Effect of Intrauterine and breast-feeding nicotine exposure on oocytes quality and reproductive capacity of adult rats

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Nicotine, the main component of cigarettes, is a potent pro-oxidant in biological samples. It is capable of altering the fertility potential of men and women, inducing the occurrence of defects in gametes. In oocytes, it affects the meiosis and maturation. As oocytes are susceptible to oxidative stress, this drug can provoke damage to cellular membrane, to change the oocyte maturation delaying the meiosis and also to induce problems during the chromosome segregation. Besides, it can cause significant increase of DNA fragmentation, affecting the oocyte quality. In addition, as the development of the gonads begins in the intrauterine phase and women are already born with the established oocyte reserve, exposure to drugs during gestation can compromise fertile offspring. Considering the high number of women who smoke during pregnancy and the importance of the events that take place in the embryonic development stage for future offspring fertility, our group has been studying the exposure of rats to nicotine during the intrauterine and lactation phases. In recent work, we found that nicotine exposure changes male germinal cells at different stages of development, increasing the number of spermatozoa with abnormal morphology and the DNA fragmentation index of these gametes in adulthood. Therefore, the objective of this work is to verify the quality of female gametes and the reproductive capacity of rats (F1 generation) exposed to nicotine during the intrauterine and lactation phases, besides evaluating the quality of pre and post implantation embryos (F2 generation) generated from these gametes.

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Effect of the uterine tube epithelial cells culture supplemented with progesterone and human chorionic gonadrotophin on the preimplantational development *in vitro* culture bubalin embryos

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Tube epithelial cells are responsible for producing signaling molecules that interacts with gametes and early stages of embryonic development. The tube epithelium secretion influences the oocyte maturation, sperm capacitation, and also promotes the embryo early development. Secretion is strongly influenced by the stradiol and progesterone hormones, in different phases of the ovulatory cycle. In order to understand tube epithelium secretion dynamics, this study evaluate the effect of progesterone (P_4) and human chorionic gonadotrophin (HCG) supplemented in the tube epithelial cells culture upon the embryonic development. For this, epithelial cells cultured with progesterone (10 ng/mL) and HCG (10 IU/mL) were pretreated with estradiol (10 ρg/mL) for 24 hours. For *In Vitro* Embryos Production (IVEP), the ovaries were obtained from a slaughterhouse located in the Amapá's State and immediately sent to the laboratory, also located in Amapá, Cumulus-Oocyte Complexes (COCs) were selected and matured *in vitro* in TCM199 medium. After maturation, 18 hours, the mature CCOs were fertilized *in vitro* (IVF) in drops containing SOF - FIV medium. Using semen from a single bull. Approximately 21 hours after IVF, the probable zygotes were transferred to beads with SOF (Synthetic fluid oviduct) medium and distributed according to the following experimental groups, Control: SOF culture medium with monolayer of granulosa cells; Treatment 1: SOF culture medium with tube epithelial cells progesterone-free and HCG-free; Treatment 2: SOF culture medium with tube epithelial cells and addition of HCG (10 IU/ml); Treatment 3: SOF culture medium with tube epithelial cells and addition of progesterone (10ng/mL). The embryos were analyzed for the cleavage rate on the second post-fertilization day (D2) and blastocyst rate and embryonic kinetics on the 6th post-fertilization day (D6). The statistical analysis was performed by ANOVA variance test, significance level of 5% and Student's post-test, using the Sigma plot 8.0 software. No statistical difference was observed in the cleavage and blastocyst rates among the experimental treatments ($P>0.05$). In regards to the good quality embryo rates (grade 1), the Control (34 ± 13.5) and Treatment 1 (22 ± 20.2) were not statistically different from each other ($P>0.05$), but were different ($P<0.001$) compared to the Treatment 2 (80 ± 5.3) and Treatment 3 (43 ± 36.7). Thus, HCG and progesterone improved embryo quality in treatments 2 and 4 compared to the control group. Although treatments 2 and 3 did not present statistical difference ($P>0.05$) on embryo quality, ($P<0.001$), comparing grade 1 (80 ± 5.3; 43 ± 36, respectively) and grade 3 (20. ± 5.9; 58 ± 36.7, respectively) within the treatments 2 ad 3, was observed that treatment 2 showed high number of good quality embryos. The treatment in which SOF culture medium with tube epithelial cells and addition of HCG (treatment 2) did not statistically affect the rates of embryo development and cleavage, but it was superior to the other treatments in relation to the rate of good quality embryos. Thus, we concluded that the epithelial cells of the uterine tube in the presence of HCG positively affect the pre - implantation development of buffalo embryos.

