Animal Reproduction

Administration of two doses of recombinant bovine somatotropin reduces cell damage in vitrified/warmed embryos from superovulated Pelibuey ewes

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Recombinant bovine somatotropin (rbST) has been extensively explored in animal breeding programs and the results of applying it have not been consistent. According to published information, the success of using this hormone depends on multiple factors such as the reproductive aspect studied, the species, the time of application, doses, etc. This study aimed to evaluate the effect of applying rbST during the superovulation (SOV) of Pelibuey sheep on the cryotolerance of vitrified embryos. A 14-day SOV protocol was used. Donors were randomly divided into two groups: rbST and control (C). Sheep in the rbST group were given 100mg of rbST at D0 and another equal dose at D14. In both groups of animals, an intravaginal sponge of 60 mg medroxyprogesterone was inserted at D0, in the D6 the sponge was replaced and 75 µg of D-Cloprostenol was injected. Between D12 and D15, 200mg of p-FSH (Folltropin-V, Vetoquinol, Canada) was applied in 8 decreasing doses with an interval of 12 hours. On D14, 400UI of eCG was injected and the sponges were removed. At 24 and 36 hours later, two controlled matings were performed with Pelibuey males of known fertility. Six days after the first mating, all donors were flushing and the embryos were recovered by laparotomy and vitrified in open pulled straws. Twenty-one embryos (morulae, early blastocysts, blastocysts, and expanded blastocysts) from each group were warmed and randomly selected to assess re-expansion and hatching at 2, 24, 48, and 72 hours (rBST, n=11 and C, n=10), and to determine the number and vitality of blastomeres by differential staining (Hoechst, Propidium Iodide) (rBST, n= 10 and Control, n=9). Rates of embryo re-expansion and hatching were compared by chi-square test. Differences in the number of total and altered cells and the percentage of intact blastomeres were analyzed by ANOVA. Re-expansion rates at 2, 24, 48 and 72 hours were 90.9, 72.7, 81.8 and 72.7% for rbST-, and 100, 90, 90 and 90% for control-embryos (P>0.05). Rates of hatching at 24, 48 and 72 hours for rbST-embryos were 45.4, 54.5, and 72.7%, and for control-embryos 40, 90, and 90% (P>0.05). The number of total blastomeres did not differ between experimental groups (93.1±5.1 and 95.4±5.1 for rbST- and control-embryos respectively). Embryos derived from rbST donors had a lower number of altered cells (5.1±1.4 v. 10.0±1.5 respectively; P=0.0316) and a greater percentage of intact cells than control embryos (95.5±1.4 v. 89.2±1.6% respectively; P=0.0284). In conclusion, administration of two doses of rbST to superovulation protocol of Pelibuey sheep did not affect rates re-expansion and hatching at 2, 24, 48, and 72 hours, but reduced plasma membrane damage in blastomeres after embryo vitrification/warming.

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Animal Reproduction

Analysis of LH receptor mRNA in granulosa cells and extracellular vesicles present in the ovarian follicle of bovine females

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During folliculogenesis, small extracellular vesicles (sEVs) are released into the follicular fluid (FF). Through their bioactive content, they act in intercellular communication and contributes to the oocyte maturation process. In cattle, follicular growths after 9 mm in diameter requires endogenous LH, as well as its own receptors. Based on that, the hypothesis of this study is that sEVs delivery LH receptor (LHr) mRNA among granulosa cells (GCs) in FF, that facilitates the LHr acquisition during follicular growth. To carry out this experiment, the bovine ovaries were obtained from slaughterhouse, selected according to the estrous cycle stage (early and middle diestrus). The ovarian follicles were dissected individually and separated in experimental groups according to the diameters (small: 3-5 mm; medium: 5,1-7 mm; and large: 7,1-9 mm). Next, follicles were ruptured individually in Petri dishes using a stereomicroscope for later collection of GCs and FFs. For the gene expression analysis, approximately 8 pools (n= 4 follicles/pool) were assembled containing the GCs and FFs for each experimental group referring to its follicular diameter. The GCs were submitted to two centrifugations at 2.5 rpm for 5 minutes to form a purer pellet. The sEVs were isolated from the FF by serial centrifugation at 4 ºC to remove live cells (300 × g for 10 min), cell debris (2.000 × g for 10 min), large sEVs (16.500 × g for 30 min) and by two ultracentrifugations (119.700 × g for 70 min) to obtain a pellet enriched with sEVs of 30-100 nm. The pellet of GCs and sEVs were submitted to RNA extraction, cDNA synthesized and RT-PCR analysis, in order to quantify the expression levels of LHCGR, BAX and BCL-2. The data were normalized by the geometric mean of the GAPDH and YWHAZ for GCs and GAPDH as endogenous genes for sEVs. The statistical analysis was performed by ANOVA and Tukey test (Graphpad Prism) with a significance level of 5%. The analysis of *LHCGR* levels with different follicular diameters showed higher levels of the gene transcripts in GCs of large follicles (0,01105 ± 0,00428) compared to small (0,00312 ± 0,00205) and medium (0,00310 ± 0,00219). Conversely, higher relative expression of the LHCGR transcripts were observed in sEVs from small (0,00472 ± 0,00074) and medium (0,00469 ± 0,00121) follicles when compared to large (0,00216 ± 0,00055). For the BCL-2 transcripts, a higher expression was noted in GCs from small (0,01213 ± 0,00649) follicles when compared to medium (0,00212 ± 0,00076) and large (0,00052 ± 0,00013) follicles. No statistical differences were found among sizes for the BAX transcripts in GCs. The present study was the first to demonstrate that sVEs contain *LHCGR*. Also, these nanoparticles possibly delivery LHr mRNA from GCs to the other GCs through the FF during the follicular growth. Finally, this mechanism can be involved in the acquisition of LHr by other granulosa cells, allowing homogeneous levels throughout the follicle. However, more studies are still needed to validate that *LHCGR* from sEVs are secreted and internalized by granulosa cells in a stage-dependent manner. Funding: FAPESP 2014/22887-0 and 2019/21028-7.

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Animal Reproduction

Angiotensin-converting enzyme inhibitor in in vitro production of bovine embryos

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Enalapril is a prodrug that needs to be hydrolyzed in the liver to enalaprilat to have the angiotensin-converting enzyme (ACE) inhibitor effect.Therefore, in this study, with oocyte culture in vitro, instead of enalapril, enalaprilat, which is the active form of enalapril, was used. Enalapril was used to verify its action on the ovary of different species such as rats, rabbits, sheep and goats. There was also an increase in the number of transferable goat embryos and an improvement in their quality, in addition to an increase in the number of pregnancies and products born by embryo transfer. In the present study, we aimed to verify the influence of enalaprilat on the production of bovine embryos and on the quality of these embryos when added in maturation, fertilization and in vitro culture. The culture media used in the protocol for in vitro production of bovine embryos in the stages of maturation, fertilization and in vitro culture were the same and the enalaprilat was used, at different concentrations, in all stages of in vitro embryo production. In the experiment, 480 oocytes were cultured in the absence or presence of enalaprilat, in the stages of maturation, fertilization and cultivation. The CCOs were divided into four groups or treatments with 6 repetitions, with each repetition having 20 to 25 CCOs. The groups were divided according to the concentrations of enalaprilat used: control group absence of enalaprilat in all phases of IVP; enalaprilat group 1 µM; enalaprilat group 2 µM and enalaprilat group 4 µM, with the addition of enalaprilat in all stages of IVP. The quality of the structures found in D7 was assessed using the parameters established by the International Embryo Technology Society (IETS). The design was completely randomized, and the non-parametric data were evaluated by the Kruskal Wallis test at the level of 5% probability, whereas for the parametric data ANOVA was performed and for the comparison of means the Holm-Sidak test was performed. The results were expressed as mean for parametric data and percentage for nonparametric data. The data were evaluated using the Sigma Stat version 3.5 program (Systat Software, Inc). The influence of enalaprilat in the concentrations of 1, 2 and 4 µM added to the media of maturation, fertilization, and in vitro culture of bovine oocytes under the cleavage rate was evaluated, which did not show any significant difference between the enalaprilat treatments (1 µM - 81%; 2 µM - 78% and 4 µM -66%) compared to the control (77%). The same was observed in the results in the blastocyst rate, evaluated on the seventh day of culture (D7), the data showed that there was no significant effect ($P > 0.05$) of the addition of enalaprilat (1 µM - 68.83%; 2 µM - 65.9% and 4 µM - 53.5%) compared to the control (76,66%) for the means of in vitro embryo production**.** The structures evaluated in D7 were of excellent quality, however the results were not significant ($P < 0.05$) between treatments: control (10.00 ± 1.25), 1 µM enalaprilat (11.33 ± 1.25), 2 enalaprilat µM (10.83 ± 1.25) and 4 µM Enalaprilat (8.66 ± 1.25). It is concluded that enalaprilat did not influence the production of bovine embryos *in vitro*, but this does not elucidate the results observed in vivo and indicates the need for further study. Acknowledgements: CNPq (processes nº 429648/ 2016-2) and Laboratory of Physiological Sciences of the Federal University of Piauí, Laboratory of Animal Biotechnology and Reproduction.

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Animal Reproduction

Antral follicle count affects fertility in Nelore Heifers Contagem de folículos Antrais afeta a fertilidade de novilhas Nelore

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Antral follicle count (AFC) has gained merit as a predictor of fertility, with important correlations for quality and quantity of embryos, ovarian follicular reserve, as well as ovulatory follicle size and conception rates. AFC presents high repeatability and it is easily measured. However, there have been differences between *Bos taurus* and *Bos indicus* regarding pregnancy rates and reproductive quality. In the present study we aimed to identify the traits that most correlated with AFC in *Bos indicus* Nellore females. A total of 223 heifers with 24 - 30 months, 379±33kg of weight were induced to puberty following with synchronization of ovulation protocol and time artificial insemination (TAI). At the beginning of the TAI protocol (D0), the ovarian follicles with diameter ≥ 3 mm were counted by ultrasound. In addition, were measured dominant follicle diameter (DFD), ovary diameter (OD), conception rate at 30 days (CR30), final conception rate (FCR) and pregnancy loss (PL). The AFC was determinate by an ovarian ultrasound exam with SonoScape Vet A5, which was used by an operator, who needed to rotated the probe 180º in ovarian region and accounted all visible follicles on a unique ovarian. Data for the present experiment were analyzed by SAS University. The AFC average was 26,2 ± 10,7 follicles, conception rate (CR) was 37,67%, final conception rate (FCR) 79,2%, and pregnancy loss (PL) 3,6%. Pearson's correlation coefficients were evaluated for all variables in relation to the AFC. Correlations were not significant for AFC with BCS, DFD and OD ($p>0.05$). A logistic procedure was also made for AFC and pregnancy rate at 30 and 60 days, and pregnancy loss. No significant correlations ($p>0.05$) were found for AFC and CR, AFC and FCR, AFC and PL. However, a tendency ($p=0,07$) was observed for FCR and AFC, greater pregnancy rate is present in Nelore heifers with lower AFC. There was no effect of AFC in pregnancy lost ($p>0.05$). In brief, female fertility can be predicated by AFC, were those with lower AFC can be selected to improve the reproductive performance of the herd.
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Animal Reproduction

Are the GDF-9 and BMP-15 presents in testicles of the bovine fetuses?

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Germ cells and somatic gonad cells synthesize several growth factors belonging to the TGFβ superfamily (β Transforming Growth Factor) which contains more than 40 multifunctional polypeptide growth factors including TGFβ, activin, BMPs, GDF-9, and Anti-Müllerian Hormone (Itman et al., 2006). GDF-9 and BMP-15 are synthesized in mammalian oocytes at various stages of follicular development and are considered an essential factor for folliculogenesis (McGrath et al., 1995, Dong et al., 1996). In the testicles, GDF-9 acts in the regulation of spermatogenesis with its expression at specific stages (Zhao et al., 2011) and BMP-15 has a role in regulating gonadal development (Aaltonen et al., 1999), and its expression is observed in human and bovine testicles (Pennetier et al., 2004) and although their signaling pathways are well known in oocytes, their role in the spermatogenic lineage is poorly understood. The aim of this work was to evaluate whether these proteins are present during testicular development in bovine fetuses. Testicles were collected from 25 bovine fetuses between 4 and 8 months, determined by measurements of crown-rump length (CRL). The testicles were fixed in 10% formalin for 24 hours and subjected to routine histological processing and paraffin embedding. Hematoxylin and Eosin staining was performed to assess the testicular structure and for immunolocalization, the primary polyclonal antibody anti-GDF-9 (SC-514933, 1:50) and the anti-BMP-15 antibody (SC-271824, 1:50) were used according to the manufacturer's instructions. The slides were counterstained with hematoxylin and mounted with Entellan for analysis under a photomicroscope (NIKON Eclipse Ci-E, Nikon Corporation, Tokyo, Japan) coupled to a digital camera (NIKON DS-Ri, Nikon Corporation, Tokyo, Japan), being evaluated the aspects of immunostaining and the cell type marked at different fetal ages. The histological structure of the testes was observed with the presence of seminiferous cords (without tubular lumen) containing germ cells (gonocytes) and Sertoli cells, however, the immunostaining of GDF-9 and BMP-15 in the analyzed testicles was not observed. suggestive that these proteins were not present in the gonadal development of bovine fetuses at the evaluated ages, agreeing with the literature that cites their expression only during the meiotic activity of germ cells.

Keywords: GDF-9, BMP-15, testis bovine fetus

Animal Reproduction

Assisted calving associated with uterine disease occurrence and reproductive efficiency of crossbred dairy cows

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Occasional assistance during calving is important to ensure cow and calf survival, and the high prevalence of uterine postpartum diseases is a challenge in terms of optimizing the reproductive efficiency of dairy herds. In this study we hypothesized that crossbred lactating dairy cows that had a single live calf without assistance would have a healthy postpartum period and better future reproductive performance than cows that need calving assistance. Based on that, the aim was to evaluate in crossbred lactating dairy cows that had a single live calf, the association between type of calving (normal or assisted) on uterine disease occurrence, and its effects on number of artificial inseminations (AI) per conception and pregnancy rate at 150 days postpartum (DPP). Cows were monitored during calving and the type of calving was classified as normal or assisted. Uterine diseases evaluated were retention of the fetal membranes, metritis, and clinical endometritis. 801 calving of a single and live calf were analyzed, of which 766 were normal and 35 were assisted. Most of the cows with normal calving had a healthy postpartum period (73.89%), while most of the cows with assisted calving had uterine diseases (74.29%). Number of AI per conception was similar in cows that had normal or assisted calving (P = 0.16), and there was no evidence of an influence of type of calving on the pregnancy rate at 150 DPP (P = 0.44). Healthy cows had a higher pregnancy rate at 150 DPP than cows affected by uterine diseases (51.65 vs. 42.92%). In conclusion, the majority of lactating crossbred dairy cows undergoing assisted parturition have uterine diseases, and healthy cows have a higher pregnancy rate with 150 DPP.

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Animal Reproduction

Associations among infrared thermography and sperm parameters of collared peccaries raised in a semiarid region

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Climate change has a major impact on ecosystems and the survival of various species. In this scenario, heat stress acts as one of the main factors that cause deleterious effects on male fertility. Understanding how an animal's body temperature affects the sperm parameters can be a key factor in the early diagnosis of low- quality semen. Thus, this study aimed to evaluate the potential associations among temperatures of different body regions and some sperm parameters of collared peccaries (*Pecari tajacu*), based on the hypothesis that high body temperatures, caused by heat stress, are related to low sperm quality. The data collections were carried during November 2019 at the Wild Animal Multiplication Center, in a semiarid region located in Mossoró, RN, Brazil (latitude 5°12'48"S, longitude 37°18'33"W, and 16 m above sea level). During the experimental period, environmental conditions were monitored twice a day (07:00 and 14:00), and the data obtained were: air temperature (T: 27.27 and 31.03 ºC), humidity (H: 65.11 and 53.89%), wind speed (W: 3.65 and 5.73 m/s), and solar radiation (SR: 223.8 and 832.19 W/m^2), respectively. The ejaculates from nine sexually mature males were obtained by electroejaculation. Semen samples were assessed for sperm motility kinetic parameters using a computer-aided system (CASA - IVOS12.0, Hamilton-Thorne, Beverly, USA), and the following average data were obtained: Total motility (92.4%), Progressive motility (64.6%), Velocity average pathway (VAP: 63.8 mm/s), Velocity straight line (VSL: 50.6 mm/s), Velocity curvilinear (VCL: 113.8 mm/s), Amplitude lateral head (ALH: 5.4 mm), Beat cross frequency (BCF: 37.1 Hz), Straightness (STR: 74.7%), Linearity (LIN: 44.7%), and subpopulations Rapid (75.8%), Medium (16.4%), Slow (2.9%), and Static (4.7%). Sperm plasma membrane integrity was determined (79.4%) by epifluorescence microscopy using the fluorescent probes carboxyfluorescein diacetate (0.46 mg/1 mL of dimethylsulfoxide) and propidium iodide (0.5 mg/1 mL of 0.9% saline solution). Moreover, the sperm binding ability was evaluated through the egg perivitelline membrane binding assay (224.1 number of bound sperm). In parallel, thermal images of different body regions were obtained on the day before the semen collection using infrared thermography. The following body areas were selected, which obtained their mean temperatures at 07:00 and 14:00, respectively: hindlimbs (Thind: 36.18 and 41.39 ºC), forelimbs (Tfore: 35.69 and 40.05 ºC), body trunk (Tbody: 36.11 and 42.64 ºC), eye (Teye: 36.32 and 41.94 ºC), ear (Tear: 36.11 and 41.29 ºC), muzzle (Tmuz: 29.68 and 36.14 ºC), and head (Thead: 36.32 and 41.94 ºC). To determine the relationship among sperm metrics, environmental data, and body surface temperatures, Spearman's correlation test was applied considering significant when P <0.05. At 7:00, Teye was negatively correlated with STR (ρ = -0.70) and LIN (ρ = -0.72); ALH was positively correlated with Teye (ρ = 0.862) and Tbody (ρ = 0.81); solar radiation presented a negative relationship with the VCL (ρ = -0.68). At 14:00, BCF was negatively correlated with Thind (ρ = -0.70). At the same time of day, mean air temperature (ρ = -0.739) and Thead (ρ = -0.65) were correlated with membrane integrity. At 14:00, Tbody was negatively correlated with the number of sperm bound to the perivitelline membrane (ρ = -0.87). Maximum air temperature also correlated negatively with membrane integrity (ρ = -0.69). In summary, stressful thermal conditions that could provoke body temperature increase would impair semen quality in collared peccaries. This study demonstrates for the first time that infrared thermography is a useful tool for early detection of a decrease in sperm metrics in this species. Financial support: CNPq and CAPES

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Animal Reproduction

Biochemical constituents of the seminal plasma of agoutis (*Dasyprocta leporina*) collected during the dry and rainy periods of a semiarid region

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The knowledge of how environmental variables can influence the reproductive aspects of wild species is of fundamental importance for the development of adequate strategies for their management and conservation, since climate change has had a great impact on their survival. Among the reproductive aspects, understanding the biochemical composition of seminal plasma helps on the development of assisted reproductive techniques that safeguard the sperm of a given species, like the agouti (*Dasyprocta leporina* Linnaeus, 1758). These are ecologically important wild hystricognath rodents, as they act in seed dispersal and soil aeration, in addition to being important links in the food chain in their habitats. Therefore, we aim to describe the organic and inorganic biochemical constituents present in the seminal plasma of agouti, analyzed during the peak of the dry and the rainy periods of a semiarid region. For this purpose, the seminal plasma of six adult males was collected, through electroejaculation, during the peaks of the dry (September, October and November 2019) and rainy (February, March and April 2020) periods of the caatinga biome. The samples obtained were analyzed under light microscopy to confirm the absence of sperm, since the purpose of the procedure was to obtain only the seminal plasma. Then, they were centrifuged at 700 g speed, to separate solid residues, and the supernatant was refrigerated at -20 °C until the occasion of the biochemical analysis. Seminal plasma was analyzed for the presence of organic (total protein, albumin, cholesterol, triglycerides, fructose and glucose) and inorganic (phosphorus, magnesium, calcium, iron, chloride, sodium and potassium) biochemical components using commercial biochemical kits. The values obtained were correlated with the climatic variables of the studied environment. To characterize the peak of the dry and rainy periods of the semi-arid climate in the Caatinga, total precipitation data (in mm) were obtained from the National Institute of Meteorology (INMET) station, located in Mossoró, RN, Brazil. The climatic variables related to air temperature, wind speed, global radiation and humidity in each period were analyzed, measured by a meteorological station close to the location of the animals' pens. Data were expressed as the mean and standard error. To assess potential seasonal differences on seminal plasma biochemical parameters and thermal environment, a one-way ANOVA was performed using the PROC GLM of SAS. Spearman's correlation test was applied to determine associations among studied variables. All the biochemical components investigated were found in the agouti seminal plasma. Higher glucose concentrations were identified in the dry period (88.24 mg/dl) than in rainy period (26.27 mg/dl). Phosphorus (66.40 mg/dl vs. 3.67 mg/dl) and potassium (92.67 mmol/L vs. 19.68 mmol/L) values were higher in the dry season than in the rainy season. However, chloride concentrations (43.04 mEq/L vs. 201.40 mEq/L) were higher in the rainy season compared to dry season. There were significant correlations ($P < 0.05$) among the climatic variables and the analyzed biochemical constituents. Rainfall was positively correlated to chlorides ($r = 0.83$), but negatively correlated to glucose concentrations ($r = -0.69$) and phosphorus ($r = -0.79$). On the other hand, humidity was positively correlated to both glucose ($r = -0.67$) and chlorides ($r = 0.678$), but negatively correlated to phosphorus ($r = - 0.82$). The global radiation was positively correlated with glucose ($r = 0.78$) and phosphorus ($r = 0.81$) but negatively correlated to chlorides ($r = -0.64$). Similarly, the air temperature positively influenced glucose ($r = 0.74$) and potassium ($r = 0.80$) concentrations but presented a negative effect on chlorides ($r = -0.54$). Finally, the wind speed was also positively correlated to glucose concentrations ($r = 0.72$) and phosphorus ($r = 0.77$), but negatively influenced the chlorides ($r = -0.71$). In summary, environmental variables can significantly influence seminal plasma composition in agoutis, and the concentration of some biochemical components varies between different climatic periods of a semiarid region. We emphasize that this is the first extensive description of the biochemical composition of seminal plasma in *Dasyprocta leporina*, thus contributing for the knowledge related to reproductive physiology of the species. **Financial support:** CAPES and CNPq

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Animal Reproduction

Bisphenol A (BPA) increases oxidative stress on *in vitro*-matured oocytes and impairs bovine embryo production

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Bisphenol A (BPA) is a monomer widely used in the plastic industry for production numerous consumer products is a endocrine disruptor and is associated with serious effects capable of affecting the reproductive systems, due to its binding to estrogen receptors (ERs). Exposure to this chemical is ubiquitous, and occurs mostly via the oral, respiratory, and dermal routes in human and animals. BPA is was detected in human urine, salive, and human fluids such as blood, amniotic fluid and even follicular fuid. Animal studies have shown that BPA cause meiotic abnormalities, acting on the spindle disorganization in oocytes and on the alignment of the chromosomes, decreasing the percentage of oocytes that progress to metaphase II and increasing the percentage of oocytes that degenerate during *in vitro*. Based on that, here we aimed to investigate the effects of BPA during oocyte *in vitro* maturation on oxidative stress and the subsequent impact on early *in vitro* embryo development in cattle. For this purpose, 5 replicates containing 20 cumulus-oocyte complexes (COCs) each were *in vitro*-matured with 1000 µM of BPA (0.1%DMSO) and subsequently submitted to analysis of oxidative stress, mitochondrial membrane potential, meiosis progression and embryo yield. The control group were *in vitro* matured with basal medium (0.1%DMSO). The effect of 1000 µM BPA was tested by unpaired T-test. Differences were considered significant when $P < 0.05$. We observed that oocytes treated with 1000 µM BPA exhibited toxicity and cell damage due to the high production of reactive oxygen species and high levels of mitochondrial membrane potential. Also, none oocyte reached to metaphase II. Due to that, BPA blocked fertilization and further embryo development. Our findings suggest that BPA has a complex mechanism of action that has a deleterious effect on the fertility of exposed animals. In a study with granulosa cells, dose-dependent effects were detected with reduced cellular viability and antioxidant capacity and a significant increase in ROS production under high doses of BPA. In general, we concluded that the addition of 1000 µM of BPA during oocyte *in vitro* maturation blocks meiotic resumption and increases oxidative stress in bovine oocytes, which led to the impediment of *in vitro* development of bovine embryos. Acknowledgements: National Council for Scientific and Technological – CNPq (grant 140620/2020-6).

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Animal Reproduction

Canine sperm quality in fast freezing protocol with melatonin

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The oxidative stress is a recurring problem related to sperm freezing, due to many process involved in this biotechnology of reproduction. Therefore, new techniques are frequently evaluated to reduce pos-thawing cellular damage, such as addition of antioxidants to protocol of sperm cryopreservation. The aim of this study was evaluate the addition of melatonin, in different concentrations, in a protocol of canine sperm fast freezing, with the purpose of reducing oxidative stress and improve the pos-thawed sperm quality. Six males dogs, adults, with age of two to five years old were used in the study, with three sperm collection, every fifteen days. The extender used was composed by Tris-egg yolk and ethylene glycol 5%, and melatonin added in the semen in five different concentrations, being a control group without addition of melatonin (T0), and the others groups with 1 mM, 1.5 mM, 2 mM, 2.5 mM and 3 mM of melatonin. The semen was thawed in water bath at 37ºC for one minute and evaluated for the kinetic parameters with computer analysis in software CASA-SCA, morphology with Rose Bengal staining and membrane integrity with eosin-nigrosin staining. Parameters of oxidative stress were evaluated, such as catalase (CAT) by consumption of H2O2, superoxide dismutase (SOD) due to pyrogallol autoxidation, and malondialdehyde (MDA) by lipid peroxidation rates. Kinetic parameters, morphology, membrane integrity, MDA or antioxidant activity of CAT and SOD in different concentrations with melatonin were compared, and there is no significant correlation ($p>0,05$) among different treated groups and control group. However, this study made possible to evaluate the melatonin addition in cryopreservation protocol to canine, and it didn't provide significant changes in quality or oxidative stress when semen was thawing. Acknowledgments: Coordination of Superior Level Staff Improvement (CAPES, Brazil).

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Animal Reproduction

Characteristics of the Boer goat ejaculate after refrigeration at 5ºC with BotuSêmen Special and BotuSêmen Gold

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For the refrigeration of goat ejaculates, it is necessary to use diluting media that suit the particularities of this species. The purpose of this biotechnique is to lower the temperature enough to reduce sperm metabolism, which can be at 15 or 5ºC, keeping them viable for up to 72 hours, but the fertility rates of does inseminated with refrigerated semen decrease from 6 hours onwards. Before refrigerating the semen, it is necessary to evaluate the breeder animal, which must be healthy and over seven months of age, in addition to evaluating the ejaculate for macro- and microscopic parameters, including volume (variable from 0.3 to 5 mL), color (white or yellowish), appearance (aqueous to milky), and microscopic evaluation, such as sperm concentration (between $2.5x10^9$ to $5.0x10^9$), mass movement (between 3 and 5) and morphological evaluation. Thus, the objective was to analyze the effects of refrigeration at 5 ºC on the characteristics of the Boer goat ejaculate, using two different diluents. Fifteen breeders belonging to properties located in the municipality of Guarapuava, state of Paraná, and region were used, during the spring and fall months. Animals were aged between one and three years, body condition score between 2.5 and 3.5, were previously subjected to a general physical examination, including determination of heart rate and respiratory rate, and oral mucosa coloration. The examination and measurement (length, width and thickness) of the testicles and scrotal circumference were also performed. The ejaculate was collected using an electroejaculator specific for small ruminants and, immediately after collection, the ejaculate was analyzed for volume (mean 1.0±0.5mL), color (white to yellowish), appearance (milky) and odor (*sui generis*) by means of direct observation, and microscopically evaluated for mass movement (mean 3.50±0.81), motility (mean 75.6±10.83%) and vigor (mean 3.60± 0.63). Later, the sperm concentration (mean $2.28±0.71x10^9$), major defects (mean 1.13±0.43%) and minor defects (mean 7.63±0.88%) were determined, and hyposmotic test (mean 97±0.33%) was performed. In this experiment, two diluents were used for refrigeration, BotuSêmen Special®, based on powdered milk, sugars, preservatives and excipients (Botupharma®, Botucatu, SP, Brazil), and BotuSêmen Gold®, based on casein, sugars, preservatives and excipients (Botupharma®, Botucatu, SP, Brazil). In this way, samples were divided into two groups, in group 1 (G1, n=15) 100 µL semen was added with 600 µL BotuSêmen Special® in plastic tubes, and in G2 (n=15) 100 µL semen was added with 600 µL BotuSêmen Gold® in plastic tubes, and later stored at 5ºC, in a box for semen transportation BotuFlex® (Botupharma®, Botucatu, SP, Brazil) for 24 hours. Motility, vigor parameters and hyposmotic test were evaluated after the refrigeration period. The results obtained were tabulated and tested by analysis of variance ANOVA, at 5% significance. The mean and standard deviation for G1 were 28.66±13.86%[a] motility, 1.6±1.0 vigor and 95.54±1.24%[a] in the hyposmotic test; whereas in relation to G2, values were 53.33±14.90%[a] motility, 3.06±0.59 vigor and 95.87±1.06%[a] in the hyposmotic test. Although no significant difference was detected between the groups ($p>0.05$), the sperm parameters of G2 were numerically higher compared to G1, within the normal range for refrigerated semen. The season effect was not evidenced in this study, since goat breeds native to Brazil, such as the Boer, do not suffer changes in their reproductive activity during the year. Based on the above, it can be concluded that the BotuSêmen Gold® dilution medium preserved better the sperm under these experimental conditions, however, more studies are needed to evaluate the fertilization capacity of processed semen under these conditions. **Acknowledgments:** Botupharma for providing the dilution media for this experiment.

Key words: semen, cryopreservation, goats.

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Animal Reproduction

Characterization of spermathecal spermatozoa from *Melipona subnitida* (APIDAE, MELIPONINI)

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The Jandaira (*Melipona subnitida*) is a neotropical bee with significantly economic, environmental, and agricultural relevance for the Brazilian semiarid region, but reproductive biotechnologies that help the multiplication of this species have not been developed yet. We aimed to identify the basic characteristics of *M. subnitida* sperm stored in queens spermathecae. Twelve *M. subnitida* queen bees (fertile females) with one month old were collected from an urban meliponary of Mossoró city, Brazil. These females keep the sperm viable for long-term usage inside the spermatheca organ. The animals were dissected for spermathecae collection. The collected material was gently homogenized in 20 µl of saline solution, then sperm quality indicators were analyzed such as viability, motility, concentration, and morphometry. To identify sperm viability, 5 µl of the original semen solution were added to 4 µl of Hoechst 33342 solution at 0.04 µg/mL and after 5 minutes 1 µl of 0.5 µg/mL propidium iodide solution was added and after 3 minutes it was analyzed in a fluorescence microscope (Olympus® DP72), one hundred spermatozoa per sample were analyzed and the percentage of viable and non-viable spermatozoa was calculated. For sperm motility analysis, the percentage from 0% to 100% of motile spermatozoa was calculated throughout the observation of the complete visual field in optical microscopy. For sperm concentration analysis, a 5 µl aliquot of the original solution was diluted in 995 µL of formalin saline solution and analyzed in a Neubauer chamber. For the analysis of morphometric characteristics 5 µl of the semen was added in 45 µl of Rose Bengal staining. The slides were analyzed under an optical microscope with a camera attached (LEICA DM 2500), photomicrographs were taken, and one hundred spermatozoa per slide were analyzed, their length was measured using the LAS V4.0 software. The data obtained were analyzed through descriptive statistics using mean and standard deviation. An average of 80 ± 10.12% of sperm viability was found, which indicates these females could storage the sperm cells for long-term usage and how they achieve this feat must be deeply investigated. The mean values of motility found was 54 ± 16.11%, which had a variation of 30% to 70%, these mean values may be related to the reproductive system of *M. subnitida*. The queen bees only use small amounts of semen to fertilize their eggs so there is no competition between sperm over egg fertilization and, consequently, high motility rates are not imperative for the reproductive success. An average of $1{,}7x\ 10^6 \pm 8{,}0x10^5$ spermatozoa per spermatheca was found, in drone bees from other related species similar results were found. It is noteworthy that all animals used in this study were newly fertilized queen bees, therefore they carried the entire sperm load of only one male. The measured spermatozoa had a total length of 95.2 ± 4.3 µm, it was possible to identify that the *M. subnitida* spermatozoa have shape, dimensions, and structures like other Hymenoptera, they were characterized as a filamentous cell that both head and tail have the same diameter and a structural difference cannot be distinguished. In conclusion, the study of spermatozoa in *Melipona subnitida* queen bees spermathecae is a potential tool for understanding the species reproductive biology also can be used as a basis for the development of reproductive biotechnologies for stingless bees. Acknowledgements: CNPq and CAPES.

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Animal Reproduction

Comparative of morphokinetics bovine embryo development between time-lapse monitoring in microwells and conventional droplets in vitro culture

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Currently, embryo selection is based on morphological evaluation (shape, color and texture). However, this method is subjective and results in unreliable embryo selection. Nowadays, time-lapse monitoring in microwells is a non-invasive method that performs individual kinetics development monitoring and allows the evaluation of embryo morphology and development. Offering the opportunity to explore an improved method for embryo selection. Thus, our aim was to compare morphokinetics bovine embryo development in microwell with time-lapse monitoring and conventional droplet culture system. Cumulus-oocyte complexes surrounded by more than three layers of cumulus granulosa cells were selected for in vitro maturation and fertilization (IVF). At approximately 18 hours post-fertilization, presumptive zygotes (n=1214) were divided randomly into two groups: i) conventional droplet (n=926 in 19 replicates; 10 embryos/70 uL) and ii) time- lapse monitoring in microwell (n=288 in 18 replicates; one embryo/microwell). Both in vitro culture systems were cultured in an incubator at 38°C in a humidified atmosphere of 6% CO2 for seven days. Embryonic development evaluation was performed at three moments post IVF (IVF=Day 0): cleavage (day 3), morulae (day 5) and blastocyst rate (day 7). In addition, the time-lapse monitoring in microwell and a computer software was used to calculate the timing of following events: 2, 3, 4, 5, 8 cells (t2, t3, t4, t5, t8) and when the embryo formed into a morula and all cell boundaries are unobvious (tM), start of a cavity forming (tSB). Statistical analyses were performed using Statgraphics Centurion. The embryonic development was evaluated by analysis of variance (ANOVA). Statistically significant at $P<0.05$. Cleavage, morulae and blastocyst rates in microwell with time-lapse monitoring were higher ($P<0.05$) than conventional droplet system (83.7±7.6%; 54.9±13.2 and 42.7±13.4 versus 76.6±12.1%; 35.2±8.7% and 25.5±8.6% respectively). In addition, 121/288 (42.7%) arrived at the blastocyst stage. Being, the average times for t2 (29.5 ± 3.2 h); t4 (43.3 ± 6.9 h); t8 (67.2 ± 11.2 h); tM (111.2 ± 9.6 h) and tB (164.5 ± 10.7 h). Furthermore, we have recorded that a first division (t2) before 29.5h post IVF has a very high (65%) probability of reaching the blastocyst stage. In conclusion, in vitro embryo culture with time-lapse monitoring in microwell showed a better rate of blastocyst and allowed an objective, constant and non-invasive embryo assessment, which also could help discriminate competent embryos. Acknowledgements: This research was supported by Peru Grant FONDECYT 143-2020.

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Animal Reproduction

Comparison between two technics to assess bull sperm membrane integrity

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The use of bovine cryopreserved sperm in reproductive biotechnologies requires the use of straws containing spermatozoa of high quality. However, cryopreservation methods may lead to sperm damages such as plasmatic membranes lesions. The plasmatic membrane of sperm cells plays important role in the sperm capacitation and oocyte fertilization, besides exerting metabolic exchanges to the extender medium. For these reasons, integrity of sperm membrane is related to sperm motility, and so the evaluation of sperm membrane integrity is considered a relevant part of andrological exam. The sperm membrane integrity may be accessed by using fluorescent probes, which are expensive and requires equipment such as flow cytometry. As a cheaper and easier alternative, the use of vital dye, as eosin-nigrosin, stains the DNA of sperms presenting membrane damages, and only requires an optical microscope. Therefore, this study aimed to compare the assessment of plasmatic membrane integrity under eosin-nigrosin dye and propidium iodide performed in flow cytometry. Seventeen frozen thawed sperm straws from Nelore bulls we used. To the group 1 (G1), an aliquot of 3µL of each sperm sample were used to produce slides smears stained with eosin-nigrosin. Past five minutes, 200 sperm cells from each smear were analyzed under optical microscopy with a magnification of 100X, considering membrane damages when sperm was colored in pink. To group 2 (G2), 50µL of each sperm sample were diluted in TALP medium and 15µM of propidium iodide (PI) were added. To assess the sperm membrane integrity of G2, the samples were evaluated under flow cytometry equipped with argon (488nm pass) and red (640nm range) lasers. To compare the results of sperm membrane integrity from both groups, the Student's T test, with significance level set to $P<0.05$ was performed. To G1 (eosin-nigrosin) the membrane integrity mean was 92.17%±3.41. Yet, to G2 (flow cytometry), the mean of membrane integrity was 90.30%±4.62. Thus, no significant difference between the groups assessing the sperm membrane integrity was detected ($P=0.188$). Since there was no difference in using eosin-nigrosin or PI under flow cytometry, we can conclude that the eosin-nigrosin is a valid and reliable method to assess the integrity of plasmatic membrane of bulls' sperm. Acknowledgment: To CAPES for granting the post-graduation scholarship.

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Animal Reproduction

Comparison of Tris-egg yolk and INRA-96® extenders in the short-term preservation of canine sperm functional parameters

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Due to the low availability of commercial extenders for canine short-term preservation in South America, the search for alternative media is yet necessary, mainly by the adaptation of products previously used for other species. At this sense, the INRA-96® extender (IMV Technologies, L'Aigle, France), commercialized for equine semen preservation, raises as an option to be tested for dogs. Therefore, we aimed to evaluate the efficiency of INRA-96®, compared to the Tris-egg yolk extender, on the short-term preservation of canine semen functional parameters. Two ejaculates from nine dogs were collected by digital manipulation at a 15- days interval, totalizing 18 ejaculates. Sperm fractions were immediately evaluated for sperm motility kinetic parameters using a computerized system (CASA – IVOS 12.0, Hamilton-Thorne, Beverly, EUA), sperm membrane functionality using distilled water as the hypoosmotic solution (0 mOsm/L) and sperm-binding ability using a perivitelline membrane assay with the yolk of fresh and unfertilized chicken eggs. Samples were fractioned in different aliquots that were diluted in Tris-based extender plus egg yolk (20%) or the commercial diluent INRA-96®, at a final concentration of 200 x 10^6 sperm/mL, and incubated at 4 °C during 48 h. Samples were then rewarmed at 37 °C, and evaluated as reported for fresh semen. Data were expressed as mean ± SEM. The effect of incubation time (fresh vs. 48 h) during chilling on sperm parameters was evaluated by variance analysis (ANOVA) for repeated measures ($P < 0.05$). Comparisons among treatments were evaluated by ANOVA followed by the Student's t test ($P < 0.05$). Fresh samples presented 97.9 ± 0.8% motile sperm. After 48 hours, the total motility was better preserved ($P < 0,05$) in Tris (97.1 ± 0.5%) than in INRA-96® (88.1 ± 2.9%). Preservation in both Tris and INRA-96® extenders for 48 h provided a decrease ($P < 0.05$) on the values of velocity average pathway – VAP (130.0 ± 3.9 vs. 113.7 ± 7.6 µm/s), velocity straight line – VSL (105.5 ± 5.3 vs. 97.2 ± 5.8 µm/s), beat cross frequency – BCF (27.9 ± 1.1 vs. 28.6 ± 0.9 Hz) and linearity – LIN (48.9 ± 3.9 vs. 52.6 ± 2.5%) when compared to fresh semen (VAP = 157.5 ± 4.3 µm/s, VSL = 144.2 ± 4.2 µm/s, BCF = 34.5 ± 0.8 Hz e LIN = 72 ± 2.2%), but no differences between extenders were evidenced. Moreover, rapid sperm subpopulation was better preserved ($P < 0.05$) in Tris (70.9 ± 3.2%) than in INRA-96® (52.2 ± 8.3%), besides a higher proportion ($P < 0.05$) of static sperm was observed for INRA-96® (11.9 ± 2.9%) than in Tris (2.9 ± 0.5%). Both extenders were similar in the preservation of sperm membrane functionality with values of 80.6 ± 3.4% for Tris and 73.9 ± 3% for INRA-96® after 48 h, being these values lower ($P < 0.05$) than those observed for fresh samples (94.6 ± 0.6%). For the egg perivitelline membrane assay, fresh samples presented 283.8 ± 27.5 bound sperm. After 48 h, INRA-96® (256.2 ± 21.1 bound sperm) was effective in maintain canine sperm ability in values similar ($P > 0.05$) to those observed for fresh samples, but a significant reduction ($P < 0.05$) in this parameter was verified for Tris (215.2 ± 21 bound sperm). Despite a reduction in some parameters was observed, both extenders provided adequate values for total motility, membrane functionality and sperm-binding ability during canine semen short-term preservation. In conclusion, we demonstrated that the commercial extender INRA-96® could be used as an efficient alternative to the Tris-egg yolk extender for the preservation of canine semen parameters during 48 h under refrigeration.

