo oocytes were cultured with bovine somatic cells after the vitrification process and observed that only those exposed to intact bovine cumulus oocytes (CCO) complexes presented the restoration of initial developmental capacity. It is possible that co-cultivation in these systems fails to mimic the association between oocytes and radiate corona cells provided by gap junctions. Thus, it is concluded that the use of buffalo embryo co-culture with bovine cumulus cells originated from IVM does not provide preimplantation development increments, and we recommend that the strategy of co-culture of buffalo embryos with intact bovine CCOs also be investigated.
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Pattern of luteinizing hormonal secretion in prepubertal Gyr heifers

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The objective of the present study was to identify the pre-peak release pattern of LH, to associate with follicular growth and ovulatory response in Gyr prepubertal heifers after hormone protocol of ovulation induction. Twelve prepubertal Gyr heifers (18 ± 1.4 months of age) created in the same conditions (*Brachiaria Decumbens* pasture and free access to water and mineral salt) received an intravaginal device (day zero = d0) containing 1.0 g of P4 (Sincrogest®, Ourofino, Cravinhos, Brazil) for 12 days. In d12, the device was removed and 0.5mg of Estradiol Cypionate (ECP®, Zoetis, Campinas, Brazil) and 200IU of eCG (Folligon®, Intervet Schering-Plow, Cotia, Brazil) were injected (I.M). After 24h of this treatment (d13), blood samples were collected every 15min for 6h to identify the pre-peak pattern of LH secretion. The animals were submitted to two evaluations (d-15 and d-8) of diameter and uterine tone (by US / DP-2200 Vet®, Mindray, China), which associated with absence of corpus luteum (CL), confirmed the sexual immaturity. Measurements of ovarian follicular diameter were performed from d11 to d15. The presence of CL was followed up to d18 to verify the occurrence of ovulation. Plasma LH concentrations were assessed by double antibody radioimmunoassay using purified bovine LH. The hormonal protocol induced ovulation in 41.6% of the heifers (5/12), which occurred between d15 and d16. After the ovulatory response, the females were grouped: "Ovulated" (G1, n= 5) and "Non-ovulated" (G2, n= 7). The LH concentrations were analyzed by deconvolution using the Pulse algorithm (Software Pulse_XP, version 20090124, University of Virginia, VA, USA). The mean follicular diameters (5%) and the data obtained from the LH analyzes (as this was a preliminary study, with a small number of animals, the level of significance was 10%) were compared by the t-test (SAS, Institute Inc., Cary, USA). The mean concentration (ng/mL) of LH (G1: 0.80 ± 0.08, G2: 0.96 ± 0.09, p= 0.22), estimated basal secretion (pg/mL) (G1: 16.9 ± 1.9, G2: 21.6 ± 2.3, G2: 25.4 ± 2.3, p= 0.16) and the mean amplitude (pg/mL) of the secretory pulses (G1: 16.9±1.9; G2=19.4 ± 2.3, p = 0.26) did not differ between groups. However, the frequency (pulses/h) of secretion pulses (G1: 0.30 ± 0.06, G2: 0.48 ± 0.06, p= 0.07) and the total mass (ng) secreted in pulses (G1: 0.57 ± 0.14; G2: 1.07 ± 0.19; p= 0.08) was higher in G2. The follicular diameter (mm) at d13 (same day of LH collection) did not differ between groups (G1: 11.2±0.81; G2: 10.4±0.8), but it was higher (p<0.05) in G1 (G1: 11.3 ± 0.93, G2: 9.7 ± 1.01) at d14. The results suggest that the ovulatory response to the hormone protocol used in prepubertal Gyr heifers is related to a lower frequency of pulsatile secretion of LH at the time of ovulation induction and not to the follicular diameter. However, further studies should be conducted to better understanding the dynamics of LH pre-peak secretion. Acknowledgments: FAPEMIG, EMBRAPA, CNPq.