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Effects of blocking Na^+/K^+-ATPase on horse blastocyst

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Currently, one of the challenges on equine reproduction is the low survival rate of horse embryos cryopreserved with diameter > 300 µm, using either vitrification or classic methods. Researches about the influence of sodium pump (Na^+/K^+-ATPase) on horse embryo development have more than 30 years and nowadays it is known that participates on embryo cavitation, changes ionic gradient of trophoblastic cells, as well as influences formation, distribution and permeability of tight junctions (TJ) between trophoblastic cells. Seeking for a medium able to reduce horse embryo diameter in the field, to support blastocyst vitrification, the goal of this study was to evaluate the influence of blocking Na^+/K^+-ATPase on morphometric characteristics of D7 and D9 horse embryos. Sixteen Mangalagar Marchador mares were monitored by rectal ultrasonography in alternated days during estrous cycle, and were inseminated with 5 x 10^8 of motile sperm using routine procedures after induced ovulation (deslorelin, 1mg IV). Ovulation was confirmed by daily ultrasonography and ovulation day was defined as D0. On D7 and D9, the mares underwent transcervical flushing and recovered embryos were evaluated regarding morphology and size, with aid of an inverted microscope and a reticle ruler. Thirteen blastocysts were harvested, eight D7 and five D9, all scored as Grade 1. Two D7 blastocysts were maintained only in control medium (EquiHold, Minitube, Germany) during 24 h at 37°C in a bacteriological incubator, to evaluate the influence of control media and temperature on embryo quality. The remained blastocysts were maintained during 1h after flushing in control medium, followed by exposition to a specific Na^+/K^+-ATPase inhibitor added to control medium (ouabain, 10^{-6} M). Images from blastocysts before and throughout ouabain exposition were recorded and further processed using ImageJ software, to determine blastocyst diameter and morphological features. Both D7 blastocysts maintained in control medium preserved their morphological characteristics during 24 h. All blastocysts exposed to ouabain reduced diameter after six hours of treatment. D7 blastocysts had an average reduction of 42.69% (ranging from 35.97 to 50.90%), whereas D9 blastocysts reduced 33.61% (ranging from 19.79 to 54.52%). However, all blastocysts underwent undesirable morphological features, such as: irregular shape (36.36%, 4/11) and texture 100% (11/11), detachment between capsule and embryo (81.82% 9/11), total blastocoel collapse 100% (11/11), and intense cellular extrusion (100%, 11/11). Due to morphological alteration, two D7 blastocysts were removed from ouabain treated medium and incubated on control medium, but no subsequent re-expansion was recorded, confirming the lack of viability. It was concluded that blocking Na^+/K^+-ATPase effectively reduced horse blastocyst diameter, but this event is associated to compromising of embryo viability. We inferred that interactions between Na^+/K^+-ATPase and Na^+/Ca^+ pump could lead to dysregulation of E-caderin/catenin complex and TJ on trophoblastic cells, but further studies are needed to better understand how Na^+/K^+-ATPase influences horse embryo viability.

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Endogenous intoxication indices in cows with preeclampsia as predictors of respiratory diseases development in their offspring

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Scientific interest in such pathologies of pregnancy as functional deficiency of the fetoplacental system, intrauterine growth retardation of embryo and fetus, and preeclampsia (gestosis), is caused not only by their wide spread occurrence among productive animals, but also by negative impact on postnatal growth and the health of offspring. Preeclampsia (PE) is a pathological state of the pregnant animals that manifests itself by endogenous intoxication (EI), generalized endotheliosis, vascular spasm and multiple organ functional insufficiency. The aim of this study was to determine the diagnostic value of biochemical markers of EI in cows with PE for predicting the development of respiratory disease in their offspring. 45 red-motley cows (31 with PE) with single gestation and their calves were examined at a gestation length of 248-255 days. The serum concentrations of medium molecular peptides (MMP), "effective" (ECA) and "total" albumin (TCA) were determined in the cows. The intoxication coefficient (IC) was calculated as IC = (MMP / ECA) x 1000. The diagnostic cut-off point of the analyzed factors was determined using ROC-analysis. MMP (0.55 ± 0.16 OD210, median 0.55) and IC (23.4 ± 7.4, median 22.5) in cows with PE were higher than the median values in animals with a physiological pregnancy by 71.9% ($P < 0.05$) and 67.9% ($P < 0.05$) respectively. Significant correlations between probability of bronchopneumonia (BP) development in neonatal calves and MMP ($r = +0.35$, $P < 0.05$), and IC ($r = +0.38$, $P < 0.05$) in their mothers were detected. The ROC-analysis revealed that these parameters of EI in cows can be used as predictors of BP development in their calves: they have good diagnostic value (AUC - 0.782 and 0.812, respectively), a high sensitivity (85.7% and 85.7%, respectively) and specificity (81.8% and 59.1%, respectively). For the cut-off point MMP > 0.555 OD210, and IC > 18.08 in cows, the development of BP was predicted in calves. The normal values were MMP 0.229-0.554 OD210, and IC 7.22-18.07. Thus, the determination of MMP, ECA and IC in cows with PE within 25-32 days before calving, allows not only to objectively evaluate the level of EI, but also to predict the development of BP in their offspring.