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Animal Reproduction

Cryopreservation of collared peccary semen using Tris-based extender supplemented of sodium dodecyl sulfate detergents

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Semen cryopreservation represents the main tool used for the conservation and maintenance of the genetic material of wild species. Use of sodium dodecyl sulfate (SDS) detergents has been highlighted for improving sperm longevity after thawing in various mammals, but its effects lack to be investigated in collared peccaries (*Pecari tajacu* Linnaeus, 1758). Therefore, we aim to evaluate the effects of SDS detergents on the longevity of peccary frozen-thawed semen. Ejaculates from 10 mature individuals from the Wild Animal Multiplication Center (CEMAS-UFERSA) were obtained by electroejaculation (Autojac®, Neovet, Campinas, SP, Brazil) under anesthetic restraint (5 mg/kg; Propovan®, Cristália, São Paulo, Brazil). Fresh semen was evaluated for sperm motility using a computerized system (CASA – IVOS12.0, Hamilton-Thorne, Beverly, USA), membrane integrity and mitochondrial activity using fluorescent probes (Hoechst 342 – Sigma-Aldrich, St Louis, MO, USA; Mito Tracker red® – CMXRos, Molecular Probes; propidium iodide – Sigma-Aldrich, Co., St Louis, MO, USA), and membrane functionality using distilled water as the hypoosmotic solution (0 mOsm / L). Samples were diluted in a Tris-based extender plus egg yolk (20%) and divided in different aliquots, among which, one remained without detergent supplementation, as a negative control group. The other aliquots were supplemented with Equex STM 0.5% that is a commercial SDS-derived detergent (positive control), or with purified SDS at different concentrations (0.1%, 0.3% and 0.5%). They were then refrigerated to 5 ºC, added of 3% glycerol to reach a final concentration of 100 x 10^6 sperm/mL, packed in 0.25-mL plastic straws, and stored in liquid nitrogen. After one week, samples were thawed in a water bath at 37°C/1 min and subjected to a thermo-resistance test (TRT) at 37 ºC for up 60 min, being reevaluated every 30 min. Data was expressed as means ± SEM. Treatments were compared by ANOVA followed by Tukey's test ($P < 0.05$). Fresh samples presented 87.8 ± 4.4% motile sperm, with 87.6 ± 2.2% membrane integrity, 87.4 ± 2.1% mitochondrial activity and 86.0 ± 3.3% functional membrane. Immediately after thawing, all the treatments provided a sperm motility higher than 40%, except the group containing SDS 0.5% that presented lower values (31.2 ± 6.3%) than controls ($P < 0.05$). During TRT, a significant decrease on sperm motility values was found for all the groups ($P < 0.05$) that provided values lower than 10% after 60 min. Regarding other parameters, all the treatments provided proximately 40% sperm presenting membrane integrity, mitochondrial activity, and membrane functionality immediately after thawing. At 30 min, these parameters were effectively preserved at the presence or absence of SDS-derived detergents, but after 60 min, lowest values for membrane integrity (26.6 ± 5.4%) and mitochondrial activity (12.9 ± 3.3%) were observed at the use of the SDS 0.5% ($P < 0.05$). Apparently, the peccary sperm presents a short longevity after thawing and the use of Tris-extender supplemented with SDS detergents is not effective to provide an improvement on its qualitative parameters. On the contrary, the increase of SDS concentration to 0.5% impairs peccary post-thawing sperm quality. Financial support: CAPES and CNPq

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Animal Reproduction

Cryopreservation of ram semen with resveratrol associated or not to antifreeze protein type I

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Artificial insemination is a biotechnology that allows a greater impact in genetic improvement of all species and can be done using cryopreserved semen. For this process, cryoprotectants are used to reduce damage caused to the cells due to low temperatures. Among potential cryoprotectants, there are the antifreeze proteins (AFP), that protect cell membranes by reducing recrystallization and freezing temperature beyond fusion point; and resveratrol, which is a polyphenol and act as an antioxidant. Therefore, this study aimed to evaluate the effect of the synergic effect of of using resveratrol and AFP type I at cryopreservation media of ram semen. The study was approved by Ethics Committee for Use of Animals (5956101218) of Universidade Federal Fluminense. Four rams were used to semen collection by artificial vagina, for six days (replicates), after having their extra gonadal reserves previously renewed. Ejaculates were individually assessed for macroscopic characteristics and kinetic parameters, and those with ≥70% motility were pooled. The pool was then diluted in Tris-Egg Yolk-Glycerol extender with one of the six treatments: AFP – standard AFP concentration (0.1 µg/mL); R10 (10 µM resveratrol); AR10 (AFP I and 10 µM resveratrol); R50 (50 µM resveratrol); AR50 (AFP I and 50 µM resveratrol); and CONT (control group, containing only the extender). After dilution in each treatment, samples were evaluated for sperm kinetics (by objective Computer Assisted Semen Analysis, CASA), plasma membrane integrity (PMI), hypoosmotic test and mitochondrial activity and, then, semen was placed into plastic straws of 0.25 mL and cryopreserved in liquid nitrogen. Immediately after thawing, the same evaluations were performed, including sperm binding to egg perivitelline membrane test, lipoperoxidation quantification and capacitation test. Data were subjected to Shapiro-Wilk normality test and Levene homoscedasticity test. Parametric data were analyzed by ANOVA and Tukey test, while non- parametric data were analyzed by Kruskal Wallis and Dunn's test. The general linear model (GLM) was used to check the interaction between resveratrol and AFP I. Values were presented as mean ± standard error of mean. Regarding kinetics, there were differences ($P<0.05$) in sperm with rapid velocity between R50 and AR50 groups (0.3% ± 0.1 vs. 0.0% ± 0.0 respectively), being deleterious in AR50 group. There was no difference ($P>0.05$) among groups for PMI, however, the hypoosmotic test had better ($P<0.05$) results in R10 compared to AR10 (28.8% ± 2.3 vs. 20.5% ± 1.9 respectively). The AR10 (11.7% ± 1.6) and AR50 (13.0% ± 2.3) had lower values ($P<0.05$) of capacitated sperm when compared with AFP group (23.2% ± 3.9). Also, AR10 and AR50 had more reacted acrosomes (80.3% ± 2.3 and 81.8% ± 1.8 respectively) compared to AFP (69.6% ± 4.2), with R50 having better results when compared with AR50 (72.4% ± 2.9 vs. 81.8% ± 1.8 respectively). The sperm binding to egg perivitelline membrane test showed that R50 had better results ($P<0.05$) when compared to CONT (1750.0 mm2 ± 185.2 vs. 773.3 mm2 ± 214.0 respectively). Regarding mitochondrial activity, no differences ($P>0.05$) were found, but higher lipoperoxidation values were observed in AR50 treatment (659.6 ng/mL ± 137.3) when compared to R50 (474.7 ng/mL ± 67.6), and AFP (493.9 ng/mL ± 104.8). Results pointed to an improvement in fertilizing capacity, without changes in membrane integrity and mitochondrial activity, but when resveratrol was associated with AFP I, parameters such as sperm kinetics, cytoplasmatic membrane functionality, sperm capacitation and lipoperoxidation were reduced. Therefore, it is possible to conclude that the association of resveratrol with AFP I may not have beneficial effects, but resveratrol could be employed singly as potential agent in ram semen cryopreservation. Acknowledgements: This study was supported by CAPES (Finance Code 001), CNPq (Project 434302/2018- 0).

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Animal Reproduction

Cushioned centrifugation before cryopreservation of epididymal stallion spermatozoa

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Unexpected death, orchiectomy or traumatic injuries can prematurely end the stallion's reproductive life, since sperm harvesting from the cauda epididymis is the last chance to preserve spermatozoa. For cryopreservation, the centrifugation step aims to increase sperm concentration, although this may induce detrimental effects on sperm quality and also lead to loss of spermatozoa. In order to protect the cell and reduce sperm loss during centrifugation, semen can be under laid with dense colloid, called cushioned centrifugation. The purpose of this experiment was to determine the effect of cushioned centrifugation of frozen-thawed epididymal stallion sperm quality. Ten stallions were submitted to bilateral orchiectomy and the harvest of epididymal cauda (n=20) was performed by retrograde flushing with cooling extender. Epididymal sperm samples were submitted to two centrifugation protocols: Conventional centrifugation (20 minutes at 600x*g*) and Cushioned centrifugation (20 minutes at 1000x*g*). The pellet was resuspended in freezing extender at a final concentration of $100x10^6$ spermatozoa/mL, frozen in liquid nitrogen and thawed. After thawing, total motility and progressive motility were evaluated with computer-assisted semen analyses (CASA). Sperm morphology was evaluated by examining a wet mount preparation of unstained samples fixed in formol saline. Mitochondrial functionality, membrane integrity and DNA integrity were evaluated in a fluorescence microscope with specific fluorescent dyes. Data was evaluated by descriptive statistics (expressed by mean ± standard error mean, Kruskal-Wallis one-way test, analysis of variance (ANOVA) followed by comparison between means by Tuckey test. No significant difference was observed between treatments Conventional and Cushioned regarding total motility, progressive motility, sperm morphology, mitochondrial functionality, membrane integrity and DNA integrity. In conclusion, cushioned centrifugation performed before cryopreservation had neither a beneficial nor a detrimental effect on thawed epididymal stallion sperm quality. Acknowledgements: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Brasil – Finance code 001 for scholarship.

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Animal Reproduction

Description of sperm morphometry and morphology in rhea (*Rhea americana*)

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Rhea (*Rhea americana*) is a large bird of the order Rheiform, native of South America, distributed from northeastern Brazil to southern Argentina. Nowadays, the species is classified as almost threatened, which justifies the development of studies on the preservation of its germplasm. At this sense, knowledge related to its sperm morphology and physiology would provide us essential data for the comprehension of Rhea reproductive performance and the development of strategies for its conservation. This study aims to describe the sperm morphometry and morphology of the rhea sperm. Three adult males, weighting 25.1 kg on average, from the Centre for Multiplication of Wild Animals (CEMAS/UFERSA) were used. The individuals were part of a programmed slaughtered conducted for this research. For the experiments, individuals were premedicated with intramuscular (IM) administration of 2% xylazine hydrochloride (1mg/Kg) plus 10% ketamine hydrochloride (15mg/kg), anesthetized with IM thiopental 1g (150mg/Kg), and euthanized with intracardiac administration of potassium chloride 19.1% (2.56mEq/Kg), according to the institutional ethical committee recommendations (09/2020). The sperm were obtained from the deferens ducts reservoir by the flotation technique using the Ovodyl™ (IMV technologies, l'Aigle, France) extender. Suspension containing sperm cells was evaluated for concentration using a Neubauer counting chamber, motility and vigor by light microscopy. After sperm collection, slides stained with Bengal Rose (Chromato®, SP, Brazil) were made and 200 sperm per animal were evaluated. Images were captured using light microscopy (100x) connected to image analyzer software LAS EZ Ink. For morphometry analysis, sperm structures as head, mid-piece, and tail were separately measured. Morphologic defects were classified according to the region of the sperm, as head, midpiece or tail defects. Data were expressed as mean and standard error. The values found for concentration were 202 ± 80.40 x 10^6, while motility and vigor were respectively 46.67 ± 4.22% and 2.83 ± 0.40. Regarding sperm morphometry, filiform-shaped sperm with sharp acrosomes measuring about 0.96 ± 0.11 µm in length and long heads measuring 7.36 ± 0.43 µm in length and 0.56 ± 0.004 µm in width were observed. Connected to the head, there was a small midpiece with about 2.01 ± 1.38 µm and a long thread-shaped tail with 30.86 ±0.13 µm totaling about 40.26 ± 0.14 µm. A total of 82.09 ± 5.04% sperm presented normal morphology, while 17.92 ± 5.04% presented morphological defects. In the head region, defects were classified as 4.50 ± 3.00% bent heads, 0.37 ± 0.12% hooked heads and 4.25 ± 2.10% detached heads. For the midpiece, we found 0.75 ± 0.25% sperm with bent midpieces and 0.5 ± 0% cytoplasmic drops. The greatest number of morphological defects was found in tails as 6.58 ± 1.67% bent tails, 1.42 ± 0.92% broken tails and 1.42 ± 0.82% double tails. Some sperm were not yet fully matured but presenting a transition phase between spermatid and sperm cell, thus presenting a rounded head, representing about 0.25 ± 0%. In summary, this study provided novel data related to the standard values for the morphometric and morphological classification of the male gametes of rheas, which may become useful as a basis for future works that focus on ways to preserve the species. **Financial support:** CAPES and CNPq

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Animal Reproduction

Description of the CASA system configuration setup for giant anteater (*Myrmecophaga tridactyla*)

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The aim of the study was to develop and describe a preset for giant anteater's semen evaluation using the CASA system for a better understanding of the species and further studies. The semen was collected through urethral catheterization from two captive giant anteaters maintained at the Zoológico Municipal de Guarulhos (Guarulhos, SP, Brazil; 23°26'33.5"S 46°33'11.8"W). Semen samples were diluted with OptiXcell (IMV Technologies) and then, 3µL of the sample was loaded into Leja 4 chamber 20µL slides. The tests were conducted on the CEROS II Computer Assisted Semen Analysis (CASA) system with the Animal Breeders II software (Hamilton Thorne, USA) and saved for future preset standardization. The semen setup was made on an IVOS II computer using the same software, Animal Breeders II, at the subsidiary of IMV Technologies in Campinas, São Paulo, Brazil. Cell detection (head and tail recognition) was done through the "Live Overlay" tool. The parameters for kinematics and morphology were defined by adjusting the items on the playback screen of each video analysis. Closest to ideal numbers for cell recognition we found: Elongation Max = 65%, Elongation Min = 1%, Head Brightness Min = 121, Head Size Max = 52 µm^2, Head Size Min = 5 µm^2 , Tail Brightness Min = 81; for kinematics parameters: Cell Travel Max = 10 µm, Progressive STR = 80%, Progressive VAP = 70 µm/s, Slow VAP = 20 µm/s, Slow VSL = 30 µm/s, Static VAP = 4 µm/s, Static VSL = 1 µm/s, Static Width Multiplier = 0.5; and for morphology parameters: DMR Confidence = 50%, DMR Droplet To Tail End Max = 7 µm, DMR Tail Length Max = 20 µm, Droplet Confidence = 50%, Droplet Distal Distance Min = 4 µm, Droplet Proximal Head Length = 10.5 µm, Min Tail Length = 8 µm, Tail Bend Angle Averaging Length = 5 µm, Tail Bending Angle Rate Min 20 °/µm, Tail Bent Confidence = 50%, Tail Confidence = 20%. Having a specific configuration for species allows a better analysis of the sperm and true results. This is the first report of a specific setup on CASA system for giant anteaters. Agradecimentos: Zoológico Municipal de Guarulhos. IMV Technologies Brasil. O presente trabalho foi financiado em parte pelo Instituto Reprocon.

Palavras-chave: computer analysis, myrmecophagids, assisted reproduction, biotechnologies, spermatozoa, morphology.

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Animal Reproduction

Description of the CASA system configuration setup for grey short-tailed opossum (*Monodelphis domestica*)

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The aim of the study was to develop and describe a preset for grey short-tailed opossum's semen evaluation using the CASA system for a better understanding of the species and further studies. The semen was collected from one captive grey short-tailed opossum maintained at the Central Vivarium of the Federal University of Mato Grosso do Sul (UFMS; 20°30'22.4"S 54°36'53.9"W). The animal was anesthetized with ketamine (100mg/kg; IP) and xylazine (10mg/kg; IP) and euthanized. For sperm recovery, the tail of the epididymis and part of the *vas deferens* was removed and placed into a 35mm petri dish. A 1mL syringe containing EasiBuffer B (IMV Technologies) coupled with a 32g needle without the bevel was inserted into the *vas deferens*, and 500µL was injected into each duct. Then, 3µL of the sample was loaded into Leja 4 chamber 20µm slides. The tests were conducted on the IVOS II Computer Assisted Semen Analysis (CASA) system with the Animal Breeders II software (v 1.11.9; Hamilton Thorne, USA) and saved for future preset standardization, performed in the same model equipment, at the subsidiary of IMV Technologies in Campinas, São Paulo, Brazil. Cell detection (head and tail recognition) was done through the "Live Overlay" tool. The parameters for kinematics and morphology were defined by adjusting the items on the playback screen of each video analysis. Closest to ideal numbers for cell recognition we found: Elongation Max = 90%, Elongation Min = 1%, Head Brightness Min = 165, Head Size Max = 70 µm^2, Head Size Min = 6 µm^2 , Tail Brightness Min = 96; for kinematics parameters: Cell Travel Max = 15 µm, Progressive STR = 60%, Progressive VAP = 40 µm/s, Slow VAP = 20 µm/s, Slow VSL = 2 µm/s, Static VAP = 5 µm/s, Static VSL = 1 µm/s, Static Width Multiplier = 0.5; and for morphology parameters: DMR Confidence = 50%, DMR Droplet To Tail End Max = 5 µm, DMR Tail Length Max = 15 µm, Droplet Confidence = 50%, Droplet Distal Distance Min = 4 µm, Droplet Proximal Head Length = 12 µm, Min Tail Length = 4 µm, Tail Bend Angle Averaging Length = 5 µm, Tail Bending Angle Rate Min 20 °/µm, Tail Bent Confidence = 50%, Tail Confidence = 20%. Two-tailed sperm were found, but most had only one tail. This is an initial setup to be used as a base. Improvement will be needed by evaluating semen from more males. Having a specific configuration for species allows a better analysis of the sperm and true results. This is the first report of a specific setup on the CASA system for grey short-tailed opossum. Acknowledgment: The present study was performed with support from the Federal University of Mato Grosso do Sul - UFMS/MEC - Brazil, with support from the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 and from the Central Vivarium - INBIO/UFMS and was funded in part by the Reprocon Institute and IMV Technologies.

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Animal Reproduction

Do different salinities influence sperm activation in salt-water rays' species?

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According to the IUCN (2014), 25% of all elasmobranch species are threatened with extinction. Reproductive technologies will become an increasingly important component of breeding programs for elasmobranchs, emphasizing self-sustaining aquarium populations and conservation efforts. The use of reproductive technologies in many terrestrial and aquatic species is widely described, but little is known about rays' artificial reproduction. Although there is little information available about the proper handling conditions required for observing the activity of elasmobranch sperm, studies to date have used ionic solutions composts to raise the osmotic pressure. Additionally, it is being known that some elasmobranchs species go to estuarine areas for activities that ensure the perpetuation of the species. The aim of this study is to describe methods for sperm activation and semen evaluation of two batoids species – whitespotted eagle ray (*Aetobatus narinari*) and spiny butterfly ray (*Gymnura altavela*) – maintained at the AquaRio (Rio de Janeiro, RJ, Brazil; 22°53'36.0"S 43°11'31.8"W; SISGEN #A0884E4). The semen was collected by exerting gentle pressure on the distal reproductive tract, proximal to the cloaca. To assess the sperm motility, fresh seminal fluid samples were tested to compare the activation of the spermatozoa with dilutions made of hypersaline water (56, 45, 42, 37, and 28±1 ppt) by adding commercial salt (Instant Ocean, USA) to rearing tank water, as well with rearing tank water dilutions (27, 13, 9, 7, 5 and 0±1 ppt), achieved by deionized water addition. Semen samples were diluted for proper concentration for CASA analysis at a ratio of 1:20 (v/v) for *A. narinari* and 1:2 (v/v) for *G. altavela.* Then, 3µL of the sample was loaded into Leja 4 chamber 20µm slides. The tests were conducted on the CEROS II Computer Assisted Semen Analysis (CASA) system with the Animal Breeders II software (v 1.13; Hamilton Thorne, USA) and saved for future preset standardization at IMV Technologies Brazil. Cell detection (head and tail recognition) was done through the "Live Overlay" tool. For *A. narinari,* results of sperm motility (SM) and progressive movement (PM) are 20,2% SM and 1,9% PM, 21,4% SM and 1,1% PM, 24,5% SM and 1,6%PM, 31,1% SM and 3,4% PM, 25,7% SM and 1,7% PM, 25,4% SM and 1,1% PM, 15% SM and 1,3% PM, 16,9% SM and 1,6% PM, 8,4% SM and 0,9% PM, 7,5%SM and 1,0% PM, 4,8% SM and 0,0% PM for 56ppt, 45ppt, 42ppt, 37ppt, 28ppt, 27ppt, 13ppt, 9ppt, 7ppt, 5 ppt and 0ppt, respectively. For *G. altavela* results of total sperm motility (SM) and progressive movement (PM) are 4,3% SM and 0,7% PM, 5,1% SM and 0,3% PM, 11,5% SM and 0,0% PM, 55,5% SM and 6,3%PM, 63,3% SM and 8,7% PM 64,1%SM and 26,6% PM, 64,6% SM and 25,3% PM, 45,5% SM and 3,1% PM, 22,5% SM and 0,9% PM, 9,5% SM and 0,3% PM, 7,0%SM and 1,0% PM for 56ppt, 45ppt, 42ppt, 37ppt, 28ppt, 27ppt, 13ppt, 9ppt, 7ppt, 5 ppt and 0ppt, respectively. In conclusion, for *A. narinari*, water salinity between 27 and 42 ppt results in good total motility of the spermatozoa, but at 37ppt, it presents the best progressive motility. In *G. altavela,* the total sperm motility achieves its best between 13 and 37ppt, but the best progressive motility was between 13 and 27 ppt. These findings demonstrate the diverse adaptative physiology of elasmobranchs, and it is the first report of how salinity could activate the spermatozoa of rays. Acknowledgment: AquaRio. IMV Technologies Brazil. This work was partially funded by Instituto Reprocon, IMV Technologies Brasil, and Grupo Cataratas.

Key words: Myliobatiformes; Batoidea; sperm viability; sperm motility; progressive movement; salinity; CASA

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Animal Reproduction

Effect of all-trans retinal (RAL) on *in vitro* maturation of sheep oocytes and *in vitro* embryo production: preliminary results

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All-trans retinal acid (RAL) is a vitamin A precursor that has not been explored well as other retinoids on *in vitro* maturation (IVM) media. This study aimed to evaluate the effect of including RAL as an antioxidant on *in vitro* production of sheep embryos. For this purpose, oocytes were aspirated from abattoir ovaries of sheep by using a vacuum pump. After oocyte classification, the best quality cumulus-oocyte complexes (COCs) were kept in the follicular fluid until maturation. The total of selected COCs was equally divided into four maturation groups: CIS group, composed of oocytes matured in a medium composed of TCM-199, supplemented with antibiotics and antimycotic, 0.2 mM sodium pyruvate, 10% (v/ v) fetal bovine serum (FBS), 10 ng/ml EGF, 10 µg/ml FSH, 10 µg/ml LH and 10 µg/ml estradiol and 100 mM cysteamine; and, in RAL1.5, RAL3 and RAL6 groups, with the same medium as CIS group, but without adding cysteamine and including 1.5 µM, 3.0 µM and 6.0 µM of RAL, respectively. COCs were placed in MIV medium, under mineral oil, for 24 hours in a CO_2 incubator, at 38.5ºC, in a humidified atmosphere with 5% CO_2 and saturated humidity. Mature oocytes were evaluated from cumulus cell expansion; after that, the oocytes went to *in vitro* fertilization (IVF), together with selected and trained semen for a period of 18 to 20 hours, in a CO_2 incubator, at 38.5ºC, with a humidified atmosphere, containing 5% of CO_2. Presumptive zygotes (PZ) were evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, the presumptive zygotes proceeded to *in vitro* embryo culture (IVC) with SOF medium supplemented with 3 mg/mL of BSA, and cleavages were evaluated at D1 and D2. The conditions of the IVC were the same used in the IVM and IVF. One-way ANOVA was used to compare the parameters between groups, followed by the Tukey test. Percentage data were submitted to the Fisher's exact test ($P < 0.05$). A total of 91 oocytes were submitted to IVM, there was no significant difference between the treatment groups when compared to CIS group regarding the rate of expansion of cumulus cells (CIS: 75.00%; RAL1.5: 94.74%; RAL3.0: 100.00%; RAL6.0: 92.00%). Likewise, there was no significant difference ($P>0.05$) between treatment groups for any degree of cumulus cell expansion. However, comparing the degrees of expansion of cumulus cells within each treatment group, a significantly higher number of oocytes with high expansion was observed in the group of oocytes matured in the presence of 1.5 µM retinal (High: 5.50± 0.35; Moderate: 2.50±0.35; Mild: 1.00±0.00), with no significant difference observed for the other treatment groups. As for the successful fertilization, the RAL6.0 group (RAL6.0: 5.50±1.06) had a significantly higher number of presumptive zygotes when compared to the RAL1.5 group (RAL1.5: 1.50±0, 35), no difference between the other groups were observed. However, there was no difference between the treatment groups regarding the number of cleaved structures (CIS: 0.50±0.35; RAL1.5: 0.50±0.35; RAL3.0: 0.00±0.00; RAL6.0: 0.50±0.35). Based on the above, it is concluded that, to date and with preliminary results, the addition of all-trans-retinal acid, in none of the concentrations tested, interferes with the *in vitro* maturation of ovine oocytes compared to the use of cysteamine; however, the use of RAL at a concentration of 6 µM showed superior performance in the number of presumptive zygotes after *in vitro* fertilization. Further repetitions and studies are needed to elucidate the possible mechanisms of using RAL as an effective alternative to cysteamine.

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Animal Reproduction

Effect of an exogenous Galectin-1 dose on the beef cattle cow's pregnancy rate

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Galectins are a family of evolutionarily conserved proteins distributed from lower invertebrates to mammals and found in several tissues and cells and they are cited in the literature as multifunctional molecules that participate in several biological processes such as adhesion, proliferation and cell cycle, apoptosis, RNA processing, control of the inflammatory processes, and physiological mechanisms of reproduction. One of them, the Galectin-1 is a 14kDa lectin, with high affinity to carbohydrates (beta-galactoside and lactose), expressed different tissues (cardiac, smooth, and skeletal muscle, neurons, thymus, kidney, hematopoietic cells) and with a considerable mRNA and protein expression in endometrium and placenta (in trophoblasts, stromal cells, villous endothelium, syncytiotrophoblast apical membrane and villous stroma, maternal decidua and fetal membranes), and in fetal membranes (amnion, chorioamniotic mesenchyma and chorion). Gal-1 is implicated to maternal-fetal tolerance, both innate and adaptive, is associated with regulating and modulating the embryo elongation events' immunological responses and adherence to the endometrium, and it also contribute to placentation as they regulate the development, migration, and trophoblastic invasion, essential in early gestational development. Because of that GAL-1 has been cited as a mediator involved in preventing early embryonic death in mammals. An effective dose of exogenous GAL-1 (eGAL-1) means 01 (one) dose of Tolerana® (Inprenha Biotecnologia®, MAPA register no. SP 000104-0.000001) and its administration is similar to the semen deposition procedure, during a Timed artificial insemination (TAI) procedure. One dose of eGAL-1 contain 200±10µg of recombinant GAL-1, diluted in 200µL of sterile PBS 1X pH 7.0 solution with 0.25mg. mL^{-1} kanamycin sulfate in a 0.25mL French-type straw. The goal of this study was to evaluate whether eGAL-1 can improve the pregnancy rate after a complementary AI procedure. The pregnancy rate was analyzed by determining the pregnancy status by ultrasound diagnosis (UD), 25 to 35 days after TAI of dams (n=3,469 beef cows). The effectiveness of eGAL-1 was evaluated by comparing the pregnancy rate of two groups (Treatment and Control Groups) in 107 contemporaries' groups (YG) established by the statistical model formed. The experiments were conducted on 17 commercial beef cattle farms, located in different municipalities in Brazil and considering only dams that keep the body score condition (BSC) from 3.5 to 2.5 between TAI and UD. The YGs were composed of animals inseminated by the same technician, within the same farm, same animal category (M=multiparous; P=primiparous and H=heifers), into the management group (identified by date of TAI procedure + FARM code + management group code); using the same semen batch and breed's dam (N = Nellore and CB = crossbreed). Only YG with more than 5 animals or with phenotypic variation (pregnancy rate different from 0 or 100%) were included on the statistical analyses. To perform the analyses a Generalized Linear Model (GLM) was applied, with GENMOD procedure of SAS (version 9.3), assuming a binomial distribution (pregnant or not pregnant) for the residual effect and a logarithmic link function (PROBIT). The model included the fixed effect of YG and treatment (dose 0 or dose 200). Based on this, the single dose of eGAL-1 and correct administration can increase the probability of obtaining the pregnancy rate in beef cattle up to 8.68% ($p < 0.0001$). The recommendation to use a dose of eGAL1 during a TAI procedure was reasonable in beef cattle routine. In average, the whole procedure, using eGAL-1, spent 10 ± 5 seconds more time than the conventional procedure.

Keywords: Tolerana®, Lectins, Galectin-1, Pregnancy Rate, Clinical trial

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Animal Reproduction

Effect of cilostazol on *in vitro* production of sheep embryos: preliminary results

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Cilostazol (CIL) is a cyclic adenosine monophosphate (cAMP) modulator that influences and improves nuclear and cytoplasmic *in vitro* maturation. However, its use on *in vitro* maturation media (IVM) of ovine oocytes has not yet been reported. This study aimed to evaluate the effect of using cilostazol in the maturation medium of ovine oocytes at different concentrations (0.3 µM, 1 µM, and 10 µM), on the oocyte maturation rate, on the quality of maturation, and the *in vitro* embryo production. For this purpose, ovine ovaries were collected from local abattoir and oocytes were aspirated using a vacuum pump, then poured in Petri dishes, analyzed under a stereomicroscope and taken to *in vitro* maturation (IVM), being divided into four groups: CIS group where cumulus-oocyte complexes (COCs) were immersed in a medium composed of TCM-199, supplemented with antibiotics and antimycotic, 0.2 mM sodium pyruvate, 10% (v/v) fetal bovine serum (SFB), 10 ng/ml EGF, 10 µg/ml FSH, 10 µg/ml LH and 10 µg/ml estradiol and 100 mM cysteamine; and CIL0.3; CIL1 and CIL10 groups, where COCs were immersed in the same medium as the CIS group, without adding cysteamine but including 0.3; 1 and 10 µM of cilostazol, respectively. COCs were placed in a Petri dish with 15 COCs per drop of 75 µL of IVM medium, for 24 hours, in a CO2 incubator, at 38.5ºC in a humidified atmosphere with 5% CO2. Matured oocytes were evaluated from cumulus cell expansion, being classified as High, Moderate, and Mild. After that, oocytes were proceeded to *in vitro* fertilization (IVF), together with selected semen for a period of 18 to 20 hours, in a CO2 incubator, at 38.5ºC, with a humidified atmosphere, containing 5% of CO2. Presumptive zygotes were denuded by successive pipetting, evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, presumptive zygotes were followed for *in vitro* embryo culture (IVC) with SOF medium supplemented with 3 mg/mL of BSA, and cleavages were evaluated at D1 and D2. The conditions of the IVC were the same used in the IVM and IVF. One-way ANOVA was used to compare the parameters between groups, followed by the Tukey test. Percentage data were submitted to Fisher's exact test ($P < 0.05$). A total of 70 oocytes were submitted to IVM. There was no significant difference of expansion rate ($P < 0.05$) between oocytes treated with 0.3 µM, 1.0 µM and 10.0 µM of cilostazol when compared to control group with cysteamine (CIS: 100%, CIL 0.3: 100%, CIL 1: 100%, CIL 10:100%). Likewise, there was no difference ($P < 0.05$) also regarding the degrees of expansion, slight (CIS: 1.00 ± 0.70, CIL 0.3: 3.50 ± 0.35, CIL 1: 2 .50±1.06, CIL 10: 3.00±0.00), moderate (CIS: 3.00±2.12, CIL 0.3:4.00±1.41, CIL 1: 3.00 ±1.41, CIL 10: 4.00±1.41) and high (CIS: 2.00±1.41, CIL 0.3: 2.00±0.70, CIL 1: 3.00±0.70, CIL 10: 4.00±0.70). Regarding fertilization, it was possible to verify that there was no difference ($P < 0.05$) in relation to the number of presumptive zygotes (CIS: 2.50±1.76, CIL 0.3: 0.50±0.35, CIL 1: 0.00±0.00, CIL 10: 1.00±0.70) and also on the cleavage rates (CIS: 3.50±2.47, CIL 0.3: 0.50±0.35, CIL 1: 0.00±0.00, CIL 10: 2.00±1.41). Thus, it is possible to conclude that, to date with preliminary results, cilostazol at different concentrations (0.3 µM, 1.0 µM, and 10 µM), can replace cysteamine in oocyte maturation medium of sheep, promoting quality oocyte maturation and *in vitro* production of sheep embryos.

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Animal Reproduction

Effect of Estradiol Benzoate on oxidative status of healthy and endometritis-affected mares

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Since estrogen has been pointed out as a possible antioxidant, this study aimed to verify the effect of estradiol benzoate administration on oxidative stress parameters quantified in the serum of mares affected by endometritis and healthy ones. For this purpose, 12 mares of the Mangalarga Marchador breed aged between 5-10 years were used. Females were divided into two groups, being classified as non-affected (G1, *n*=6) and sick (G2, *n*=6), according to the diagnosis for endometritis, performed prior to the start of treatment with estrogen and carried out through the association of cytological examination and bacteriological/fungal culture. The experimental design used was completely randomized, with six replications. The treatments were arranged in a 2 x 3 factorial scheme**,** being the first factor constituted by the uterine status and the second by sampling moments. The experiment was carried out in the city of Visconde de Rio Branco-MG, Brazil (Latitude: 21° 1' 2" South, Longitude: 42° 50' 16" West). Intramuscular (IM) 10mL of Estradiol Benzoate (Ric-Be®, Agener União Saúde Animal, São Paulo, SP, Brazil) were administered at a concentrate on of 1mg/mL for all mares. Blood samples were collected in both groups at three different times: immediately before drug application (M1), after 48 hours (M2) and after 96 hours (M3). For this purpose, plastic tubes with clot activator were used in the collection, performed through jugular puncture. The blood samples obtained were centrifuged at 3000 rpm for 10 minutes to obtain serum, aliquoted and then frozen at -20°C for further analysis. All quantifications related to the activity of oxidizing and antioxidants substances (superoxide dismutase-SOD, reduced and oxidized glutathione, catalase-CAT, thiobarbituric acid reactive substances, total protein, total antioxidant capacity and nitric oxide- NO) were carried out at the Laboratory of Immunochemistry and Glycobiology at the Department of Biology, in the Federal University of Viçosa, Viçosa, MG. Data were subjected to analysis of variance and means compared by Duncan's test ($p \leq 0.05$). Higher CAT values (133.42U/mg protein**)** were obtained in the analysis of serum samples from G1 in M3, whereas in M2, high values of SOD (54.45 U/mg protein) were verified in the group of sick mares. In the analysis between groups, difference in SOD values and NO were found, with the highest means observed in G2, M2 (54.45 U/mg protein and 4.03µM respectively). The analyzed results indicate that mares with endometritis undergo an oxidative process, evidenced in the present work by the increase of NO and SOD in G2. Activation of the immune system in response to endometritis associated with excess of NO production is part of the body's defense mechanism. The excess of pro-oxidants, in turn, is responsible for the imbalance of the antioxidant pathway, possibly associated with the observed increase in SOD. The enzymes CAT and SOD are key factors in the control of reactive oxygen species, and agents capable of modulating them may be useful as therapeutic intermediates in disorders associated with oxidative stress, such as endometritis. In the study, it was shown that Estradiol Benzoate (10mL, 1mg/mL, IM) has a direct effect on the serum antioxidant enzyme activity of CAT and SOD, however, further investigations on the subject are needed. Acknowledgments: This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001

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Animal Reproduction

Effect of follicular wave moment on AFC and its repeatability of Nelore and Caracu prepubertal heifers

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Antral Follicle Count (AFC) is the evaluation of the ovarian antral follicles number (≥ 2 mm) presents in the ovaries at the same time. This is an attribute that doesn't follow the same pattern among bovine genetic groups and is highly variable between individuals. Despite this, it has high repeatability in the same animal (Burns et al. 2005. Biol Reprod 73:54-62; Ireland et al. 2007. Hum Reprod 22:1687-1695) but regarding this characteristic in prepubertal heifers and its repeatability at different follicular wave moment, there're conflicting reports. The objective of this study was to evaluate the effects of antral follicular wave moment (emergence and dominance) on the AFC and its repeatability in prepubertal heifers of different genetic groups. Nelore (n = 30) and Caracu (n = 28) heifers were previously selected by B-Mode ovarian ultrasound evaluations that confirmed the condition of prepubertal anestrus (absence of corpora lutea). The mean age of Nelore heifers was 468 ± 25 days (between 395 and 518 days) and for Caracu heifers, 468 ± 20 days (between 404 and 504 days). Nelore heifers weighed between 186 and 284 kg (average of 236.73 kg), while Caracu heifers had body weight between 214 and 317 kg (average of 282.14 kg). A sequence of eleven managements was conducted every 48 hours (Day 0 – random day of the antral follicular wave, until Day 20) to study the AFC of both ovaries. Antral follicles were identified and quantified by mapping the location of each structure. Data obtained were compared according to the moments of the antral follicular wave, which were defined by the absence or presence of a dominant follicle (DF) in at least one of the ovaries, with a diameter ≥ 6.2 mm for Nelore and ≥ 8.5 mm for Caracu breed. Statistical analysis included fixed effects of evaluation days, breeds and their statistical interaction, being performed by the MIXED, GENMOD, CORR and GLM procedures of the SAS statistical program ($P < 0.05$). Regardless of the antral follicular wave moment, the total AFC was greater in prepubertal Nelore heifers compared to Caracu ones ($P = 0.0004$ and < 0.0001, respectively) in all study days. The follicular wave moment also influenced the evaluation, and the total AFC was greater ($P < 0.05$) in females with DF (dominant phase of the antral follicular wave). The AFC repeatability in prepubertal Nelore and Caracu heifers were 0.76 and 0.74, respectively ($P < 0.0001$). In conclusion, although the genetic group and the antral follicular wave moment exert effects on the AFC, it has high repeatability.

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Animal Reproduction

Effect of glycine on *in vitro* production of sheep embryos: preliminary results

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Reactive oxygen species (ROS) are involved in a series of degenerative processes, due to the property of generating free radicals. The formation of ROS in the *in vitro* embryo production (IVP) is a significant obstacle, requiring the use of antioxidants such as glycine. Therefore, the objective of this study was to test the effect of three concentrations of glycine (3, 6 and 12 mM) in the *in vitro* maturation medium of ovine oocytes on the *in vitro* production of embryos. Oocytes from ovaries from a local slaughterhouse were collected. For the oocyte collection, the follicular aspiration method was used, with an 18G needle coupled to a vacuum pump. Right after collection, the oocytes were poured into petri dishes, analyzed under a stereomicroscope and forwarded to *in vitro* maturation (IVM), where four experimental groups were tested: CON group, where the COCs were immersed in TCM-199, supplemented with sodium pyruvate, fetal bovine serum, antibiotic-antimycotic solution, FSH / LH and 100 mM cysteamine; GLI3, GLI6 and GLI12 groups, where the COCs were included in the medium of the CON group, replacing cysteamine by 3 (GLI3), 6 (GLI6) and 12 (GLI12) mM of glycine, respectively. The drops of IVM were covered with mineral oil and incubated for 24 hours, at 38.5°C, with 5% of CO2. Matured oocytes were evaluated for the degree of expansion of the cumulus cells, being classified as: High, Moderate and Mild and then destined for *in vitro* fertilization (IVF), where they were placed in drops with IVF medium, together with selected spermatozoa and capacitated in SOF medium supplemented with 10 µg/mL of sodium heparin and 10%(v/v) of FBS. After the evaluation of the presumptive zygotes, they were switched to *in vitro* culture medium (IVC) composed of SOF medium supplemented with BSA and cleavages in D1 and D2 were evaluated. The conditions of the IVC were the same as for IVM and IVF. Data were submitted to Analysis of Variance, followed by Tukey's Test and Fisher's Exact Test, with a significance level of 5%. Considering the comparison between the treatment groups regarding the presence and absence of expansion of cumulus cells, there was no significant difference. Regarding the comparison within the treatment groups, there was no significant difference regarding the presence and absence of cumulus cells for the CON (with expansion: 11.00±2.82; without expansion: 0.50±0.35), GLI3 (with expansion: 7.50±2.47; without expansion: 3.00±2.12) and GLI12 (with expansion: 9.00±1.41; without expansion: 1.50±1.06) groups. However, within the GLI6 treatment group, a significant increase in oocytes with expansion of cumulus cells was observed (with expansion: 9.50±1.06; without expansion: 1.00±0.70). Analyzing the comparison between the treatment groups regarding the degree of High, Moderate and Mild expansion, there was no significant difference. Regarding the comparison within each treatment group, there was no significant difference in the CON (High: 4.5±1.76; Moderate: 5.0±1.41 and Mild: 1.5±0.35), GLI3 (High: 2.0±0.70; Moderate: 4.5± 1.76 and Mild: 1.0±0.0) and GLI12 (High: 1.5±0.35; Moderate: 6.0±0.70 and Mild: 1.5±1.06) groups. However, within the GLI6 treatment group, a greater number of oocytes with degree of expansion mild was observed (High: 1.5±0.35; Moderate: 2.0±0.0 and Mild: 6.0±0.70). Regarding the number of presumptive zygotes (CON: 5.00±0.70; GLI3: 3.00±0.70; GLI6: 5.00±0.70 and GLI12: 2.00±0.0) and number of cleaved structures (CON: 8.50±2.47; GLI3: 6.00±2.82; GLI6: 6.50±0.35 and GLI12: 2.00±0.0), there was no significant difference. With these results, it can be concluded that, to date, the addition of glycine in the IVM medium does not improve the oocyte rate with expansion of cumulus cells. With regard to presumptive zygotes and cleaved structures, glycine may become an option for improving the quality of oocytes and, consequently, the in vitro production of ovine embryos. Later sessions will likely elucidate some questions that have not yet been answered.

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Animal Reproduction

Effect of heat shock protein A5 (HSPA5) on maturation oocyte during bovine in vitro embryo production

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The oviduct provides the required structural environment and nutritional support for fertilization and early embryonic development. During ovulation, cumulus-oocyte- complexes (COCs) are released in the oviduct where the final oocyte maturation and fertilization occurs. Proteins derived from oviductal fluid (OF) have shown an active role in the reproductive physiological process and, the HSPA5 protein is found in high abundance in bovine OF. Then, the present study aimed to investigate if the addition of HSPA5 in the last four hours of COCs in vitro maturation (IVM) could impact the further embryo produced. For this, COCs were recovery from ovaries obtained in a slaughterhouse (n= 20 COCs/group) and in vitro matured for 20 hours in a basic medium (BM). After that, the medium was partially replaced with BM + recombinant rHSPA5 (rHSPA5; 100 ng/ml) or to a new BM (control) and the IVM was carried out until to completed 24 hours. The matured COCs were followed to the in vitro fertilization (IVF) and in vitro culture (IVC) until 7 days. We investigated the blastocyst rate and the gene expression of the embryos. We did not find any difference in the blastocyst rates (P= 0.13); however, we figure out the upregulation of 18 genes involved in embryo quality, DNA methylation, cell growth, cellular development, and oxidative stress in the embryos produced from COCs treated with rHSPA5. Taken together, these results show that the addition of rHSPA5 in the final stage of oocyte IVM might play a control role on embryo development modulating in low-scale the embryonic transcriptional profile.

Keywords: GRP78, heat shock protein, gene expression, cattle, embryo production.

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Animal Reproduction

Effect of myo-inositol on *in vitro* production of sheep zygotes: preliminary results

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The success of *in vitro* embryo production (IVP) is hampered by oxidative processes in oocytes, caused by free radicals, requiring the use of antioxidants, such as myo-inositol (MI). Myo-inositol (MI) is defined as a stereoisomer of inositol, acting in the maintenance of cell osmolarity by presenting an antioxidant action and being considered an osmotic regulator, showing an important role in oocyte maturation and sperm capacitation, through its role in the regulation of intracytoplasmic calcium and, when used in culture medium, promotes notable advances in pre-implantation development in mammalian species. In this way, the objective was to evaluate the effect of including three concentrations of myo-inositol (20.0, 30.0 and 40.0 mM) in the oocyte *in vitro* maturation medium on the oocyte maturation rate, oocyte maturation quality and the *in vitro* production of ovine embryos. Oocyte collection was performed by follicular aspiration, using a vacuum pump and from ovaries obtained from a local slaughterhouse. The oocytes were poured into a petri dish, analyzed under a stereomicroscope and forwarded to *in vitro* maturation (IVM), being divided into four groups: CON group, oocytes were matured in medium containing TCM-199, sodium pyruvate, fetal bovine serum, antibiotic-antimycotic solution, FSH / LH and 100 mM cysteamine; groups MIO20, MIO30 and MIO40, the oocytes were matured in the medium of the CON group, supplemented with 20.0, 30.0 and 40.0 mM myo-inositol, respectively. The drops of IVM were covered with mineral oil and incubated for 24 hours, at 38.5°C, with 5% of CO_2. After that, the oocytes were evaluated for the degree of expansion of the cumulus cells, being classified as: High, Moderate and Slight and then destined for *in vitro* fertilization (IVF), where they were placed in drops with IVF medium, together with selected spermatozoa and capacitated in SOF medium supplemented with 10 µg/mL of sodium heparin and 10%(v/v) of FBS. After evaluating the presumptive zygotes (PZ), they were switched to *in vitro* culture medium (IVC) composed of SOF medium supplemented with bovine serum albumin and cleavages at D1 and D2 were evaluated. The conditions of the IVC were the same as for IVM and IVF. Data were submitted to Analysis of Variance, followed by Tukey's Test and Fisher's Exact Test, with a significance level of 5%. Taking into account the comparison between the treatment groups regarding the presence and absence of expansion of cumulus cells, there was no significant difference ($P>0.05$). Regarding the comparison within the treatment groups, there was no significant difference ($P>0.05$) regarding the relationship between the presence and absence of cumulus cells for the CON group (with expansion: 8.00±3.07; without expansion: 1.00±0.76). However, within the treatment groups MIO20, MIO30 and MIO40, a significant increase in oocytes was observed with expansion of cumulus cells (MIO20: 11.33±1.28; MIO30: 11.00±1.15; MIO40: 10.33±2.05) and for oocytes without cumulus cell expansion (MIO20: 0.66±0.51; MIO30: 1.00±0.76; MIO40: 1.66±0.64). Regarding the degree of expansion of cumulus cells between the treatment groups, there was no significant difference ($P>0.05$). As for the comparison within each treatment group, there was no significant difference in the CON groups (High: 2.00±0.76; Moderate: 3.33±1.28 and Slight: 2.66±1.02), MIO20 (High: 2.00±0.76; Moderate: 5.00±0.00 and Slight: 4.33±0.51), MIO30 (High: 3.66±0.51; Moderate: 4.00±0.76 and slight: 3.33±1.28) and MIO40 (High: 2.00±1.15; Moderate: 4.00±1.53 and Slight: 4.33±1.28). As for the number of PZ (CON: 3.00±2.12; MIO20: 5.50±0.35; MIO30: 4.50±1.06; MIO40: 4.50±1.76) and to the number of cleaved structures (CON: 0.00±0.00; MIO20: 3.00±1.41; MIO30: 3.50±1.76; MIO40: 2.00±0.00), there was no significant difference ($P>0.05$). Based on this information, it can be concluded that, to date and in possession of preliminary results, myo-inositol showed an effective antioxidant action, in addition to acting in a relevant way in oocyte maturation, maintaining and improving the quality of oocyte maturation. Furthermore, it can also be used in the *in vitro* production of sheep zygotes. Later sessions will likely elucidate some questions that have not yet been answered.