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Immunolocalization of aromatase in the developing gonad of *Podocnemis expansa*

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Podocnemis expansa is a turtle specie that is known for its large size, high prolificacy, rusticity and the high economic value attributed to its meat and by-products. The specie has been exploited commercially, however little is known about the physiology of reproduction, especially on gonadal development. Aromatase is the enzyme that converts androgens into estrogens. Estrogens, in turn, are essential for the differentiation and gonadal sex determination in vertebrates. Furthermore, *P. expansa* shows the determination of temperature-dependent sex (TDS), in which case the incubation temperature for a period of development thermosensitive determines the descending sex. One egg was collected from each of the 5 nests, located nests on a beach near the Center for Research and Conservation of Aquatic Chelonians (CPPQA) of Eletrobrás, near the Balbina hydroelectric power plant on the Uatumã River, Amazonas, Brazil (01o54'56.9" S, 59o28'18" W) (SISBIO/IBAMA 39472-4), in the period between 20º and 64ºday of incubation. The incubation temperature in the nests was measured using a remote temperature sensor, Hobo data loggers (Onset ™ Computer Corporation). After opening the egg shell, embryos were euthanized with an overdose of thiopental (50 $mg.ml^{-1}$) in combination with lidocaine (4 $mg.kg^{-1}$) intraperitoneally, then dissected under a stereoscopic to obtain gonads (CEUA/INPA nº025/2013). Gonads were analyzed at three different stages of development: undifferentiated (between days 22 and 29), thermosensitive period (30° to 36° day) and differentiated gonad (37° and 64° days). The gonads were fixed in 10% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 at 4 °C and after, histological processing was performed for paraffin inclusion. Histological sections were subjected to immunohistochemistry using anti-aromatase antibody (1:100, ab18995, ABCAM) and secondary antibody anti-rabbit (1:500; ab6720, ABCAM). The mean incubation temperature of the nests was 30.3°C. In the undifferentiated gonad, intense immunostaining was observed in the interstitial region, between the sexual cords and in the cells located in the transition region between mesonephro and gonad; moderate reaction in the cytoplasm of primordial germ cells (PMC's) and no immunolocalization on the germinal epithelium. In the thermosensitive period, immunoreactivity was observed weakly in the region of the germinal epithelium and intense in the interstitium, sexual cords and in the cells located in the transition region between mesonephro and gonad. As for the differentiated gonads, in the male, there was intense reaction to the aromatase in the interstitial region, around the seminiferous tubules, due to the presence of the Leydig cells, as well as in the germinal epithelium, and weak reaction in the cell transition region. The female gonads showed intense immunoreaction in the medullar region and below the germinal epithelium, however this imunorreaction was weak in this epithelium. The intense marking in the interstitial cells, at all stages of development, confirms the participation of these cells in the synthesis of estrogen in both sexes. There was a decrease in immunoreactivity in CGPs during gonadogenesis. In the testicles and ovaries, aromatase is concentrated in the interstitial region, however in the ovaries mainly in the medulla. It is concluded that the variation in the aromatase expression in the gonad regions and during the gonadogenesis confirms its involvement in the gonadal differentiation of *P. expansa*.

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Immunolocalization of estrogen receptors during gonadal development in *Podocnemis expansa*

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Animals with temperature-dependent sex determination (TSD) are vulnerable to temperature oscillations during the incubation period, more significantly in the middle third, where the temperature-sensitive period occurs, whose temperature directly influences the sex ratio of the offspring. In this process, enzymes such as aromatase, convert androgens into estrogens, triggering the development of ovaries in embryos. For this, estrogen receptors α (ERα) and β (ERβ) are capable of altering DNA transcription, binding to genes that trigger fundamental biological responses to ovary establishment. Thus, this research aims to evaluate the immunolocalization of the receptors in the gonad of the *Podocnemis expansa* turtle, during the embryonic development. This study (CEUA / INPA no. 025/2013) used 50 embryos from five different nests, collected from the 22nd to the 64th incubation period, on a beach at the Eletrobras Center for Research and Preservation of Aquatic Chelonia (CPPQA), located near to the Balbina Hydroelectric Power Plant on the Uatumã River - Amazonas, Brazil (01o54'56.9 "S, 059o28'18" W) (SISBIO / IBAMA 39472-4). The incubation temperature in the nests was measured using a remote temperature sensor, Hobo data loggers (Onset ™ Computer Corporation). After the collecting of the eggs, the embryos were euthanized with an overdose of thiopental (100 $mg.kg^{-1}$) in combination with 4 $mg.kg^{-1}$ lidocaine intraperitoneally. The embryos were dissected and the gonad-mesonephros complex removed under stereoscopic. Fragments of the complex were submitted to histological processing and immunohistochemical assays for estrogen receptors, using Anti-Rabbit polyclonal antibodies ERα and ERβ (AB75635 and AB3577 from ABCAM, respectively) and the secondary Anti-Rabbit IgG H & L / HRP (AB205718). Gonads were analyzed at three different stages of development: undifferentiated or bipotential (between days 22 and 29), differentiation (30th to 36th day) and differentiated gonad (37th and 64th days). The average incubation temperature of the nests was 30.3ºC. It was observed that α and β receptors appear together with some differences in tissue location according to the progression of the embryonic development. In the bipotential phase, there was moderate ERα and ERβ labeling in the germinal epithelium, but it was more poorly expressed in the primordial germ cell cytoplasm (PGC). During the differentiation period, the expression of both receptors was shown to be weak in the germinal epithelium and moderate in the PGC cytoplasm. However, at this stage, for β-type, more marked markings were seen in the medullar region. In the differentiation phase, there were strong immunoblots in the male for ERα in the germinal epithelium and in the seminiferous tubules in formation, whereas in the female there was a variable ranging from weak to moderate in the germinal epithelium and moderate in the PGC. In this same phase, ERβ were scored in both sexes, the females presented moderate marking in the medullar region, and strong marking in the PGC. While in males, intense marking can be observed in PGC and seminiferous tubules in formation. Positive cells for both receptors were observed in the transition region between mesonephros and gonad in all phases. Expression of the ERα and ERβ receptors were identified in the cells that make up the gonads of both sexes, being more expressive in the differentiation phase. In females, the presence of these receptors is linked to the establishment of the ovaries and development of the oocytes, seen through PGC and medulla positively marked. In males, positive marking of the seminiferous tubules suggests that estrogen is involved in the development of germ cells and the establishment of Sertoli cells.

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Impact of oxytocin on reproductive rates of lactating Girolando cows

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The use of oxytocin has become a common practice in dairy herds in Brazil, with the aim of increasing dairy production. Its effect, however, is to promote contraction of the mammary gland as well as the contractions of the smooth muscles of the uterus. The increase in the concentration of oxytocin receptors in the endometrium, during ovulation, is directly related to the augmentation of the luteolytic process, by increasing the uterine secretion of Prostaglandin F2α and therefore their daily use could cause embryonic death. In this regard, in the present study we have addressed the effect of intravenous oxytocin at the moment of milking on the reproductive performance of lactating cows. For this, the reproductive histories of 60 Girolando animals were evaluated. The animals were divides into two groups: Control Group (CG) whose animals that did not receive treatment with oxytocin at the time of milking and Group Treatment (GT) with animals that received oxytocin at the time of milking. In the animals of the treated group, oxytocin (1000 IU; 2,0 ml) was applied twice daily at each milking intravenously, on all days of lactation. Each animal had the follow up two lactations and were evaluated the time from calving to the subsequent conception (days) GC = 171,2 x GT = 186,2, interval of calving (days) GC = 446.4 x GT = 463.2 and number of services per gestation GC = 2,0 x GT = 2,3. There was a statistical difference ($P <0.05$) in the time from calving to the subsequent conception and the interval of calving. The daily application of oxytocin had a negative impact under the time from calving to the subsequent conception and interval of calving of the animals. According to (1) the effects of oxytocin on embryo loss was evaluated and concluded that in cows treated with oxytocin (100 UI; 5 ml) on days 4 to 7 after artificial insemination, the risk of embryo loss increased. (2) and (3) report that Treatment of cows with oxytocin at days 5 to 8 after artificial insemination reduced the pregnancy rate from 80% to 32%, but this effects was reversed when an antiluteolytic agent was given along with oxytocin. According to (4), the administration of oxytocin during ovulation and onset of the luteal phase delays the secretion of progesterone by the corpus luteum and presents the establishment of gestation, besides favoring the contraction of the musculature of the reproductive tract, interfering in the transport of the embryo. These results suggest that an adequate pattern of oxytocin secretion may also be necessary in establishing gestation. However, (5) presented on average the time from calving to the subsequent conception of 59.9 days in the group treated with oxytocin, in the results there was no negative effect of the daily application of oxytocin on the percentage of pregnant cows, as well as on other reproductive parameters evaluated, such as the time from calving to the subsequent conception and interval of calving. It should be noted that a large part of the studies use supra-physiological doses of oxytocin (100 UI), which is much higher than that used in the work in question, of only 2 UI per cow.