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Animal Reproduction

Effect of pre-maturation culture using NPPC on cumulus cells gene expression profile and acquisition of embryo developmental competence

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Prematuration (PM) of oocytes in the presence of drugs that temporarily block meiosis gives additional time to improve the acquisition of oocyte competence *in vitro*, as this strategy avoids early chromatin condensation, which favors the accumulation of transcripts and cytoplasmic reorganization. The aim of this study was to evaluate the effect of the meiotic inhibitor C-type natriuretic peptide precursor (NPPC) during a PM culture of bovine oocytes on: 1) progress of nuclear maturation; 2) gene expression in cumulus cells and 3) acquisition of competence for *in vitro* embryo development. COCs (n=240) were PM for 8h in TCM-199 with 100 nM NPPC. Then, the COCs were washed to remove NPPC and cultured for 22h in *in vitro* maturation medium (IVM) (TCM-199 with bicarbonate, 0.5 mg/mL FSH, 100 IU/mL hCG and 10% FCS); therefore, the total culture time in this treatment (NPPC group) was 30h. Control oocytes were cultured in IVM medium in the absence of NPPC for 22h (group C22) and for 30h (group C30). After maturation, oocytes were evaluated for progress in nuclear maturation and cumulus cells for relative mRNA expression. Other oocytes were fertilized and cultured until day 7 to assess blastocyst rates. Expanded blastocysts (*n*=148) were evaluated for total cell number and apoptotic index. Data were analyzed by ANOVA followed by Tukey's test ($P<0.05$). No difference was observed between groups (C22 vs. NPPC vs. C30) in nuclear maturation, with the majority of oocytes having completed meiosis (83.23% oocytes - on average - reached metaphase II stage; $P>0.05$); however, higher rates of degenerated oocytes were observed in C30 compared to NPPC and C22 groups (11.6% vs. 2.5% and 3.6%, respectively; $P<0.05$). The relative levels of cumulus cells expansion related-genes differed between treatments: *PTX3* was up-regulated in NPPC compared to C22 and C30 groups ($P<0.05$), as it was also up-regulated in C30 compared to C22; the *PTGS2/COX2* gene was up-regulated in NPPC and C30 compared to the C22 group ($P<0.05$), but there was no difference between NPPC and C30 groups ($P>0.05$). The relative expression of the oocyte maturation related-genes *ADCY6* and *NOS3* was higher ($P<0.05$) in C30 compared to C22, and both were similar to NPPC ($P>0.05$). *PDE5A* levels were higher ($P<0.05$) in C30 than in C22, and both were similar to NPPC ($P>0.05$). *AREG* and *BDNF* levels were higher ($P<0.05$) in NPPC than in C30, and both were similar to C22 ($P>0.05$). *BMP15* levels were higher in NPPC compared to C22 group ($P<0.05$). The relative levels of embryonic development related-gene *LUM* were up-regulated in NPPC compared to C22 and C30 ($P<0.05$). Blastocyst rates did not differ between NPPC and the other groups ($P>0.05$), but there was a difference between C22 and C30 (69.3 vs. 37.4%; $P<0.05$). There was no difference between groups in the total number of blastomeres and percentage of apoptotic cells ($P>0.05$). In conclusion, the PM culture with NPPC allowed the maintenance of oocytes in culture for a longer period of time (up to 30h) than the usual 22-24h of IVM, without increasing the aging and degeneration rates. As a consequence, the embryonic development capacity of NPPC-treated oocytes was not altered by prolonged culture. The adoption of this strategy in the practice of *in vitro* embryo production protocols can be beneficial in different situations, for example, in the transport of oocytes obtained by follicular aspiration from donors kept on farms located long distances from the laboratory and also when there is interest from the laboratory to synchronize the time of *in vitro* fertilization of oocytes collected at different times, aiming at optimizing the use of semen straws of high commercial value. Financial support: FAPESP (#2019/11174-6), CNPq (#314136/2018-5) and CAPES (Financial Code 001).

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Animal Reproduction

Effect of repeated manipulations of the genital tract before insemination on the conception rate of dairy cows

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Ultrasound monitoring of ovarian follicular dynamics in cows is not a routine practice, and its use is mostly related to research aimed at clarifying events related to follicular dynamics and the development, maintenance and regression of the corpus luteum. This information is fundamental and serves as a basis for understanding the entire estrous cycle, as well as for the development and application of techniques related to animal reproduction. However, repeated manipulations of the genital tract performed during ultrasound evaluations can stimulate the release of prostaglandins as well as cause endometrial changes, interfering with luteal dynamics and impairing the establishment of pregnancy. Thus, the aim of the present study was to evaluate the effect of constant manipulations of the genital tract during transrectal ultrasound evaluation on the conception rate of timed inseminated dairy cows. For this purpose, 69 Girolando cows were used, with body scores between 2.5 and 3.5 (scale from 1 to 5) in a randomized block design. They were randomly distributed into two groups: experimental (EG; n=34) and control (CG; n=35), and EG females were submitted to ultrasonographic evaluations (Mindray D2200 vet) every 12 hours from removal of the progesterone device (P4) until ovulation and those in the control group were not manipulated. To perform ovulation synchronization, on D0, all animals received an intravaginal device with 1g of P4 and an intramuscular (IM) application of 2.0 mg of estradiol benzoate (EB). On D8, the device was removed and the females received (IM) 500µg of cloprostenol. On D9, 1.0 mg of BE (IM) was administered. Inseminations occurred on D10, with frozen semen, using two bulls (Holstein and Gir), equally distributed in both groups. Pregnancy diagnosis was performed using ultrasound (Mindray D2200 vet) or rectal palpation, 30 and 50 days after insemination, respectively. Conception rates were analyzed using Fischer's exact test (Statdisk software), with a significance level of 5%. The conception rates of the EG and CG groups were 26.5% (9/34) and 54.3% (19/35), respectively, with a significant difference (p=0.027). There was no effect of the bull on conception rates. The literature in the field of ultrasound manipulation affecting the conception rate is not very extensive. As ultrasonography is an extremely important technique for understanding follicular dynamics, it can be used in research work. However, its routine use in the periods that precede the AI procedures should be avoided, due to the possibility of reduction of conception rates, and consequently of financial losses for the farmer. It is concluded that ultrasound manipulation performed every 12 hours prior to artificial insemination causes negative effects on conception rates. However, research should be carried out to elucidate the mechanisms by which this manipulation impairs fertility, which may be related to ovulation, oocyte uptake, fertilization, early embryonic death, as well as genital tract reactions in the proestrus period (edemaciation, increase in the volume of uterine fluid and delay in its elimination or absorption).

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Animal Reproduction

Effect of testicular heat stress in sperm kinetics and resistance during storage at 4 ºC in rams (*Ovis aries*)

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Our objective was to investigate effects of testicular heat stress on sperm kinetics and survival post-cooling storage at 4 ºC. Eight post-pubertal, reproductively sound rams were used. Testicular temperature was increased by scrotal insulation (disposable diapers) for 51 h, increasing scrotal surface temperature ~3-5 ºC, but not affecting rectal temperature. Semen was collected by artificial vagina before (Control; CO) and at days (D) 7, 14, 21, 28, 35, 47, and 62 post insulation. Semen was evaluated immediately after collection (M0), diluted with BotuBOV® (400 x 10^6/mL), cooled to 4 ºC, and assessed at 72 h. At M0, for CO there was (mean ± SEM): total motility (TM): 90.4±2.1%; progressive motility (PM): 54±2.2%; VLC: 178.4±14.1 µm/s; VAP: 109.2±6.8 µm/s; and VSL: 91.8±4.2 µm/s. All motility end points were decreased (P<0.05) on D7, with further reductions (P<0.05) on D14: TM: 7.3±3.9%; PM: 2.5±1.6%; VLC: 100.4±33 µm/s; VAP: 52.9±17.7 µm/s; and VSL: 43.8±15.6 µm/s. Motility end points reached nadir at D21 and D28, began to improve on D35 (were similar to D14) and on D47 were TM: 59.5±7%; PM: 39.4±4.4; VLC: 177.7±17.5 µm/s; VAP: 107.64±3.1 µm/s; and VSL: 93.3±5.4 µm/s. On D62, they were similar to CO. As a percentage of pre-chilling values, after 72 h of storage at 4 ºC, there was no difference in the rate of motility maintenance in CO, D7 and D47: TM: 87, 94 and 85%, and PM: 78, 73 and 95%, respectively (P>0.05). Furthermore, despite significant reductions in motility end points on D7 and D47, TM and PM were preserved after 72 h of semen storage at 4 ºC. Therefore, we inferred that D7 and D47 sperm were resistant to cooling, due to preservation of mitochondrial function and energy metabolism. Acknowledgments: CAPES (process nº 88887.486411/2020-00), CNPq, FAPESP (grant#2018/02007-6) and Botufarma®.

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Animal Reproduction

Effect of trans-ferulic acid on *in vitro* production of sheep zygotes: preliminary results

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The *in vitro* embryo production (IVP) occurs by exposing the oocytes to oxidative stress, being essential the use of antioxidants, as well as transferulic acid, especially in the *in vitro* maturation of oocytes (IVM). This study aimed to evaluate the use of transferulic acid in IVM on the IVP of ovine embryos. Oocytes were collected from ovaries obtained from a local slaughterhouse. A vacuum pump was used to collect oocytes, which, soon after, were classified and taken to the IVM, where they were divided into four groups: CIS group, with medium containing TCM-199, supplemented with 0.2 mM of pyruvate sodium, 10% fetal bovine serum, 1% antibiotic-antimycotic solution, 20 mg/mL FSH/LH and 100 mM cysteamine; in ATF10, ATF50 and ATF100 groups, oocytes were matured in the presence of a medium with the same composition as the CIS group, but without the addition of cysteamine and including 10 µM, 50 µM and 100 µM of transferulic acid, respectively. The oocytes with the medium were incubated for 24 h at 38.5 °C, with 5% CO_2. Then, *in vitro* fertilization (IVF) was performed, where IVF medium was used, together with selected spermatozoa for a period of 18 to 20 hours, under the same conditions as the MIV. The presumptive zygotes were evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, the presumptive zygotes went to CIV, with SOF medium supplemented with 3 mg/mL of BSA and, from that, were evaluated for the occurrence of D1 and D2 cleavage. ANOVA and Tukey's test were used to compare parameters between the studied groups. One-way ANOVA was used to compare the parameters between groups, followed by the Tukey test. Percentage data were submitted to the Fisher's exact test ($P < 0.05$). A total of 97 oocytes were used for *in vitro* maturation (IVM), so it was observed that there was no significant difference between the groups with transferulic acid and the CIS group, in relation to the expansion rate of cumulus cells (CIS: 84.00%; ATF10: 83.33%; ATF50: 83.33%; ATF100: 87.50%). Likewise, no significant difference ($P>0.05$) was observed within each moderate and mild expansion treatment group. However, a significant smaller number of oocytes with high expansion was observed in the group of oocytes matured in the presence of 50 µM transferulic acid (High: 1.00±0.70; Moderate 3.00±0.7; Mild: 6.00±0.00). As for the success of fertilization, there was no significant difference between the treatment groups in relation to the presumptive zygotes (CIS: 4.50±0.35; ATF10: 5.00±0.70; ATF50: 5.00±0, 70; ATF100: 5.50±0.35). Likewise, no significant difference was observed between the treatment groups regarding the number of cleaved structures (CIS: 1.50±1.06; ATF10: 3.50±1.06; ATF50: 1.50±0.35; ATF100: 5.00±0.00). Based on the preliminary results, it is possible to conclude that the addition of transferulic acid does not interfere in the *in vitro* maturation of ovine oocytes when compared to the use of cysteamine. More studies are needed to see the real effect of trans-ferulic acid on *in vitro* maturation of sheep embryos, which may prove to be an effective alternative to cysteamine.

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Animal Reproduction

Effect of two different feeding strategies on prepubertal Nellore Heifers' growth performance and oocytes and in vitro embryo production

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The younger the embryo donors, the greater the impact on breeding programs, since it promotes a reduction in the interval between generations, which positively influences the genetic gain in cattle herds. It is known that oocytes from pre-pubertal heifers and calves have a different composition from the oocytes of cows, because they have different enzymatic and metabolic activity, besides changes in the composition of proteins and hormones, which leads to a lower oocyte competence for in vitro embryo production. In this context, the objective of the study was to investigate the effects of two nutritional plans on Nellore pre-pubertal heifer growth performance and development of follicles, oocytes quantity and quality, as well as in vitro embryo production. Thirty four Nellore heifers (experimental unit) with average initial age of 8.5 months were used throughout the experiment in a completely randomized design, distributed in two treatments: T1- nutritional diet with pasture and 650 g of concentrate with 21.9% of crude protein and 79.1% of total digestible nutrients per day; T2- nutrition diet with pasture and 1.8 kg concentrate with 21.7% of crude protein and 80.8% of total digestible nutrients per day. Withers height and body weight of all animals were measured monthly. Subcutaneous fat deposition was measured at the end of the study by ultrasonography. Heifers were aspirated every 15 days without prior follicular wave synchronization for 4 months. Immediately before OPU, all follicles were quantified and follicles above 3 mm were punctured. The oocytes were subjected to IVF with semen from the same bull with known fertility. Oocytes from adult females from slaughterhouses were fertilized as control group. The animals from treatment 2 showed greater growth performance than the animals from treatment 1, where it was observed greater height at the withers (1.37 ± 0.09 m vs 1.33 ± 0.03 m), monthly body weight gain (0.650 kg and 0.550 kg), fat subcutaneous rib thickness (3.7 ± 0.58 mm vs 3.11 ± 0.48 mm) and subcutaneous rump thickness (5.23 ± 0.56 mm vs 4.11 ± 0.99 mm). This higher growth performance did not affect the population of follicles on the surface of the ovaries, but influenced the number of retrieved oocytes (87.3 ± 25.73 vs 129.17 ± 35.17 oocytes for T1 and T2, respectively; p=0.04), number of viable oocytes (71.33 ± 20.87 vs 98.16 ± 30.80 oocytes for T1 and T2, respectively; p=0.038) and number of blastocysts in D7 (16.67 ± 12.50 vs 25.66 ± 15.54 embryos for T1 and T2 respectively; p=0.0176). There was no difference in the blastocyst rate between T2 and control with oocytes from slaughterhouse female adult (25.11% vs 37.11%; p>0.05), but T1 was lower than the control (21.43 vs 37.16; p = 0.0264). Prepubertal heifers usually present a greater number of follicles available for aspiration, but in vitro embryo production in this category is lower than in adult animals. In this study, it was observed that embryo production of prepubertal heifer group that received more concentrated feed was equal to the control group with adult cows. This information demonstrates that nutritional supplementation contributes to the body development and reproduction of pre-pubertal Nellore heifers. Financial support: SEG Embrapa and CNPQ.

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Animal Reproduction

Effects of anethole supplementation during IVM and IVC on oocyte maturation and embryo development in cattle

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In vitro production (IVP) has shown significant growth that surpassed in vivo production in 2017 and, in 2018, reached 68,7% of the total embryos produced in the world. Despite its commercial viability, IVP in cattle has low efficiency; thus, increased embryo production rates and quality would provide substantial gains in livestock. The production of reactive oxygen species (ROS) and oxidative stress can impact the development of oocytes and embryos *in vitro* produced. Previous studies showed that antioxidants participation in oocyte metabolism helps to neutralize excessive amounts of ROS and prevent cell damage, ensuring better oocyte and embryo development. This study aimed to evaluate the effect of anethole supplementation, which is a natural antioxidant, in the maturation and culture medium on oocyte maturation and bovine embryo development. In the first stage, the oocytes were divided into 3 experimental groups: the control group (MC) (IVM medium); group M300 (IVM medium supplemented with 300 µg/ml anethole); and group M3000 (IVM medium supplemented with 3000 µg/ml anethole). Oocytes were collected from ovaries obtained from slaughterhouses, and 2-8 mm follicles were punctured and subjected to maturation with in vitro maturation (IVM) medium. The IVM medium consisted of TCM199 with Earle's salts, BSA, pyruvate, amikacin, and rhFSH, supplemented with 10% fetal bovine serum (FBS). After IVM, nuclear maturation was evaluated to obtain metaphase II (MII) results with the best concentration to be used in the next step of the experiment. In the second stage, IVM and in vitro culture (IVC) were performed with respective controls medium supplemented with 300 µg/ml anethole or not, which was the best concentration previously found, resulting in 4 groups: C-C (controls IVM and IVC), C-300 (control IVM and treated IVC), 300-C (treated IVM and control IVC), 300-300 (treated IVM and IVC). For this, the IVM was performed in the same manner as the first experiment for 24 hours and after, the oocytes were subjected to in vitro fertilization (IVF) for 18 hours and in vitro culture (IVC) for seven days After these stages, the cleavage (D3) and blastocyst (D8) rates were calculated in relation to total oocytes subjected to IVM, and blastocyst cell numbers were assessed by Hoechst 33342 staining. Data were arcsine transformed and compared with Tukey (parametric data) or Kruskal-Wallis (non-parametric data) tests. Data are presented by mean ± SEM and differences were considered significant when $P \leq 0.05$. When we added 300 µg/mL anethole to the IVM medium, there was no difference in MII rates compared to those in the control group, however, the addition of 3000 µg/mL anethole significantly reduced the percentage of oocytes that reached MII in relation to the other groups ($P < 0.05$; MC: 77.86±2.67, M30: 77.23±5.62, M300: 28.71±8.81). Regarding the cleavage index, the addition of 300 µg/ml anethole during IVM and IVC increased the percentage of cleaved embryos when compared to the other groups ($P < 0.05$; C-C: 81.24±4.49, C-300: 82.13±5.22, 300-C: 83.48±5.86, 300-300: 94.03±3.88). Nevertheless, no difference was found between the groups on embryo production, assessed by the blastocyst production rate ($P > 0.05$; C-C: 51.02±5.37, C-300: 47.51±4.53, 300-C: 48.74±6.54, 300-300: 49.17±6.18), nor on embryo quality, obtained by counting the total number of embryonic cells ($P > 0.05$; C-C: 131.9±16.41, C-300: 128.8±13.49, 300-C: 156.3±15.87, 300-300: 149.0±9.88). In summary, these results suggest that anethole addition to the IVM and IVC medium does not positively impact on bovine IVP. Further studies are needed to assess the impact of anethole on oxidative stress and gene expression profile. Acknowledgements: CAPES

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Animal Reproduction

Effects of cryopreservation of somatic cells of puma, *Puma concolor* (Linnaeus, 1771) aiming at the formation of cryobanks

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The puma is one of the largest felids in the Americas, widely distributed on the continent and with important contributions to the ecosystem, mainly as a predator. Puma populations have reduced in size mainly due to hunting in retaliation for the predation of domestic animals, burning, and being run over. A tool that is yet to be developed for this species is the establishment of somatic resource banks, such as cryobanks of somatic cells. To obtain a bank of somatic cells, the cryopreservation of these cells is essential for adequate application. Therefore, we aimed to assess the effects of cryopreservation on the viability, metabolism, proliferative activity e levels of reactive oxygen species (ROS) in somatic cells derived from four pumas. All procedures were approved by the ethics committee of UFERSA (no. 23091.010755/2019-32) and Institute Chico Mendes for Conservation of Biodiversity (no. 71834-1). Thus, skin samples were obtained from puma belonging to Brazil' zoos and cells recovered in third passage were cryopreserved by slow freezing using Mr. Frosty system®. A solution constituted of Dulbecco's Modified Eagle medium supplemented with 1.5 M dimethyl sulfoxide, 0.25 M sucrose and 10% fetal bovine serum was used for cryopreservation. Cells were evaluated before and after the cryopreservation for viability using the trypan blue, metabolism using the (3-(4.5-dimethylthiazole-2yl)- 2.5-diphenyl tetrazoline bromide, proliferative activity by determination of population doubling time (PDT), ROS levels using the fluorescent probe 2',7' dichlorodihydrofluorescein diacetate, and mitochondrial membrane potential (ΔΨm) using MitoTracker Red®. Data were expressed as mean ± standard error (one animal/repetition) and analyzed using StatView 5.0 software. Since data did not show a normal distribution, they were arcsine transformed and analysed by ANOVA followed by Tukey test ($P < 0.05$). The cryopreservation did not affect the viability (79.2% ± 5.2% vs. 79.8% ± 4.6%) and metabolism (100% ± 13.7% vs. 100% ± 3.6%) of cells. There were no differences in the PDT of cryopreserved and non-cryopreserved cells (31.4 h ± 8.5 h vs. 68.1 h ± 18.9 h). The ROS levels in cryopreserved cells (1.0 ± 0.1) did not differ from those in non-cryopreserved cells (1.0 ± 0.04). When comparing non-cryopreserved cells (1.0 ± 0.09) with cryopreserved cells (0.7 ± 0.04), a reduction in ΔΨm was observed. In summary, cryopreservation did not affect the viability, metabolic activity, or proliferative activity of the somatic cells after slow freezing. Nevertheless, cryopreservation changed ΔΨm, indicating the need for optimization of the cryopreservation protocol. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Effects of high and low intrafolicular progesterone concentration on in vitro embryo production rate and follicular small extracellular vesicles during the same estrous cycle stage in bovine

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During follicular development, the ovarian follicle is exposed to different progesterone (P4) levels. At different stages of the estrous cycle, P4 levels modulate the content of small extracellular vesicles (sEVs). These molecules are small nanoparticles that carry bioactive materials within biofluids such as follicular fluid (FF). Based on that, our hypothesis is that corpus luteum (CL) proximity attributes different intrafolicular P4 concentrations during the same stage of the estrous cycle, and this difference is capable to alter the sEVs profile and the in vitro-produced embryos rates. For that, local slaughterhouse ovaries were collected in pairs (ipsilateral and contralateral to the CL) and classified according to the estrous cycles stage (middle diestrus- 11 to 17 day of estrous cycle), according to CL morphology (Ireland et al. 1980. J Dairy Sci. 63:155–160). The 3-6mm follicles (n~10) were aspirated from three ovaries per group and separated in two experimental groups (ipsilateral and contralateral) to collect cumulus–oocyte complexes (COCs) and FF. The FF (n= six replicates) was used to measure P4 and estrogen (E2) concentration (ng/mL) by chemiluminescence assay as well as to isolate sEVs. The COCs of six replicates were morphologically classified and submitted to in vitro embryo production. To isolate the sEVs, first the FF was centrifuged at 300 x g for 10 min to remove live cells, 2000 x g at 10 min to remove cellular debris and finally centrifuge at 165000 x g to remove large vesicles (100 a 1000nm). To obtain an enriched pellet of sEVs the supernatant was filtered (0.20µm) and ultracentrifuged twice at 119700xg for 70 minutes at 4°C. The sEVs size and concentration were determined using nanoparticle tracking analysis (NTA). The analysis was performed capturing five videos of 30 s each. All data followed the criteria of normality. The statistical analysis were performed using Student *t*-test (GraphPad Prism) with a significance level of 5%. The intrafolicular progesterone concentration was 3.8 higher in follicles localized ipsilateral (371.36 ± 60.26 ng/mL) to the corpus luteum when compared to the contralateral (97 ± 11.33 ng/mL) counterparts (p=0.0018). In regarding E2 concentration, no difference was observed between ipsilateral (0.013 ± 0.0003 ng/mL) and contralateral (0.017 ± 0.002 ng/mL) groups. The COCs morphological analysis demonstrate no difference in the rate of viable oocytes between ipsilateral (75.54 ± 6.54%) and contralateral (82.26 ± 4.12%) groups. Conversely, the rate of in vitro-produced embryos also showed no difference according with the CL proximity (ipsilateral: 30.64 ± 2.95% vs contralateral: 28.40 ± 2.74%). The ipsilateral follicles demonstrate a concentration of $2.20 \times 10^{11} \pm 3.98 \times 10^{10}$ particles/mL and the contralateral follicles demonstrate a concentration of $1.53 \times 10^{11} \pm 2.23 \times 10^{10}$ particles/mL. In regarding the diameter of FF sEVs, the ipsilateral group showed a mean size of 166.11 ± 2.42 nm and the contralateral group a mean size of 160.40 ± 4.15 nm, both analysis with no statistical difference between groups. Based on the results, we demonstrated that the follicular environment at the same estrous cycle stage are exposed different progesterone concentrations according to the proximity of the CL. Although non-differences were observed among the analysis performed, we believe that a compensatory molecular mechanism of different P4 follicular environment is mediated by sEVs content during follicular dynamics process. For this, the next steps of this study is to characterize the molecular contents of sEVs modulate by high or low P4 concentrations caused by CL proximity. Funding: FAPESP 2014/22887-0. FAPESP 2020/08478-0. CAPES - financial code 1.

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Animal Reproduction

Effects of supplementation of *Citrus sinensis* essential oil in maturation medium on bovine oocytes and oxidative status

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The use of natural antioxidants in maturation medium can be an alternative to reduce the negative effects of oxidative stress produced during *in vitro* embryo production (IVEP). *In vitro* tests have demonstrated the ability of essential oil from *Citrus sinensis* (EOCS) to decrease reactive oxygen species (ROS) and could be an interesting antioxidant agent during *in vitro* maturation (IVM) of bovine oocytes. Therefore, we aimed to evaluate the antioxidant effect of the EOCS on bovine IVM, and levels of ROS. Initially, EOCS was extracted by hydrodistillation of the peels using a Clevenger-type apparatus. The chemical composition of EOCS was performed using a gas chromatography coupled to mass spectroscopy. EOCS presented as constituents D- limonene (48.5%), α-terpineol (40.2%) and other compounds (11.3%). Then, viable oocytes derived from slaughterhouse individuals were matured *in vitro* under three sets of conditions: EOCS0 (without antioxidants), EOCS30 (30 µg/mL of EOCS), and CYS (100 µM of cysteamine). The concentration of 30 µg/mL of EOCS was defined according to biochemical studies of the major constituents of EOCS. After IVM, oocytes were evaluated to determine their IVM according to the expansion of *cumulus* cells, and presence of the first polar body (1PB). Moreover, oocytes were assessed to verify their oxidative status based on the levels of ROS using H2DCFDA probe. All data are expressed as the mean ± standard error and analyzed using the StatView 5.0 software ($P < 0.05$). ROS levels were altered with arcsine and analyzed by ANOVA followed by a Tukey test. All other data were compared with a chi-squared test. A total of 287 ovaries were used to acquire 1113 viable immature oocytes (3.9 viable oocytes/ovary) that were selected and distributed in twenty repetitions. Initially, for *cumulus* cell expansion, EOCS30 (99.4% ± 0.9; 363/365) and CYS (99.7% ± 0.4; 374/375) were increase to that of EOCS0 (96.2% ± 3.0; 359/373). No difference was observed in the IVM rates obtained from 1PB among EOCS30 (84.6% ± 2.4; 270/319), EOCS0 (79.8% ± 2.9; 265/332), and CYS (82.8% ± 3.2; 279/337). Additionally, oocytes derived from the EOCS30 (0.50 ± 0.14), and CYS (0.39 ± 0.24) groups showed significantly lower ROS compared to the EOCS0 group (1.00 ± 0.65). These results demonstrate the antioxidant potential of EOCS in controlling oxidative stress, which may be acting on the synthesis of ROS regulatory enzymes. In summary, IVM medium supplementation with 30 µg/mL of EOCS improves the expansion of *cumulus* cells, possibly through antioxidant effects due to the decreased oocyte ROS. The results for EOSA30 are comparable to those with cysteamine in terms of parameters evaluated. Therefore, EOCS, added to the IVM medium, could be an interesting alternative for the reduction of damage caused by the oxidative stress in bovine oocytes during IVEP. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Effects of supplementation of different concentrations of eugenol in maturation medium on bovine oocyte oxidative status and parthenogenetic embryonic development

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In vitro embryo production requires competent oocytes for embryonic development. This competence can be acquired during the *in vitro* maturation (IVM); however, at this stage, oocytes may be exposed to oxidative stress. In this context, eugenol, considered a bioactive compound with a role in reducing oxidative stress, could be used in bovine IVM. Therefore, we aimed to evaluate the antioxidant effect of different concentrations of eugenol on bovine levels of reactive oxygen species (ROS) in matured oocytes and parthenogenetic embryonic development. Then, viable oocytes were allocated into five groups: control (without antioxidant), CYS (100 µM cysteamine), E83 (83 µM eugenol), E100 (100 µM eugenol), and E120 (120 µM eugenol). After IVM, 60 oocytes per group was assessed to verify their oxidative status based on the levels of ROS using H2DCFDA probe. Oocytes with the first polar body were artificially activated with activated with 5 µM ionomycin for 4 min and 2 mM 6-dimethylaminopurine for 3 h. After activation, the structures were washed and cultured for eight days. Blastocysts were incubated with Hoechst 33342 for 15 min, washed in PBS/BSA and the number of nuclei was counted using ImageJ software. All data are expressed as the mean ± standard error and analyzed using the StatView 5.0 software ($P < 0.05$). ROS levels and number of nuclei were altered with arcsine and analyzed by ANOVA followed by a Tukey test. All other data were compared with a chi-squared test. A total of 193 ovaries were used to acquire 790 viable immature oocytes (4.1 viable oocytes/ovary) that were selected and distributed in eight repetitions. Initially, ROS levels were lower ($P < 0.05$) in E83 (0.44 ± 0.10) and CYS (0.43 ± 0.09) groups in comparison with oocytes matured in E100 (0.66 ± 0.13) and E120 (0.55 ± 0.11). All treatments had better results than the control (1.00 ± 0.16, $P < 0.05$). The cleavage rate on D3 was similar among all groups, ranging from 69.5% to 79.0% ($P > 0.05$). On D8, the percentage of blastocyst/total oocytes was higher in oocytes matured in E83 (32.2% ± 9.4, 27/87); however, it was also similar to the CYS (26.0% ± 6.0, 18/69) and E120 (22.6% ± 5.0, 17/75) treatments ($P < 0.05$). Moreover, E83 improved the blastocyst/cleavage oocyte rates on D8 (47.3% ± 8.2, 27/57) compared with the control (23.4% ± 4.6, 15/64) and E100 (25.0% ± 5.0, 14/56) and the effects were similar to the CYS (37.5% ± 8.5, 18/48) and E120 (30.9% ± 9.9, 17/55, $P < 0.05$). Additionally, 83 µM of eugenol promoted better embryo quality (128.9 ± 6.2 nuclei/blastocyst) when assessed by the comparison of cell counts in relation to the control (90.3 ± 7.1 nuclei/blastocyst) and E120 (102.0 ± 8.8 nuclei/blastocyst). The CYS (111.1 ± 12.4 nuclei/blastocyst) and E100 (108.2 ± 8.0 nuclei/blastocyst) were like all groups ($P > 0.05$). Therefore, 83 µM eugenol lowered ROS production, increased the rate of blastocyst production on D8, and improved quality as indicated by a higher number of blastocyst cells. In summary, the use of the eugenol with a defined concentration of 83 µM can yield more positive results for IVEP in cattle, thus increasing the efficiency of the technique. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Efficiency of superovulation protocols using 250, 333 or 400 IU pFSH in White Dorper ewes during seasonal anestrus

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This study aimed to evaluate the superovulatory efficiency of three superovulation protocols using 250, 333 or 400 IU of pFSH. A total of 48 Dorper ewes received intravaginal progesterone device (P4; 0,36 g; Primer®, Agener União Saúde Animal, São Paulo, Brazil) for nine days and six decreasing doses (25, 25, 15, 15, 10, 10%) of 250 (G250, n=16); 333 (G333, n=16) or 400 IU (G400, n=16) of pFSH i.m. (Pluset®, Biogénesis Bagó, Curitiba, Brazil) plus two doses of 37.5 µg of d-cloprostenol (Prolise, Agener União Saúde Animal, São Paulo, Brazil) i.m., concomitantly with the 5th and 6th doses of pFSH. Animals also received 50 µg of analog of GnRH (Gestran Plus®, Agener União Saúde Animal, São Paulo, Brasil) i.m. 24 h after the P4 removal. Ewes were checked for estrus behavior and were naturally mated twice a day during 72 h. All animals received a cervical relaxation containing 37.5 µg of d-cloprostenol i.m. plus 0.0 or 0.5 mg of estradiol benzoate (RIC- BE®, Agener União Saúde Animal, São Paulo, Brazil) i.m. 16 h before embryo recovery and 50 IU of oxytocin (Ocitocina Forte UCB®, UCBVet, Brazil) i.v. 20 min before embryo recovery. Non-surgical embryo recovery (NSER) was performed eight days after progesterone device withdrawal. Corpora lutea (CL) were counted by transrectal ultrasonography 24 h before NSER. Data express as percentage and mean and standard error were analyzed, respectively, by Fisher exact test and analysis of variance, followed by Tukey test, at 5% significance level. A total of 97.9% of animals presented estrus, being 93.7 (15/16), 100 (16/16) and 100% (16/16) in G250, G333 and G400, respectively (P>0.05). The mean number of CL/ewe was higher in G333 and G400 groups in comparison to G250 (P<0.05), totaling 6.1±1.7 (G250), 9.8±1.5 (G333), and 12.4±1.5 (G400) considering all animals, but was similar in animals successfully flushed (P>0.05), being 6.0±1.2 (G250), 11.4±2.4 (G333), and 12.0±1.4 (G400). NSER was successfully performed in 50.0% (24/48) of animals. The mean number of structures recovered from successfully flushed animals were 2.7±1.3, 6.6±2.5 and 6.0±1.3, being 2.3±1.1, 5.4±2.4 and 4.4±1.3 viable embryos, in G250, G333 and G400 groups, respectively (P>0.05). Following data suggest that the use of pFSH at concentrations of 333 or 400 IU are more effective for superovulation in White Dorper ewes. Financial support: Embrapa (Project 20.19.01.004.00.03.001) and CNPq (Project 314952/2018-7).

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Animal Reproduction

Estimation of the preantral follicles ovarian population in six-banded armadillos (*Euphractus sexcinctus*) infected or not by the *Mycobacterium leprae*

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The six-banded armadillo (Linnaeus, 1758) is a wild mammal with a wide distribution and a stable population. The species is intensively exploited by sport hunting, especially due to cultural habits of local communities in Northeast Brazil. However, they are natural reservoir hosts for the Hansen's bacillus that causes leprosy (*Mycobacterium leprae*), which emphasizes the importance of studying the species. At this point, the acquisition of knowledge about the effects of *M. leprae* on armadillo reproduction is essential for the comprehension of the species reproductive aspects. Therefore, we aimed to estimate the population of preantral ovarian follicles (PAFs) in six-banded armadillos, which were carrying or not the *M. leprae*. Five sexually mature adult female six-banded armadillos were captured from rural locations in the state of Ceará (CE) and euthanatized (IBAMA Authorization nº 50564-2). They were investigated for the presence of *M. leprae* by enzyme-linked immunosorbent assay (ELISA), which identified two positive and three negative individuals. The ovaries from all the individuals were collected and washed in 70% alcohol and saline solution to remove dirt and contaminants. The gonads were fixed for 12 hours, followed by dehydration in alcohol, cleared in xylene and embedded in paraffin blocks. The blocks were sectioned in series of 5 µm, and every 120 sections were mounted for histological slides, and later stained with hematoxylin-eosin. Samples were evaluated under light microscopy for the PAFs identification, measuring and counting, according to morphological category (primordial, primary, and secondary). The values obtained were given to the formula for estimating the follicular population: PAF population = [(number of follicles x number of sections x slice thickness) / (number of observed sections x mean diameter of the oocyte nucleus)]. Data were expressed as means and SEM, and the statistical analyzes were performed using the ANOVA test followed by Fisher's least significance difference test. For the healthy animals, we found 3668 ± 1067.8 primordial follicles, 2860.6 ± 910 primary follicles, and 147.2 ± 41.2 secondary follicles, totalizing 6175.8 ± 1923.5 PAFs per ovarian pair. For the individuals carrying the *M. leprae*, we found 2040.6 ± 542.5 primordial follicles, 2024.8 ± 112.6 primary follicles, and 46.7 ± 21.6 secondary follicles, totalizing 3112.1 ± 615.1 per ovarian pair. Despite infected individuals presented proximately the half of the total PAFs population in comparison to healthy animals, no significant differences were evidenced ($P > 0.05$). We emphasize, however, that the infectious agent presents the capability of acting infiltratively in tissues and organs, and lepromatous infiltrate has already been found in the ovaries of another armadillo species, the *Dasypus novemcinctus*. Possibly, the low number of individuals could have interfered on the results. Besides it, we provided important data related to follicular population of the six-banded armadillo. We verified that the majority of PAFs found were classified as primordial follicles, which are known for forming the reservoir pool in the ovary, similar as described for other mammals. In summary, we provide new data related to the reproductive morphology and physiology of the six-banded armadillo, demonstrating, for the first time, the estimation for its PAFs population in individuals infected or not with *M. leprae*. This knowledge is of fundamental importance for the effective reproductive management of the species. **Financial support:** CAPES and CNPq

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Animal Reproduction

Evaluation of arachidonic acid supplementation in the diluent on acrosomal membrane integrity in post-freezing goat sêmen

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Seminal cryopreservation has become notable due to its benefits, such as seminal storage for a long period of time. However, sperm manipulation causes damage to the integrity of sperm membranes, caused by oxidative stress, thus impairing their biological functions. The maintenance of the acrosomal membrane integrity after cryopreservation is essential for the acrosomal reaction to occur, with consequent release of hydrolytic enzymes, resulting in successful fertilization, besides indicating good freezability of the semen. Therefore, it is necessary to add substances that can improve the antioxidant defenses of biological systems, fighting the high production of ROS. In this sense, the addition of arachidonic acid, a polyunsaturated fatty acid (PUFA), also present in membrane phospholipids, was able to improve membrane fluidity, reduce the action of free radicals and maintain the integrity of sperm membranes of other species. Thus, we aimed to evaluate the effect of arachidonic acid supplementation in the TRIS-gem diluent on acrosomal integrity during cryopreservation of goat spermatozoa. Six ejaculates/ animal were collected from four Anglo-Nubian goats, using an artificial vagina. After immediate analysis, the "pool" of these was made, diluted in TRIS-Gema and divided into the treatments: control and supplementation with 0.5 µM, 5 µM and 50 µM of arachidonic acid. Then, the semen was cryopreserved. After thawing, acrosomal membrane integrity analysis was performed using the fluorescein isothiocyanate dye conjugated to Peanut agglutinin (FITC-PNA). Aliquots of 20µL of FITC-PNA were placed on smears of slides containing spermatozoa, incubated for 20 minutes in a humid chamber at 4°C in the absence of light. Then the slides were rinsed twice in refrigerated PBS (4°C) and dried in the dark. Immediately before evaluation, 5µL of UCD mounting medium (4.5mL glycerol, 0.5mL PBS, 5mg sodium azide and 5mg p- phenylenediamine) was placed on the slide and covered with a coverslip. We evaluated 200 spermatozoa/ slide under epifluorescence microscope (400x), using LP 515nm emission filter and BP 450-490nm for excitation. Cells classified with intact acrosomes showed the acrosomal region stained with green fluorescence, and cells with reacted acrosomes showed a green fluorescent band in the equatorial region of the sperm head or no green fluorescence in the entire head region. The results were submitted to analysis of variance, followed by Duncan's test, with a significance level of 5%. The supplementation with 50 µM of arachidonic acid (32.00 ± 17.17) differed significantly ($P<0.05$) from the other treatments (control= 60.00 ± 7.84, 0.5 µM of arachidonic acid=53.20 ± 9.85 and 5 µM of arachidonic acid= 51.60 ± 7.50) regarding acrosomal integrity. A possible explanation for the lower values after addition of 50 µM of arachidonic acid would be the excessive concentration of this, considering that arachidonic acid, through the action of cyclooxygenase, is converted into prostaglandin E2, inducing the influx of Ca2+ across the sperm membrane, leading to membrane fusion. It is concluded that the addition of 0.5 µM and 5 µM of arachidonic acid to the diluent maintained the acrosomal membrane integrity in post-cryopreservation goat semen, being beneficial for sperm capacitation and fertilization process, suggesting further research aiming a greater accuracy of arachidonic acid concentrations in the antioxidant function.

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Animal Reproduction

Evaluation of epidermal growth factor during *in vitro* maturation on meiotic status and embryonic development of red-rumped agouti (*Dasyprocta leporina* Linnaeus, 1758) oocytes

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The red-rumped agoutis are considered one of the most important species for seed dispersal, soil aeration, and reforestation. Although its populations are considered stable, conservation strategies need to be employed, aiming to develop research on expanding the knowledge on this species, and to manage captive populations. An interesting conservation strategy would be the *in vitro* embryo production (IVEP). *In vitro* maturation (IVM) is a fundamental step for the development of IVEP, being the establishment of medium composition essential for the success of the IVM and *in vitro* embryo development (IVD). Studies in different mammals have shown the positive effect of 10 ng/mL of epidermal growth factor (EGF) on IVM and IVD rates. Therefore, we aimed to evaluate the EGF on the IVM and IVD of red-rumped agouti oocytes. All procedures were approved by the ethics committee of UFERSA (no. 21/2019) and Institute Chico Mendes for Conservation of Biodiversity (no. 71837-1). Thus, six red-rumped agoutis (two females per session) derived from the Centre of Multiplication of Wild Animals (CEMAS/UFERSA) were used. After a programmed slaughter, *cumulus*-oocyte complexes were recovered by ovarian slicing. Oocytes were classified with a stereomicroscope and only oocytes with more than one layer of *cumulus* cells and homogeneous cytoplasm were used for IVM. Oocytes were matured in TCM-199 contained 10 mIU/mL of pFSH, 10% of fetal bovine serum, 100 µM of cysteamine, 1% of antibiotic-antimycotic solution and in the absence (group without EGF) or presence of 10 ng/mL of EGF (group with EGF). Oocytes were matured for 24 h at 38.5 °C and 5% CO2. After the IVM, oocytes were evaluated for expansion of *cumulus* cells and *cumulus* expansion index (CEI). Addittionally, oocytes were denuded and assessed for the presence of first polar body (1PB). Subsequently, oocytes were activated artificially using 10 mM of strontium chloride and 5 µg/mL of cytochalasin B for 6 h. Afterwards, the IVD was evaluated after 168 h. All data were expressed as mean ± standard error and analysed by the Fisher exact test ($P < 0.05$). CEI was compared between using ANOVA followed by a Tukey test. In total, 114 immature oocytes were obtained from all ovaries with nineteen oocytes per female. Of these, 104 oocytes were considered viable and used for IVM. After the IVM, no difference ($P > 0.05$) was observed between oocytes matured in absence and presence of EGF for expansion of the *cumulus* cells [93.3% ± 4.5 (42/45) vs. 100% ± 0.0 (44/44)], and rates of 1PB [37.3% ± 7.7 (19/51) vs. 52.1% ± 13.6 (25/48)]. Nevertheless, oocytes matured in the presence of EGF had a higher CEI (3.6 ± 0.1) when compared to oocytes matured in the absence of EGF (2.7 ± 0.3). This index identifies that oocytes close to full expansion will present values close to 4, showing greater oocyte viability. After IVD, no difference ($P > 0.05$) was observed between oocytes matured in absence and presence of EGF for cleavage rates [43.2% ± 14.7 (19/44) vs. 30.0% ± 8.7 (12/40)] and morulae/cleaved rates [18.8% ± 20.5 (3/16) vs. 20.0% ± 14.7 (2/10)]. In conclusion, we describe initial data on the effects of EGF on meiotic potential and *in vitro* embryo development in red-rumped agoutis, suggesting that EGF is an interesting supplement for optimizing IVM media in this species. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Evaluation of extended *in vitro* culture of somatic cells from six-banded armadillos (*Euphractus sexcinctus* Linnaeus, 1758) as a step towards for cloning by nuclear transfer

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The six-banded armadillo is a wild mammal with an important ecological role because it is an intermediary link in food networks, being responsible for the balance of termite and ant colonies, as well as for the cycling of nutrients. Although its populations are considered stable, conservation strategies need to be employed, aiming at the conservation of biodiversity. An interesting conservation strategy would be one aimed at the development of cryobanks. In general, the characterization of the cells during prolonged culture represents an important step in the formation of these banks. Therefore, we aimed to evaluate the effects of culture time (fourth, seventh, and tenth passages) on the functional parameters of somatic cells derived from six-banded armadillos. All procedures were approved by the ethics committee of UFERSA (no. 36/2020) and Institute Chico Mendes for Conservation of Biodiversity (no. 76655-1). Then, ear skin samples were obtained from five adults six-banded armadillos and were transported in minimal essential medium modified by Dulbecco (DMEM) supplemented with 10% fetal bovine serum and 2% penicillin and streptomycin solution at 37 °C. In the laboratory, fragments (9.0 mm^3) were cultured under controlled atmosphere (38.5 °C, 5% CO2) and evaluated every 24 h. The cells were harvested when they reached 70% confluency, subcultured until the fourth, seventh, and tenth passages for the analysis. All cells were evaluated for viability using trypan blue assay, metabolic activity using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay, and proliferative activity by determining the population doubling time (PDT) over 168 h. Data were expressed as mean ± standard error (one animal/ one repetition) and analyzed using StatView 5.0 software. Since data did not show a normal distribution, they were arcsine transformed and analyzed by ANOVA followed by Tukey test ($P < 0.05$). The viability remained high throughout the three different passages evaluated, with values above 85% (fourth: 90.6% ± 3.3 vs. seventh: 95.1% ± 2.3 vs. tenth: 87.1% ± 3.6). Additionally, high metabolic activity was maintained throughout the different passages (fourth: 100.0% ± 21.9 vs. seventh: 99.2% ± 8.9 vs. tenth: 100.0% ± 5.7). No difference was observed among the passages for proliferative activity (fourth: 15.2 h ± 2.0 vs. seventh: 14.3 h ± 0.9 vs. tenth: 21.5 h ± 2.7). In summary, we were able to isolate somatic cells derived from six-banded armadillo skin, associated with high viability rates, and high metabolic activity after extended *in vitro* culture. These results provide security for the application of these cells in reproductive biotechnologies in the species. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Evaluation of follicular development by the expression of proliferative cell nuclear antigen (PCNA) in the queen

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The knowledge regarding the follicular development mechanisms responsible to regulate an ovarian cycle in companion animals is still fragmented and controversial. Additionally, more studies are needed to try to understand this complex physiological mechanism. Thus, the aim of this study was to determine if ovarian follicles cell proliferation has related with follicle recruitment and development along the estrous cycle of queens. The study evaluated ovaries collected from 11 queens with ages ranging from 5 months to 2 years old. The ovaries were grouped according to the animal estrous cycle phase, determined by vaginal cytology, plasma progesterone concentration (P4) and histological observation of the ovaries. The study was designed to use the expression of Proliferative Cell Nuclear Antigen (PCNA) as a marker of cell proliferation in ovarian follicles. The statistics analysis was performed using SPSS (Statistical Package for the Social Sciences) version 24.0. The average percentage of granulosa/pre-granulosa cells with PCNA expression in the different follicular and estrous cycle phases were analyzed by multifactorial ANOVA (P≤ 0,05). Image analysis and processing was performed using ImageJ®. As well as follicular cells were considered positively stained when PCNA immunostaining was evidenced. Although samples of ovaries from several different animals were used, the experimental unit of analysis was the follicle. A total of 238 follicles was obtained from queen and subjected to analysis. In the early stages of follicular development, primordial follicles exhibited PCNA expression in all stages of the estrous cycle. This expression was more specifically detected in the granulosa cells and oocytes. Although not all granulosa cells of primordial follicles express PCNA with the same intensity, there are a significative difference in the percentual of stained cells along the estrous cycle limited to proestrus and anestrus, with the percentage of stained cells being higher in the anestrus(p=0.024). No significant difference in percentual of highly stained cells along the estrous cycle in primordial follicles. The PCNA expression in secondary follicles was similar in different estrous cycle phases, with a slight decrease in stained intensity in the anestrus phase. In the granulosa cells of preantral and antral follicles PCNA expression does not show significant variations in the percentual of stained and highly stained cells throughout the estrous cycle phase. However, in antral and preantral follicles there is a higher percentual of marked cells in the anestrus compared to the diestrus. Regarding the granulosa cells of the antral follicles, was evidenced that the corona radiata (CR) and cumulus oophorus (CO) zone have different markings depending on the estrous cycle phase. Also,it is possible to observe that the proestrus phase has a greater number of cells with positive and negative immunoreactions. In estrus, it is possible to verify that the CR and CO zone offer cells marked as unmarked. A diestrus phase expresses itself similarly to that observed in the estrus phase The results obtained allowed to conclude that cell proliferation varies according to follicular development and estrous cycle phase in queens, with both these variables having a significate interaction. In this context, the regulation of cell proliferation in the follicle could be a therapeutic route for fertility control (stimulation or contraception). However, this work will essentially serve as a basis for subsequent studies to better uncover the physiological processes that regulate folliculogenesis.

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Animal Reproduction

Evaluation of the vascular and morphostructural changes of the preovulatory follicle

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In equine reproduction usually is used ovulation induction for obtained better pregnancy rates, this treatment produced vascular and morphological changes identifiable at ultrasonography of the preovulatory follicle. This study aimed to investigate mares vascular dynamics of follicles' with or no ovulation induction using color Doppler ultrasonography to identify vascular and morphostructural changes of the preovulatory follicle (FP) until ovulation. Thirty healthy cyclic mares were subjected to daily ultrasonography examination during two estrous cycles. The inclusion criteria of the animals were on the observations by the estrus, rectal palpation of the ovaries, the follicular dynamics, and edema classification (no pathology edema). After estrus detection and follicle dominant, the measurement of the follicles and the classification of the uterus were carried out. The animals selected had dominant follicle (diameter more than 35 mm) and edema 3. Two groups were performed: G1 (15 animals) received saline solution (1 mL) and G2 (15 animals) received 2.2 mg deslorelin acetate (1 mL). After ovulation induction, ultrasonography evaluation was performed each 6 hours until ovulation. The parameters assessment were FP size, follicular growth rate, time of ovulation after induction, appearance time serrated granulosa, and subjective ovary vascular change (score 1 low – 4 high). The mean diameter of the PF in the G1 was 43.5 mm ± 4.08 with daily follicular growth of 2.5±0.93 mm, post-treatment ovulation time of 86.8±40.56 hours, and appearance time serrated granulosa of 61,4 ± 40.5 hours. In the G2 mean diameter of the PF was 39.3mm ± 5.0, with daily follicular growth of 3,02 ± 2,41 mm, post-treatment ovulation time of 39,60±34 hours, and appearance time serrated granulosa 48.0 ± 33.9 hours. All mares presented serrated granulosa in the PF. Vascularization of the PF was: Score 1 mares 2/30 or 6,6% (1/30 mares G1 and 1/30 mares G2), Score 2 mares 13/30 mares or 42.9% (6/30 mares G1 and 7/30 mares G2), Score 3 mares 11/30 mares or 36.3% (7/30 mares G1 and 4/30 mares G2) and Score 4 mares 4/30 or 13.2% in the G2. Results suggested that the ultrasonographic changes of the wall of the preovulatory follicle were associated temporally with ovulation induction. The effectiveness of ovulatory induction can be affected by the stage of the estrus cycle, follicle size, and maturity, also likely, appearance time serrated granulosa also may be another important factor for predict ovulation.

KEYWORDS: blood flow, preovulatory follicle, doppler, ultrasonography, ovulation

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Animal Reproduction

Expression of heterologous bovine Spemadhesin-1 and functional assays in post-thawed sperm cells

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Bovine seminal plasma contains several compounds which maintain proper conditions for the successful fertilizing process. These compounds are not only related to the normal sperm function, but also some of them can protect sperm cells from the heat shock response, oxidative stress, and female tract immune response. In this sense, some seminal plasma proteins have a relevant role in this biological protection system and their protective effect has been described in cryopreservation procedures. Spermadhesin-1, also known as acidic fluid seminal protein (aSFP), has been recognized as one of the main proteins to mitigate the deleterious effect of the oxidative stress derived from the cold shock of the cryopreservation process. This protective effect is mainly due to his structural features. The aim was the production of recombinant aSFP using *Escherichia coli* as a microbial cell factory. Then, to use purified protein to evaluate its effect on the quality of post- thawed sperm cells. For that purpose, the cDNA sequence encoding for bovine aSFP (120 AA) reported in Gene Bank® (Accession number NM_1746.3) was inserted into a pDass expression vector containing an ampicillin resistance cassette. In addition, a 6xHis-tag was included at the N- terminus of the aSFP gene sequence to obtain raSFPH6. The obtained plasmid was used to transform competent cells of the BL21(DE3) *E. coli* strain, and the positive colonies were grown in LB Broth medium. To the expression trials, bacterial cultures were performed with different inducer concentrations (1.0, 0.5, 0.1, and 0.01 mM IPTG) and post-induction temperatures (20, 30, and 37°C). The obtained raSFPH6 was released from bacterial cells by ultrasonic disruption and confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli method, and Western blotting Afterward, raSFPH6 was purified through affinity columns to perform the biologic function assays by adding different concentrations in post-thawed sperm cells (7.5, 15, and 30 µg per million sperm cells). The post-thaw seminal quality parameters as sperm motility and membrane integrity were evaluated in post-thawed semen of ten bulls (Three semen straws per bull) after one hour of incubation at 37 °C with raSFPH6 and then were compared against a control sample with no recombinant protein added. Results showed 0.01 mM IPTG and 37°C as the best set of conditions for raSFPH6 production using E. coli as a bio-factory. The purification procedure using affinity columns showed a high nickel affinity, being necessary up to 200 mM imidazole to elute raSFPH6. In addition, the pDass vector has a translocation signal peptide which promotes the formation of disulfide bonds. Translocation also improves the right folding and reduces the inclusion bodies formation obtaining 15 kDa raSFPH6. Regarding the sperm quality trials, raSFPH6 did not affect the post-thaw sperm motility or viability. However, there was a clear tendency for decrease motility when protein amount was increased. These results suggested an anti-motility effect of raSFPH6, possibly related to the natural regulation function of motility attributed to aSFP. This regulation function could be related to a reserve mechanism of bovine semen to avoid premature energetic consumption before sperm cells reach the oocyte fertilizing site. Protein quantities lower than 7.5 µg per million sperm cells should be considered in further experiments. Finally, the effect of adding raSFPH6 directly into the cryopreservation medium is an interesting alternative strategy for the development of protein-based additives.

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Animal Reproduction

First attempt at sperm cryopreservation in rhea (*Rhea americana*) - Preliminary results

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The rhea *(Rhea americana)* is a native bird of South America, which is globally classified as an almost threatened species. In this context, research on its reproductive physiology as well as the development of assisted reproductive techniques are essential for its conservation. Despite the importance, protocols for sperm cryopreservation of Rhea sperm were not yet stablished. In this initial study, we aim to investigate the use of a commercial extender for sperm recovery and dilution, as well as the effect of dimethyl sulfoxide (DMSO) as a cryoprotectant agent for the cryopreservation of Rhea sperm. For this purpose, we used two adult males, weighting 25.1 Kg on average, from the Centre for Multiplication of Wild Animals (CEMAS/UFERSA). Individuals were part of a programmed slaughtered conducted for research. For the experiments, individuals were premedicated with intramuscular (IM) administration of 2% xylazine hydrochloride (1mg/Kg) plus 10% ketamine hydrochloride (15mg/kg), anesthetized with IM thiopental 1g (150mg/Kg), and euthanized with intracardiac administration of potassium chloride 19.1% (2.56mEq/Kg). Sperm were obtained from the deferens ducts reservoir by the flotation technique using the Ovodyl™ (IMV technologies, l'Aigle, France) extender. Suspension containing sperm cells was evaluated for concentration using a Neubauer counting chamber, motility and vigor by light microscopy, morphology using a smear stained with Bengal Rose, membrane integrity using fluorescent probes [Hoechst 33342 (40 µg/ml in DPBS) and Propidium iodide (0.5 mg/ml in DPBS)] and membrane functionality using distilled water as hypoosmotic solution (0 mOsm/L). For freezing, samples were diluted in Ovodyl™, equilibrated at 5 ºC for 30 min, and added of different DMSO concentrations (6%, 10%, 14%, 18%), reaching a final concentration of 75 x 10^6 sperm/ml. Samples were then packed in 0.25 ml plastic straws, exposed do liquid nitrogen vapors for 15 min, and plunged into liquid nitrogen for storage. After one week, samples were thawed at 5 ºC for 40 s and reevaluated. Data were expressed as mean and standard error. Fresh samples presented a concentration of 140 ± 10.0 x 10^6 sperm/ml, being 77.5 ± 2.5% motile sperm with vigor 3.5 ± 0.5, with 72 ± 1.7% normal morphology, 66.5 ± 4.5% membrane integrity and 78.5 ± 14.5% functional membrane. After thawing, treatments containing different DMSO concentrations (6%, 10%, 14% and 18%) presented: 15.0 ± 10.0%, 10.0 ± 5.0%, 7.5 ± 2.5%, and 7.5 ± 2.5% motile sperm with vigor 1, respectively. We highlight that post-thawing sperm motility increasingly varied between the two individuals, which could indicate an individual variation related to sperm freezability in the species. For the same treatments, post-thaw samples presented 75 .0 ± 3.5%, 77.5 ± 9.5%, 83.5 ± 2.5% and 81.0 ± 9.0% for morphology, as well as 15.5 ± 1.5%, 19 ± 4.0%, 15 ± 7.0%, and 9.5 ± 6.5% for membrane integrity, and values of 29.0 ± 3.0%, 35.5 ± 0.5%, 39.5 ± 3.5% and 28 ± 6.0% for membrane functionality, respectively. Notably, this study is the first description related to cryopreservation of Rhea sperm. In summary, we have had promising results showing that Ovodyl™ is an effective means of sperm retrieval and dilution; however, more studies are needed to define the appropriate concentration of cryoprotective agents to improve the freezing protocol for Rhea sperm.
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Animal Reproduction

Fractal and stereological analysis of oviduct from cows submitted to ovarian superstimulation.

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Oviduct is a dynamic organ that changes each estrous cycle, presenting different transcriptome and tissue remodeling profiles. These events are mediated mainly by the sex steroids estradiol (E2) and progesterone (P4). In this context, fractal dimension (FD) is used to quantify tissue structural changes. Also, it is a useful technique for quantifying the organization in an image from fractals that describes the amount of space and the self-similarity of the structure. Several studies highlight the effects of ovarian superstimulation (OVS) associated with equine chorionic gonadotropin (eCG) and follicle stimulating hormone (FSH) on regulation of gene expression in the oviduct and changes on oviductal cells differentiation. Here our objective was to investigate morphological and fractal changes in the ampulla and isthmus from cows submitted or not to OVS with FSH or FSH combined with eCG. Multiparous non-lactating Nellore cows, aged between 5 and 7 years, were submitted to OVS protocols with FSH (n=5) or FSH/eCG (n=5). Control group was not submitted to OVS protocol (n=5). Histological slides from the ampulla and isthmus region were stained with Hematoxylin- Eosin and photographed at 5X and 40X magnification, with subsequent morphometric analysis and fractal dimension using Image J software. The effect of OVS on the stereological characteristics and fractal dimension of the bovine oviduct was investigated by parametric ANOVA. Means were compared by Tukey-Kramer test. Differences were considered significant when $P<0.05$. In the ampulla, use of FSH/eCG was able to increase the area of the muscle layer of the oviduct ($p=0.04$), as well as the height of the mucosa ($p=0.02$). On the other hand, in the isthmus, use of FSH combined with eCG decreased the mucosal height ($p = 0.006$), epithelium area ($p = 0.01$), and minimize lumen area ($p = 0.004$). Regarding, fractal analysis, ovarian OVS decrease fractal values in the ampulla ($p=0.0116$), but not in isthmus of bovine oviducts ($P> 0.05$) compared to non- superstimulated cows. The evaluation of the morphological parameters of the oviduct is extremely important to understand tissue remodeling during physiological adaptations against hormonal variations resulting from ovarian overstimulation, since the extracellular matrix plays an important role in preparing the oviductal environment for gametes, fertilization and early embryo development. In general, we figure out that super stimulatory approach of FSH combined with eCG modifies the ampulla and isthmus stereological phenotype in divergent ways in the bovine oviduct. Furthermore, we suggest that alterations on fractal analysis caused by OVS could impacts the cellularity and cellular distribution pattern of the bovine oviduct with possible changes on extracellular matrix remodeling. Acknowledgements: FAPESP (grant #2018/06674-7) Email: castilho.anthony@gmail.com

Animal Reproduction

Gene expression of the antioxidant enzyme Peroxiredoxin 1 (PRDX-1) from in vitro produced bovine embryos in culture media with essential oil of Lippia origanoides

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The *in vitro* production (IVP) of bovine embryos is one of the most widespread reproductive biotechniques in Brazil in the last decade. Nevertheless, during IVP, the embryo is exposed to several external agents that generate excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H2O2), which can modify biological molecules (lipids, proteins, and nucleic acids), inducing abnormal development and embryonic death. To circumvent this damage, the addition of antioxidants to the embryonic culture medium has become routine. In addition, antioxidants have the effect of directly influencing gene expression through genetic reprogramming, preventing DNA and histone methylation, and modulating the expression of antioxidant enzymes such as peroxiredoxins (PRDXs). This study evaluated the influence of the addition of essential oil obtained from the plant *Lippia origanoides* to the *in vitro* bovine embryo culture medium on the gene expression of the antioxidant enzyme Peroxiredoxin 1 (PRDX-1). Oocytes obtained from slaughterhouse ovaries were matured and fertilized *in vitro* according to standard procedure for bovine species. After 20 hours of fertilization, the possible zygotes were cultivated in SOF*m* medium supplemented with antioxidant, constituting five different treatments: **T1**: without addition of antioxidants; **T2**: addition of 50µM/mL of Cysteamine; and **T3**, **T4** and **T5** with addition of 2.5µg/mL, 5.0µg/mL and 10.0µg/mL of antioxidant obtained from the essential oil of *Lippia origanoides*, respectively. On the seventh day of culture (D7), blastocyst (Bl) stage embryos were washed in DPBS added with 0.1% PVP and transferred to 1.5 mL RNA-free cryotubes in a volume of 5µL. Cryotubes were immediately immersed in liquid nitrogen (-196°C) and stored at -80°C until the moment of gene expression analysis by RT-PCR. The RNA samples were immediately reverse transcribed using the TaqMan® Reverse Transcription Reagents kit (Applied Biosystems®, Carlsbad, CA, USA) with Oligo dT, to obtain complementary DNA (cDNA) for the target gene PRDX-1. The gene expression of PRDX- 1 in bovine embryos produced *in vitro* did not differ in the amount of transcripts between the control group without supplementation, T2 (1.28 ± 1.27) containing antioxidant Cysteamine, T4 (0.75 ± 0.58) and T5 (1.07 ± 0.99) ($P>0.05$). However, embryonic culture medium containing a lower concentration of antioxidant obtained from essential oil of *Lippia origanoides* (2.5µg/mL; T3) had lower ($P<0.05$) expression of PRDX-1 (0.47 ± 0.43) which may be considered underexpressed in embryos cultured at these concentrations. The present study demonstrated for the first time the low amount of PRDX transcripts in embryonic culture media supplemented with essential oil of *Lippia origanoides*, mainly at a concentration of 2.5µg/mL. These results suggest that the difference in the expression of this gene reflects in the intracellular situation, producing a lower protection against oxidative stress in embryos cultivated in this treatment.
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Animal Reproduction

Gerbil's ovarian xenotransplantation in NSG mice

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The NSG mice (NOD scid gamma, The Jackson Laboratory, USA) have a severe immune deficiency due to the absence of T, B, and natural killer (NK) cells. Therefore, they are commonly used in research involving immunology, oncology, and stem cell biology. The immunological absence allows its use to perform xenotransplants or xenografts. Thus, the objective of the present study was to evaluate the viability of ovarian transplantation from a female gerbil (*Meriones unguiculatus*) into female NSG mice (*Mus musculus*), and the response of the transplanted ovary to hormonal superovulation. The study was conducted in the Central Vivarium of the Federal University of Mato Grosso do Sul (UFMS; 20°30'22.4"S 54°36'53.9"W) and was previously approved (CEUA n° 1.176/2021). All animals were anesthetized with ketamine (100mg/kg; IP) and xylazine (10mg/kg; IP). After anesthetized, the female gerbil (n=1) was euthanized, and the ovaries were removed and placed in a sterile petri dish containing saline solution. NSG females (n=2), after anesthetized, underwent antisepsis and trichotomy of the left flank region. An incision was made to locate and expose the left ovary, which was completely removed from the ovarian bursa through a small opening made, and the gerbil ovary was transplanted through this opening. Afterward, the structures were returned to the anatomical position and the synthesis of the musculature, and the skin was performed with individual simple stitches. After the surgical procedure, Tramadol (5mg/kg; SC) and meloxicam (2mg/kg; SC) were administered for analgesia. After 21 days, vaginal cytology was performed for four consecutive days to monitor the estrous cycle phase. After this period, females were superovulated with 7 IU of eCG (Novormon; IP), and after 48 hours, 7 IU of hCG (Chorulon; IP) was applied and were mated with vasectomized males. The ovaries and their structures evaluation were performed 12h after mating, after euthanasia of the females by cervical dislocation (according to normative resolution nº37, January 27, 2018 by Concea). The monitoring of vaginal cytology demonstrated that the females were normally cycling, presenting the stages of diestrus, proestrus, estrus, and metestrus. A vaginal plug was observed in both females on the day after the mating. Both transplanted ovaries responded to the hormonal protocol, confirmed by visualizing follicles, corpus luteum, and hemorrhagic body. It was possible to obtain a total of 11 oocytes from the gerbil ovaries after slicing. The remaining mice's ovaries presented oocytes in the oviducts. Studies involving ovarian xenotransplantation of GFP mice transplanted into another genetic lineage of immunodeficient mice (SCID) showed success rates in offspring. Still, it is noteworthy that only the lines differed, not the species. Another study that addresses the ovarian xenotransplantation of sheep in CB17 and Balb/c nude mice, performed in the renal capsule, did not present follicles, making the xenotransplantation performed in the ovarian bursa more applicable so far. Therefore, this technique is an alternative for obtaining oocytes from other animal species, especially those threatened with extinction or those presenting reproductive problems to conventional techniques. The present study was successful in ovary transplantation from one species to another, resulting in a positive response to the hormonal protocol. Both the gerbil and the mice female's ovaries presented ovulation. Acknowledgment: The present study was performed with support from the Federal University of Mato Grosso do Sul - UFMS/MEC - Brazil, with support from the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 and from the Central Vivarium - INBIO/UFMS and was funded in part by the Reprocon Institute and IMV Technologies.

Palavras-chave: xenograft, vaginal cytology, superovulation, rodent

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Animal Reproduction

Global proteomic analysis of tertiary follicles from sheep

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Folliculogenesis is the process of formation, activation, growth and maturation of ovarian follicles, the functional unit of the ovary composed of an oocyte surrounded by somatic cells. The present study was conducted to identify the major proteome signature tertiary follicles from ewes. Ovaries from five adult, cross-breed ewes were collected and under sterile conditions, the ovarian cortex was sliced and antral follicles were dissected individually. Fifty tertiary follicles were pooled and resuspended in lysis buffer for protein extraction. Then, proteins were digested with trypsin, desalted and lyophilized. Next, tryptic peptides were loaded into LC-MS/MS, followed by analysis of raw data using PatternLab's software, with *Ovis aries* protein database downloaded from UniProt. Identified proteins were analyzed for gene ontology terms using FunRich software, protein clusters and metabolic pathways using DAVID software. There were 1.536 proteins detected in the tertiary ovine follicles, based on the current methods. Among all listed proteins, 1.305 proteins were characterized according to Uniprot Database and 231 were still defined as non- characterized proteins. Based on enrichment score and spectrum count, vimentin and actin cytoplasmic were the most abundant proteins in the ovine tertiary follicles, followed by 78 kDa glucose-regulated protein, albumin, disulfide isomerase, HATPase_c domain-containing protein, heat shock protein alpha, ATP synthase subunit beta, malate dehydrogenase mitochondrial, decorin, histones (H2B, H3, H4 and H2A), collagen-binding protein, ribonucleoproteins A2/B, gluthatione S transferase, among others. The most abundant proteins found in our study are cytoskeleton compounds that contribute to the structural integrity of cells and participate in cell-to-cell binding, differentiation and proliferation events. Important biological processes linked to follicle proteins were cellular response to zinc ion (11,8%), translation, glycolytic process and cell aging (5,7%). The most expressive cellular components were defined as cytoplasm (36,2%), nucleus (27,7%), cytosol (23,4%), extracellular region (12,8%) and cytoskeleton (6,4%). Molecular functions were mainly reported as metal ion binding (24,4%), zinc ion binding (12,2%), oxygen binding (9,8%) and ATP binding (9,8%). Analysis based on DAVID platform detected 34 enriched functional clusters with potential association with follicle development, such as translation, metabolic process, protein stabilization, glycolysis and some cellular components, nucleosome and structural components. Proteins involved in these clusters play essential roles in regulated and constitutive transcription, and participate in synthesis of different endogenous substrates, including steroids and prostaglandins. The current study is the first comprehensive description of the proteome of tertiary follicles in the ovine species. We also describe a detailed method for extraction and characterization of follicle proteins using HPLC-coupled mass spectrometry and bioinformatic tools. The present results should contribute to the identification, in future studies, of genes and functional proteins important for follicular development and oocyte maturation.

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Animal Reproduction

Heat stress impairs kinetics and morphology of epididymal sperm in rams (*Ovis aries*)

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Our objectives were to investigate impacts of testicular heat stress (HS) on kinetics and morphology of epididymal sperm from rams. Testicular HS was induced by scrotal insulation (disposable diapers). Rams were allocated into 3 groups (n=5), according to duration of insulation: Control (CO - not insulated); Insulated 24 h (24H); and Insulated 48 h (48H). Immediately after treatment, rams were castrated and epididymides dissected and washed in warm PBS (37 ºC). Then, incisions were made in the *cauda epididymides* and recovered sperm were either diluted with PBS and kept at 37 ºC until kinetic evaluation, or stored in 4% formalin for morphology assessment. Treatment increased scrotal temperature ~5ºC, irrespective of the duration of insulation, with no significant effect on body temperature. All motility end points were decreased (P<0.05). For CO, 24H and 48H, there was (mean ± SEM): total motility (TM): 72±7.6, 39.5±8.2 and 36.1±11%, respectively; progressive motility (PM): 33.5±5.6, 5.0±0.9 and 6.8±2.3%; VAP: 114.4±7.6, 64.3±9.8 and 73.5±10.1 µm/s; and VSL: 93.55±8.8, 42.0±2.6 and 51.4±1.9 µm/s. In CO, 24H and 48H, there were progressive reductions in morphologically normal sperm (92.5±3.7, 55.2±5.2 and 32.4±4%, respectively (all differed from each other, P<0.05), primarily due to coiled tails (4.8±2.9, 35.0±8.3 and 53.5±3.8%; all differed, P<0.05). Therefore, short-term scrotal insulation in rams had a rapid and severe adverse impact on kinetics and morphology of epididymal sperm. Acknowledgments: CAPES (processes nº 88887.571133/2020-00 and nº 88887.486411/2020-00), FAPESP (grant#2018/02007-6), CNPq, and Botufarma®.

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Animal Reproduction

Heat stress modulates endoplasmic reticulum stress and heat shock proteins protective response in peripheral blood mononuclear cell in early pregnant cows

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Heat stress (HS) is one of the major limiting factors in dairy bovine production and contribute to economic losses on dairy industry. It also impairs reproduction rates, immune defenses and induces the occurrence of oxidative stress, which is a precursor and a major contributor to the occurrence of endoplasmic reticulum (ER) stress and heat shock proteins (HSPs) protective response. Furthermore, HS induces pregnancy losses, which are more frequently in the first 30 days in bovine and coincides to maternal recognition of pregnancy period. Consequently, we aimed to verify early pregnancy modulation in ER stress and HSPs response in peripheral blood mononuclear cells (PBMCs) from dairy cows, and whether this modulation is affected by HS. Cows had their estrous cycle synchronized and assigned to a non-heat stress or heat stress group. Blood samples were collected at the moment of artificial insemination (AI) and also on Days 10, 14 and 18 following AI. Pregnant cows were pregnancy checked by ultrasound on Day 30 and confirmed on Day 60 post-AI. *HSF, HSP70, HSP90, HSPA5, ATF6, uXBP1, sXBP1* and *CHOP* were evaluated by RT-qPCR on Days 10, 14 and 18 in PBMCs. Gene expression data were analyzed by repeat measures (JMP7 Software). Results are presented as mean ± SEM. Temperature-humidity index (THI) was considered to sort cows, and on non-heat stressed period was 68.77±1.55 and on heat stressed period was 83.02±1.37. High THI affects physiological parameters like rectal temperature, heart and respiratory rates in dairy cows ($P<0.05$), which were evident at all days along the season, confirming the experimental model. Estrous occurrence and pregnancy rates were not different ($P>0.05$). However, pregnant cows had endoplasmic reticulum (ER) stress pathway increased ($P<0.05$) on Day 10 and HSP90 response on Days 10 and 18 following artificial insemination (AI) when compared to non-pregnant cows. Considering the HS, ER stress pathway was increased ($P<0.05$) in heat stressed cows independent of pregnant status, except the *ATF6* gene, which was greater only in pregnant cows. HSPs expression was different only on pregnant cows, which were upregulated in heat stressed cows ($P<0.05$). Taken together, out data demonstrate that ER stress occurs on Day 10 in pregnant cows; Considering cows in HS or non-HS environment, heat stressed cows present greater expression of ER stress on Day 10, regardless of pregnancy status, except *ATF6* gene expression; and pregnancy induces *HSF* and *HSP90* mRNA transcription, but not *HSP70*. In conclusion, early pregnancy modulates ER stress and HSPs response in PBMCs from dairy cows, and this modulation in pregnant cows is affected by the occurrence of HS.

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Animal Reproduction

Histological characterization and immunolocation of *Trypanosoma vivax* in sexual glands of young goats

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Trypanosoma vivax is one important protozoon which causes economic loss and affects the genital system causing testicular and epididymal damages. However, it is important to emphasize that the seminal characteristics are also a result of male sexual glands contribution, and there is scarce information regarding how trypanosomiasis affects these organs in ruminants. In the present study the aims are to characterize the sexual glands of young goats experimentally infected with *T. vivax* by histology and to localize the parasite in these glands by direct immunofluorescence assay. Twenty Saanen goats, aging 6 to 9 months, weighting (± EPM) 30.2 ± 0.5 kg were assigned in infected group (G-INF, n = 15) and control group (G-CON, n = 5). In G-INF, one mL of blood containing $1x10^4$ trypomastigotes of Miranda strain were inoculated i.v. Animals were euthanized 47 days after inoculation and fragments of seminal glands, prostate, and bulbourethral glands were collected for histological processing (5 µm). Part of slides were stained with hematoxylin-eosin and the other part was submitted to direct immunofluorescence assay. For this purpose, slides were deparaffinized in xylol and decreasing alcohol solutions, followed by antigen recovery by heat and protein blocking. After protein blocking, slides were washed with PBS solution and two areas were delimited with hydrophobic pen in each slide to receive in one of them, bovine serum from an animal infected with *T. vivax*, and in the other, bovine serum from an animal not infected (negative control). Slides were incubated, washed, and co-incubated with rabbit anti-bovine IgG-FITC antibody (Sigma-Aldrich, Missouri, EUA). After this step, the slides were prepared with buffered glycerin under a coverslip and observed in an epifluorescence microscope (Olympus BX60). In the presence of parasite, tissue emitted green fluorescence. Only descriptive statistics was used in the present study. Regarding microscopic aspect, G-INF showed presence of mononuclear inflammatory infiltrate predominantly perivascular and/ or difuse varying from discrete to moderate in seminal vesicles of 60.0% (9/15) of animals, in the prostate of 40.0% (6/15) and in bulbourethral glands 6.7% (1/15), inversely to G-CON, in which no alterations were observed. Direct immunofluorescence assay was effective in detect the presence of *T. vivax*, in a diffuse pattern (interstitium and parenchyma), in seminal vesicles of 80.0% (12/15), in prostate of 60.0% (9/15) and bulbourethral glands of 60.0% (9/15) of G-INF, while 100.0% of G-CON were negative in all tissues. Also were estimated in seminal glands, prostate, and bulbourethral glands the specificity (100% for all tissues), sensitivity (80%, 60%, and 60%, respectively), and and accuracy (90%, 80%, 80%, respectively), demonstrating reliability of this assay. This is the first report in which microscopic characterization and location of *T. vivax* in tissues of sexual glands were described in small ruminants. In cattle, *T. vivax* infection produced several degrees of seminal vesicles and prostate degeneration, and no lesions in bulbourethral glands, which could result in male infertility or sterility (Sekoni et al., 1990 Br Vet J, 146:175-80). The location of *T. vivax* in these reproductive organs indicates direct involvement in the pathogenesis of infection, in addition to systemic effects of trypanosomiasis, affecting male fertility. In conclusion, experimental infection with *T. vivax* in male goats produce inflammatory damage in sexual glands, and for the first time the presence of the parasite in these tissues was demonstrated by direct immunofluorescence assay. Acknowledgements: To FAPESP (PROC 2019/ 22695-7; PROC 2020/06493-2), Alessandra Regina Carrer, Letícia Castro Fiori, Beatriz Eustachio Boarini, Gwenever Camargo Moraes, Maísa Pansani Santos e Viviane Bobadilha Morelli.

Keywords: trypanosomiasis, histology, sexual accessory glands, direct immunofluorescence assay, bucks

Animal Reproduction

Histological characterization of bovine corpus luteum from females treated with different PGF2α and doses during metestrus or diestrus

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Prostaglandin F2α (PGF2α) analogues are widely used in the control of estrous cycle, promoting luteolysis and, consequently, apoptosis. Although there is knowledge about ultrasonographic aspects, corpus luteum morphology and profile of progesterone, there are few reports regarding the histological pattern of luteal cells. Thus, the aim of the present study was to evaluate the luteolytic efficiency of dinoprost tromethamine (DT) and sodium cloprostenol (SC), administered in a dose of 100% or 50% of recommended by label, during metestrus or diestrus (D4 or D11 post-ovulation, respectively), regarding the histomorphometric characterization of small luteal cells (SLC) and large luteal cells (LLC). For this purpose, 40 *Bos indicus* or crossbred cows were submitted to an ovulation synchronization protocol. The time of ovulation was monitored by ultrasound evaluation and only animals which ovulated between 72 and 84 hours were allocated to the experimental groups. A total of 23 cows were selected for containing corpus luteum (CL) and were divided into the following treatments, in a factorial arrangement 2 (days of the estrous cycle) x 2 (luteolytic agents) x 2 (doses): D4SC50%, D4SC100%, D4DT50%, D4DT100%, D11SC50%, D11SC100%, D11DT50% and D11DT100% (n = 3/ group, except D11DT50% in which n = 2). After two days of treatment, the animals were slaughtered, their ovaries collected and the CLs isolated. Six histological slides per animal were prepared using hematoxylin-eosin staining. In each histological section, 50 CLP + 50 CLG were analyzed, totaling 600 cells per animal. For statistical analysis, data were described as mean ± SEM and ANOVA followed by Tukey's test (SAS) with 5% significance was used. As fixed effects were included in the model: estrous cycle day, type of prostaglandin and dose, as well as interactions, and animal was included as random effect. There was no significant difference for the main effects in the measurement of SLC (D4: 452,1 ± 2,2 μm^2; D11: 443,7 ± 2,6 μm^2; DT: 447,4 ± 2,6 μm^2; SC: 448,6 ± 2,1 μm^2; 50%: 435,2 ± 2,15 μm^2; 100%: 462,1 ± 2,6 μm^2). Regarding LLC, no significant difference was shown for the main effects of drug and dose (DT: 6109,1 ± 34,2 μm^2; SC: 5551,9 ± 33,2 μm^2; 50%: 5887,1 ± 33,8 μm^2; 100%: 5743,3 ± 34, 2 μm^2), only according to the day of estrous cycle (D4: 6377,7^B ± 36,1 μm^2 vs. D11: 5208,1^A ± 27,7 μm^2, P = 0,02). When analyzing the moment D4, the mean of the area obtained indicates that the application of any luteolytic agent, regardless of the dose, has no predicted result (induction of total luteolysis); this is because at this stage the CL is refractory to PGF, as it expresses vasoactives peptides (Levy N et al. 2000. Biol. Reprod., 63:377-382) and because of its specificity in signal conversion mediated by gene expression of PGF receptors at this stage (Goravanahally M et al. 2009. Biol. Reprod., 80:980-988). In conclusion, the luteolytic efficiency is similar for both luteolytic agents regardless of the dose, being able to cause structural luteolysis. However, when analyzing the time of administration, the best luteolytic response occurred, as expected, in diestrus. **Acknowledgments:** FAPESP (process: 2019/03690-4) and Ourofino Animal Health.

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Animal Reproduction

Identification of reproductive diseases in beef cattle in south of Brazil

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The detection of reproductive diseases in cattle herds is extremely important for reproductive efficience. The objective of this study is to report the pregnancy rate, loss gestational and infectious agents detected in beef cows (n=12,320) and bulls (n=493) in Rio Grande do Sul. Samples from females (n=717) cervical mucus were sent for analysis through Polymerase Chain Reaction (PCR) to *Campylobacter fetus* subsp*. venerealis; Ureaplasma diversum; Mycoplasma bovis; Mycoplasma bovigenitalium*. Blood samples collected from the tail vein were forwarded for detection of anti-Leptospirosis spp antibodies (microagglutination) and anti- Neospora caninum antibody (Elisa or Indirect Immunofluorescence). The bulls (n=177) were submitted to preputial shaving for direct search of agents through PCR for *Campylobacter fetus* subsp. *venerealis*; *Ureaplasma diversum; Mycoplasma bovis; Mycoplasma bovigenitalium*. The model of reproductive management used in these farms was the Fixed-Time Artificial Insemination (FTAI) or Natural Breading (NB), therefore 3FTA (n=1); 2FTAS+NB (n=3); FTAI+NB (n=9) and NB (n=4). The average pregnancy rate was 80.18%, and pregnancy loss reported on farms was 9.98%. The farms that used NB obtained gestational loss, numerically superior to the farm that uses only 3FTAS (13.34% vs 4%; P=0.719). In females, the rate of seroreagents to Leptospirosis 30.68% and *Neospora caninum* was 14.35%. The PCR detection for *Ureaplasma diversum* was 10.97%, *Campylobacter fetus* subsp *veneralis* 8.84%, *Mycoplasma sp*. 5.96%. Thus 70.80% of females who had pregnancy loss were of positive. The handling preventive vaccine for reproductive diseases was used in 9 of the 17 farms. The pregnancy loss was 12.1% on farms not use reproductive vaccines and 8.1% in farms who use vaccine. The bulls had 57.9% of positive, being 17.0% for *Ureaplasma diversum*, 24.9% for Campylobacter and 16.0% for Mycoplasma sp. The results of this study emphasize the existence of a considerable number of animals affected by infectious agents that cause reproductive losses in beef cattle in South of Brazil. The bulls must have special sanitary management during Breading Season Exam and annual on the farm, recommended submitted to preputial scraping. This study showed various agents in beef cattle in RS and emphasizes special attention to agents such as *Neospora caninum*, *Mycoplasma sp., Ureaplasma diversum* who have few reports in beef cattle. It is concluded that the identification of agents and FTAI intensification are tools to carry out sanitary management suitable for reducing pregnancy losses and achieving reproductive efficiency.

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Animal Reproduction

Immunolocalization of melatonin receptor type 1 in the sheep ovary and involvement of the PI3K/Akt/FOXO3a signaling pathway in the effects of melatonin on survival and *in vitro* activation of primordial follicles

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Melatonin plays an important role in the early folliculogenesis, and its actions can be mediated through interaction with type 1 receptor (MT1) and subsequent activation of the phosphatidylinositol-3-kinase/protein kinase B/forkhead box O3a (PI3K/Akt/FOXO3a) pathway. However, there are no data on the immunolocalization of the MT1 receptor protein in preantral and antral follicles and on the melatonin effects in the survival and activation of primordial follicles in sheep. The expression of the MT1 receptor in ovine ovaries was investigated by immunohistochemistry. Furthermore, ovine ovarian fragments were fixed for histology (fresh control) or cultured in α-MEM$^+$ (control medium) or α-MEM$^+$ supplemented with different concentrations of melatonin (100; 500 or 1,000 pg/mL) for 7 days. After *in vitro* culture, ovarian fragments were destined to histological (follicular survival, activation, and growth) and immunohistochemical (cleaved caspase-3: apoptosis marker) analyses. To evaluate the possible involvement of the PI3K/Akt/FOXO3a pathway in the melatonin actions, PI3K inhibition was performed in fragments cultured with LY294002 and immunostaining for phosphorylated Akt and FOXO3a was analyzed after culture in the absence or presence of PI3K inhibitor. Data regarding normal (survival), cleaved caspase-3-positive (atretic), primordial and growing (activation) follicles were compared by Chi-squared test. Data of follicular and oocyte diameters were evaluated by ANOVA and Tukey´s tests ($p<0.05$). The immunohistochemical localization of the MT1 receptor protein was observed in oocytes and granulosa cells of all stages of follicle development, and in theca cells of antral follicles. After culture, follicular survival was greater ($p<0.05$) in 100 pg/mL melatonin (60%) than in the α-MEM$^+$ (46%) and other melatonin concentrations (41.33% and 37.33% for 500 and 1,000 pg/mL melatonin, respectively). In addition, culture with 100 pg/mL melatonin maintained the percentage of atretic follicles (26.08%) similar ($p>0.05$) to that observed in the fresh control (20.8%) and lower ($p<0.05$) that other treatments (50%, 55% and 72.7% for α-MEM$^+$, 500 and 1,000 pg/mL, respectively). Although all treatments induced a reduction ($p<0.05$) in the percentage of primordial follicles and an increase ($p<0.05$) in the percentage of growing follicles compared to the fresh control, 100 pg/mL melatonin showed greater ($p<0.05$) follicular activation than α-MEM$^+$ and other melatonin concentrations. *In vitro* culture of ovarian cortex with PI3K inhibitor plus 100 pg/mL melatonin increased ($p<0.05$) the follicular atresia (60% of cleaved caspase-3- positive follicles) and inhibited ($p<0.05$) the primordial follicle activation compared to the medium without PI3K inhibitor (containing only 100 pg/mL melatonin). Furthermore, follicles cultured with 100 pg/mL melatonin in the absence of LY294002 showed a moderate staining for p-Akt, while those cultured with the PI3K inhibitor showed a markedly reduced staining. The percentage of p-FOXO3a-positive oocytes was lower ($p<0.05$) in the presence of PI3K inhibitor (30%) compared to the culture without the inhibitor (90%). These results suggest that melatonin effects on ovarian follicular cells can be via its antioxidant properties and/or mediated by its interaction with MT1 receptor. In conclusion, MT1 receptor protein is present in all follicle categories in the sheep ovary. After *in vitro* culture of sheep ovarian cortex, 100 pg/mL melatonin reduces atresia and promotes primordial follicle activation through the modulation of the PI3K/Akt/FOXO3a pathway. Studies on the activation of primordial follicles are important for the development of an *in vitro* culture system that maximize the pool of growing follicles, providing a large supply of mature female germ cells.

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Animal Reproduction

Immunostaining of progesterone and α estrogen receptors in the uterus of pseudopregnant bitches treated with Pyridoxine Hydrochloride

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Pseudopregnancy is a common syndrome that affects diestrus bitches, triggering signs similar to pre- and postpartum, such as enlarged mammary glands and milk production. Pyridoxine hydrochloride has recently been shown to be a safe and effective substance for the alternative treatment of pseudopregnancy. As the female reproductive physiology is dependent on the presence of hormonal mediators, the evaluation of these factors associated with alternative treatment may be a way to elucidate the mechanism of action of this substance in the uterus. Thus, we evaluated the effect of pyridoxine hydrochloride on uterine immunostaining of progesterone (PR) and estrogen (ERα) receptors. Eighteen bitches with pseudopregnancy were used, divided into three groups: 1) untreated control, 2) treated with pyridoxine hydrochloride (50 mg/kg/day; n=6) and 3) treated with cabergoline (5µg/kg/day; n=6) for 20 days. Serum prolactin was measured on day zero, 2 hours before the administration of the first dose of the drug and 120 hours after the start of treatment, to control hyperprolactinemia. At the end of the treatment, the animals were submitted to ovary-salpingo-hysterectomy and uterine fragments were collected, fixed in formaldehyde (10%) for 24 hours and embedded in paraffin. Histological sections were obtained for immunohistochemical evaluation using anti-PR (1:2000) and anti-Erα (1:50) antibodies by the streptavidin-biotin-peroxidase technique. A quantitative and qualitative evaluation of PR and ERα immunostaining in the uterus was performed and the proportion and intensity determined in five regions: epithelial surface, glandular ducts, endometrial glands, stroma and myometrium. Data were subjected to analysis of variance (ANOVA) followed by the Kruskall-Wallis test. Both pyridoxine hydrochloride and cabergoline reduced serum prolactin concentrations in pseudopregnant bitches compared to control ($P<0.05$), showing similar efficacy ($P>0.05$). Regarding hormone receptors, all experimental groups exhibited strong uterine PR immunostaining in all regions evaluated, with no difference between treatments ($P>0.05$). Erα, unlike PR, showed weak or null marking in the uterus and no differences were observed between groups ($P>0.05$). In conclusion, we demonstrate that treatment with pyridoxine hydrochloride is efficient in reversing the hyperprolactinemia of pseudopregnant bitches, but its beneficial effects do not come from a direct modulation in the expression of hormone receptors of progesterone and estrogen in the uterus.

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Animal Reproduction

Impact of the use of Butophosphan and Cyanocobalamin associated with FTAI protocols in beef cattle

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The objective of this study is to evaluate the impact of the use of Butophosphan and Cyanocobalamin during a fixed-time artificial insemination (FTAI) protocol in nulliparous, primiparous and multiparous *Bos taurus* females. A total of 388 females with mean body score 2.95 was submitted to insert of an intravaginal progesterone device (P4) (Sincrogest, Ouro Fino) plus 2mg of estradiol benzoate (IM) (Sincrodiol, Ouro Fino) on the first day (D0). On D9, the animals received 250µg of Cloprostenol Sodium (Sincrocio, Ouro Fino), 1mg of estradiol cypionate (Sincro CP, Ouro Fino), 300UI of equine chorionic gonadotropin (ECG) (Sincro eCG, Ouro Fino) and P4 was removed. The animals were allocated into two groups randomized by category, ECC and presence of CL at D0: heifers – control group (G1N, n=59) and treatment group (G2N, n=58); primiparous – control group (G1P, n=74) and treatment group (G2P, n=73); multiparous – control group (G1M, n=62) and treatment group (G2M, n=62). All the females in the treatment group received 1g of Butophosphan and 0.5mg of Cyanocobalamin (Catofos®) IM on D0 and D9. Cows had their tail-heads marked with chalk (Raidl-Maxi, RAIDEX GmbH,Dettingen/Erms, Germany) at the removal of the P4 insert (Day 9). The occurrence of estrus was evaluated at TAI and was determined based upon the removal of the tail-head mark. Estrus was deemed to have occurred in cattle without a tail-head chalk mark at TAI.. Artificial insemination (AI) was performed 48h after P4 removal with semen of high fertility bull. US was performed to assess the diameter of the dominant follicle on D9 and on the day of FTAI (D11). Pregnancy diagnosis was performed 30 days after AI through transrectal ultrasonography. The mean of the dominant follicle among all categories in D9 was 8.27 ±1.24 mm in G1 and 8.48 ±0.69 mm in G2 (P=0.12) and varied from 9.06 ±1.2 mm in G1M to 10.7 ±0.84mm in G2M. On the AI day, the mean of the dominant follicle was 12.25 ±1.61 mm in G1 and 13.36 ±2.1 mm in G2 (P=0.001), ranging from 11.99 ±2.14 mm in G1P to 13.30 ±2.01 mm in G2P. The estrus rate was 70.7% (138/195) in the G1, 84.9% (164/193) in the G2, 72.6% (45/62) in the G1M and 88.7% (55/62) in the G2M (P=0.001). The overall conception rate was 57.5% (111/193) in the treatment group and 47.7% (93/195) in the control group (P=0.038). In the multiparous category, comparing the conception rate between the treatment and control groups, there was a considerable difference with supplementation with Catofos® (64.51% versus 50.0%; p=0.038). In summary, primiparous and multiparous treated cows presented larger follicular diameter on the day of artificial insemination, higher estrus demonstration rate and higher conception rate, signaling that Catofos® is a potential tool to be used in association with FTAI protocols in categories with greatest nutritional challenge to obtain better reproductive rates.

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Animal Reproduction

In silico reference genes evaluation for real-time qPCR in oocytes and embryos of buffalos

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The use of reference genes is a simple and popular method to normalize data in Real-Time Quantitative Polymerase Chain Reaction (qPCR), so the selection of reliable reference genes is essential to correctly interpretation of qPCR results. Currently, *in silico* methodologies have been developed to identify new reference gene candidates, that are based on RNA-seq analysis, and performed to select genes which presents low variability in expression across tissues and experimental conditions and presents essential cellular functions. Thus, this study aims to identify new reference genes candidates in oocytes and in vitro blastocysts from buffalo using a combination of *in silico* methodologies. Initially, RNA-seq data were obtained from buffalo oocytes and blastocysts and normalized in Reads Per Kilobase Million (RPKM). Then, using Excel and R studio software, genes were classified as stable expression among the samples considering the following criteria: 1) RPKM value; 2) coefficient of variation values, CV; 3) Maximo fold change, MFC (J et al. 2007. Plos One, 2:1-5); 4) Gini index, GI, which measures inequality in the expression levels of each gene between samples (Oet al. 2018. CellSystems, 6:230-240). In the second step, we compared the variation in expression levels of genes frequently used in the literature to normalize qPCR data in buffalo tissues. As a result, of the total of 8,649 genes expressed in the RNA-seq of buffalo oocytes and embryos, 18 genes (*NACC2, ZNF106, DDX28, MED4,THUMPD1, IRF2BP2, ELK1, QKI, EFTUD2, RAPGEF1, RAPGEF1, CCNYL1, PJA2, NUP58 , GOLT1B, TTC4, GNL3 and PRR14L*) showed stable expression values and are considered candidates for reference genes. The 18 candidate genes were selected in all 4 methodologies used, as they presented: RPKM values >40 (moderate/high expression), CV <3%, MFC <2 and GI ranging from 0 to 1. Finally, the biological functions of genes were researched on the Genecards platform (https://www.genecards.org/) being related to RNA transcription and processing, protein regulation, protein folding and stem cell proliferative capacity, that is, they exert essential cellular functions. In the second step, standard reference genes such as GAPDH, YWHAZ, SDHA, G6PD, B2M and A2M that presented CV>100%, MFC >2 and IG >4 were evaluated, thus it was considered that they did not exhibit stable expression between the samples analyzed. The *in silico* methodologies listed here have already been used in the literature to select stable genes in humans, bacteria and fish and, in the present study, they were used in a combined way to increase the selection rigor. The reference gene selection is usually neglected in many studies that use a limited variety of genes considered standard, and, it is well know that these genes can show variations in expression level when compared between different situations and/or tissues. So the combination of *in silico* methodologies can be a useful selection tool as long as RNA-seq data are available and further validation by qPCR is performed, in order to confirm the gene expression values. It is concluded that, the 18 genes selected in this study are promising new gene reference candidates to be used in real-time qPCR assays to quantify gene expression in oocytes and buffalo embryos, but prior validation by qPCR is required.

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Animal Reproduction

In vitro culture of bovine embryos with C-type natriuretic peptide change their cryotolerance?

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The *in vitro* production of bovine embryos has increased technologically in the last two decades. However, cryopreservation of these embryos is not consistent with general improvements in the technique. One of the main factors related to the low post-cryopreservation survival rate are the different lipid content and profile in *in vitro* produced embryos (IVP). They are present in the form of lipid droplets in the cytoplasm and in the composition of the cell membrane of embryos. Thus, some attempts to add and remove molecules in the *in vitro* culture medium (IVC) have already been tested. Although the use of C-type natriuretic peptide (CNP) - a modulator of intracellular concentrations of cAMP and cGMP - has already been described in *in vitro* maturation or pre-maturation, its use in the modulation of the lipid profile of embryonic cells in the IVC of bovine embryos is still poorly reported. This study aimed to evaluate the effect of two increasing concentrations of CNP (Control; 100 nM - CNP-100; 400 nM - CNP-400) since the first day (D1) of IVC with high oxygen tension (20%), in bovine blastocyst rate (*Bos taurus indicus*) and intracytoplasmic lipid content of embryonic cells by Sudan Black B analysis. Eight replicates were performed with approximately 1,000 presumptive zygotes/group. Data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk test. For the analysis of blastocyst rate, the data were normally distributed, so they were submitted to analysis of variance by one-way ANOVA. In the Sudan Black B analysis, there was a non-parametric distribution, so the Kruskal-Wallis test was applied (significance when $P \leq 0.05$). On day 7, the Control, CNP-100 and CNP-400 groups produced, respectively, 34.13; 33.63 and 32.55% of blastocyst. On days 7 and 8, in the evaluation of the embryos for lipid content, the treatment with CNP-400 had the lowest intracytoplasmic lipid content (gray intensity analyzed by Software Image J) when compared to the Control group (respectively, 2.184×10^{-6} and 2.341×10^{-6}; $P = 0.007$). However, CNP-100 had an intermediate lipid content compared to CNP-400 and the Control group (2.219×10^{-6}; $P \geq 0.05$). The results suggest, as recently reported in the literature, that the use of CNP in IVC can alter the lipid content of bovine embryos. Our study showed that the concentration of 400 nM was the most efficient concentration in this function. Nevertheless, further studies are needed to investigate the mechanism by which CNP interacts with lipid metabolism and whether there was a change in the profile of these intracytoplasmic lipids and in the cell membrane. Work in progress are focusing in the *in vitro* cryopreservation survival of the embryos derived from CNP, to evidence any improvement of their cryotolerance. Acknowledgements: FAPESP (processes nº 2019/10732-5) and CAPES- Financing Code 001.

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Animal Reproduction

In vitro culture of fresh and vitrified preantral ovarian follicles of *Dasyprocta leporina*

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The agouti (*Dasyprocta leporina*) is a histricognate rodent, widely spread throughout South America, with important ecological functions for acting as a seed disperser and contributing to the balance of the food chain. Among the 12 different species of the genus Dasyprocta, *D. leporina* is the one with a stable population, which highlights its importance as an experimental model for the development of assisted reproductive technologies to be applied to endangered individuals. In this sense, research aimed at the conservation of male agouti germplasm is in rapid development, but females have still been neglected. Therefore, we aimed to stablish a system for the in vitro culture (IVC) of fresh and vitrified ovarian preantral follicles (PAFs) from agoutis by the use of TCM-199 medium supplemented with porcine follicle stimulating hormone (pFSH). After a programmed slaughter at the Centre for Multiplication of Wild Animals (CEMAS/UFERSA), conducted according to the recommendations of the ethics committee of UFERSA (no. 21/2018), six pairs of ovaries from sexually mature females were collected, washed in 70% alcohol and Minimal Essential Medium (MEM) and fragmented (9.0 mm^3 = 3 × 3 × 1 mm). Two fragments were destined to a fresh control group, while other fragments were vitrified through a solid-surface (SSV) procedure using a vitrification solution consisting of MEM supplemented with 3 M ethylene glycol (EG), 10% fetal bovine serum (FBS), and 0.25 M sucrose. Vitrified samples were rewarmed after one week and subjected to a 6-days IVC in an incubator at 38.5 °C and 5% CO_2, using TCM-199 supplemented with 50 ng/mL pFSH, ITS (10 µg/mL of insulin, 5.5 µg/mL transferrin and 5.0 ng/mL selenium), 0.23 mM sodium pyruvate, 2 mM glutamine, 2 mM hypoxanthine, and 1.25 mg/mL albumin bovine serum. Culture medium changes were performed every other day. Fresh, vitrified and cultured samples were evaluated for morphology and development (proportion among growing and primordial follicles) by a hystological procedure, and viability through a Trypan blue assay. Data were expressed as means (± SEM) and evaluated by ANOVA followed by PLSD Fisher ($P < 0.05$). Fresh samples presented 71.8 ± 2.1% (71/98) morphologically normal follicles, similarly ($P > 0.05$) as verified for vitrified (67.5 ± 13.9%; 76/119) and vitrified-cultured (76.2 ± 7.2%; 61/82) samples. Regarding PAFs development, we found the proportions of 73.9 ± 18.7%, 52.2 ± 13.5% and 68.0 ± 9.4% developing PAFs in the fresh, vitrified and vitrified-cultured samples, respectively. No differences were found among treatments ($P > 0.05$). For viability, fresh samples presented 88.3 ± 4.8% (106/120) viable follicles, similarly ($P > 0.05$) as found for vitrified samples 65.8 ± 11.4% (79/120); however, a decrease ($P < 0.05$) on the percentage of viable follicles was verified for vitrified-cultured samples (60.0 ± 9.2%; 73/120) in comparison the the fresh control. In summary, we provide initial data regarding the establishment of a IVC system for agouti PAFs, essential for the utilization of female germplasm stored in biobanks. The use of TCM-199 medium supplemented with pFSH seems to be adequate for this proposal, but further studies are needed to improve the protocol in order to provide efficient PAF development during IVC. **Financial support:** CNPq, CAPES

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Animal Reproduction

In vitro culture of testicular tissue from prepubertal collared peccaries using different culture media supplemented with various GDNF concentrations

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Populations of collared peccaries (*Pecari tajacu* Linnaeus, 1758) have been threatened by anthropic devastation in Caatinga and Mata Atlantic Brazilian biomes. Studies to preserve the germplasms therefore are critical to protect that species from extinction. To that effect, the in vitro culture of gonadal tissues is an essential biotechnology to produce gametes from gonadal tissues stored in germplasm banks (especially from prepubertal individuals). The aim of the study was to evaluate the influence of different media supplemented with various concentrations of Glial cell line-derived neurotrophic factor (GDNF) on the *in vitro* culture (IVC) of the testicular tissues from prepubertal collared peccary. Testes from 5 prepubertal individuals were collected at the Wild Animal Multiplication Center/UFERSA. From each individual, four tissue fragments (1x1x1 mm) were used as fresh controls, while 48 fragments were cultured for 28 days at 34 °C in a humidified atmosphere with 5% CO2 in air. Culture media were StemPro-34 SFM or the Dulbecco's modified Eagle medium (DMEM), both supplemented with various concentrations of GDNF (0, 10, or 20 ng/mL). Tissue fragments were cultured on the flat surface of 0.75% agarose gel (w/v) previously submerged in the culture medium (2 tissue fragments per gel). Medium was changed every 48 hours. Tissues in each treatment group were evaluated every 7 days for viability via fluorescence microscopy using propidium iodide (0.5 mg/mL) and Hoechst 33342 (40 µg/mL) staining. Evaluation of tubular cell swelling, tubular cell loss, rupture from basal membrane, shrinkage from basal membrane, and tubular structure according to scores (3 – adequate; 2 – regular; 1 – poor) were assessed by histology. Proliferative activity was evaluated through quantification of nucleolar organizer regions (NOR) technique. Data were expressed as mean ± standard error (SEM) and analyzed by ANOVA followed by the Tukey test. After 7 days of IVC, DMEM plus GDNF at 10.0 ng/mL (73.7±3.7%) and 20.0 ng/mL (73.3±4.5%) led to percentages of cell viability that were similar to the fresh controls (88.7±1.4%) while values were lower in the other groups ($P<0.05$). After 28 days, a similar cell viability (~70%) was verified for all treatments ($P>0.05$). Regarding tissue morphology, the StemPro-37 SFM medium plus 10.0 ng/mL GDNF provided higher scores at all the evaluating times (7, 14, 21 and 28 days) in comparison to DMEM using any GDNF concentration ($P< 0.05$). For the spermatogonia proliferative capacity after 7 days IVC, DMEM without GDNF, and StemPro-34 SFM using any GNDF concentration provided values similar to the controls (4.0 ± 0.12; $P > 0.05$). After 14 days, however, only StemPro-34 SFM plus any GDNF concentration remained similar to the controls ($P > 0.05$). For Sertoli cell proliferative capacity, both DMEM and StemPro-34 SFM without GNDF were similar to the controls; but only StemPro-34 SFM 10.0 ng/mL remained similar to the controls after 14 days ($P>0.05$). At 28 days IVC, there were no differences among treatments regarding cell proliferative capacity for both spermatogonia and Sertoli cells ($P > 0.05$). In summary, the StemPro-34 SFM seems to be the most adequate medium for IVC of prepubertal peccary testicular tissue. Moreover, the supplementation of media with GDNF, especially at a 10.0 ng/mL concentration, seems to be essential for the maintenance of cell survival and proliferation. Financial support: CNPq.

Keywords: Pecari tajacu, Testicle, Germplasm, Wildlife, Biobank

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Animal Reproduction

In vitro oocyte maturation with eugenol antioxidant improves the quality of bovine embryos

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In vitro production of bovine embryos is used worldwide, although only 30 to 40% of selected oocytes reach the blastocyst stage. It occurs due to the excessive formation of reactive oxygen species (ROS), especially in the *in vitro* maturation (IVM) stage. Therefore, new potent antioxidants such as eugenol (EU; 4-allyl-2-methoxyphenol) represent a promising strategy to reduce ROS, improve oocyte maturation status and blastocyst formation. EU is a natural phenolic compound present in essential oils of some aromatic plants, such as cloves. It has several pharmacological properties, including antioxidants. Due to its great antioxidant importance, the EU has been used in the culture of different cell types. However, it has not yet been tested in the IVM medium. Thus, this work aimed to investigate the impact of EU supplementation to the IVM medium during *in vitro* production of bovine embryos. Therefore, a total of (n = 516) *cumulus*- oocyte complexes were *in vitro* matured for 22-24h in TCM-199^{+} alone (control treatment) or supplemented with EU at concentrations of 10 (EU-10), 20 (EU- 20) or 40 µM (EU-40). After IVM, the oocytes were submitted to *in vitro* fertilization (IVF) for 18-22h and further culture of embryos for 3, 7 and 10 days. The maturation (22-24h), cleavage (day 3), blastocysts (days 7 and 10) and hatching rates (day 10), as well as the total number of cells/expanded blastocyst (day 7), was observed. The experiment was repeated three times. The variables were analysed by one-way ANOVA followed by chi-square, Fisher's exact, post hoc or Pearson's correlation tests. Statistical significance was defined as $P < 0.05$. Resulting no significant ($P < 0.05$) difference was observed between treatments for the maturation, blastocyst and hatching rates. However, the addition of 40 µM EU to the IVM medium improved ($P < 0.05$) the cleavage rate than the control treatment. Furthermore, a positive correlation ($r = 0.61$, $P < 0.03$) was observed between cleavage rate and EU concentration. The EU-10 and EU-20 treatments had higher ($P < 0.05$) total number (mean) of cells/expanded blastocyst than the control and EU-40 treatments. A previous study showed that lower EU concentrations (10-12.5 µg/mL) increased cell viability and the expression of genes related to cell survival and proliferation after murine mesenchymal stem cell culture. By considering the antioxidant effect of EU reported previously during *in vitro* culture of isolated cells i.e., human mononuclear and murine macrophages. We suggest that EU increased cleavage rate and embryo quality may be due to maintaining the redox balance in the IVM medium. In the present study, although EU at the highest concentration (40 µM) increased the cleavage rate without improving embryo quality. Previous studies have shown that the EU is a competitive inhibitor of α and β 17-β-estradiol receptors. Therefore, it may be due to the higher EU concentration during IVM may have reduced the efficiency of estradiol in improving oocyte maturation rate and embryo quality. In conclusion, the addition of EU to the IVM medium did not affect the oocyte maturation rate and blastocyst formation, but improved cleavage rate and bovine embryo quality in a concentration-dependent manner. Suggesting, the EU can be used as a potential supplement to improve *in vitro* embryo production in other species, a matter of research.

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Animal Reproduction

In vitro production of hybrid embryos derived from collared peccary semen selected by different methods and swine oocytes

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The collared peccary (*Pecari tajacu* Linnaeus, 1758) is a wild ungulate that adapt well to captivity and its population can benefit from the use of assisted reproduction techniques, such as *in vitro* fertilization (IVF). Sperm selection is an important step in the success of IVF and different selection methods can influence sperm quality. Percoll gradient (PG) and washing by centrifugation (WC) techniques have distinct selection mechanisms and are routinely used in the IVF of domestic mammals, but not yet stablished for the peccaries. Therefore, we aimed to compare the efficiency of PG and WC in the sperm selection on the production of hybrid embryos using collared peccary semen and swine oocytes. All procedures were approved by the ethics committee of UFERSA (no. 30/2019) and Institute Chico Mendes for Conservation of Biodiversity (no. 71834-1). Fresh semen was collected from six collared peccaries by electroejaculation and diluted for selection (100 x 10^6 sperm/mL) in SPTL medium (100 mM NaCl, 3.1 mM KCl, 0.4 mM NaH_2PO_4, 21.6 mM sodium lactate, 25 mM $NaHCO_3$, 0.5 mM caffeine, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 0.6% albumin bovine serum, 1 mM sodium pyruvate, and 1% antibiotic– antimycotic solution). Immediately after collection (control group) and selection (PG or WC), sperm were evaluated for kinetic motility patterns. For selection by PG, 250 µL of semen was added over a 45/90% Percoll gradient and centrifuged at 900*g* for 15 min. The pellet obtained was centrifuged (300*g*/ 3 min) to remove the Percoll. For selection by WC, 1.0 mL of semen was subjected to three centrifugations of 100*g* for 3 min. After selection for both methods, sperm concentration was adjusted, and sperm (3 × 10^5 sperm/mL) were co-incubated for 6 h with swine oocytes previously submitted to *in vitro* maturation for 42–44 h. The IVF medium consisted of 114 mM NaCl, 3.2 mM KCl, 0.35 mM NaH_2PO_4, 10 mM sodium lactate, 5 mM glucose, 25 mM $NaHCO_3$, 2 mM caffeine, 4.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM Hepes, 0.3% albumin bovine serum, 0.11 mM sodium pyruvate, and 1% antibiotic–antimycotic solution. After IVF, the presumed zygotes were cultured *in vitro* for six days in synthetic oviduct fluid. On Days 2 and 6, the cleaved embryos and morula were quantified, respectively. Data were expressed as mean ± standard error and kinetic motility patterns were analyzed by ANOVA followed by Tukey test ($P < 0.05$). All other data were assessed with a chi-squared test ($P < 0.05$). All kinetic patterns of motility were similar between the control and WC groups. Already PG improved parameters of straight-line velocity - VAP (98.7 µm/sec *vs*. 61.7 µm/sec *vs*. 72.6 µm/sec), amplitude of lateral head displacement - ALH (4.2 µm *vs*. 7.3 µm *vs*. 7.2 µm), straightness - STR (92.0% *vs*. 71.3% *vs*. 72.5%) and linearity (72.3% *vs*. 40.3% *vs*. 46.0%) when compared to control group and WC, respectively. However, PG significantly increased the number of static sperm compared to control group and WC ($P < 0.05$). The cleavage rate did not differ between selection methods, with a total of 26.3% ± 3.4 (35/133) for WC and 25.2% ± 4.5 (34/135) for PG. More than 88% ± 4.1 (30/34) of cleaved hybrid embryos were classified as having more than 3 cells. The total percentage of embryos that reached the morula stage was 10.5% ± 2.6 (14/133) and 8.1% ± 2.5 (11/135) for the WC and PG groups, respectively. The rate of morula per cleaved embryo was 40% ± 14.2 (14/35) with WC selection and 32.3% ± 13.5 (11/34) using PG. There was no difference in the development of morula among the experimental groups. In summary, although PG improves some motility kinetic parameters, both selection methods were efficient to isolate motile sperm capable of fertilizing porcine oocytes *in vitro*. PG and WC allowed the development of hybrid embryos and can be used for selection of collared peccary sperm. These results are essential and initial for the development of embryo production by IVF in collared peccaries. **Acknowledgements:** CAPES, CNPq

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Animal Reproduction

Influence of fragment size on the morphology of cryopreserved goat testicular tissue

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The cryopreservation of testicular tissue is the main option for the creation of germplasm banks for endangered species and domestic animals of high productive value such as the goat species, which has great economic importance in several regions of Brazil, especially in the northeast region (7.6 million). Testicular tissue cryopreservation has been employed in several domestic species, however, in goats cryopreservation protocols still need to be established. Therefore, this study aimed to evaluate the influence of three sizes of goat testicular tissue on the morphology after vitrification and slow freezing (Mr. Frosty). For this purpose, testicles from five prepubertal males (approximately 2 months old) were collected by orchiectomy, from each pair of testicles nine fragments (n=9) were obtained and randomly distributed in three groups: 1 mm, 5 mm and 9 mm fragments. In each group, one fragment was destined for fresh control, vitrified and another frozen, after heating and thawing these fragments were also fixed for further histological evaluation. The data were analyzed by the Kolmogorov-Smirnov normality test and the Kruskal-wallis multiple comparison test. The results showed that basal membrane retraction was significantly higher ($p < 0.05$) in the vitrified tissue at 5 and 9 mm size, although slow freezing at 1 mm showed higher basal membrane retraction, however, this had no difference from the control ($p > 0.05$). On the other hand, the rupture of the basement membrane, organization and loss of the cells of the seminiferous tubule showed no difference between the three sizes in both slow freezing and vitrification, with respect to the organization of the peritubular cells vitrification showed no difference between the sizes, but the 5 mm slow freezing showed better results. Thus, we can conclude that the best size to vitrify testicular tissue in pre-pubertal goats is 1 mm and for slow freezing, 5 mm.

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Animal Reproduction

Influence of heat stress on *in vitro* oocyte and embryo production in high-yielding Holstein cows

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The introduction of biotechnologies such as *in vitro* embryo production (IVP), has been the key for increasing productivity in dairy cattle by maximizing the reproductive potential. However, high production Dutch matrices, when subjected to hyperthermic conditions, show a decrease in IVP. Given this, the objective was to evaluate the influence of temperature on the quality of oocytes and *in vitro* embryo production in high-yielding Holstein bovine females, which were subjected to heat stress on the day of follicular aspiration (OPU; 0), 30, 60 and 90 days before the OPU. From the average temperature on day 0 and at 30, 60 and 90 days before, were classified into comfort (TC; up to 15°C) and heat stress (HS - above 15°C) groups. Through the Anova and T- Student tests, a negative influence was observed on viable oocytes and embryos (total and grade I). The submission to heat stress in the periods of 30 and 60 days prior to the OPU resulted in lower production of viable oocytes (P=0.0028; P=0.0092, respectively). When thermally stressed on the OPU day (HS- OPU), cows showed no reduction in the amount of viable oocytes (P=0.5497), as well as no influence of temperature was observed for the stressed group 90 days before OPU (P=0.8287). For total embryos, the difference occurred only in the ET-30 group (P=0.0317), where the HS-OPU, HS-60, HS-90 groups showed, respectively, P=0.1987, P=0.0596 and P=0.4580. Regarding the production of grade 1 embryos, there was no difference for the HS-OPU (P=0.2291) and HS-90 (P=0.2868) groups, however there was a reduction for HS- 30 (P=0.0143) and HS-60 (P=0.0253). In summary, heat stress had a negative impact when it occurred 30 or 60 days before follicular aspiration. Furthermore, 30 days seems to be the period of greatest susceptibility and that causes the greatest deleterious effects on oocyte viability and IVP.

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Animal Reproduction

Influence of sperm subpopulations of Angus and Nelore bulls on pregnancy rates by FTAI

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The heterogeneity of ejaculate indicates that fertility is still variable among bulls and that more stringent evaluation methods have needed to identify the ejaculates suitable for AI. Thus, the aim of this study was to identify and characterize sperm subpopulations in thawed semen doses of Nelore and Angus bulls to evaluate the influence of these sperm subpopulations on pregnancy rate in cows submitted to FTAI. A dose of post- thawed semen from each bull (*n*=18; Angus *n* = 9 and Nelore *n* = 9) was analyzed for: sperm kinetics; morphology; plasma membrane integrity; and sperm subpopulations identification. Differences between the groups were estimated with t-test considering a significance level of <5%. There was no influence between breeding bulls for sperm morphology (Angus 14.55 ± 7.65 vs. Nelore 19.88 ± 8.44; *P* = 0.179), plasma membrane integrity (Angus 81.00 ± 4.74 vs. Nelore 82.22 ± 6.47; *P* = 0.654) and pregnancy rate (Angus 49.54 ± 8.77 vs. Nelore 56.64 ± 8.03; *P* = 0.092). Regarding the kinetic parameters evaluated by the CASA system, Nelore bulls presented greater values, for cells with slow velocity (Angus: 16.44 ± 5.41 vs. Nelore: 21.66 ± 3.35; *P* = 0.028). In contrast, Angus bulls presented greater values for static cells (Angus: 27.22 ± 22.24 vs. Nelore: 9.33 ± 7.08; *P* = 0.048). Based on CASA system data and clustering procedures, four sperm subpopulations were statistically established; SP1: Fast and progressive spermatozoa (Characteristic of sperm movement: Fast, progressive, linear sperm with low oscillation), SP2: Slow and progressive spermatozoa (Characteristic of sperm movement: Slow, progressive and linear sperm with low sinuous trajectory), SP3: Fast and nonlinear spermatozoa (Characteristic of sperm movement: Slow, Hyperactive pattern, sinuous sperm with low linearity), SP4: Slow and nonlinear spermatozoa (Characteristic of sperm movement: Slow sperm with low progressivity and linearity and high oscillation). In Angus bulls, higher percentage of SP3 (33.25%) fast and nonlinear spermatozoa were found, followed by SP1 (32.66%) with fast and progressive spermatozoa. Whereas, Nelore bulls had greater percentage of SP1 (33.82%) with fast and progressive spermatozoa, followed by SP3 (32.20%) with fast and nonlinear spermatozoa. Thus, it is concluded that both breeds of bulls presented similar proportions of sperm subpopulations, therefore pregnancy rate in cows submitted to the IATF programs on a large scale was similar.

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Animal Reproduction

Is it possible to immunolocalize Trypanosoma vivax in testis and epididymis of young goats?

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Trypanosoma vivax causes great economic losses and important damage to affected animals. Clinical signs include weight loss, anemia, hyperthermia, loss of milk quality and, in chronic cases, infertility, abortion and severe testicular and reproductive tract organ degeneration. The aim in present study is to localize the parasite in testis and epididymis of young goats, using direct immunofluorescence technique. Twenty Saanen goats, aging 6 to 9 months, weighing (± EPM) 30.2 ± 0.5 kg, were assigned in infected group (G-INF, n = 15) and control group (G-CON, n = 5). In G-INF, one mL of blood containing $1x10^4$ trypomastigotes of Miranda strain were inoculated i.v. Animals were euthanized 47 days after inoculation, testis and epididymis fragments were collected for histological processing (5 µm), and slides submitted to direct immunofluorescence assay. For this purpose, slides were deparaffinized in xylol and decreasing alcohol solutions, followed by antigen recovery by heat and protein blocking. After protein blocking, slides were washed with PBS solution and two areas were delimited with hydrophobic pen in each slide to receive in one of them, bovine serum from an animal infected with *T. vivax*, and in the other, bovine serum from an animal not infected (negative control). Slides were incubated, washed, and co-incubated with rabbit anti-bovine IgG-FITC antibody (Sigma-Aldrich, Missouri, EUA). After this step, the slides were prepared with buffered glycerin under a coverslip and observed in an epifluorescence microscope (Olympus BX60). In the presence of parasite, tissue emitted green fluorescence. Only descriptive statistics was used in the present study. Direct immunofluorescence assay was effective in detect the presence of *T. vivax*, in a diffuse pattern (interstice and parenchyma), in testis of 73.3% (11/15) and in epididymis of 86.6% (13/15) of infected goats, while 100.0% of control animals were negative in both tissues. In addition, for testis and epididymis, respectively, sensitivity was 0.73 and 0.86, specificity was 1.00 in both organs, and accuracy was 0.8 and 0.9, demonstrating reliability of this assay. The effects of trypanosomiasis in reproductive organs had been previously described in ruminants, and indicate inflammatory and degenerative lesions in histopathology. However, despite DNA of *T. vivax* had been found in testis, indicating a participation of this parasite in etiopathogenesis of infection (Bezerra N.M. et al. 2018. Vet Res Commun, 42: 131-5), until this moment it was unknown the location of this parasite in the organ. This is the first report in which location of *T. vivax* in tissues of testis and epididymis was described, and can contribute to additional comprehension of this disease. In conclusion, the location of *T. vivax* by direct immunofluorescence assay was described for the first time in testis and epididymis of experimentally infected goats. Acknowledgements: To FAPESP (PROC 2019/ 22695-7; PROC 2020/06492-6), Alessandra Regina Carrer, Letícia Castro Fiori, Beatriz Eustachio Boarini, Gwenever Camargo Moraes, Maísa Pansani Santos and Viviane Bobadilha Morelli.

Keywords: trypanosomiasis, direct immunofluorescence assay, male reproductive organs, bucks

Animal Reproduction

Levels of miRNAs in corpus luteum of beef cows treated with different doses of PGF2α at metestrus or diestrus

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In FTAI protocols PGF2α analogues are used to induce complete luteolysis and promote ovulation. In this study we aimed to characterize the expression pattern of 40 miRNAs present in bovine corpus luteum after induction of luteolysis with 50% or 100% of PGF2α dosage, in metestrus or diestrus stages of the estrous cycle (4 or 11 days after ovulation (D0), respectively). For this purpose, 23 cycling beef cows, with body condition score ≥2,75 (1-5 score), were randomly assigned in four groups (D11-100%: n = 5; D11-50%: n = 6; D4-100%: n = 6; D4-50%: n = 6). *Corpora lutea* were obtained after 48 hours of PGF2α administrated and conserved in liquid nitrogen. The miRNAs chosen had functions in apoptosis or/and angiogenesis. For analyses of miRNAs expression in all samples we performed qPCR using reverse transcribed with miScript HiSpec Buffer. The relative levels were evaluated and data normalized by miR-99b. For statistical analysis, one way ANOVA and post-hoc Student's t-test were used (JMP® program; $p \leq 0.05$), including the effects of dosage, phase of estrous cycle and interactions. As results a total of 32 from 40 miRNA were detected in both groups and were analyzed. No differences on expression have been found, but when observing frequency of expression, results were described as follows. A greater frequency of 14 miRNAs (miR-183, miR-202, miR-210, miR-126-5p, miR- 17-5p, miR-181a, miR21-5p, miR-20a, miR30a-5p, miR455-3p, miR 503-3p, miR92a and miR let-7g) was observed on D11 with dosage of 100%, however, when 50% of dosage was used on this same day none of miRNAs were observed. On D4, using 100% of dosage three miRNA were found with more frequency (miR21-5p, 30a-5p and miR-186), and two with 50% of dosage (miR-182 and miR210). Nine of 14 miRNA with higher frequency on D11-100% have association with apoptosis and oxidative stress, while the others have functions of cellular proliferation and CL maintenance. Even when PGF2α was administered in diestrus, miRNAs associated with apoptosis inhibition were noted. Besides, in D4-50%, the two more frequent miRNAs had the function of modulate gene expression of cellular proliferation and recruitment of immune cells, what is expected in this phase. However, when we use 100% of dosage on D4, one of the three miRNAs (miR-186) is correlated with suppression of cell proliferation and initiation of apoptosis. In conclusion, frequency of miRNAs obtained in the present study it was what was expected. The dosage of 100% of PGF2α on D11 increase the chance to obtain a complete luteolysis, while in the dosage of 100% on D4 there are more miRNAs responsible for CL maintenance and with 50% of dosage on D4, only miRNA associated to cell proliferation and recruitment of immune cells were observed. Acknowledgements: CAPES and Ourofino Animal Health.

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Animal Reproduction

Magnetic Nanoparticle Hyperthermia as a Non-surgical Method for Neutering Male Animals: a short and a long-term study

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Non-surgical neutering strategies for male animals have been long pursued. The present study investigated the localized application of magnetic nanoparticle hyperthermia (MNH) to the testicles as a potential non-surgical sterilization method for animals. First, a short-term experiment was conducted. Wistar rats (6-7 weeks old) were divided into 4 groups according to the treatment: 1) Saline Group (N=2) – received an intratesticular injection of sterile saline solution; 2) MNP Group (N=2) – received an intratesticular injection of magnetic nanoparticles; 3) AC Group (N=3) – were subjected to an alternate magnetic field only, and 4) MNH Group (N=15) – received an intratesticular injection of magnetic nanoparticles and were subjected to an alternate magnetic field. MNH treated animals were divided into 3 subgroups (N=5 per sub-group), to be evaluated at different time-points and were euthanized 7 (MNH-D7 Group), 28 (MNH-D28 Group), and 56 (MNH- D56 Group) days after the treatment. Animals did not present signs of pain and the only side effect was a mild skin lesion on scrotal skin observed on the first few days after treatment. On the euthanasia, the testicles of MNH- D7 group animals were dark and adhered to the scrotal skin. Testicles of MNH-D28 group were atrophied, and absence of the left testicle was observed in one animal. In animals from the MNH-D56 group, 3 testicles had disappeared (30% loss of testes - 3/10 testicles), while the remaining testicles were atrophied. A significant decrease ($P<0.05$) in relative testicular weight (testis weight/body weight) and testicular volume was observed in the MNH-D56 (0.13% and 0.14 cm^3) and MNH-D28 (0.34% and 0.53 cm^3) groups compared to the MNH- D7 (0.81% and 0.77 cm^3), AC (0.83% and 2.78 cm^3), MNP (0.76% and 2.25 cm^3) and Saline (0.59% and 1.84 cm^3) groups. Testicular histopathology of MHT treated groups showed progressive degeneration of seminiferous tubules, which were completely replaced by connective tissue after 56 days. Then, a long-term study was conducted to verify the efficacy and safety of the method. Wistar rats (12 weeks old) were treated with testicular MNH (MNH Group - N=13) or with an intratesticular injection of sterile saline solution (Saline Group - N=5). Animals' health was evaluated monthly by blood tests and abdominal ultrasound for 12 months. Testicular atrophy was observed in all MNH treated animals and only 3 animals still had a testicular structure by the end of the experiment, which proved to be an afunctional tissue on the histopathological analysis (absence of seminiferous tubules). MNH treated animals showed significantly decreased serum testosterone as soon as 30 days after the treatment (0.28 ± 0.4 ng/mL), compared to their own levels 8 days before treatment (1.52 ± 1.7 ng/mL) and to saline treated animals 30 days after injection (2.48 ± 1.7 ng/mL). Serum testosterone levels remained low in MNH treat animals thereafter. Treated animals presented no long-term side effects. Hemogram, ALT, AST, urea, and creatinine were within normal parameters for Wistar rats over the 12 months. The abdominal ultrasound exams also revealed liver, spleen, kidneys, urinary bladder and stomach with normal structure over the 12 months. Histopathology of liver, spleen, kidneys, and lungs also showed normal structure 12 months after the treatment. In conclusion, testicular magnetic nanoparticle hyperthermia caused irreversible infertility in rats, with few side effects to general animal's health. Acknowledgements: FAP-DF (grant nº 0193.001378/2016); CNPq (grant nº 304922/2017-0); INCT- Nanobiotecnologia (grant nº 573.880/2008-5); (CAPES (Finance Code 001).

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Animal Reproduction

Metabolic activity in cryopreserved and grafted ovarian tissue using high-resolution respirometry

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Ovarian tissue cryopreservation followed by transplantation has become a promising strategy to restore ovarian function and fertility. However, the mitochondria could be seriously impacted during the cryopreservation- thawing processes can lead to alterations and/or damage your structure and functionality. Using high-resolution respirometry (HRR) and histological analysis, were assessed the effect of the cryopreservation (fresh tissue vs. cryopreserved tissue) and transplantation (transplanted tissue vs. non-transplanted tissue) on the O2 consumption rates (OCR) and the follicular viability. All groups were able to respond to the substrates- uncoupler-inhibitor protocol. However, the impact of cryopreservation on mitochondrial metabolism was less intense than observed in transplanted hemi-ovaries, since the transplantation affected all of the mitochondrial states analyzed (Basal; Leak; OXPHOS; Oligo; ETS; ATP-linked, and Spare capacity). Only the transplantation significantly affected the oxygen consumption linked to Complex II ($p<0.01$) and to the OXPHOS state ($p<0.05$). Also, there was a significant effect for transplantation and cryopreservation interaction ($p<0.05$). Only the transplation procedure had a significant effect on the ATP-linked OCR ($p<0.01$). However, both factors, transplantation ($p<0.01$) and cryopreservation ($p<0.01$), showed significant impact on the Spare capacity. A total of 2644 follicles were analyzed in all ovarian tissue samples, of which 2198 were classified as morphologically normal. The percentage of morphologically normal primordial and growing follicles was significantly lower in the Cryopreserved transplanted group when compared to the Cryopreserved non-transplanted group ($p<0.05$) and the Fresh transplanted group ($p<0.05$). Moreover, both transplanted groups (Fresh and Cryopreserved transplanted) showed a lower number of follicles compared to the non- transplanted groups (Fresh and Cryopreserved non-transplanted). Despite the decrease in follicular viability, it was possible to find morphologically normal follicles in all analyzed groups. Antral follicles were also found after fresh and cryopreserved tissue transplantation. It is well known that ovarian tissue cryopreservation poses a major challenge, especially relating to tissue composition, with different cell types and follicles at different stages of development, which may demand different conditions for the technique. The mitochondrial damage can occur as a consequence of alterations in the electron transport chain resulting from the loss of membrane potential caused by exacerbated ROS generation and/or decreased ATP synthesis. Oximetry data of all samples confirmed that there was oxygen consumption, responsive to all reagents used, even though a significant reduction of mitochondrial function occurred. These findings suggest that the damage caused by transplantation is greater than the damage caused only by cryopreservation, since it affected all analyzed mitochondrial states and the cryopreservation disturbed only three of them. This may be due to the period of ischemia and hypoxia the ovarian tissue endures immediately post-transplantation, until local neovascularization can be completely established. In conclusion, the cryopreservation of ovarian tissue has an impact on mitochondrial metabolism and follicular viability, however, the transplantation has a greater impact. Furthermore, cryopreserved tissues are even more sensitive to transplantation than fresh tissues. Despite decreased follicular viability and mitochondrial activity, the cryopreservation technique followed by transplantation of ovarian tissue proved feasible, both morphologically and metabolically, for attempts to restore ovarian function.

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Animal Reproduction

Micro RNAs isolated from the sperm head and the efficiency of in vitro embryo production in cattle

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In vitro embryo production (IVEP) is a reproductive biotechnology, highly exploited in cattle production and needs studies to improve it even more. MicroRNAs are small, non-coding RNA molecules that participate in epigenetic processes in various biological functions, including reproductive physiological pathways. The aim of this study was to correlate, through in silico analyzes, the microRNAs isolated from sperm heads of crossbred bulls (n = 6), using the mirVana ™ miRNAisolation kit, with the efficiency of these sires in the in vitro embryo production. A transcriptomic analysis was performed through of two normalization and filtering software (DESeq2 and edgeR) and PIVE routines of bull sperm with different levels of efficiency. It was found that a high expression of miR-425-5p (P = 0.01 DESeq2; P = 0.04 edgeR) positively affected a blastocyst formation rate in PIVE and that a high expression of miR-11972 (P = 0.03 DESeq2; P = 0.008 edgeR) and miR-11975 / miR-11976 (P = 0.05 DESeq2; P = 0.03 edgerR) negatively affected the rate of blastocyst formation in PIVE. Therefore, it was concluded that these microRNAs have the potential to be used as fertility biomarkers of bulls submitted to in vitro embryo production, in addition to being identified as future study targets for a better understanding of the process of fertilization and earlier embryonic development and mainly to improve the technology in question.

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Animal Reproduction

MiRNA profile of bovine blastocysts produced in sodium alginate hydrogels 3D culture

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In vitro embryo production (IVP) is a relevant and widely used technique. However, IVP still have some limitations, such as reduced pregnancy rates compared with *in vivo* produced embryos. Embryos obtained through IVP also differ from embryos produced *in vivo* in epigenetic factors, which can affect pregnancy establishment and postnatal health. Importantly, *in vitro* embryos receive different external stimuli which generate different biological intracellular responses. One of the reasons is that the IVP embryos are cultivated in culture plates that can be six times more rigid than the *in vivo* maternal environment. An alternative is the three-dimensional (3D) cultures that are known for inducing changes in proliferation and development pathways in different cells. These pathways can be modulated by mRNAs as well as microRNAs (miRNAs). miRNAs are small non-coding RNA molecules involved in post-transcriptional regulation of target transcripts. In this view, our objective was to analyze the differences in miRNAs between the conventionally bi-dimensional *in vitro*-produced embryos, *in vitro*-produced embryos in an alginate overlay system, and an alginate encapsulation system. Bovine ovaries were collected from a local slaughterhouse, the Cumulus–oocyte complexes (COCs) were *in vitro* maturated, fertilized, and on day 1 of culture, the presumable zygotes were denuded and divided into three groups: Control, Alginate Overlay (AO) and Alginate Encapsulation (AE). The alginate hydrogels had 1.5% alginate in a crosslinking calcium solution ($CaCl_2$ 50mM and NaCl 140mM). The presumable zygotes were cultivated in groups of 30 embryos for 7 days in SOFaaci medium. On day 7, the blastocysts were collected by snap-freezing for further analyses. Prior to total RNA isolation 10 blastocysts of 8 different routines were pooled to form the experimental groups. Total RNA was extracted according to QIAzol Lysis Reagent. MiRNA reverse transcription was performed using miScript II RT Kit (HiSpec). Relative levels of 380 bovine miRNAs were determined by RT-qPCR (n=3 pools of 10 blastocysts/group) with SYBR Green PCR kit (QIAGEN) and the miR-99b was used as housekeeping. Differences in relative levels were determined by ANOVA with posthoc Tukey Test using Jmp15 Statistics Software. A $p<0.05$ was considered as a statistical difference. We identified a total of 28 miRNAs in all three groups, among these, 2 miRNAs (miR-1246 and miR-1260b) were upregulated in the control group comparing to the AO group and 1 miRNA (miR-541) was upregulated in the control group comparing to the AE group. There were no differences between groups AO and AE. MirWalk software (version 3.0) was used for functional enrichment analysis of the pathways that can be modulated by these miRNAs. The miRNAs miR-1246 and miR-1260b are associated with the regulation of pathways, such as Ras (16%), Phospholipase D (16%), MAPK (13%), Rap1 (13%), Adherens junction (19%), PI3K-Akt (11%), cGMP-PKG (13%), Insulin (14%), Hippo (13%), Wnt (12%), cAMP (11%), Signaling pathways regulating pluripotency of stem cells (13%), AMPK (13%), mTOR (12%), VEGF (16%), HIF-1 (13%), and ErbB (13%). Also, the miRNA miR-541 which was higher in control compared with the AE group can modulate signaling pathways, as Insulin secretion (20%), Ras (14%), MAPK (13%), Wnt (15%), Fluid shear stress and atherosclerosis (14%), Fatty acid metabolism (19%), Rap1 (13%), Apoptosis (14%), and Signaling pathways regulating pluripotency of stem cells (13%). Furthermore, the miR-1246 was found to be upregulated in heat-stressed spermatozoa, and also decreased in small extracellular vesicles secreted by *in vivo* blastocysts in day 9 compared to *in vitro*. miR-1246 overexpression in LPS treated cells decrease cell viability and reversely its suppression alleviated LPS-induced inflammatory damage. Interestingly, miR-1260b is downregulated under hypoxia and its overexpression promoted cell proliferation and cell cycle. Similarly, increase of miR-541 is associated with cell proliferation and differentiation in cancer cells. The results demonstrate that different embryo culture systems can modulate miRNAs secreted by blastocysts. These miRNAs are predicted to regulate important pathways that are crucial for embryo development. Funding: FAPESP 2014/22887-0 and 2019/25675-7. Acknowledgments: Alta Genetics.

Animal Reproduction

Morpho-histological evaluation of the *Epicrates cenchria* sexual segment of the kidney.

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Snakes correspond to the second most diverse group within reptiles, with reproductive aspects not understood in several species. There are morphological, physiological and reproductive differences in snakes, an example of this is the sexual segment of the kidney (SSK). The SSK is present in the kidney of the snakes and lizards, presenting importance in the reproductive dynamics of these individuals. The aims of this study are to evaluate morpho-functional aspects of the *Epicrates cenchria* SSK. For these study kidneys fragments of five male *E. cenchria* specimens were fractionated and incorporated into paraffin and metacritalo glycol plastic resin. Subsequently, the samples were cut at 3µm, dyed with Toluidine Blue (plastic resin samples), and Periodic Acid-Schiff (PAS) and Alcian Blue (paraffin samples), photodocumented and analyzed. The reproductive stages were determined through the histological evaluation of the testicles. The specimens in the reproductive period (mature sperm in the lumen of the seminiferous tubules) presented the SSK with simple epithelium, glandular, composed of cylindrical cells with evident nucleus and nucleolus, and granular structures inside the cytoplasm. In some of them the granules have different sizes, in others presenting absence of granules in part of the cylindrical cells, assuming that their release occurs in different periods of the reproductive phase. In Toluidine Blue, the granules demonstrated metachromasia, indicating secretory activity formed by acid compounds. These also demonstrated positivity in PAS staining, and together with the metachromasia observed in Toluidine Blue, confirms the sulfated acid polysaccharide compounds production. In the Alcian Blue coloration, the granules are not colored, evidencing absence of acid glycosaminoglycans in the SSK secretion. In cells without granules no metachromasia was observed in Blue Toluidine staining, however demonstrated positive in PAS and Alcian Blue. In non-reproductive periods, the SSK presents cylindrical cells without secretion granules inside the cytoplasm. In Toluidine Blue, SSK cells presented metachromasia, and was positive in PAS staining, evidencing the sulfated acid polysaccharide compounds production. The positivity in the Alcian Blue staining evidences the presence of acid glycosaminoglycans secretion in individuals outside reproduction. We concluded that the histological aspects of *Epicrates cenchria* SSK presents variations when comparing individuals in reproductive and non-reproductive periods, but with SSK secretory activity in both periods. However, the secretion product is different according reproductive period, and even, among individuals in the same reproductive period, as evidenced by the different staining techniques showing the dynamic in the SSK secretory function in different periods of the same reproductive phase.

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Animal Reproduction

Morphology of the Goby peacock gonad (*Ctenogobius boleosoma)*

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Descriptive and comparative analysis of the fish reproductive system allows evaluating their reproductive process, the viability of the offspring, identifying morphological variations and their implications for the reproductive physiology of the species. Goby (*Ctenogobius boleosoma*) is an estuarine species that adapts to freshwater, where it is cultivated by aquarists. Oviparous, presents very evident sexual dimorphism, differentiating males from females, with territorial behavior. Reproductive behavior reflects the environment in which they live, and can be used as a basis for stock maintenance mechanisms. Therefore, this study aimed to describe the morphology of the gonads of male and female Goby peacock (*Ctenogobius boleosoma*). Five adult animals of each sex were selected from the Tanganyika fish farm, located in the city of Aquiraz/CE. After euthanasia with benzocaine by immersion, the gonads were removed from the coelomic cavity and fixed in 4% paraformaldehyde for further histological processing and microscopic analysis. In males, it was observed that the specimens were able to release the gametes, presenting discontinuous germinal epithelium and dilated seminiferous tubules lumen completely filled by sperm. Moreover, in the seminiferous tubules, large cells were seen, marked by eosinophilic cytoplasm and a distinct nucleus containing condensed chromatin, characteristic of primary spermatogonia. Secondary spermatogonia were smaller than the primary, with large, slightly basophilic nuclei and little cytoplasm. In addition, they grouped together to form an encapsulated cyst. In the testicular interstitium, polymorphic cells with spherical nuclei, characteristic of leydig cells were found. It was observed a continuous germinal epithelium along the entire length of the seminiferous tubules. In females, ovaries with layers composed of germinal and follicular epithelia supported by a vascular connective tissue stroma were observed. Furthermore, it was possible to visualize oocytes in different stages. Stage I and II oocytes were located in the germinal epithelium; respectively with mildly eosinophilic to basophilic cytoplasm. Stage III oocytes were ruptured from the germinal epithelium, being surrounded by a simple squamous follicular epithelium and nucleoli located in the periphery of the nucleus. Stage IV ones were at the beginning of vitellogenesis, with the appearance of yolk granules and fat vacuoles in the ooplasm, presence of a distinct chorion under the follicular epithelium. In stage V, there was an increase in the vitelline vesicles that filled almost the entire ooplasm. In addition, the nuclear envelope was in the process of degeneration and also had a nucleus located in the peripheral region. Showing that females were able to spawn Therefore, it is concluded that males of Goby peacock have testes organized in cysts and females have ovaries with oocytes in five stages of development. Key words: Histology, fish, microscopy.

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Animal Reproduction

Morphometric analysis of *ex situ* giant anteater (*Myrmecophaga tridactyla)* sperm

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The giant is currently classified as "Vulnerable" related to its risk of global extinction. Habitat loss, roadkill and fires are the main causes of population decline. Therefore, reproductive biotechnologies are an important tool for this species conservation. The objective of the present study was to assess sperm morphometry measures of giant anteaters using the CASA system. Two captive giant anteaters (M1 and M2) housed at Zoológico Municipal de Guarulhos (Guarulhos, SP, BRAZIL; 23°26'33.5"S 46°33'11.8"W) were used and pharmacological semen collection was performed through urethral catheterization. 671 sperm cells were analyzed (M1 = 371; and M2 = 300) were assessed using IVOS II Computer Assisted Semen Analysis (CASA) system with the Animal Breeders II software at IMV Technologies Brazil (Campinas, São Paulo). The parameters assessment were Average Head Length, Average Head Width, Average Head Perimeter, Average Head Area, Average Tail Length and Average Tail STR. The general mean and standard deviation from the evaluated parameters were then calculated and are expressed as mean ± standard deviation. The parameters of each animal was statistically compared by the unpaired t test (GraphPad Software). As result, we obtained the following averages: Average Head Length = 17.4 ± 5.3 µm (M1 = 17.9 ± 5.4 µm; M2 = 16.8 ± 5.1 µm; p = 0.0058), Average Head Width = 4.1 ± 0.8 µm (M1 = 4.1 ± 0.7 µm; M2 = 4.1 ± 0.9 µm; p = 0.5336), Average Head Perimeter = 40.6 ± 11.2 µm (M1 = 41.7 ± 11.3 µm; M2 = 39.4 ± 11.0 µm; p = 0.0092), Average Head Area = 29.8 ± 7.6 µm² (M1 = 30.9 ± 7.0 µm²; M2 = 28.4 ± 8.0 µm²; p = 0.0001), Average Tail Length = 20.4 ± 8.9 µm (M1 = 19.7 ± 8.2 µm; M2 = 21.2 ± 9.6 µm; p = 0.0361) and Average Tail STR = 67.7 ± 22.0% (M1 = 67.1 ± 21.7%; M2 = 68.6 ± 22.3%; p = 0.3697). By conventional criteria of unpaired t test, the difference between the two males is not statistically significant for Average Head Width and Average Tail STR, is considered to be statistically significant for Average Tail Length, very statistically significant for Average Head Length and Average Head Perimeter, and extremely statistically significant for Average Head Area. Gathering information about giant anteater spermatozoa is useful for future studies aiming for the conservation of the species. Agradecimentos: Zoológico Municipal de Guarulhos. IMV Technologies Brasil. O presente trabalho foi financiado em parte pelo Instituto Reprocon.

Palavras-chave: CASA, conservation, biotechnologies, urethral catheterization, pharmacological semen collection.

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Animal Reproduction

Neuregulin 1 modulates meiotic resumption during amphiregulin-induced IVM in cattle

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Oocyte *in vitro* maturation (IVM) has been recognized as a major bottleneck of assisted reproductive technologies (ART) applied in animal production. The induction of the ovulatory cascade with EGF-*like* ligands instead of supraphysiological concentrations of FSH during IVM has provided promising results in cattle IVP, although it may accelerate meiotic resumption potentially compromising nuclear/cytoplasmic tuning. Neuroregulin 1 (NRG1) is a particular EGF-*like* factor that modulates EGFR-induced pathways during oocyte maturation in mice. The objective of the present study was to assess the effects of NRG1 on meiotic dynamics of bovine oocytes undergoing IVM using amphiregulin (AREG) as the maturation trigger. Three graded concentrations of NRG1 (1, 10 or 100 ng/mL), were added to IVM media containing FSH and steroids at approximately physiological levels and AREG [follicular system (FS); Soares, A. C. *et al.* 2017. Reprod. Fertil. Dev., 29:2217-2224]. The FS medium consisted of TCM199 (with Earle's salts, BSA, pyruvate, and amikacin) supplemented with 10^{-2} UI/mL rhFSH, 100 ng/mL AREG, 50 ng/mL 17β-estradiol and 150 ng/mL progesterone. Five replicates were performed comparing four groups: 0; 1; 10; 100. Ovaries were obtained from a slaughterhouse and COCs were aspirated and submitted to IVM for 6, 9, 12, 20, and 24h in controlled and humidified air. All the oocytes were denuded by pipetting, fixed in 60% methanol and stained with 1 µg/mL Hoechst 33342. Chromatin status and meiotic progress were determined by epifluorescence microscopy. Oocytes at the germinal vesicle (GV) and germinal vesicle breakdown (GVBD) stages were classified according to rupture of GV and the meiotic phase was classified as metaphase I, metaphase II, or degenerated. Data were arcsine transformed and compared with Tukey *post-hoc* test using JMP software (SAS Institute Cary, NC). Data are presented as means ± SEM and differences were considered significant when P≤0.05. The addition of 1 ng/mL NRG1 delayed germinal vesicle breakdown, as indicated by a reduced percentage of GVBD oocytes at 6 hours of IVM as compared to the control treatment (P<0.05; 0: 70.37±5.1; 1: 52.24±4.7; 10: 53.99±7.4; 100: 62.40±5.5), but did not change later meiotic dynamics at 9h (P>0.05; 0:57.20±7.0; 1: 64.93±3.0; 10: 52.43±6.3; 100: 53.59±7.3), 12h (P>0.05; 0: 90.42±3.7; 1: 88.57±4.2; 10:88.73±4.3; 100: 89.63±4.6), 20h (P>0.05; 0: 72.04±3.7; 1: 80.95±4.1; 10: 74.87±3.1; 100: 78.42±2.3) or 24h of IVM (P>0.05; 0: 79.30±5.6; 1: 80.75±4.5; 10: 79.16±3.0; 100: 81.13±4.3). The present study provides novel evidence that NRG1 is involved in the mechanisms that control meiotic resumption in cattle, being a potential useful tool to improve the efficiency of current IVM/IVP protocols. Acknowledgements: FAPESP (process nº 2019/14588-6) and CAPES.

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Animal Reproduction

Nutraceutical supplementation during transition period increase steroidogenic competence of the first postpartum dominant follicle

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Several studies have highlighted the importance of nutrition in regulating bovine reproductive efficiency. The aim of this study was to determine the effect of nutraceutical supplementation during the transition period in dairy cows on the steroidogenic potential of the first postpartum dominant follicle. 36 Holstein cows were randomly assigned to one of four treatments from -45 days before calving to 30 ± 3 days in milk (DIM). Treatments were: Methionine (**MET**; n = 9) fed the basal diet + rumen-protected methionine (Smartamine® 15g/cow/day) from -15 days before calving until 30 DIM; Treatment selenium+tocoferol (**SeE;** n = 9) two injections of selenium (Toco- sel® 8 ml, im) + vitamin E (Betapherol E® 15 ml, im) were given two weeks before calving (- 2 and -1), and at weeks 1, 2, 3 and 4 postpartum; Treatment essential oil (**AOR**; n = 9) fed the basal diet + origanum *Origanum vulgare hirtum* (Oregostim®; 12g/cow/day) from -15 days before calving until 30 DIM; Control (**CTL**; n = 9), no supplementation, all cows were fed with *ryegrass* and water *ad libitum.* Follicular development was monitored via ultrasound every three days starting at eight DIM until aspiration of the first postpartum dominant follicle (DF). Follicular fluid was aspirated, and cells were retrieved immediately by centrifugation. Expression of 3 beta- hydroxysteroid dehydrogenase (*HSD-3B*), Cytochrome P450 Family 19 Subfamily A Member 1 (*CYP19A1*), Steroidogenic acute regulatory protein (*STAR*), Luteinizing Hormone/Choriogonadotropin Receptor (*LHCGR*), in the follicular cells of first postpartum dominant follicle. Total RNA was extracted using Trizol reagent (Life Technologies, Inc.,) according to the manufacturer's instructions. Total RNA was treated with DNAse I (DNAse Amp Grade, Invitrogen®) to remove genomic DNA contamination and primed with oligo dT to synthesize single strand cDNA (SuperScript III First-Strand Synthesis Supermix, Invitrogen®). The PCR amplifications and fluorescence detection were performed in duplicate in the ECO System, using the SYBR Green detection chemistry (Power SYBR Green, Life Technologies), as recommended by the manufacturer. The 18S Ribosomal 1 (*RN18S1*) gene was used as an internal control. In addition, the expression of each target gene of interest was calculated relative to *RN18S1* using the equation: relative target gene expression = (1/Etargetˆ CTtarget)/(1/E- *RN18S1*ˆCT-*RN18S1*), where E was the reaction efficiency and CT was the cycle threshold. Statistical analyses were performed using SAS 9.4. *LHCGR* mRNA expression was higher in **MET** and **SeE** treatments (by 7.6 ± 0.3 and 7.8 ± 0.4 fold, respectively) in comparison to **CTL** cows ($P < 0.05$). The other evaluated target genes *STAR, HSD-3B,* and *CYP19A1* mRNA were not differently expressed between treatments ($P > 0.05$). In conclusion, peripartum nutraceutical strategies in dairy cows increased expression of *LHCGR* mRNA in follicular cells of the first postpartum wave, which can potentially increase the chance of ovulation of the first postpartum follicular wave. The authors would like to thank the Ministerio de Agricultura y Desarrollo Rural (MADR), for the financial support.

Key words: gene expression, dairy cattle, reproduction

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Animal Reproduction

Obtaining oocytes by hormonal stimulation in NOD mice

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The non-obese diabetic mouse genetic lineage (NOD/ShiLtJ – The Jackson Laboratory, JAX stock #001976) is an experimental model for autoimmune type I diabetes studies. Females are more used than males as they develop an earlier decrease in pancreatic insulin, around 12 weeks of age, whereas in males, this occurs later. Because they are animals with a short lifespan, about 30 to 40 weeks, it is necessary to use assisted reproduction to preserve the lineage, generate a genetic bank of animals and meet the principles of the 3R's (Replacement, Refinement, Reduction). Thus, the objective of the present study was to evaluate the obtainment of oocytes by hormonal stimulation in NOD females. The study was conducted in the Central Vivarium of the Federal University of Mato Grosso do Sul (UFMS; 20°30'22.4"S 54°36'53.9"W) and was previously approved (CEUA n° 1.176/2021). Two groups of females (n=10) were used: group A (n=5) as control and group B (n=5), which received hormones for superovulation. Females from group A were placed in a box with wood shavings pre- used by males to synchronize the estrous cycle for three and a half days and then mated with vasectomized males (1:1). In group B, females were superovulated with 7 IU of eCG (Novormon; IP), and after 48 hours, 7 IU of hCG (Chorulon; IP) was applied and were mated with vasectomized males. The animals were then euthanized by cervical dislocation (according to normative resolution No. 37, January 27, 2018, by Concea). An incision was made in the abdominal cavity, and the oviduct was located and removed. It was then placed in a heated petri dish with a bit of medium to prevent dryness. The ampoule was incised with the bevel of a needle, and the oocytes were retrieved and counted. A vaginal plug was observed in both groups on the day after the mating. A mean of 8 and 55.4 oocytes were obtained in groups A (control) and B (hormonal stimulus), respectively. Studies performed by The Jackson Laboratory recorded an average of 31 oocytes for hormonally stimulated females of the NOD lineage. Through hormonal stimulation of the ovaries, it was possible to obtain ~6.9 times more oocytes than the control group and ~1.8 times more than the Jackson laboratory studies. Historical data from this vivarium report eight newborns/females as the average genetic lineage birth rate, corroborating the number of oocytes retrieved in the control group. These results demonstrate that NOD females responded satisfactorily to the hormonal protocol for ovarian stimulation, being a viable alternative to obtaining oocytes. Acknowledgment: The present study was performed with support from the Federal University of Mato Grosso do Sul - UFMS/MEC - Brazil, with support from the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 and from the Central Vivarium - INBIO/UFMS and was funded in part by the Reprocon Institute and IMV Technologies.

Key words: non-obese diabetic, superovulation, oocytes, rodent.

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Animal Reproduction

Oocyte exposure to genistein impairs development and quality of *in vitro*-produced bovine embryos

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Human and animal contact with genistein is through the consumption of soy-based products and their derivatives. It is known that genistein, the main isoflavone present in soy, affects the reproductive processes due to a potent steroidogenic action. Intrauterine exposure to genistein can affect the reproductive system of offspring, stimulates uterine cancer, and cause changes in the testicular epithelium. To understand the effects of genistein on embryonic development, we aimed to evaluate the effects of genistein during cumulus-oocyte complexes (COCs) maturation on production and quality of bovine *in vitro*-produced embryos. Therefore, ovaries from local slaughterhouse were transported to the laboratory under recommended conditions. The COCs were recovered and divided into three groups: control group (no genistein addition); GEN 100 (100 µM of genistein); and GEN 500 (500 µM of genistein). Only COCs of excellent quality, grades I and II, were used. All the experimental groups contained the same *in vitro* maturation base medium with 0.1 mM dimethyl sulfoxide (DMSO), as the genistein dilution takes place in this solution. The COCs were *in vitro* matured for 24 hours. After maturation, we submitted COCs to *in vitro* fertilization for 18 hours. Further, presumptive zygotes remained to *in vitro* culture for seven days. To evaluate the effects of genistein on *in vitro* embryo production (IVEP) we analyze the blastocyst yield and expression of genes related to embryo quality. The genes related to embryo quality: *OCT4* (Octamer-binding transcription factor 4), *PLAC8* (Placenta associated 8), and *CDX2* (Caudal type homeobox 2); normalized with *PPIA* (Peptidylprolyl isomerase A - housekeeping gene) were observed. We analyzed the effect of oocyte exposure to genistein using ANOVA. Means were compared by orthogonal contrast and we considered different when $P<0.05$ and biological tendency when $0.051>P<0.10$. Data were demonstrated by mean ± S.E.M. Regarding *in vitro* embryo production, we figure out ($P<0.0001$) that 500 µM of genistein during oocyte maturation decreases blastocyst yield (13.05%) compared to GEN 100 group (46.11%) and control group (46.87%). Furthermore, COCs from GEN 500 group demonstrated lower mRNA abundance of *OCT4* compared to the control group ($P<0.05$). Likewise, *PLAC8* showed a biological tendency to reduce its expression in blastocysts from GEN 500 group ($p=0.09$). No differences were found when we analyzed *CDX2* mRNA abundance ($P=0.21$). The *in vitro* embryo production was decreased when sheep oocytes were matured with genistein; we found similar results with bovine oocytes. Taken together, we concluded that the addition of 500 µM of genistein during COCs maturation impairs *in vitro* embryo yield and down-regulates a key gene related to inner cells mass differentiation. Acknowledgments: National Council for Scientific and Technological - CNPq (grant 140619/2020-8).

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Animal Reproduction

Oxidative stress in bovine oocytes in the presence of melatonin and/or gonadotropins

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The antioxidant action of melatonin makes possible to reduce reactive oxygen species (ROS), which are produced by oocytes, embryos and cumulus cells during normal metabolism. However, when there is an imbalance between pro-oxidant agents and antioxidant agents, a condition called oxidative stress sets in, which can lead to blockage in development. As melatonin seems to have the ability to reduce the intracellular amount of ROS and appears to be a substitute for gonadotropins in promoting oocyte maturation, the objective of this work is to evaluate the effect of melatonin supplementation in the presence or absence of gonadotropins under oxidative stress in *in vitro* matured bovine oocytes. For that, bovine oocytes were submitted to maturation in TCM-199 media, supplemented with bicarbonate and 10% of FCS (C∅ group) or plus 0.5 mg ml^{-1} of FSH and 100 UI ml^{-1} of hCG (C group), resulting in the treatments without and with gonadotropins, respectively. Furthermore, to assess the effect of melatonin, these media were supplemented with 10^{-5}, 10^{-7} or 10^{-9} M melatonin (MT5∅, MT5, MT7∅, MT7, MT9∅, MT9 groups, without and with gonadotropins, respectively). Thus, the experiment followed a 2x4 factorial design (use or not of gonadotropins and 4 concentrations of melatonin). For oxidative stress evaluation, the matured oocytes were stained with 5 µM CellROX®Green (Molecular Probes, Eugene, OR, USA), to determine the intracellular ROS levels, and evaluated immediately under epifluorescence microscope. To quantify the fluorescence emission, the images were analyzed by ImageJ software. The total fluorescent intensity emitted by the oocyte was subtracted by the background and then normalized by the oocyte area. Data were analyzed by 2-way ANOVA, followed by Tukey or Bonferroni test, for multiple comparison of means, and are presented as mean ± SEM. There was no significant interaction (P=0.548) of melatonin and gonadotropins in the amount of ROS in oocytes, therefore, the results are presented considering the isolated effects. Regardless of gonadotropin supplementation, a reduction (P<0.05) of ROS was observed in oocytes treated with 10^{-9} M melatonin (25.89 ± 0.94 and 31.54 ± 1.77 - MT9∅ and MT9 groups) compared to oocytes that did not receive melatonin supplementation (29.62 ± 1.31 and 34.97 ± 1.32 - C∅ and C groups). Regarding the effect of gonadotropin, a greater (P<0.05) accumulation of ROS was observed in the groups with the presence of gonadotropins supplemented with 0 (C∅ and C groups), 10^{-7} (28.59 ± 1.31 and 35.12 ± 1.36 - MT7∅ and MT7 groups) and 10^{-9} M (MT9∅ and MT9 groups) melatonin and no differences (P>0.05) were found in the group supplemented with 10^{-5} M melatonin (29.35 ± 1.34 and 29.06 ± 1.14 - MT5∅ and MT5 groups). These results demonstrate that 10^{-9} M melatonin supplementation is able to reduce oxidative stress in *in vitro*-matured bovine oocytes, regardless of whether it is performed with or without gonadotropins, but it is necessary to investigate the effects of the absence of gonadotropins on oocyte competence. Furthermore, the results demonstrate that the presence of gonadotropins causes a greater accumulation of ROS, even in oocytes treated with melatonin, except in those that received 10^{-5} M of melatonin, which seems to be an interesting dose to reduce oxidative stress in *in vitro*-matured oocytes under conventional conditions, that is, in the presence of gonadotropins. Therefore, we can conclude that although no interaction was observed between the effect of melatonin and gonadotropins on the amount of ROS in bovine oocytes, further studies are needed in order to elucidate these mechanisms and identify the combination of factors that result in less oxidative stress, which should contribute to an increase in embryo production due to better oocyte quality. Acknowledgements: UNIFIMES (Edital nº 002/DIP/PIBIC–2020) and Laboratory of Reproductive Physiology – FMVA – UNESP.

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Animal Reproduction

Oxidative stress patterns in mares with endometritis

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Oxidative stress can be described as an imbalance between pro-oxidants and antioxidants in the body. In cattle, the relationship of such imbalance in cases of endometritis has already been described, however few studies on the subject in the equine species are available. Since it is known that the increase in free radicals directly influences reproductive performance, this study aimed to verify the effect of endometritis on parameters of oxidative status in mares, evaluated in blood serum samples (A1), low-volume uterine flush (A2) and uterine cytology (A3). For this purpose, 12 mares of different breeds with ages ranging between 5 and 10 years were used. These were divided into two groups, being classified as healthy (G1, n=6) or affected by endometritis (G2, n=6), through the association of cytological examination and bacteriological/fungal culture, performed prior to sample collection. The experimental design used was completely randomized with six replications. The treatments performed were arranged in a 2 x 3 factorial scheme, the first factor being constituted by the uterine status and the second by the sampling methods. The experiment took place in Visconde do Rio Branco-MG, Brazil (Latitude: 21° 1' 2" South, Longitude: 42° 50' 16" West). The three types of samples were collected in a single moment when in presence of uterine edema, verified by ultrasonography, and cervical relaxation. Endometrial cytology was performed with a commercial disposable collector introduced through cervix and placed in contact with the endometrium through rotational movements. The contents of each brush were depleted in 5 mL of alkaline phosphate buffer (PBS), aliquoted and frozen at -20°C. Low- volume uterine flush was performed by infusion of 250 mL of 0.9% saline through a transcervically placed sterile silicon tube. The fluid was dispersed throughout the uterus via transrectal manipulation and returned by gravity, being separated into 5 mL aliquots and frozen at -20°C for further analysis. In the laboratory, samples from both collections (A2 and A3) were added with 1% Triton® X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) and centrifuged at 3000 rpm for 10 minutes to obtain the supernatant and perform the analyses. For blood collection, performed by jugular puncture, plastic tubes with clot activator were used. The samples were centrifuged for 10 minutes at 3000 rpm to obtain serum, aliquoted and frozen at -20°C until the quantification was carried out. Tests regarding the activity of pro-oxidant and antioxidant substances (superoxide dismutase, catalase - CAT, malondialdehyde, total protein, nitric oxide, total antioxidant capacity) were performed at the Laboratory of Immunochemistry and Glycobiology of the Department of General Biology of the Federal University of Viçosa, Viçosa, MG, Brazil. Data were subjected to analysis of variance and means compared by Duncan's test ($p \leq 0.05$). Higher serum catalase means were observed in G1 (133.4 U/mg protein). CAT is an enzymatic antioxidant essential for homeostatic balance. This enzyme is responsible for catalyzing and decomposing H_2O_2 into water and oxygen, which protects cells from oxidative damage. The inflammatory response associated with endometritis generates an imbalance in the oxidative status, evidenced by the lower antioxidant capacity, demonstrated in the lower serum averages of this enzyme in G2 (90.2 U/mg protein). Statistical differences were not observed in the oxidative status analyzes in A2 and A3. Mares affected by endometritis have reduced antioxidant response capacity, and such difference can be detected by means of blood serum analysis. Acknowledgments: This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001

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Animal Reproduction

Parthenogenetic activation of buffalo oocytes aspirated by ultrasound guided transvaginal ovum pick up from less fertile Murrah buffaloes (*Bubalus bubalis*)

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Parthenogenetic activation means the production of embryos from oocytes without sperms. This way of embryo production can help to understand the potential of oocytes or reproductive efficiency of females because it only has maternal chromosomes. In the previous studies, the different methods or quality of oocytes was accessed by activation of buffalo's oocytes using the slaughter ovaries. To our knowledge, this is the first study that compares the competence of the less and normal fertile Murrah buffalo's oocytes (obtained by ovum-pick-up: OPU) by parthenogenetic *in vitro* embryo production. A total of 15 females belonging to the Laguna farm located in the municipality of Paracuru-CE (Brazil) were selected based on their reproductive history. Females that became pregnant after 100 days of delivery were classified as low fertile (LF, n=8) and less than 100 days with normal fertile (Cr, n=7). The average body condition score of the Cr and LF group animals was 3.57 (range 2.75 - 4) and 3.34 (range 2.75 - 4.5), respectively. The oocytes were collected by transvaginal OPU technique and classified as grade A, B, C and D. Grade A, B and C were submitted to *in vitro* maturation at 38.5 °C, 5% CO2, 5% O2 and 90% N2 in a portable incubator. After 24 hours of *in vitro* maturation, the oocytes were denuded and activated by ionomycin (5 µM) for 5 minutes followed by incubation with 6-DMAP (2 mM) for 4 hours in SOF medium. Finally, the presumptive parthenotes were *in vitro* cultured in synthetic oviductal fluid (SOF) medium at 38.5 °C in 5% CO2, 5% O2 and 90% N2 for 6 or 7 days. The recovery rate, morphological classification of oocytes, maturation, cleavage and blastocysts rates were observed in the groups and compared using the chi-square test, considering a 5% significance level, using the SPSS software, version 22.0. The aspiration rate of Cr was 66% (72/109) that was similar (P>0.05) to the LF group of 73.7% (118/160). The rates of oocytes grade A, B, C and D were similar (P>0.05) for Cr vs LF groups ie., 5.5 (4/72), 31.9 (23/72), 33.3 (24/72) and 29.1% (21/72) *vs* 5.9% (7/118), 35.5% (42/18), 32.2% (38/118) and 26.2% (31/118), respectively. The maturation rate was similar (P>0.05) between the Cr (51.1%, 22/43) and LF (44.4%, 36/81) groups. The cleavage and blastocyst rates for Cr vs LF groups were 62.1% (23/37) and 45.9% (17/37) vs 45.2 (33/73) and 30.1% (22/73), respectively, no difference (P>0.05) was observed between groups. Studies revealed that the genetics and bull factor affect the fertility of buffalo. Perhaps these factors may have influenced the results observed. It was concluded that oocytes obtained from less fertile buffaloes could develop into blastocysts after *in vitro* chemical activation. However, additional studies are needed to better understand the factors that affect the fertility of these female buffaloes.

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Animal Reproduction

Passive immune transfer of preterm lambs subjected to prenatal or immediate postnatal corticosteroid therapy

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In sheep, the placenta impedes the adequate transfer of immunoglobulins to the fetus, thus neonatal protection is extremely dependent on colostrum ingestion to ensure passive immunity. Furthermore, prematurity negatively affects passive immune transfer, as the reduction in gestational length reduces the quantity of immunoglobulins transferred to the mammary gland, increasing the chance of neonatal death. To minimize the adverse effects of prematurity, antenatal or postnatal corticosteroid therapy are alternatives to induce fetal-neonatal systemic maturity. Therefore, the aim of this study was to evaluate the efficiency of colostral immunity transfer in preterm lambs (born at 135 days of gestation) subjected to prenatal or postnatal corticosteroid therapy, and also the quality of colostrum in ewes submitted to antenatal corticosteroid therapy. For this study, 20 ewes were allocated to the following experimental groups: Term Group (GTERM; n=7, spontaneous delivery at term), Untreated Premature Group (GPREM; n=8, induced delivery at 135 days, without corticotherapy) and Prenatal Corticosteroid Premature Group (GPRE; n=5, maternal IM administration of 0.5 mg/kg of betamethasone at 133 days and induced delivery at 135 days). The respective neonates (n=26) were allocated into: GTERM Group (n=10); GPREM Group (n=6), GPRE Group (n=4) and Postnatal Corticosteroid Premature Group (GPOS; n=6, neonatal IM administration of 0.5 mg/kg of betamethasone, 15 minutes post-birth). Blood samples from the lambs and colostrum/milk were collected at birth (0h), 2h, 4h, 12h, 24h, 48h and 72h postpartum to determine the concentrations of total protein and γ- globulins, the blood concentration of gamma-glutamyl transferase (GGT), blood glucose and also the percentage of solids (%Brix) in colostrum *in natura*. Data were analyzed by LSD test ($P<0.05$). All experimental groups showed a progressive drop in %Brix in colostrum, with a significant reduction at 12h postpartum. The GPRE had lower %Brix at birth and after 2h, when compared to the other groups. Compared to GTERM, GPRE showed a lower concentration of γ-globulins in colostrum at 2h and total protein at 4h, while GPREM had a decrease only later (24-48h). Up to 24h, all premature lambs had lower serum concentration of total protein compared to the term lambs. GTERM showed a significant increase in total protein at 4h of life, while the others only at 12h (GPREM and GPOS) and 24h (GPRE). GPRE lambs had a higher serum concentration of γ-globulins than GTERM and GPREM at birth and at 2h. GPREM lambs, on the other hand, had a lower concentration of γ-globulins (less than 0.5 g/dL) up to 4h, when compared to the other groups. Among the preterm lambs, treated lambs had a higher serum concentration of γ-globulins than untreated preterm ones. The serum concentration of GGT was higher in GTERM until 12h of life. There was a progressive increase in GGT levels between 12h and 24h of life in the other groups. Term and preterm lambs subjected to corticosteroid therapy remained normoglycemic at all times, while GPREM showed hypoglycemia at 12h. In conclusion, despite the negative influence on the concentration of total protein and γ-globulins in colostrum, corticosteroid therapy promoted better glycemic stability in preterm lambs and increased globulin absorption in the first hours of life. Prematurity reduced the immunological quality of colostrum; however, the transfer of passive immunity occurred satisfactorily, regardless of maternal or neonatal corticotherapy. Acknowledgement: FAPESP (process nº 2015/17105-5)

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Animal Reproduction

Perspectives of gene editing use by the CRISPR tool in livestock production

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According to the Food and Agriculture Organization, despite the likely negative effects on global milk production, due to the COVID-19 pandemic, the forecast for the year 2021 is 921 million tons, which represents an increase in 1.6% compared to 2020. In addition, it is estimated that the per capita consumption of milk will increase, worldwide, by around 25% by the year 2050. Despite the great nutritional benefits associated with milk and its derivatives consumption, some people may suffer from allergic or intolerance reactions when consuming dairy products. The main components of milk associated with food hypersensitivity are α- lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins and caseins. In this sense, the growing demand for milk and dairy products leads to the search and development of effective solutions to health and food safety problems related to their consumption. In this context, A2A2 milk was obtained, spread internationally and produced through classical genetic selection, which contains only β-casein A2, facilitating its digestion and reducing the possibility of immune reactions. Innovative methodologies in the field of gene- editing biotechnology break frontiers and announce promising prospects for the consumption of animal origin products. Genetic modifications can represent a significant increase in the production of milk and other products with different therapeutic properties intended for human consumption. The CRISPR tool emerged as a disruptive alternative and provided major advances in the field of gene editing, such as speeding up the process, higher on-target rates, lower off-target rates, in addition to lower cost and greater accessibility. For these and so many other advantages, in 2020 the technique was awarded with the Nobel Prize in Chemistry. The CRISPR tool applied to gene editing for animal production involves several steps, including the need for extensive expertise in *in vitro* embryo production, the basis for the success of the technique. In a simplified way, we separated the gene editing biotechnology in eight steps: I. *In silico* study of target genes; II. RNA guide design and construction of the CRISPR vector; III. *In vitro* fertilization of bovine oocytes; IV. Zygote gene editing; V. *In vitro* culture of edited embryos; VI. Confirmation of the gene editing success in produced blastocysts; VII. Embryo transfer to recipients; VIII. Birth of the edited animal. In this sense, having knowledge about the genes already studied within the approach to food allergy to milk, we can highlight the casein, α- lactalbumin and β-lactoglobulin genes as the main targets to be explored. Since 2020, the CRISPR technique has been used in this field in Brazil, and resulted in the birth of a heifer lacking β-lactoglobulin, known to be the first animal born from an *in vitro* produced genetically modified embryo in the country. Thinking about the near future, we can also highlight the use of CRISPR as a tool for multigene editing, which will allow the production of animals with more than one edited target at the same time, reducing laboratory time, accelerating genetic gain and conferring greater merit to animals. With this, we can produce high quality and precise genetics, as well as establish frozen embryo banks for direct transfer, or even for exportation. However, we cannot fail to mention the legal aspect of this activity. Although gene editing has been known for many decades, worldwide there is a restricted number of genetically modified animals released for commerce, contrary to what is observed in agriculture, for example. Therefore, in order to achieve significant progress in fact, the legal field and technological development need to advance together to show the full potential of Brazil, which is already world-renowned in livestock production, to be in frontier Biotechnologies and in generation of products with high biomedical and commercial value as well.

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Animal Reproduction

Pharmacological semen collection and sperm morphometric evaluation in Silky anteater (*Cyclopes didactylus*) Linneus, 1758

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Pharmacological semen collection by urethral catheterization after anesthesia induction using α2-agonists has been a potential tool for semen conservation of endangered species, considered a less invasive alternative, with a capability to dismiss specialized equipment as an electroejaculator and resulting a more concentrated semen. So far, very likely the α2-agonists will be already administered to animal restrain in field for other procedures. Here we report an unintentional semen collection from a healthy silky anteater restrained with ketamine 5mg/Kg and 0,02mg/Kg medetomidine and an unpublished study of sperm morphometric. Sperm were collected from a drop of semen visualized in the external ostium of the urethra using a slide and making three smears. The slides were air dried and sent to the Animal Reproduction Laboratory of UESC where they were stained with fast panoptic stain. Morphometric measurements were performed from images captured in light microscopy (Leica Microsystems inc, DM2500, 1000x) and 150 normal sperms were evaluated using the software Micrometrics SE Premium v.2.8 (Princeton, MN). A statistical analysis was performed with Graphpad prism 8 software (version 8.0.2) using Shapiro-Wilk test for normality. The results are presented as mean ± SEM and Coefficient Variation (CV, %). Sperm morphometric measurements parameters were: 4.98 ± 0,04µm of head length (CV: 8.98%.); 2.91 ± 0.02µm of head width (CV: 8.37%); 6.2 ± 0.04µm of midpiece length (CV: 7.36%); 22.82 ± 0.28µm of tail length (CV: 15.24%); 34.04 ± 0.3µm of total sperm length (CV: 10.75%); 11.49 ± 0.12µm² of head area (CV:12.85%); 13.53 ± 0.07µm of head perimeter (CV: 6.47%); 1.71 ± 0.01 of head ellipticity (CV: 9.03%); 0.26 ± 0.00 of head elongation (CV: 16.81%); 0.79 ± 0.00 of head rugosity (CV: 0%); and 0.99 ± 0.00 of head regularity (CV: 7.95%). Morphologically, the sperm heads were oval with discrete elongated form. The midpiece is inserted centrally to the posterior region of the sperm head. The maximal head width was roughly in the middle of the head, with a narrower base and apex. The midpiece was found at the anterior portion of the sperm tail and presented a distal narrow segment at the transition region into the principal tail piece. The sperm tail tapered gradually distally. When we compare parts of sperm cell measurements in proportional percent, we have: head width µm corresponding to 58.75 ± 0.45% of the head length µm (CV: 9.34%); head length and sperm length (14.73 ± 0.16%; CV: 13.03%); head length and midpiece with tail length (17.34 ± 0.22; CV 15.49%); midpiece with tail length compared to sperm length (85.27 ± 0.16%; CV: 2.25%); head area and sperm length (34.11 ± 0.45%; CV: 15.98%); head perimeter and sperm length (40.09 ± 0.39%; CV: 12.43%). Due to the unexpected sperm collection during capture, the morphological abnormalities of sperm could not be adequately investigated on air-dried slides, since the smear method, without prior dilution, resulted in a slide very concentrated that directly impacted the evaluation of sperm morphology abnormalities. Therefore, the considered morphologically normal sperm, found separately near the edges of the slides, were used for morphometric analysis. Efforts to conserve this and other species are very important for conservation, as many habitats are suffering from increasing deforestation. Furthermore, the preservation of biodiversity is also closely linked to human health through of unique health concept. The use of α2-agonists for pharmacological collection of semen has been quite efficient for some species of wild animals, as it conducts the smooth muscle contraction of the vas deferens, promoting the emission of semen in the urethra, which can be collected by a urethral catheter, resulting in a highly concentrated sample. For silky anteaters, the smallest known anteater and an endangered species, knowledge about the characteristics of the semen is a big step towards cryopreservation of gametes and formation of germplasm banks for use in assisted reproduction techniques in the future.

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Animal Reproduction

Position of the reproductive tract of Bos taurus heifers impact fertility

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The aim of this study was to evaluate association between the anatomical position of the reproductive tract of Bos taurus (Angus) heifers on pregnancy rate (P/AI) during the reproductive season. A total of 360 heifers were enrolled at one farm of Rio Grande do Sul – Brazil were used. The heifers were submitted to exam between 25 and 50 days before de beginning of reproductive season and classified in one of three groups according to the position of the reproductive tract: Pelvic (smaller reproductive tracts with uterine horns resting within the pelvic cavity; n=174), Intermediate (cow reproductive tracts were intermediate between cervical and uterine horn diameter with longer uterine horns resting partially outside the pelvic cavity; n=84), or abdominal (reproductive tracts that were larger and resting mostly outside the pelvic cavity; n=102). On the first day (D0) of reproductive program, the females were submitted to insert of an intravaginal progesterone device 0.5g (P4) (DIB®, Zoetis) plus 2mg of estradiol benzoate (IM) (Gonadiol®, Zoetis) and 12,5mg of Dinoprost Tromethamine (Lutalyse®, Zoetis). On D8, the heifers received 12,5mg of Dinoprost Tromethamine, 0,7 mg of estradiol cypionate (ECP®, Zoetis), 300IU of equine chorionic gonadotropin (eCG; Novormon®, Zoetis) and P4 was removed. The heifers were artificially inseminated at 48 h after P4 removed. Semen from one sire was used. All batches of sire were analyzed previously. Moreover, tail heads of the cows were marked with chalk. On the day of AI, occurrence of prior estrus was evaluated based on chalk removal and the fluid of uterus was evaluated by transrectal ultrasound. Pregnancy diagnosis was performed 30 days before AI by transrectal ultrasound. The data were submitted to variance analysis, followed by Tukey Test, with a significance level of 5%. At the time of AI, the estrus demonstration rate and fluid accumulation in the uterus by ultrasound were evaluated. Regarding the demonstration of estrus, heifers of the pelvic (75.86%) and intermediate (78.57%) groups presented a higher percentage of heat compared to the abdominal group (49.02%) (P=0.0001). When the accumulation of fluid in the uterus was evaluated, heifers of the abdominal group (41.18%) presented a higher percentage in relation to the pelvic (18.39%) and intermediate (16.67%) groups (P=0.0001). Regarding the pregnancy rate at 30 days, heifers of the pelvic (61.49%) and intermediate (69.05%) groups presented a higher pregnancy rate compared to the abdominal group (41.18%) (P=0.0001). The final pregnancy rate performed of the reproductive season showed that heifers of the pelvic (82.76%) and intermediate (83.33%) groups presented higher final pregnancy rate compared to the abdominal group (70.59%) (P=0,03). In summary, relationship exists between the reproductive tract position and fertility parameters in beef heifers. In the present study, heifers classified as abdominal group increase fluid of uterus at AI , reduced estrus and low P/AI. Therefore, these data suggest underlying biological mechanisms associated with a abdominal uterus and further research is necessary to elucidate the mechanistic actions responsible for this reduced fertility.

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Animal Reproduction

Pre-ovulatory follicular diameter and ovulation rate of Pantaneiro breed cows after ovulation induction using two different estradiol esters (benzoate and cypionate)

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The Pantaneiro breed cattle descend from eleven European taurine cattle breeds. Over three centuries, these animals were able to develop high adaptability and rusticity, as well as, to maintain good reproductive rates, even in the harsh conditions of the Pantanal biome. However, in detriment to the introduction of the Zebuine cattle, the Pantaneiro breed is critically endangered. Thus, aiming the preservation and multiplication of the herd, as well as the genetic improvement of the breed through reproductive biotechnologies, the objective of the present study was to evaluate the potential of two estradiol esters, estradiol benzoate and estradiol cypionate, for ovulation synchronization in Pantaneiro cows. Eleven females with average age of 29.07 ± 9.53 months were used. On a random day of the estrous cycle, all animals received a single-dose intravaginal progesterone device (P4) and 2.0 mg of estradiol benzoate intramuscularly (IM) on D0. On D8, the P4 device was removed and PGF2α (25 mg dinoprost tromethamine) was administrated IM. The animals were divided into 3 groups: BE (estradiol benzoate); EC (estradiol cypionate) and Control, in a crossover experimental design. In the EB and EC groups, 1 mg of EB and 1mg of EC, respectively were administered IM, on the day of P4 withdrawal. In the Control, no ovulation inducer was applied. Regardless of the group, 73.3% (22/30) of the animals showed synchronization of the follicular emergence, which resulted in 81.81% of ovulations. For the EB and EC treatments, the ovulation rate was 81.71% (6/7) and 88.88% (8/9), respectively. For all treatments, the average size of the dominant follicle (DF) on D8 was 9.72 mm. Similar findings were noticed for pre-ovulatory follicle size for EB (10.7±0.64), and EC (11.98±0.95) treatments. Lastly, the interval for ovulation was 7.4 hours shorter for the EB group compared to the EC treatement. In conclusion, the base protocol led to synchrony of follicular emergence and treatments with EB and EC were efficient in inducing ovulation in Pantaneiro cows.

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Animal Reproduction

Prostaglandin E2 and F2α secretion is reduced by conjugated linoleic acid supplementation during *in vitro* bovine trophoblastic cells culture

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Early embryo mortality leads to reproductive failure in animals resulting in reduced pregnancy rates. Reproductive failure and embryonic loss in cattle are some of the largest economic burdens to cattle producers. Strategies that can benefit the maternal-fetal recognition, such as decrease prostaglandin F2α (PGF2α) synthesis and increase synthesis of prostaglandin E2 (PGE2), are fundamental to establishment of pregnancy. Conjugated linoleic acid (CLA) supplementation in cell culture medium affects the synthesis of prostaglandins however, the effect of CLA supplementation on cultured bovine trophoblast cells (CT1) has not been determined. Our hypothesis is that CLA supplementation on *in vitro* culture medium of CT1cells increase synthesis of PGE2 and decrease synthesis of PGF2α, benefiting the establishment of pregnancy. The objective was to determine the effects of varying concentrations of CLA supplementation (Sigma-Aldrich, USA, Cat N°. O5507) on PGE2 and PGF2α synthesis by *in vitro* culture of CT1 cells. The CT1 cells were cultured for 22 days in a humidified incubator at 38.5°C with 5% CO2 until they reached 100% confluence. On the 23rd day they were transferred to six-well plates with DMEM (1X) + GlutaMAX medium supplemented with 10% of fetal bovine serum (FBS), 1% of non-essential amino acids, 1% of antibiotic-antimycotic and 0.001% of β-mercaptoethanol, to be cultured for another 5 days until reaching 50% confluence. Twenty-four hours before CLA supplementation (day 6 of culture), the medium was replaced with a new medium without FBS, and on day 7 medium without FBS was supplemented with varying CLA concentrations (10, 20, 50 or 100 μM) and an additional control group for a 72-hour culture period. Collected medium was stored at -20°C until analysis. A total of five culture replicates were performed. Concentrations of PGE2 and PGF2α on day 8 were determined by enzyme-linked immunosorbent assay. Statistical analyzes were performed using the PROC MIXED of SAS program (version 9.2, SAS Institute Inc., Cary, NC, USA) considering the main effect of treatment group and the random effect of culture replicate. Concentration of PGE2 was greater ($P = 0.02$) for control (78.43 ± 11.92 ng/mL) in comparison to 10, 20, 50 and 100 μM of CLA (47.67 ± 7.40; 54.60 ± 6.37; 52.43 ± 3.42 and 56.40 ± 3.18 ng/mL, respectively). Concentration of PGF2α was also greater ($P < 0.0001$) for control (67.87 ± 7.28 ng/mL) in comparison to 10, 20, 50 and 100 μM of CLA (27.15 ± 7.94; 21.58 ± 4.92; 16.58 ± 0.25 and 20.04 ± 2.39 ng/mL, respectively). A significant effect ($P < 0.0001$) on PGE2/PGF2α ratio was also observed, reflecting a greater ($P < 0.05$) ratio in CLA-treated groups (1.98 ± 0.29; 2.72 ± 0.24; 3.16 ± 0.18 and 2.90 ± 0.22, respectively) compared to the control group (1.14 ± 0.06) and a greater ($P < 0.05$) ratio in CTl cells treated with 100 μM compared to 10 μM of CLA. We conclude that CLA treatment for 72 hours on *in vitro* culture medium of CT1 cells decreased PGE2 and PGF2α synthesis, but a CLA dose-dependent effect was observed on PGE2/PGF2α ratio. Acknowledgement: grant #2018/24168-1 and #2019/00637-5, FAPESP.

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Animal Reproduction

Puerperium evaluation in Canindé goats

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The Canindé is a naturalized and highly adapted goat breed, playing an important role in subsistence farming in the semi-arid region. Considering that there are few studies regarding the reproductive parameters of this breed, the goal of this study was to evaluate its physiological puerperium. Five multiparous Canindé goats (body condition score = 3; # 4 years old) maintained in a semi-intensive system (9°45'31"S, 35°50'36"W) were evaluated from eutocic parturition until the first natural service. Clinical evaluations were performed daily (0 to 7d post-partum - dpp), followed by weekly evaluation. Once a week, until five weeks post-partum, vaginal smears were obtained, stained with fast panotic kit, and 100 cells were evaluated using light microscopy to determine the percentages of each epithelial cell (basal, intermediate, superficial, and anucleated). Transrectal ultrasounds (7.5 MHz probe; Mindray® M5 Vet, China) were performed, to evaluate uterine involution (a weekly measurement of the uterine body wall thickness) and to detect follicular growth and ovulation (every 2 days, beginning 35 dpp). Mean percentages of epithelial cells/weeks were compared using Dunn test (5% confidence level). The interval between parturition and final uterine involution (constant uterine wall thickness), initial follicular growing and ovulation are presented using descriptive analysis. None of the goats had clinical signs of disease throughout the experiment. The percentages of superficial and anucleated cells were similar and not influenced by time. From three weeks post-partum, there was a reduction in the percentage of basal cells, concurrently with an increasing in the percentage of intermediate cells, compared to first week ($P<0.05$). Uterine involution was completed between three and four weeks post-partum, in three and two goats, respectively. Follicular growing was detected at 41,8 ± 4,2 dpp (mean ± SD; ranging from 38 to 48 dpp), and ovulation occurred between 48 and 53 dpp, with all goats accepting natural service. In conclusion, uterine involution precedes the detection of antral follicular growing, but changes in vaginal cytology indicate the influence of reproductive hormones three weeks before first ovulation post-partum.

Key Words: goats, post-partum ovarian activity, uterine involution, vagina cytology.

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Animal Reproduction

Quercetin as an antioxidant in bovine semen cryopreservation

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In order to reduce the negative effects of cryopreservation of sperm it has been studied the possible effect of antioxidant supplementation in dilution means for preparing, maintaining and cryopreservation of these cells. In the present study we aimed to evaluate the addition of Quercetin flavonoid to the freezing diluent, aiming at reducing the cryoinjury caused to the bovine semen. Five Nellore *Bos indicus* bulls, (24-36 months) had their ejaculates collected by electroejaculation. Three collections were carried out per bull (n = 15). Ejaculate volume (mL), sperm concentration (x10^6 sperm/mL), motility (%) and vigor (1-5) were measured and those who had at least 80% of motility and vigor 3 were used. Ejaculates was distributed in group C (CONTROL - without addition of quercetin), group Q25 (25 µg/mL of quercetin), group Q50 (50 µg/mL of quercetin) and group Q100 (100 µg/mL of quercetin) and subjected to the conventional cryopreservation process. Sperm kinetics analyzes were performed using a computerized system (SCA™ - Sperm Class Analyzer, Microptic, Barcelona, Spain). The variables used were total motility (TM; %), progressive motility (PM; %), curvilinear speed (VCL; µm/s), amplitude of lateral head displacement (ALH; µm) and linearity (LIN; %). Plasma membrane integrity, acrosome integrity, plasma membrane fluidity, mitochondrial activity and susceptibility to lipoperoxidation were performed via flow cytometry. Spermatozoa were classified into: intact plasma membrane with intact acrosome (PIPNA; %), intact and non- fluid plasma membrane (YOMERO; %), intact plasma membrane and high mitochondrial potential (PIMST; %) and percentage of peroxidized cells (PERO; %). The analysis was performed immediately after thawing (moment 0) and after Rapid Thermoresistance Test (moment 30). The free software SAS™ for academics was used to perform the statistical analyzes. The analyzes were based on answering the following questions: Was there a treatment effect for each moment? And if so, where is the difference? The values were analyzed by Proc GLIMMIX for repeated measures. In case of significance, the adjusted Tukey test was used to test for differences among treatments. There was no significant difference between treatments at moment 0 and 30 for all variables analyzed by flow cytometry ($p>0.05$). However, for sperm kinetics standards, the QUER25 treatment showed higher values ($p<0.05$) of VCL at time 30 (47,22±2,67), demonstrating that at the lower concentration of quercetin, the sperm cells were probably hyperactivated. From the results presented, we conclude that quercetin at the concentrations used does not promote significant gains to frozen semen. However, it was observed that as the dose was increased from 25 to 50 and 100 µg/mL of quercetin, there was a decrease in the patterns of sperm hyperactivation. This suggests that it is necessary to test higher doses, aiming at better results of post-thaw kinetics and cell structure. Acknowledgements: This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001 “. We would also like to thank the Federal University of Mato Grosso do Sul and the Multiuse Animal Reproduction Laboratory (FAMEZ / UFMS), which provided the facilities for carrying out the experiments.

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Animal Reproduction

Regulation of LHR mRNA binding protein (LRBP) expression in bovine *corpus luteum*

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Luteinizing hormone (LH) plays an essential role in controlling physiological processes such as ovulation and luteal maintenance acting by LH receptor (LHR). Although studies report the importance of this receptor in luteal development in bovine species, the mechanisms of regulation of LHR expression in the bovine *corpus luteum* (CL) have not been completely elucidated. Studies described the regulation of the LHR by the LHR mRNA binding protein (LRBP) in granulosa cells during follicle development, but its role has not yet been described in luteal tissue in cattle. Thus, we aimed to quantify relative mRNA abundance of LHR and LRBP in bovine CL. For this, CL were morphologically classified into two stages of development: functional CL; characterized by well-developed vasculature, often visible at the apex, completely orange or yellow, and 1.6 to 2.0 cm in diameter; and CL in regression, characterized by no visible vasculature on the surface, pale yellow to white in color and less than 1 cm in diameter. Ten CLs were collected from a local slaughterhouse: five functional CLs and five CL in regression. The abundance of *LHR* and *LRBP* was investigated by RT-qPCR using bovine-specific primers and expression of cyclophilin A (*PPIA*) was used as an endogenous control. Relative expression was determined by the Pffafl's equation and means were compared by T test. Tissue progesterone concentration was also quantified in order to confirm luteal status. Differences were considered significant when $P \leq 0.05$. For characterization of bovine CL status: a higher progesterone concentration in the functional CL (102.8 ± 11.4 ng/mL) was confirmed compared to CL in regression (12.38 ± 1.95 ng/mL; $p<0.0001$). The relative mRNA abundance of LHR was also higher in the functional CLs (0.55±0.14) when compared to the CLs in regression (0.01±0.0015; $p\leq0.05$). However, the abundance of mRNA of LRBP was lower in functional CLs (0.14±0.01) when compared to CLs in regression (0.70±0.09; $p\leq0.05$). We concluded that there is *LRBP* expression in bovine CLs and it is regulated during luteal regression. Furthermore, we suggest that the suppression of *LHR* expression during luteal regression could be, in part, due to the increase in *LRBP* expression.

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Animal Reproduction

Scrotal surface thermography is associated with sperm morphology in Dorper rams

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Dorper rams have been used to obtain crossbreed animals with a better carcass. However, there are reports indicating a higher percentage of sperm defects in Dorper rams compared with native breed rams in Brazilian semi-arid regions. Considering the importance of testicular thermoregulation for sperm quality, the goal of this study was to evaluate the relationship between scrotal surface thermography and sperm parameters in Dorper rams. Scrotal surface thermographies from 12 purebred Dorper rams were obtained (Thermal imager FLIR® i7), followed by measurement of rectal temperature and semen collection with artificial vagina. The images were evaluated using FLIR tolls® software (version 5.13.18.31.2002), to obtain the temperatures of right and left sides and calculate the averages in the regions of scrotum neck (SN), medium portion of the testes (TT), and the epididymis tail (ET), as well as general testicular average (TA) was obtained using the ellipse tool, which allows determining an average temperature of both testes area. Sperm samples were evaluated regarding morphology (total, minor and major sperm defects) using phase-contrast microscopy in a humid chamber, plasma membrane integrity (PMI; eosin-nigrosin) and membrane functionality (HOST; hypoosmotic swelling test). Pearson correlations were determined using R software and $P<0.05$ was considered significant. There was a high and positive correlation ($P<0.001$) among all the thermographic values. Rectal temperature and PMI were not correlated with other parameters evaluated. There was a positive correlation between HOST and normal sperm morphology ($r=0.6$, $P=0.03$). Values of SN were positively correlated with the percentage of normal sperm ($r=0.6$, $P=0.03$) and negatively correlated with major sperm defects ($r=-0.7$, $P=0.01$). There was a negative correlation between TT and the percentage of major sperm defects ($r=-0.6$, $P=0.04$). Values of ET were positively correlated with the percentage of normal sperm ($r=0.7$, $P=0.02$) and negatively correlated with major sperm defects ($r=-0.7$, $P=0.02$). The values of TA were positively correlated with the percentage of normal sperm ($r=0.6$, $P=0.03$) and negatively correlated with major sperm defects ($r=-0.6$, $P=0.02$). In conclusion, scrotal surface temperatures in different areas, detected by thermography, are correlated with total and major sperm defects in Dorper rams.

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Animal Reproduction

Seasonal impacts on the sperm characteristics of Africanized honeybee drones (*Apis mellifera L.*) reared in the Caatinga biome

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Global warming has been highlighted as an important threat to the honeybee populations, being even more drastic in tropical areas as the Caatinga. This biome is characterized by the scarcity of rain and high temperatures, which have been provoking the abandonment of the colonies. Since honeybee drones are fundamental for the reproductive success of the colony, we aim to investigate the impacts of dry and rainy seasons of the Caatinga biome on the sperm characteristics of Africanized honeybee drones. Study was conducted during the peak of rainy (March – June 2018) and dry (October – December 2018) periods of the semiarid climate in the Caatinga. A total of 100 Africanized honeybee (*Apis mellifera L.*) drones, aging approximately 12 days old, were used in the experiment, being 50 obtained during dry season and 50 during rainy season. Each drone was trapped by the head and chest region, with its abdomen being pressed gently resulting in the eversion of the endophalus, for later extraction with aluminum forceps. This structure was macerated using tweezers in 20 µl of 0.9% sodium chloride saline. Semen was then collected and immediately evaluated for sperm concentration using a Newbauer counting chamber, motility by light microscopy, membrane integrity using the fluorescent probes Hoechst 342 and Propidium Iodide, and morphology using a smear stained with Bengal Rose by counting 100 cell under light microscopy. Using the same smears, we evaluated sperm morphometry through the ImageJ software (Wayne Rasband - National Institute of Health, Maryland, United States). Data were presented as means and SEM and compared between seasons by variance analysis followed by Tukey test ($P < 0.05$). During rainy season, samples presented higher values ($P < 0.05$) for sperm concentration ($3.8 \pm 8.0 \times 10^6$ sperm/mL vs. $3.0 \pm 6.6 \times 10^6$ sperm/mL), motility (85.6 ± 1.5% vs. 52.0 ± 2.9%) and membrane integrity (82.8 ± 1.9% vs. 70.9 ± 1.8%) than those observed at the dry season. However, higher values ($P < 0.05$) for normal sperm morphology were found during dry (36.6 ± 1.8%) than at the rainy (30.8 ± 2.3%) season. Regarding sperm morphometry, sperm presenting higher dimensions ($P < 0.05$) were found at the rainy season (262.92 ± 1.2 µm) in comparison to those evaluated during dry season (252.24 ± 4.1 µm). In summary, Africanized honeybee drones raised in Caatinga biome seems to present a seasonal variation related to its sperm parameters. This information will be useful for the development of strategies to improve the reproductive management of the honeybee colonies reared in regions with semiarid climate. Financial support: CNPq and CAPES

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Animal Reproduction

Seasonal variation of epididymal sperm parameters of agouti (*Dasyprocta leporina*) reared in the semiarid region

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The agouti (*Dasyprocta leporina*) is a forager rodent that has a habit of dispersing seeds with the intention of storing its food for later consumption. Due to this behavior, it ends up making seed dispersions, thus having great ecological importance, contributing to the diversity and maintenance of forests. Its natural habitat, however, has been suffering constant deforestation, causing a progressive decline in wild populations. Thus, attempts to obtain data related to its reproductive physiology contribute to the development of appropriate strategies for the management and conservation of the species. In this sense, we studied the influence of environmental factors on the epididymal sperm parameters of male agouti kept in captivity in different seasons of a semiarid region. To characterize the climatic seasons, the rainfall regime (mm) was obtained daily from the automatic station of the National Institute of Meteorology - INMET, located in Mossoró, RN, Brazil. Twelve animals were euthanized (according to CEUA/UFERSA recommendations – nº 11/2019), and the testicular-epididymis complexes were collected. Six of these collections were conducted during the peak of the dry season (November and October 2019) and six at the peak of the rainy season (March and May 2021). In the laboratory, epididymal sperm were obtained from the epidydimal cauda by using the slicing- floating technique. The washing containing sperm was evaluated for volume (µl) using micropipettes, and for sperm concentration (sperm/mL x 10^6), using a Newbauer counting chamber. With basis on volume and concentration, we calculated the total number of sperm obtained in each season. Total and progressive sperm motility were evaluated by a computerized system (CASA – IVOS 12.0, Hamilton-Thorne, Beverly, USA). Bengal Rose-stained smears were used for evaluating the sperm morphology, using light microscopy (1000×), counting 100 cells per slide. Data were presented as means and SEM and compared between seasons by variance analysis followed by Student's t test ($P < 0.05$). Dry season was characterized by an accumulated rainfall regime of only 1.2 mm, while a total of 568.1 mm was obtained during rainy season. Regarding sperm parameters, a significantly higher ($P < 0.05$) number of epidydimal sperm was recovered during the dry season (1911.6 ± 420.1 sperm) in comparison to those obtained during the rainy season (788.4 ± 184.2 sperm). On the contrary, rainy season provided higher values for total (93.3 ± 1% vs. 76.5 ± 6.5%) and progressive (63.7 ± 2.7% vs. 14.7 ± 2.3%) sperm motility than dry season. Regarding sperm normal morphology, similar values were obtained for dry (89.8 ± 2.4%) and rainy (90.8 ± 1.4%) seasons. In summary, our results showed that a better sperm quality of agouti reared in the semiarid region can be obtained during the rainy season when compared to the dry season. However, it is possible that there is some compensatory mechanism, since a greater number of sperm is obtained in drier periods, to the detriment of rainy periods. These are valuable data for the improvement of strategies for the reproductive management and conservation of the species. Financial support: CNPq

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Animal Reproduction

Seasonality of German Shepherd bitches bred in Brazil

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Dog is considered a non-seasonal species, as bitches cycle, conceive, and deliver in all months of the year. Sires also produce spermatozoa and are sexually active during all months of the year. Brazil has a large territory, extending from +3º50' to -33º50' latitude and, consequently, have a large range of climate conditions and photoperiod. This study aimed to verify if bitches really are non-seasonal. To this, we used the data bank of the Brazilian Confederation of German Shepherd Breeders, analyzing 6,212 births occurred from 01/01/2009 to 12/31/2018 in Brazilian German Shepherd facilities. In a first trial, it was verified the distribution of births monthly, despite of the year. In a second trial, it was compared births occurred between 0º to -10º latitude (North) or between -25º to -35º latitude (South) monthly, despite of the year. Chi-square was used for all analysis, and to avoid considering minimal numeric differences among months or between latitude groups as significant, it was used $P < 0.0001$ level of significance. The birth distribution among months was as follows: Jan (460, 7.4%); Feb (395, 6.4%); Mar (455, 7.3%); Apr (513, 8.3%); Mai (495, 8.0%); Jun (569, 9.2%); Jul (586, 9.4%); Aug (536, 8.6%); Sep (577, 9.3%); Oct (588, 9.5%); Nov (539, 8.7%); and Dec (500, 8.1%). The lowest birth incidence was in Feb, and the highest in Oct. The Feb incidence was lower than those from Mar to Nov, but not than those of Jan and Dec. High number of births during winter and low number during summer were observed, gradually increasing during autumn and decreasing during spring. Considering that estrus and conception happens 60 days before birth, estrus/conception incidence was lower during mid to late spring than in other time of the year. Comparing incidence of births occurred between North and South monthly, there was no difference ($P = 0.11$). Our results showed a discrete, but significant, seasonality in German Shepherd reproductive activity of bitches, probably not influenced by the photoperiod. Dog is a monoestral species with approximately two heats per year, and breeders cannot control the moment of heat occurrence, but only decide to breed or not a spontaneously manifested heat. On the other hand, birth seasonality may also be a result of seasonal fertility of the bitches, expressing heat but not becoming pregnant. In conclusion, at least for German Shepherd bitches, there is a seasonal birth distribution during the year, which seems not to be influenced by photoperiod, but by unknown intrinsic and/or extrinsic reasons that need future investigation.

Keywords: Dog, seasonality, reproduction.

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Animal Reproduction

Selection of bovine epididymal sperm with the use of a microfluidic device

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Sperm selection is a fundamental step for in vitro embryo production and the use of microfluidic devices can provide more appropriate methods to isolate viable sperm, with greater motility and with less cellular damage. Thus, this study aimed to perform the selection of epididymal sperm of cattle, testing different chemoattractive solutions, from the use of microfluidics. Samples obtained from 10 pairs of bovine epididymis were recovered and placed in the microfluidic device designed with an inlet reservoir and four outlet reservoirs. Each output reservoir was filled with a different medium to evaluate the best chemoattractive for sperm. Four experimental groups were formed: CG = Control Group (saline solution 0.9%); GIVM= medium only with in vitro maturation medium; GIVM+O= IVM medium + matured oocytes; GIVM+MPA = IVM medium + medroxyprogesterone acetate at 0.05%. In addition, Percoll gradient was performed as control of the selection method. The medium was placed separately in the outlet reservoirs and each sample of epididymal sperm was placed in the inlet reservoir. After 30 minutes, at a temperature of 37.5ºC, the samples were collected from the outlet reservoirs for assessment. The kinetic parameters of sperm were evaluated using the computerized analysis system (CASA), morphology was performed with Bengal Rose, and plasma membrane integrity and mitochondrial sperm activity were evaluated using fluorescent microscopy probes. The data were submitted to descriptive statistics, followed by Mann-Whitey test, with a significance level of 5%. The percentage values of total motility were higher ($p < 0.05$) in the samples collected from the control group (82 ± 22.1) and those that passed through the Percoll gradient (78.3 ± 15.7), and were still similar ($p > 0.05$) to GIVM (69.2 ± 23.9) and GIVM+O (66.7 ± 27.2). However, the GIVM+MPA presented the lowest values ($p < 0.05$) compared to the other groups. When analyzing progressive motility, it was observed that all means presented similar values ($p > 0,05$), ranging from 24.1 ± 14.7 to 47.6 ± 16.1. Regarding morphology, it was observed that the spermatozoa selected by Percoll presented a lower percentage ($p < 0,05$) of normal cells (49.2 ± 9.5) when compared to CG (58.1 ± 11.4), GIVM (62.7 ± 10.4), GIVM+O (69.2 ± 11.9), and GIVM+MPA (70.6 ± 8.6). Plasma membrane viability and mitochondrial activity showed that the samples from the Percoll, CG, and GIVM groups were similar ($p > 0,05$) and presented higher values than the other groups ($p < 0,05$). In conclusion, it was confirmed that the microfluidic device was able to select epididymal sperm with adequate total and progressive motility, being ideal to be used for in vitro fertilization. The highest percentage of morphological abnormalities of epididymal sperm selected by Percoll gradient probably occurred due to the centrifugation stage, necessary to perform the gradient. Since the plasma membrane viability of these cells was viable, there was no influence on the use or not of centrifugation. The microfluidic device showed promising results for the selection of epididymal sperm of cattle and the medium containing only in vitro maturation medium (GIVM) and IVM + oocytes medium (GIVM+O) showed greater attraction to spermatozoa.

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Animal Reproduction

Severe alterations in sperm chromatin identified by transmission electron microscopy interfere in *in vitro* development of bovine embryos

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When observed under transmission electron microscopy, bull sperm chromatin is usually extremely electron- dense and homogeneous. However, changes from small spots to larger lighter regions are also often observed. The aim of this study was to verify the importance of these sperm chromatin changes identified by transmission electron microscopy on the *in vitro* fertilization and early embryonic development. For this study, five ejaculates from five different Giroland bulls with different levels of fertility were used. Part of each semen sample was used for in vitro embryo production (IVEP) routines and part was used for evaluation of sperm chromatin by transmission electron microscopy. One hundred and twenty IVEP routines were performed according to the protocol of the Biology of Reproduction Laboratory of the Universidade Federal de Uberlândia, with approximately 40 oocytes per routine, totaling 4916 oocytes, all from slaughterhouse- collected ovaries. Cleavage rates of experimental bulls were determined 48 hours after in vitro fertilization, represented by the percentage of mature oocytes that started cleavage. Blastocyst rates were determined seven days after fertilization and were represented by the percentage of oocytes that started cleavage and reached the blastocyst stage. The rest of the semen samples were used for the evaluation of sperm chromatin by transmission electron microscopy. On average 150 sperm head images of each sample were captured and classified into five grades of defects: Grade 0, absence of chromatin defects; Grade 1, presence of up to 3 small bright spots in chromatin; Grade 2, presence of up to 6 small bright spots; Grade 3, presence of several light points (above 6) or lighter region(s) occupying up to a quarter of the sperm head; Grade 4, lighter region(s) occupying above a quarter of the sperm head or large regions with granular chromatin. Pearson's correlation test was applied between each chromatin defect type and the cleavage and blastocyst rates. The high positive and significant correlations between cleavage and blastocyst rates and the percentage of sperm without chromatin defects show that, in general, the absence of changes in sperm chromatin favors the process of fertilization and the early embryonic development. The lack of significant correlation between cleavage and blastocyst rates and milder defects (grades 1 and 2) indicates that these defects have little or no effect on the oocyte fertilization process and that when sperm carriers with this type of chromatin defect fertilized the oocyte, the initial embryonic development is not impaired. The most severe defects (grades 3 and 4) showed significant negative correlations with cleavage and blastocyst rates, indicating that these types of defects can interfere with the fertilization process and when a sperm with these defect types fertilizes the oocyte, the early embryonic development is impaired. Acknowledgements: CNPq, CAPES.

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Animal Reproduction

Study of ovarian dynamics in Holstein bovine females with low and high antral follicle count by color Doppler ultrasound

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Recently, color Doppler ultrasound has become popular in cattle reproduction. This technique is relatively new and has as characteristic be a non-invasive study method, allowing a detailed evaluation of ovarian morphofunctional structures and a better understanding of reproductive physiology. Thus, the present study aimed to analyze the ovarian dynamics and characterize the follicular and luteal blood perfusion in Holstein bovine females (*Bos taurus taurus*), grouped according to antral follicle count (AFC). Eighty cows had the number of the antral follicles counted through the use of transrectal ultrasound and scanning of both ovaries, with 9 females classified in a low group (≤ 15 follicles) and another 9 females in the high (≥ 25 follicles) AFC group. 18 females were submitted to follicular growth wave synchronization by hormonal treatment. In a random day of the estrous cycle, considered D0, the females were submitted to the application of 2 mg of estradiol benzoate (Syncrogen®, GlobalGen Vet Science, Jaboticabal, São Paulo) and 25 µg of GnRH synthetic analog (Tec-Relin® , Agener União Saúde Animal, Embu- Guaçu, São Paulo) by intramuscular (IM) via and insertion of an intravaginal slow-release implant of 2 mg progesterone (Repro sync®, GlobalGen Vet Science, Jaboticabal, São Paulo). On D7, 0.526 mg of sodium cloprostenol (synthetic analog to PGF2α, Induscio®, GlobalGen Vet Science, Jaboticabal, São Paulo) was applied IM and on the following day (D8) the intravaginal P4 implant was removed and 0.526 mg of cloprostenol and 1 mg of estradiol cypionate (Cipion®, GlobalGen Vet Science, Jaboticabal, São Paulo) via IM injection. Follicular dynamics were monitored daily by Doppler ultrasonography, from the protocol days until 11 days after ovulation, at least once a day. To ensure the constancy of the AFC in bovine females, the repeatability calculation was performed for all days of the protocol. The ovulatory follicle (OF) and *corpus luteum* (CL) blood perfusion areas were analyzed. The data obtained from the evaluation of ovarian dynamics were previously analyzed for normal distribution by the Shapiro-Wilk test and homogeneity of variances by the F test. Parametric variables were analyzed by t-test for independent samples and non-parametric variables were analyzed using the Mann-Whitney test. When $p \leq 0.05$ was considered significantive. The mean number of antral follicles found in the low AFC group was 11.22 (standard error of the mean (SEM) = 0.99), while the mean in the high AFC group was 30.22 follicles (SEM = 1.78) with $P \leq 0.0001$. It was observed that low AFC animals had a larger OF blood perfusion area (17.36 *vs.* 8.16 mm^2; $P \leq 0.005$) when compared to high AFC cows. Animals with low AFC showed a higher blood perfusion area of CL (97.16 *vs.* 68.30 mm^2; $P \leq 0.021$) when compared to animals with high AFC. In conclusion, low AFC animals had greater blood perfusion of the ovulatory follicle and *corpus luteum* compared to high AFC Holstein cows.

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Animal Reproduction

Symmetric dimethylarginine (SDMA) to identify renal injury in bitches affected by cystic endometrial hyperplasia – pyometra complex

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Cystic endometrial hyperplasia (CEH)-pyometra complex is the uterine infection with intraluminal purulent secretion. It is an emergency that can lead to death of bitches due to its important systemic changes, mainly kidney injuries, which are the most common. Serum creatinine dosage is the standard test used in veterinary medicine, but it suffers non-renal influence and increase only after 75% loss of renal function. Symmetrical Dimethylarginine (SDMA) is a renal function biomarker capable to detect renal damage with 25 to 40% of renal functionality, being considered earlier and with less extra-renal interference than serum creatinine. The aim of this study was to evaluate kidney damage by measuring serum creatinine and dimethylarginine (SDMA) in 15 bitches diagnosed with CEH-pyometra complex. Blood samples were collected from the jugular vein after antisepsis with alcohol and deposited in a dry tube to obtain blood serum, creatinine measurement by kinetic method and SDMA using the IDEXX Catalist One ® device for sample processing in conjunction with the IDEXX SDMA kit. A reference range for healthy canines considered was 0.5 to 1.5mg/dL for creatinine and 0 to 14 µg/dL for SDMA. Mean creatinine results were 1.42mg/dL, median was 1 mg/dL. The mean SDMA was 20.4 µg/dL, and the median was 17 µg/dL. Only three bitches had creatinine above the reference value, being considered azotemic, but 10 patients had SDMA measurements above the reference value. With these findings it was possible to conclude that there is presence of acute kidney injury in patients affected by CEH – pyometra complex, which can be detected by SDMA, even in the bitch not showing azotemia. This fact is important during the evaluation of renal alterations in patients with CEH – pyometra complex.

Keywords: renal biomarker, uterine infection, canine.

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Animal Reproduction

Testicular and body biometry of Crioulo stallions

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The Brazilian Association of Crioulo Horse Breeders (ABCCC – "Associação Brasileira de Cavalos Crioulos) establishes minimal and maximal body biometrics evaluation; although, nothing is mentioned about testicular measurements. About testicular biometry, the amount of sperm that a stallion produces varies according to the amount of functional testicular tissue, which is correlated with testicular size. Body and testicular growth are associated and related to age. Growing expansion and appreciation of Crioulo breed encourages scientific research in the area, since this activity has a significant economic impact in Brazil. We aimed to describe data regarding body and testicular biometry of Crioulo stallions, with a comparative study between young and adult categories. Evaluation was performed in 56 stallions, Crioulo breed, split in: youngsters (3 and 4 years-old, n=16) and adults (above 5 years-old, n=40). Body biometry included weight (weight tape), height (hypometer,) cannon bone and chest circumference (measuring tape), body score condition (according to Henneke Chart scale 1-9) and neck fat accumulation (measurement of subcutaneous fat at the neck crest by ultrasound). Testicular biometry included height, length, width (pachymeter), volume (width x height x length x 0.5333), combined volume and daily sperm output (DSO) [(combined testicular volume * 0.024) - 0.76]. Statistical analyses included descriptive statistic, Pearson correlation, comparison between means by Kruskal Wallis, being $p<0.05$ considered significant. There was no significant difference between the parameters of testicular and body biometrics between categories young and adult. In relation to height, the average was very close to the lower limit established (141.3 cm young and 141.4 cm adults). Average values of chest circumference (179.9 cm young and 175.7 cm adults) were above the minimum recommended by the breed association. Chest circumference showed a positive correlation with ECC and weight. All stallions showed values of cannon bone circumference equal or greater (19 cm young and 19.2 cm adults) than recommended by the breed association Cannon bone circumference presented a positive correlation with height. Most of stallions presented excessive body fat, with a body score condition above 8 (scale 1-9). Neck fat accumulation presented a positive correlation with body score condition. About testicular biometry, there are no data with minimum and maximum values recommended by the breed association. Testicular growth usually occurs gradually and proportional to body growth. Testicular height, length, width and volume presented a positive correlation between the ipsi and contralateral testicle, total testicular volume and DSO. In conclusion, no difference in testicular and body biometric evaluation was observed between young and adult Crioulo stallions. Testicular and body growth are associated and also related to age, so our finding suggested that after 3-4 years-old most of Crioulo stallions have already reached maximum growth. Acknowledgements: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Brasil – Finance code 001 for scholarship.

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Animal Reproduction

Testosterone levels in goats experimentally infected with *Trypanosoma vivax*

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Trypanosomiasis is a disease caused by a protozoan of the genus *Trypanosoma*, and has an important impact on farm animals, reducing productive performance. *T. vivax* also affects the reproductive system and, in males, can cause testicular degeneration and a decrease in testosterone levels. The aim of this study was to evaluate serum levels of testosterone in young goats experimentally infected with *T. vivax*. Thirteen Saanen male goats, aging between 6 to 9 months, were assigned in two groups: "infected" (INF, n = 8) and "control" (CON, n = 5). They were submitted to jugular venipuncture, and blood was collected in 10 mL vacuum tubes without anticoagulant, on days D-3, D6, D13, D20, D27, D34 and D41, in which D0 was the day of *T. vivax* inoculation (1 x 10^4 trypomastigotes of Miranda strain). Blood was centrifuged at 1500 x g for 15 minutes, kept at -20°C until testosterone was measured by radioimmunoassay (RIA Testosterone direct, Beckman Coulter; sensitivity of 0.04 ng/ mL, intra and inter-assay coefficient of variation: 0.04 and 4.39%, respectively). Data were submitted to analysis of variance, including the effects of group, day of blood collection and interaction, followed by Duncan's test. The main effects of group and day were significant, but no interaction (P = 0.48) was observed. Testosterone levels were higher in CON than in INF group (3.02±0.59 vs. 0.53±0.12 ng/ mL; P<0.01). For day effect (P = 0.03), the highest level of testosterone occurred on D-3 compared to the lowest levels on D34 and D41; from D6 to D27 levels did not differ from the other days (D-3: 2.88^{a} ± 1.10, D6: 2.45^{ab} ± 1.03, D13: 1.46^{ab} ± 0.69, D20: 1.02^{ab} ± 0.47, D27: 1.02^{ab} ± 0.47, D34: 0.72^{b} ± 0.21, D41: 0.83^{b} ± 0.31). The decrease in testosterone levels in INF group in the present study corroborates a previous report in bulls infected with *T. vivax* (Camejo MI et al., 2016. Rev Científica, XXVI:13-19). It is known that trypanosomiasis can cause testicular degeneration and, in some cases, affects not only gametes but also Leydig cells, compromising testosterone production. Inversely to occurred in the present study, some authors indicate increase (Kumbhar et al. 2019. Trop Anim Health Prod, 51:1467-80) or oscillatory pattern (Becker-Silva et al. 2021. Br J Anim Environmental Res, 4:42-51) of testosterone levels according to the age of peripubertal bucks, related to periods of greater Leydig cells proliferation during puberty and sexual maturity. Based on our results, we conclude that *T. vivax* infection decreases serum testosterone in peripubertal Saanen goats and, besides that, levels of this hormone decreased along the time, regardless of experimental group. Acknowledgements: To FAPESP (PROC: 2019/22695-7), to Devani Mariano Pinheiro of Endocrinology Laboratory of Araçatuba, to Master students (Alessandra Regina Carrer and Letícia Castro Fiori), and to under graduation students (Amanda Kassem Sammour, Beatriz Eustachio Boarini, Gwenever Camargo Moraes, Maísa Pansani Santos, Sarah Daccach and Viviane Bobadilha Morelli).

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Animal Reproduction

The effect of anethol supplementation during in vitro maturation and in vitro culture on bovine embryo development

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In vitro production (IVP) has shown significant growth, reaching 68.7% of embryos produced in the world in 2018, when the technique surpassed *in vivo* production for the first time (IETS, 2018). Despite its commercial viability, IVP has low efficiency; thus, increased embryo production rates and quality would provide substantial gains in livestock. This study aims to evaluate the effect of anethole supplementation, which is a natural antioxidant, in the maturation and culture medium of bovine embryos produced *in vitro*. Oocytes were collected from ovaries obtained from slaughterhouses, and 2-8 mm follicles were punctured and subjected to *in vitro* maturation (IVM) in medium supplemented with 10% fetal bovine serum (FBS). In the first stage of the experiment, the oocytes were divided into 3 experimental groups: the control group, composed of base medium; group M300 (base medium supplemented with 300 µg/ml anethole); and group M3000 (base medium supplemented with 3000 µg/ml anethole). After IVM, nuclear maturation was evaluated to obtain metaphase II (MII) rates. In the second stage or the experiment, IVM was performed either with base medium (control group) or supplemented with 300 µg/ml of anethole, which was the best concentration previously found. Afterward, the oocytes were subjected to *in vitro* fertilization (IVF) and *in vitro* culture (IVC), being divided into 6 groups: three of them were maturated as the control group and cultivated in base medium with no anethole addition; in medium with 300 µg/ml; and in medium with 3000 µg/ml of anethole. The other three groups were maturated with 30 µg/ml of anethole and cultivated with base medium with no supplementation; in medium with 300 µg/ml; and in medium with 3000 µg/mlof anethole. Cleavage rates were evaluated on day 3 and formation and classification of blastocysts on day 8. When 300 µg/mL anethole were added to the MIV medium, there was no difference in MII rates compared to those in the control group. However, the addition of 3000 µg/mL anethole significantly reduced the percentage of cumulus oocyte complex (CCOs) that reached MII in relation to the other groups ($P \leq 0.05$). Regarding the cleavage index, the addition of 300 µg/ml anethole during IVM and IVC increased the percentage of cleaved embryos when compared to the other groups ($P \leq 0.05$). Nevertheless, no difference was found on embryo production ($P > 0.05$), nor on embryo quality, obtained by counting the total number of embryonic cells ($P > 0.05$). The Thiobarbituric Acid Reactive Substances (TBARS) and gene expression analyses will be performed subsequently. Acknowledgements: CAPES.

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Animal Reproduction

The effect of environmental variables on production, maturation and viability of sperm in white- lipped peccary (*Tayassu pecari*)

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White-lipped peccary (*Tayassu pecari*) is classified as a vulnerable species, which is disappearing from the Atlantic Forest. As a homoeothermic animal, it is capable of maintaining body temperature within physiological limits regardless of fluctuation in environment temperature. For efficient spermatogenesis, however, the testicular temperature must be 2 to 6°C below the body temperature. Environment temperature above 35°C for a long period can cause damages to sperm, including those cells that are maturing in the epididymis. Therefore, we aimed to evaluate the influence of environmental variables on semen collection, sperm maturation and production of adult white-lipped peccaries. We made the semen collection from nine adult male peccaries, born and raised in captivity, at the Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil (14° 47′ 50″ South; 39° 2′ 8″ West). On site, the animals are maintained under natural photoperiod of 12 h, and data collection occurred between March and June, 2017. The environmental variables were obtained from a nearby weather station starting from the 55th day before up to day before the semen collection. Thereafter we calculated the environmental variables means between 51 and 55 days before the semen collection (beginning of the spermatogenesis) as well as the means of the 14 days prior to collection (sperm maturation period). After the capture with a net, the peccary was anesthetized with acepromazine (0.2 mg/kg; IM) and ketamine (5 mg/kg; IM). Semen was obtained by electroejaculation and immediately evaluated for volume, pH, concentration, sperm morphology, and sperm motion kinematic parameters obtained by a computer-aided system (Sperm Class Analyser®, Microptics S.L., Barcelona, Spain). We checked the link between semen parameters and environmental variables by Spearman rank correlation tests. The mean volume of ejaculates was 420-µL (standard error – se = 272.8), with a mean concentration of 638.2 sperm/ mL (se = 550.4), and mean pH 7.1 (se = 0.2). The morphology means observed were 63.7% (se = 18.8) of normal sperm, 19.9% (se = 14.5) of major defects, and 16.4% (se = 17.7) of minor defects, as well as 11.7% (se = 14.2) of proximal and 7.3% (se = 15.3) of distal cytoplasmic droplets. The kinematic parameters means were 77.3% (se = 17.5) for total motility, 37.8% (se = 17.8) for progressive motility, 36.3% (se = 13) for linearity, 59% (se = 12) for straightness, 45.3-µm/s (se = 12.3) for curvilinear velocity, 16.5-µm/s (se = 7.3) for progressive linear velocity, 27.5-µm/s (se = 11.7) for velocity path average, 2.4-µm (se = 0.4) for amplitude lateral head – ALH, and 6.0Hz (se = 1.7) for beat cross frequency. The ambient temperature was higher between 51-55 days before semen collection than on the other periods evaluated ($P < 0.05$). In contrast, rainfall was significantly higher in the 14-day period before collection ($P < 0.05$), which led to a higher relative humidity at this period ($P < 0.05$) compared to the other ones. There was no association between sperm parameters and environmental variables at the day before collection ($P > 0.05$). On the other hand, accumulated rainfall observed in 14 days before semen collection was positively correlated with ALH (rSpearman = 0.62, $P < 0.05$), and with the presence of morphological defects at the proximal cytoplasmic droplets (rSpearman = 0.62, $P < 0.05$). The presence of these droplets was also positively correlated with rainfall for the period of 51-55 days (rSpearman = 0.62, $P < 0.05$). In conclusion, rainfall seems to be the most important environmental variable that can influence white-lipped peccary sperm parameters, especially during the formation and maturation of the cell. This novel information can contribute for the development of strategies for the reproductive management and the conservation of this threatened species. **Financial support:** CAPES and CNPq.

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Animal Reproduction

The impact of FGF supplementation, in *in vitro* culture of bovine embryos

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During the *in vivo* or *in vitro* embryo development the pathways to initials cell differentiations are crucial for the embryo progress. After the embryo development until blastocyst, the inner cell mass (ICM) differentiates into two new cell lines: epiblast (origin of germ-layers) and hypoblast (origin of extraembryonic tissues). The members of the fibroblast growth factor (FGF) family are reported in several processes, such as: differentiation, cell survival and cell division. Interestingly, the exogenous FGF4 supplementation inhibits Nanog expression, enhancing GATA6 expression and, consequently, acts on blocking the epiblast lineage derivation and promotes the hypoblast formation. This pathway can be investigated to apply in cattle, as an alternative of the Tetraploid Complementation Assay (TCA) - largely used in mice but not feasible in the bovine species - once the embryo committed only with the extraembryonic tissue's formation could substitute the tetraploid embryo component of that assay. Thus, bovine embryos were produced *in vitro* and cultured with different FGF4 concentrations (PeproTech, cat. Number 476; 10, 100 or 1,000ng/mL) from day 5 (120 hours post insemination) to day 7. The experiment was performed 8 times, where each replicate was made with 40 *cumulus*-oocyte complexes per group. The tests performed were analysis of variance followed by the Holm- Sidak's *post hoc*. As the partial results obtained so far, the different FGF4 supplementation did not alter the blastocyst rate (Mean±Standard Error of Mean, 43%±8.4; 40.3%±9.6; 39.4%±11.4, and 38.4%±9.6, respectively for groups control, 10, 100 and 1,000 ng/mL; p=0.84). However, the FGF4 supplementation with 100ng/mL increased the embryo hatched rate compared to the 10ng/mL group ($p<0.05$), control group (p=0.06) and 1,000ng/mL group (p=0.05). Interestingly, the FGF4 does not show positive or negative effects on the blastocyst rate, but the increased hatching let open questions about the FGF4 action on the cell differentiation and, mainly, the trophectoderm's activity. The next step (in progress) is to evaluate the abundance of target transcripts (RT-qPCR) and immunolocalization for differentiation markers of cell lines. Acknowledgements: São Paulo Research Foundation (FAPESP) grant numbers: 2020/11596-5 (IMP), 2016/16841-2 (RCB), 2019/10732-5 (CBC), scholarship PIBIC CNPq 1/2020 number 690 (LEF) and fellowship of Research Productivity (PQ2-CNPq) granted by the National Council for Scientific and Technological Development, number 301912/2019-0 (MFGN).

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Animal Reproduction

The in vitro effects of n-3 fatty acids on immune response regulation of bovine ex vivo endometrial explants

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The positive effects of diets supplemented with polyunsaturated fatty acids (PUFAs) such as Omega 3 (n-3) and Omega 6 (n-6) on cows' reproductive performance have been extensively reported. Although, which cell lines these fatty acids act to protect bovine reproductive and immune systems is not yet elucidated. Studies that explore bovine *ex vivo* endometrium explants offer a potential link between the whole animal and cellular studies. Still, culture of intact endometrium explants is capable to maintain the tissue architecture providing a bridge between *in vivo* observational studies of infection and *in vitro* immune mechanisms. Some studies reported that long chain n-3 PUFAs have been proposed as a strategy to enhance innate immune function in cows. Based on this, the objective of this study was to investigate the immune effects of n-3 PUFAs as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and linolenic acid (LNA) in five concentrations against lipopolysaccharide (LPS) on bovine *ex vivo* endometrium explants by measuring the accumulation of IL-6 and IL1- β proinflammatory cytokines. Bovine female genital tracts of non-pregnant Angus heifers were selected based on their healthy and normal appearance. In the intercaruncular area of the endometrium, sterile biopsy punches were used to select around 2-mm of tissue. Around 26 explants per animal (n=5) were collected. The explants were pre-treated for 24 hours with DHA, EPA and LNA in five concentrations: 0μM, 50μM, 100μM, 200μM and 400 μM. After that, they were treated with DHA, EPA and LNA and challenged with 1 μg / mL of LPS (L2880, *E. coli* 055: B5 lipopolysaccharides, Sigma-Aldrich) for another 24 hours in those same concentrations. After a total of 48 hours supernatants were collected and explants were weighed. The concentrations of IL-6 and IL-1β were measured by ELISA. Data analyses were performed by a two-way ANOVA with *post hoc* analysis performed using Bonferroni multiple comparison test with animal as experimental unit. Data were presented as mean ± S.E.M. and difference was considered when $P < 0.05$. After treating bovine *ex vivo* endometrial explants with EPA, DHA and LNA without challenging with LPS no difference was observed with the accumulation of IL1-β and IL-6 ($P > 0.05$). In this study, the LPS was used to stimulate the innate immune system, which mediates a local or systemic inflammatory response and explants treated with EPA and challenged with LPS were stimulated by a small concentration of this acid (50 μM) as it reduced the accumulation of both cytokines IL-6 and IL1-β ($P < 0.05$), indicating an anti-inflammatory effect. Still, it could be related that the accumulation of IL-1β was dose- dependent, as 400 μM of EPA were more efficient to produce an anti-inflammatory response when compared to 50 μM of EPA, whereas, for IL-6 no difference between concentrations EPA was observed when compared to Control. When explants were treated with DHA and challenged with LPS, it was reported that 100 μM of DHA generated an an-inflammatory response by decreasing the production of IL-1β when compared to the Control ($P < 0.05$). Still, concentrations of 400 μM of DHA were more efficiency to produce an anti- inflammatory reaction by reducing IL-1β response. Also, when IL-6 was measured, 50 μM of DHA started to produce an anti-inflammatory response, and 400 μM of DHA increased this response ($P < 0.05$). Quantities of DHA and EPA are found in marine products (fish oil), however, in order to minimize the fish oil dependence in aquaculture, alternatives to this oil have been studied. In this study we tested LNA as an alternative and less expensive source of n-3. We observed that bovine explants treated with LNA and challenged with LPS showed an anti-inflammatory response by decreasing the accumulation of both IL1-β and IL-6 only at 400 μM ($P < 0.05$). In conclusion, immune response was stimulated by n-3 fatty acids in bovine endometrium *ex vivo* explants challenged with LPS, as they reduced the release of anti-inflammatory cytokines (IL1-β and IL-6). Still, a greater anti-inflammatory effect was stimulated in response to EPA and DHA compared to LNA. Acknowledgements: FAPESP (process nº 2018/16051-7).

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Animal Reproduction

The use of levamisole phosphate (Biopersol® Forte) in timed-AI protocols improves body weight and reproductive efficiency of *Bos indicus* beef females

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This study aimed to assess the influence of the administration of levamisole phosphate in association to a timed artificial insemination (TAI) protocol on productive (body weight; BW) and reproductive (pregnancy rates and loss) outcomes of *Bos indicus* (Nelore) beef females. The study was conducted during the breeding seasons of 2018/2019 and 2019/20 and allocated 631 Nelore females (485 multiparous and 52 primiparous ranging 30-55 days post post-partum, and 94 heifers aging 18-24 months old) from a commercial farm in MT State, Brazil. At random days of the estrous cycle (D0), cows and heifers were evaluated by ultrasonography for uterine condition and cyclicity (CL). At that time, a subset of 82 cows had fecal samples collected for counts of eggs per gram of feces (EPG). Subsequently, cows received an intravaginal device with 1g P4 (Cronipres® Mono Dose, Biogénesis Bagó, Curitiba, Brazil), 2 mg estradiol benzoate (EB; Bioestrogen®, Biogénesis Bagó) IM, 150 µg D-Cloprostenol (PGF, Croniben®, Biogénesis Bagó) IM and were homogenously assigned to receive (group Levamisole) or not (group Control) 2.363 g (10 mL) of levamisole phosphate (Biopersol® Forte, Biogénesis Bagó) SC. On D8, device was removed and 150 µg D-Cloprostenol, 300 IU eCG (Ecegon®, Biogénesis Bagó) and 1 mg estradiol cypionate (EC; Croni-Cip®, Biogénesis Bagó) were given IM. Cows were painted with chalk on their tailheads on D8, and removal of chalk evaluated on D10 was used as an indication of estrus. TAI was done by a single veterinary 48 hours after device removal (D10), concomitant with the administration of 10.5 µg buserelin acetate (GnRH; Gonaxal®; Biogénesis Bagó) IM. Heifers were treated with similar protocol, except for the doses of EB (1 mg), PGF (75 µg), eCG (200 IU), EC (0.5 mg), and 1.890 g (8 mL) of levamisole phosphate. Pregnancy diagnosis was done 30 and 60 days after TAI. Data was analyzed using SAS for Windows. The average EPG registered on D0 was 30,5 ± 5,7 and was similar among groups (P = 0.53). The percentage of cyclic (presence of a CL) cows and heifers at the onset of the TAI protocol was 30.7% and 88.3% respectively. However, the percentage of cyclic females was similar among the experimental groups [Control: 39.1% (124/317) vs Levamisole: 40.5% (127/314); P = 0.56). Similar body condition score (BCS; 2.92 ± 0.02 and 2.88 ± 0.02; P = 0.28) and BW (409.6 ± 2.7 and 413.4 ± 2.9; P = 0.30) were also registered for control and treated females on D0. However, between D0 and D40, females that received levamisole phosphate had greater (P = 0.02) BW gain (32.2 ± 2.4kg) than those from the control group (23.9 ± 2.5kg), consequently achieving a greater BW on D40 (442.7 ± 4.3kg vs 431.3 ± 3.8kg; P = 0.05). As for reproductive outcomes, effect of animal category was observed (P = 0.03) regarding the rate of estrus occurrence, which was greater for cows (72.4%, 406/561) than heifers (66,05; 62/94). However, no interaction was found between category and treatment (P = 0.43). Also, pregnancy loss was greater (P = 0.009) in primiparous (22,2%) than multiparous cows (2.2%) and any interaction was detected between parity and treatment for this variable (P = 0.64). Similar rate of estrus occurrence D8-D10 [72.2% (229/317) vs 69.1% (217/314); P = 0.34], and pregnancy loss 30-60d [4.3% (7/162) vs 3.3% (6/183); P = 0.80] were found for Control vs Levamisole groups, respectively. Yet, females treated with levamisole phosphate had greater P/AI 30 days [59.2% (186/314) vs 51.7% (164/317); P = 0.05] and 60 days after TAI [56.9% (177/311) vs 49.5% (155/313); P = 0.06], and greater cumulative pregnancy rate [TAI + clean up bulls; 78.7% (244/310) vs 71.6% (224/313); P = 0.04] than control females. Thus, treatment with levamisole phosphate on D0 of a TAI protocol improved both productive (BW gain) and reproductive (P/AI 30d, 60d and cumulative pregnancy) outcomes of Nelore cattle and may be considered as an accessory tool to improve livestock efficiency. Acknowledgements: Farm São José.

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Animal Reproduction

Treatment with c-type natriuretic peptide precursor (NPPC) during in vitro prematuration of bovine cumulus oocyte complex: effects on transcript profile and meiotic arrest

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Oocytes that undergo in vitro maturation (IVM) come from follicles with 3 to 8 mm in diameter; these characterize a heterogenous oocyte population, which are at different stages of development. Furthermore, when oocytes are removed from follicular environment, they spontaneously resume meiosis (from germinal vesicle (GV) to metaphase II (MII) stage). C-type natriuretic peptide precursor (NPPC) is mainly synthesized by granulosa cells and acts to maintain oocyte meiotic arrest. In this way, the use of NPPC during a pre-in vitro maturation (preIVM) culture may be helpful in promoting meiosis blockade, allowing additional time for oocytes to acquire competence. So, the aim of this study was to evaluate the transcript profile in cumulus cells derived from bovine COCs submitted to preIVM with NPPC and investigate its effects on meiosis blockade. For that, COCs obtained from follicles between 3 to 8 mm were cultured during 8h according to the treatments: (1) NPPC: cultured in TCM-199 supplemented with 0.2 mM pyruvate, 25 mM sodium bicarbonate, 50 µg/ml amikacin, 0.3% BSA and 100 nM NPPC; and (2) control: cultured in TCM-199 supplemented with 0.2 mM pyruvate, 25 mM sodium bicarbonate, 50 µg/ml amikacin, 0.5 µg/ml FSH, 100 UI/ml hCG and 10% of fetal calf serum (FCS). A sample of immature oocytes was evaluated immediately after follicle removal (C0 group). Immature and cultured oocytes (n=223) were stripped from their cumulus cells by vortexing in PBS with 0.1% hyaluronidase for 3 minutes. Denuded oocytes were then stained with Hoechst 33342 (1 mg/mL) for 15 minutes at room temperature to determine their maturation status and assess the rates of oocytes maintained in GV stage GV. The transcriptomic profile (35 transcripts) of the corresponding cumulus cells was assessed by RT-qPCR using a microfluidic platform (BioMark HD System™, Fluidigm®). Data were analyzed by ANOVA followed by Tukey's test (P<0.05). There was no significant difference (P>0.05) in GV rates (mean ± SEM) between NPPC (52.8% ± 15.5) and C8 groups (26.1% ±7.2), but both differ (P<0.05) from C0 (96,4% ± 2.4). A total of 7 genes were upregulated (↑; P<0.05) and also 7 genes were downregulated (↓; P<0.05) in NPPC group compared to C0, as follows: genes related to embryonic quality and development (↓*CLIC3*, ↑*IGF1R*, ↑*KRT8*, ↑*LUM*, ↓*PRDX3*,↓*S100A14*), oxidative stress (↓*CAT*, ↓*GPX1*, ↓*HSPA1A*, ↑*GFPT2*, ↑*GLRX2*, ↓*DDIT3*) and apoptosis (↑*TNFRSF21*, ↑*TP53*, ↑*BCL2*) (P<0.05). When N8 group was compared to C8 group, genes like *KRT8*, *CAT* and *TP53* were upregulated (P<0.05), in contrast 7 genes related to embryonic quality and development (*LUM and S100A14*) and oxidative stress (*GFPT2, GLRX2, ATF4, DDIT3* and *XBP1*) were downregulated (P<0.05). Analysis of gene ontology (GO) was performed with the R package clusterProfiler and 46 enriched GO biological processes were identified. We also identified 31 KEGG pathways. Although the meiosis progression in the NPPC group did not differ from the control group, gene expression results demonstrate that the preIVM culture may be beneficial, suggesting a higher antioxidant response and lower activation of endoplasmic reticulum stress, apoptosis, and cellular stress response pathways. Acknowledgements: FAPESP (#2015/06733-5 and #2012/50533-2), CNPq (#307416/2015-1) and CAPES (Financial Code 001).

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Animal Reproduction

Ultrasonographic evaluation of accessory sex glands of goats experimentally infected with *Trypanosoma vivax*

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Trypanosoma vivax is a hemoparasite that affects negatively the reproductive system of ruminants. Despite the importance of accessory sex glands in the composition of seminal plasma, there is no information about their involvement in *T. vivax* infections in goats. Considering that ultrasonography is an auxiliary tool to assess lesions of reproductive tissues, the aim of the present study was to identify possible alterations in sexual glands of goats and characterize the ultrasonographic attributes of these tissues (numerical pixel value: NPV, minimum pixel value: MIN, maximum pixel value: MAX and standard deviation of NPV or heterogeneity: HET). Thirteen male Saanen goats, of initial age between 6 and 9 months, initial average weight (± EPM) of 30.2 ± 0.5 kg were used. Eight animals were infected (G-INF) by intravenous inoculation of $1x10^4$ *T. vivax* trypomastigotes and the other 5 were kept as negative control group (G-CON). The animals were submitted to Mode-B ultrasound examination (Z5-, VET, Mindray; 7.5 MHz) for evaluation of the sexual glands (vesicular glands: VG, disseminated prostate: DP and bulbourethral glands: BG) on D-3, D6, D13, D20, D27, D34 and D41 (D0: day of experimental inoculation). Images obtained from these organs were analyzed using Image Pro Plus® version 4 software, in which settled circular areas were created to estimate the echotexture per area. Statistical analysis was performed by analysis of variance (PROC MIXED, SAS), which included group, day of ultrasound evaluation and the interaction between these factors as fixed effects, and animal as a random effect. Comparisons between means were performed using the Tukey test, with significance ≤ 5%. For NPV variable, no interaction between group and day (GV: P = 0.93; PD: P = 0.61; GB: P = 0.17) or main effect of day (GV: P = 0.39; PD: P = 0.56; GB: P = 0.16) were observed. For group main effect, greater NPV (MIN – MAX) was observed in GV in G-INF (106.60 ± 1.80; 71.30 – 143.05) than in G-CON (99.76 ± 2.28; 64.23 – 135.95; P = 0.04). However, no difference in the PD NPV (G-CON: 99.85 ± 2.64 (65.84 – 127.46) vs. G-INF: 100.87 ± 2.09 (64.05 – 144, 86); P = 0.77) or GB (G-CON: 118.91 ± 7.94 (50.06 - 183.37) vs. G-INF 104.60 ± 6.27 (57.71 - 177.07); P = 0.19) was detected. For HET variable, no interaction (GV: P = 0.20; PD: P = 0.95; GB: P = 0.21) or day effect (GV: P = 0.10; PD: P = 0.88; GB: P = 0.12) occurred. In GV no group effect was observed (G-CON: 14.91 ± 0.67 vs. G-INF: 13.76 ± 0.53; P = 0.20), inversely to PD (G-CON: 14 .40 ± 0.60 vs. G-INF: 16.58 ± 0.47; P = 0.02) and GB (G-CON: 19.92 ± 0.62 vs. G-INF: 17.92 ± 0.49; P = 0.03). The present study is the first report regarding the evaluation of sexual glands echotexture in goats experimentally infected with *T. vivax*. The increase in NPV is indicative of increase in reflective surfaces, which may be due to fibrin and/or fibrosis. HET, on the other hand, represents the variation of hypo and hyperechogenic areas and may be indicative of tissue modification, possibly due an inflammatory process (Kastelic JP et al. 2012. ReprodDomAnim, 47:45-51). Based on the results of the present study, we conclude that increase in NPV in GV and HET in PD and GB were indicative of tissue alterations promoted by *T. vivax*, which were detectable by ultrasonography. Acknowledgments: To FAPESP (PROC: 2019/22695-7), to PIBIC-CNPq (Grant 1/2020 – PROC: 147825/2020-2) and to graduate students Amanda Kassem Sammour, Maísa Pansani dos Santos, Sarah Daccach and Viviane Bobadilha Morelli

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Animal Reproduction

Ultrasonographic evaluation of testis and epididymis of experimentally infected goats with Trypanosoma vivax

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Trypanosoma vivax is a hemoparasite which causes economic losses and can affect the reproductive system in farm animals, producing inflammatory and degenerative lesions mainly in testis and epididymis. Information about the effects of the parasite on reproductive tract of young goats is scarce, and ultrasound evaluation as an attempt to identify alterations in the parenchyma of these organs is unprecedented. The aim in the present study was to evaluate by B-mode ultrasonography, young goats experimentally infected with *T. vivax*, in order to identify possible alterations in ecotexthure of testis and epididymis. Thirteen male Saanen goats, aging 6 to 9 months, were assigned in negative control group (G-CON, n = 5) and infected group (G-INF, n = 8). G-INF was intravenously inoculated with 1 mL of blood containing $1x10^4$ *T. vivax* metacyclic trypomastigotes/animal, and this day was considered D0. Longitudinal ultrasound images were obtained on D-3, D6, D13, D20, D27, D34 and D41, and analyzed with Image Pro Plus® software, version 4 (Media Cybernetics Inc., San Diego, CA, USA). Average numerical pixel values (echogenicity: NPV), minimum pixel value (MIN), maximum pixel value (MAX) and pixel heterogeneity (standard deviation of NPVs: HET) were obtained in circular regions of interest distributed along the organ parenchyma (testis: T; epididymis: EP). Statistical analysis was performed by analysis of variance (PROC MIXED, SAS), which included the group, the day of ultrasound evaluation and the interaction between these factors as fixed effects, and the animal as a random effect. Comparisons between means were performed using the Tukey test, with significance ≤ 5%. For NPV variable, there was no interaction between group and day (T: P = 0.81, EP: P = 0.65) and no main effect of day (T: P = 0.33; EP: P = 0, 41). For main effect of group, NPV (MIN – MAX) in T was greater in G-INF (72.50 ± 3.79; 44.83 – 126.58) than in G-CON (59.34 ± 4.80; 32.61 - 94.55; P = 0.05). However, there was no difference in EP NPV (G-CON: 52.10 ± 2.78 (32.61 – 80.74) vs. G-INF: 56.19 ± 2.20 (35.32 – 89.27); P = 0.27). For the variable HET there was also no interaction (T: P = 0.12; EP: P = 0.14). In T there were main effects of group (G-CON: 10.78 ± 0.60 vs. G-INF: 14.33 ± 0.47; P < 0.01) and day (D13 was lower than D6, D34 and D41, while the other days did not differ; P < 0.01). In EP there was no main effect of group (G-CON: 9.22 ± 0.80 vs. G-INF: 10.76 ± 0.63; P = 0.16) and neither of day (P = 0.20). The normal testicular parenchyma has a homogeneous and hypoechoic echotexture, therefore, alterations are easily detectable in this structure. As trypanosomiasis is associated with testicular and epididymal inflammation and degeneration, collectively, the increase in T NPV and T HET suggest changes in microstructure and chemical composition, due to an inflammatory process and proliferation of fibrous connective tissue. Based on the results of the present study, it can be concluded that the increase in testicular NPV and testicular HET are indicative of tissue alterations promoted by *T. vivax*, which are detectable by ultrasonography. Acknowledgments: To FAPESP (PROC: 2019/22695-7 and PROC: 2020/07152-4), Alessandra Regina Carrer, Letícia Castro Fiori, Amanda Kassem Sammour and Sarah Daccach.

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Animal Reproduction

Ultrasound pregnancy diagnosis in a free-living jaguar (*Panthera onca*) with subsequent birth: First case report

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In situ species monitoring allows long-term observation of how they behave and interact in the environment and better understand their reproductive biology. The jaguar is an apex predator, requiring a large living area. With anthropic actions' fragmentation of the habitat, the species is vulnerable to trampling and conflicts with humans. Therefore, monitoring through GPS collars ensures a better understanding of how the species interact in these modified environments and allows actions to mitigate disputes, making conservation programs more effective. In contrast, advances in reproductive technologies for livestock will enable the extrapolation of advanced veterinary equipment – such as ultra-portable ultrasound – developed for domestic animals for application in wild species. Researchers from the Reprocon Institute routinely conduct scientific capture of jaguars to form a germplasm bank in the Passo do Lontra region (Corumbá – MS – Brazil; 19°34'37.5"S 57°01'08.0"W). During the clinical examination, in addition to weighing, blood collection, and age estimation by dentition, a reproductive evaluation is performed by ultrasound (DuoScan Plus; IMV Imaging/IMV Technologies) in females and semen collection in males. Among the captured animals, the females Neliza (Nov 07th/20) and Carol (Jul 31st/21) were estimated eight and eleven years old, weighing 66 and 67 kg, respectively. The pregnancy diagnosis of both females was positive, with an estimated gestational age between ~50-55 and ~90 days, respectively. Both animals were equipped with a GPS collar (Neliza: Telonics/Iridium; Carol: Patriot Tech/North Star) and released after recovery from anesthesia. Between 12/28/20 and 01/02/21 and 08/20/21 and 08/25/21, the geographic coordinates resulted in parturition clusters for Nelize and Carol, respectively. The parturition cluster points associated with gestational age measured by ultrasound are consistent with the species' gestational period of 105-110 days. In addition to being an essential tool for obtaining data on the species' reproductive biology, GPS collars also allow the discovery of important events in the animal, such as the parturition. Even more, the use of high-tech veterinary equipment, developed for production animals – in this case, ultrasound for pigs – allows obtaining important data in often adverse situations in the field. Thus, we report the first ultrasound diagnoses of gestation in free-living jaguar females and the sequential farrowing, estimated by telemetry patterns. Acknowledgments: Passo do Lontra Parque Hotel, Inês Luíza Caracante Pandolfi, Richard Rasmussen. This work was financed in part by Instituto Reprocon and in part by IMV Technologies Brasil.

Keywords: conservation, big cats, parturition, telemetry, GPS collar

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Animal Reproduction

Use of Buserelin Acetate associated with Estradiol Benzoate in early ressynchonization in taurus heifers

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The aim of this study was evaluated the synchronization and pregnancy of heifers submitted to the association of Buserelin Acetate and Estradiol Benzoate (EB) on the 22nd day (D22) post-AI for early resynchronization protocol, as well as to measure the diameter of the dominant follicle (DF) on the D22, during the removal of the progesterone device (D30) and at the moment of artificial insemination (AI; D32). A total of 229 Angus heifers (*Bos taurus taurus*), 14 months old, with a mean body condition score of 3.7 ± 0.23, they were submitted to an FTAI (FTAI1) with a conventional three-handling protocol. On the 22nd day after the first FTAI, an intravaginal device of 0.5g of progesterone (P4; DIB, Zoetis) was inserted in all females, and the heifers were separated into two groups - by the diameter of the largest follicle. The control group (G1, n=117) receive only 1mg of of BE (Sincrodiol, Ourofino) IM and treatment group (G2, n=112) receive 10µg of Buserelin Acetate (Sincroforte, Ourofino) plus 1mg of BE (Sincrodiol, Ourofino) IM. On D30, P4 was removed and the pregnancy diagnosis was performed by transrectal ultrasonography. The heifers which were pregnant (G1, n=67; G2, n=62) were not submitted to the continuity of protocol. In the non-pregnant heifers (G1, n=49; G2, n=50), an IM application of 0.5mg of Sodium Cloprostenol (Sincrocio, Ourofino), 1mg of estradiol cypionate (SincroCP, Ourofino) and 200UI of gonadotropin equine chorionic were performed (eCG; Novormon, Zoetis), in addition the heifers their tail-heads marked with chalk (Raidl-Maxi, RAIDEX GmbH,Dettingen/Erms, Germany) at the removal of the P4 insert. The occurrence of estrus was evaluated at TAI and was determined based upon the removal of the tail-head mark. Estrus was deemed to have occurred in cattle without a tail-head chalk mark at TAI. AI was performed 48h after P4 removal, with semen of high fertility bull. An ultrasonography with a rectal linear transducer at a frequency of 8MHz (Sonoscape A5) was used to measure the DF on D22, D30 and D32 (AI), and the pregnancy diagnosis was carried out 30 days after the FTAI2. Data were analyzed by the Anova procedure using Generalized Linear Model and comparisons through the Tukey test, significance level 95% (Minitab). In 8 non-pregnant heifers from G1 and G2 follicular dynamics was performed, and it was observed that the emergence after treatment in days of G1 was 2.6±1.3 and 1.9±0.6 for G2 (P=0.15). Heifers from G1 and G2 with DF <13mm in diameter on D22 had a pregnancy rate in the FTAI2 of 56.5% (13/23) and 51.90% (14/27) respectively, while nulliparous with DF >13mm from diameter presented a pregnancy rate of 33.6% (9/26) in G1 and 52.2% (12/23) in G2 (P=0.01). The mean of DF on D30 of non- pregnant heifers was 13.57mm in G1 and 13.93mm in G2 (P=0.44). On the AI day, the mean DF was 14.9 mm in G1 and 15.3 mm in G2 (P=0.72). Anticipation of ovulation occurred in 4.1% of females in G1 and in 10% of females in G2 (P=0.07). The pregnancy rate of FTAI1, FTAI2 and accumulated of 57.3% (67/117), 44.9% (22/49) and 76.0% (89/117) in G1 were obtained; and 55.4% (62/112), 52% (26/50) and 78.6% (88/112) in G2, respectively (P=0,485). The results of this study demonstrate that females with DF >13mm in diameter at D22 had a higher pregnancy rate with the use of Buserelin Acetate associated with Estradiol Benzoate, signaling that this tool has the potential to obtain better results in early resynchronization protocols in heifers. This study is going to be continued - including a larger number of animals to assess follicular dynamics, and the use of short-term protocols for resynchronization. Acknowledgements: Ourofino Animal Health.

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Animal Reproduction

Use of in silico approach for identification of miRNAs content in buffalo

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The miRNAs are short molecules involved in the post-transcriptional regulation of gene transcripts in several metabolic pathways. Due its regulative role, elucidate the content of miRNAs in different tissues can be used to study the gene expression mechanisms. The use of bioinformatic tools are low cost and available strategy for identifying miRNAs, particularly in species with few transcriptomic data available on open access platforms such as buffaloes (*Bubalus bubalis*). Thus, this work aims to carry out an *in silico* approach to identify homologous miRNAs between cattle (*Bos taurus*) and buffaloes, through the mapping of miRNAs sequences from cattle to the buffalo genome. Herein, the *in silico* approach consisted in a combination of bioinformatic tools in the following steps: 1) selection of the *Bubalus bubalis* genome; 2) selection of *Bos taurus* miRNAs content; 3) alignment of miRNAs sequences to the buffalo reference genome; 4) prediction of the miRNAs putative target genes and its biological functions. Firstly, the buffalo genome (access code GCA_000180995.3) was obtained from the NCBI database, as well as the set of mature miRNAs from cattle (n=1025) was obtained from the miRBase V22.1 platform (mirbase.org/). Secondly, to map the cattle miRNAs in the buffalo genome, the alignment of the miRNA sequences and reference genome was performed using the galaxy platform (http://usegalaxy.org/). Three alignment tools were used: Bowtie2, BWA and Hisat2. As a result, 29 (2.83%) miRNAs were identified using Bowtie2, 78 (7.71%) using BWA and 39 (3.74%) using Hisat2. Next, the miRNAs sequences obtained from the alignments were superimposed using the bedtools intersect tool associated to galaxy, as a result 8 miRNAs were simultaneously aligned by the three different algorithms (bta-miR-11975, bta-miR-214, bta-miR -7865, bta-miR-1343-5p, bta-miR-10185-5p, bta-miR- 2449, bta-miR-12030 and bta-miR-11976). The last step was to identify the putative target genes related to the 8 miRNAs using the miRmap open source software library (https://mirmap.ezlab.org), from that was possible to identify the targets for 3 miRNAs that were available in the library, the others one were unavailable. A miRmap tool based on the strength of the resulting mRNA repression was applied to increase the accuracy of target prediction, as a result was found that bta-miR-2449 regulates at least 33 target genes, bta-miR-214 at least 32 genes and bta-miR -1343-5p at least 8 target genes. At last, was found that many target genes were related to immune and inflammatory response, female fertility, fetal development and lipid metabolism. In summary, 8 homologous miRNAs with identical sequences were identified between buffalo and cattle, whose biological functions may also be conserved. Studies comparing cichlid fishes (X et al. 2019. Sci Rep, 9: 13848) and human and murine oocytes (B et al. Biol Reprod, 95: 1–13) indicate that the miRNAs content among species may be conserved, therefore, we suggest that a comparative alignment with cattle related specie may be useful as an initial screening of miRNAs in buffalo. Due to the molecular complexity the identification of miRNA content and its associated targets in tissues and species is still challenge, to achieve that the development and improvement of bioinformatic tools are required, as well as the generation of transcriptomic data remains essential. In conclusion, the *in silico* approach used herein was able to find homologous miRNAs in cattle and buffalo, the increment of transcriptomic data may further reveal that the miRNA content shared by these species may be more extensive than this initial evaluation showed to be.

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Animal Reproduction

Using commercial pregnancy associated-glycoproteins (PAG) for pregnancy detection at day 28 of gestation in high producing dairy cows

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Pregnancy diagnosis is an important part in reproduction management of ruminants. The ruminant trophoblast produce pregnancy associated-glycoproteins (PAG) that can be used for early pregnancy detection. The aim of these study is to validate commercial PAG pregnancy detection results with transrectal ultrasonography and assessed the benefits of PAG testing. A total of 60 blood samples were taken from high producing dairy cows (>35 kg/d) (Breed: Holstein and Brown Swiss). Blood samples were collected from each cow from the coccygeal at day 28 after artificial insemination, and stored at -20°C to asses circulating blood concentration of PAG ELISA using IDDEX Rapid Visual Pregnancy test. Transrectal ultrasonography (TRUS) was performed 40 days after artificial insemination and will be used for this study as reference for assessing pregnancy. On day 28 using the PAG test, the 60 samples were classified as 21 pregnant and 39 nonpregnant. On day 40 using TRUS, 11 pregnancies of 21 and 36 non-pregnant of 39 were confirmed. Result derived from PAG test (based on receiver operating characteristic) show on PAG test as significant predictors of pregnancy in cows (AUC=76.8%). In addition, the commercial PAG test showed the following performance: sensitivity of 76.9% and a specificity of 76.6% within a 95% confidence interval. In summary, the data shows that commercial pregnancy-associated glycoproteins are a reliable marker for early pregnancy detection in high producing dairy cows. However, the high rate of pregnancy loss could be a factor that reduces the sensitivity of the method. This research was supported by Peru Grant VRI-UNALM -2020.

Key word: Pregnancy associated glycoprotein; pregnancy diagnosis; transrectal ultrasonography

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Animal Reproduction

Uterine health: The importance of clinical and subclinical endometritis early diagnosis of dairy cows in pasture-based management systems.

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The objective of this study was to evaluate the prevalence of clinical and subclinical endometritis, and to follow up cows with subclinical endometritis from calving during days open in grazing systems. Postpartum gynecological examinations (n=479) were performed on 338 Holstein cows, primiparous and multiparous, at a dairy farm in southern Santa Fe (Lat -32°50´02.2´´S, Long -61°41´38.4´´W). After the first examination, cows were distributed in three periods: 15-20, 21-28 and 29- 60 days from calving. The cows were divided into two groups: Healthy Cows (HC) and Cows with Clinical Endometritis (CE). The voluntary waiting period was 60 d. Cervical vaginal discharge samples were obtained by vaginal examination, after which the cows were divided into two groups: Healthy Cows (HC), crystalline mucus and Cows with Clinical Endometritis. Samples for endometrial cytology were taken with the cytobrush technique at HC. Subclinical endometritis (SE) was diagnosed when cytology resulted in ≥5 polymorphonuclear neutrophils (NPM-N). The variables analyzed were: days of milk, HC, cows with clinical endometritis and cows with subclinical endometritis. The dependence between the status of uterine health (health or illness) and the postpartum period (15-20, 21-28 and 29-60 days postpartum) was evaluated with a Chi-square homogeneity test ($P < 0.05$) and t-Student ($P<0.05$). The t-Student test was used for paired samples. The results showed that 48.8% were HC and 51.2% had some degree of CE, after first postpartum evaluation. A significant difference was observed regarding to the dependence between the uterine health status (HC or CE) and the postpartum period at the first evaluation ($p> 0.0001$). Endometrial cytology was performed in the first postpartum evaluation at 140 HC. Results showed that 25.7% had subclinical endometritis (SE), but in this case, there were no significant differences in dependence on uterine health status (HC or SE) and postpartum period. The evolution of SE was studied in 27 cows that received a second postpartum evaluation. Forty-four percentage (74%) of the cows had spontaneous recovery of their SE. On average, cows with SE decreased PMN-N values at the 2nd evaluation ($p> 0.0001$). Our results indicate that the prevalence of CE is high in grazing dairy cows' system. Cows with SE had a high percentage of spontaneous recovery before the end of the days open. This study could be used to investigate deeply this subject looking for facilitate the early uterine health to improve reproductive performance and fertility of grazing dairy cows in the future.

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Animal Reproduction

Pelvic area in taurine beef heifers and occurrence of parturition dystocics

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The goal of this study was to measure the pelvic area of beef heifers using a Pelvimeter of Rice (Wiltbank et al. Journal of Animal Science, 30 (1970), p. 1043) and evaluate the Reproductive Tract Score (RTS), in a scale from 1 to 5, that RTS5 contemplate animals with the presence of corpus luteum. This verification is used as a selection tool to reduce the occurrence of dystocic during birth. The evaluation was done during breeding season of 2018, 2019 e 2020. For the study it was used as total of 9670 heifers of 13 and 24 month-old [14±1 m (n=1450) and 24±2,3m (n=8220)]. In this evaluation, the animals were divided in commercial herd and genetic producing herd. The animals were submitted to a protocol of induction of cyclicity, which consists of the application of 150 mg of intramuscular injectable progesterone (IM) 50 days before Fixed-time Artificial Insemination (TAI) protocol, along with RTS and pelvimetry assessment. The TAI protocol used, consisted in the application in day 0 (D0) of 2mg of estradiol benzoate IM and an intravaginal progesterone device (P4; 0,5g). On D8 it was withdrawn the P4 device, applying 0,5 mg de cloprostenol sodium IM, 200 IU of eCG (Equine Chorionic Gonadotropin) and 1 mg of estradiol cypionate. After 48 hours of removal of the P4 implant, an insemination was done with a bull of fertility proven in the field and indicated for heifers 14m (Expected Progeny Difference (EPD): BW -1,4) and 24m (EPD: BW -0,8). The pregnancy diagnosis was made 30 days after insemination and the matrices were followed until the time of delivery, when it was observed whether the delivery occurred in a eutocic or dystocic manner, with the need of help. The data were analyzed using the Glimmix procedure of SAS. A medium pelvic area was found in heifers at 14 months of 160 cm^2, but in the commercial herd 67,8% (461/680) stayed above this number, while in the genetic breeding herd 87,5% (674/770) was above the average (P=0.001). However, in heifers at 24 months, the average found was of 190 cm^2, the commercial herd had 73,5% (3896/5300 of heifers above average and the genetic breeding herd 86,6% (2530/2920). Regarding the time of parturition, the dystocia rate for heifers paired at 14 months according to the pelvic area was 44,6% (24/54); 27,3% (9/33); 25,9% (7/27) e 25% (8/32) for the pelvic areas 100-150 cm^2, 150-200 cm^2, 200-250 cm^2 e 250-300 cm^2, respectively. In heifers paired at 24 months, the rate of some degree of dystocia was 51,3% (41/80); 29,4% (27/92); 23,3% (59/254) e 10,1% (13/129) for the pelvic areas 100-150 cm^2, 150-200 cm^2, 200-250 cm^2 e 250-300 cm^2 (P=0,001). It is concluded that heifers from genetic producing herds showed greater pelvic area when compared to the commercial herd. Therefore, heifers with a pelvic area greater than the average had higher rates of eutocic births. The results show that pelvimetry together with RTS helps in the selection of heifers, reducing dystocic birth rates. However, other factors such as nutritional management and genetic selection, which also influence the development of the heifer pelvis, need greater investigation.

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Animal Reproduction

Anticipation of prostaglandin and fractionation of eCG in FTAI protocol in anestrus *Bos taurus* cows

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The aim of the present study was to determine the effect of dose and moment of administration of Equine Chorionic Gonadotropin (eCG) and PGF in the Fixed-Time Artificial Insemination (FTAI) protocol on estrus and pregnancy of suckling *Bos taurus* cows. In the study, 470 suckled Bos taurus cows were used, subjected to a FTAI protocol four-management (0-6-8-10) and divided into two groups: the Control Group (G1, n=249), with a mean body score of 2.67 ±0.03, which received 400 IU of eCG (Novormon, Zoetis) on D8 of the protocol, and the treatment Group (G2, n=221), with a mean body score of 2.55 ±0.04, which received 200 IU of eCG on D6 and 200 IU on D8 of the protocol. On the first day of the protocol (D0), the presence or ausence of *Corpus luteum* was evaluated using ultrasonography with a rectal linear transducer at a frequency of 8MHz (Sonoscape A5). Then, a 0.5g progesterone (P4) intravaginal device (DIB, Zoetis) was inserted and 2mg of Estradiol Benzoate (BE) (Gonadiol, Zoetis) was administered intramuscularly (IM) to all females. On D6, 12.5mg of Dinoprost Tromethamine (Lutalyse, Zoetis) was applied IM to all cows, and 200 IU of eCG was applied IM to G2. On D8, the intravaginal device was removed, 1mg of Estradiol Cypionate (E.C.P., Zoetis) was applied IM to all females, 400 IU of eCG in G1 females and 200 IU of eCG in G2 females. The cows their tail-heads marked with chalk (Raidl-Maxi, RAIDEX GmbH,Dettingen/Erms, Germany) at the removal of the P4 insert. The occurrence of estrus was evaluated at TAI and was determined based upon the removal of the tail-head mark. Estrus was deemed to have occurred in cattle without a tail-head chalk mark at TAI Artificial Insemination (AI) was done 48h after the removal of P4. A pregnancy diagnosis was performed by ultrasound exam 30 days after the AI. The analysis of the results was done from the Anova pocedure, using Generalized Linear Model and comparisons through Tukey's test, with a significance level of 95% (Minitab). The estrus and pregnancy rates were 67.4% (149/221) and 52.5% (116/221) respectively in G1, and 85.5% (213/249) and 58.6% (146/249) in G2 (P/IA: P=0.18). In addition, pregnancy loss between 30 and 60 days after TAI was 8.6% (10/116) and 2.7% (4/146) for G1 and G2, respectively. The estrus rates as well as the embryonic death rates showed significant difference between the G1 and G2 groups, with P=0.0001 and P=0.03, respectively. The results obtained in this study support the hypothesis that the anticipation of prostaglandin simultaneously with the fractionation of the eCG can increases estrus, pregnancy and also reduces the pregnancy loss. Thus, this procedure can be adopted to increase the productive rates in FTAI protocols and, consequently, improve the reproductive efficiency of the herd. Acknowledgements: PROBIC-FAPERGS

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Animal Reproduction

Preliminary results on cryopreservation of in vitro produced sheep and goat embryos using ethylene glycol followed by embryo transfer

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In vitro embryo production (IVEP) in small ruminants is commercially available in Brazil. However, limitations in the cryopreservation success of such embryos limit the technology's application to fresh embryo transfer. The objective of this study was to test a cryopreservation protocol for these embryos using ethylene glycol. The procedures for oocyte collection and commercial IVEP in sheep and goats were conducted as previously described by our group (Requena et al. 2017. Anim. Reprod., 14:3:773 and Requena et al. 2017. Rev. Bras. Reprod. Anim., 41:1:368). Briefly, the females were restrained on a laparoscopy table at a 45° angle and then, using a 5mm laparoscope and an atraumatic grasping forceps to uncover the ovaries, all follicles ≥ 2 mm diameter were aspirated using a 20G needle mounted in a plastic pipette connected to a collection tube and a vacuum line. IVM and IVF were performed in 50µL drops of medium under mineral oil at 38.5°C in a humidified atmosphere with 5% CO2 in air for 24h. After ~15h in IVF, the presumptive zygotes were transferred to 25 µL drops of mSOF under oil and cultured for six days at 38.5°C in a humidified atmosphere with 5% O2, 5% CO2, and 90% N2. Blastocyst-staged embryos were cryopreserved in 1.5M ethylene glycol medium (Vigro, Vetoquinol). Sterile DT yellow straws (IMV Technologies) were loaded with the embryos, 1 per straw, in the central of 3 columns separated by air bubbles. Prior to loading into the straws, the embryos were washed in holding medium (Holding Plus, Reprodux Laboratórios) and washed in ethylene glycol medium. Shortly after, the straws with the embryos were placed in the freezing machine (Freeze Control CL-5500 Temperature Controller) at -6°C for 2 min, and seeding was performed. The temperature was then decreased at a rate of 0.5°C/min until the temperature reached -32°C. At this point, embryos were held for 5 minutes to stabilize the temperature, and then straws were quickly immersed in liquid nitrogen. The straws were placed into goblets and stored in a nitrogen tank until transfer. Eighteen goat embryos were implanted on different occasions (four Saanen, four Toggenburg, and ten Anglo Nubian) in 18 healthy crossbred goat recipients, and 10 Santa Ines sheep embryos were transferred into ten healthy crossbred sheep recipients. After ~40 days, the pregnancy check was performed by ultrasound (Duo-Scan: Go Plus, IMV Imaging), and seven pregnancies were confirmed in goats (2/4 Saanen, 1/4 in Toggenburg, and 4/10 in Anglo Nubian) and five in sheep, resulting in 38,9% and 50% of pregnancy rate for goat and sheep, respectively. Up to date, three goats gave birth, and the rest of the animals remain pregnant. We believe this is a first report demonstrating in vitro produced sheep and goat embryos can be successfully frozen in a commercial setting, including pregnancies and live births. This is expected to have a high impact on the propagation of valuable sheep and goats and allowed a wider commercial application of the technology.
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