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Influence of equine chorionic gonadotrophin (eCG) on the pregnancy rate in Nelore cows submitted to fixed-time artificial insemination protocols (FTAI) in the semi arid region of the State of Alagoas

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Brazil is rated as one of the world's leading beef exports, and holds the second largest commercial herd. According to the Brazilian Institute of Geography and Statistics (IBGE, 2015), the cattle ranching in the Northeast has been growing in the national scenario, even though it is one of the regions that has one of the smallest herds in Brazil with 29.1 million head. The state of Alagoas is also expanding, where in 2015 a herd of 1.25 million head was estimated, making Alagoas the 21st place in the ranking of the states. In cattle herds there is no doubt that reproductive efficiency is a determining factor to have a good economic return on beef cattle. With Fixed Time Artificial Insemination (FTAI), the difficulties and barriers are being overcome, thus improving the reproductive indexes on farms that adhere to the FTAI. The use of equine chorionic gonodrotonic (eCG) can further improve indices because eCG binds to LH and FSH receptors having follicle stimulating and luteinizing function. The objective of this work was to evaluate the influence of eCG on the pregnancy rate in Nelore cows submitted to FTAI protocols. The research was approved by the Committee of Ethics in the use of animals of the University Center Cesmac (Protocol: 07A2017). The experiment was conducted in a beef cattle ranch in the municipality of Paulo Jacinto, located in the semi arid region of the State of Alagoas. We selected 70 cows of the Nelore breed that were healthy to the clinical examination with age between 2 and 8 years. The bovine females were then submitted to gynecological examination by rectal palpation and ultrasonography using the 5-10 MHz linear rectal transducer (DP-2200VET®, Mindray), where females that were approved for gynecological examination were submitted the FTAI protocol described below: D0: 2 mg of estradiol benzoate (Fertilcare synchronization®, Vallée S / A Veterinary Products) intramuscularly (IM) and an intravaginal device containing 1.2 g of Progesterone (Fertilcare Implant 1200®, Vallée S / A Veterinary Products). In D7: 0.530 mg of cloprostenol sodium (Ciosin®, MSD Animal Health) by I.M. route and in D8 the progesterone device was removed and 1 mg of estradiol cypionate (ECP®, Zoetis) was given IM. At that time the cows were divided into two groups at random. Control group (GC): administered IM saline at the time of progesterone-based implant withdrawal; Test Group (GT): administered 300 IU of eCG by I.M pathway (FOLLIGON 5,000®, MSD Animal Health) at the time of removal of the progesterone-based implant. Artificial insemination was performed 52 hours after withdrawal of the progesterone device. Cows were evaluated by ultrasonography using the 5-10 MHz linear rectal transducer (DP-2200VET®, Mindray) 30 days after artificial insemination to verify the pregnancy status in each batch. The pregnancy data were submitted to chi-square and Fisher's exact tests with $p <0.05$. In the control group, 50% of pregnant females (20/40) were obtained, in the test group 53.3% (16/30) of pregnant cows, either cycling or anestrous, in which there were no significant differences ($p < 0.05$) in the pregnancy rate between the groups. In conclusion, the eCG did not increase the pregnancy rate in Nelore cows of the semi arid region of the state of Alagoas submitted to FTAI.

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Influence of Kisspeptin in the spermatic selection stage for *in vitro* production of bovine embryos

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The aim of the present study was to evaluate the addition of Kisspeptin (Kp) in the *in vitro* process of spermatic selection, regarding the viability of bovine spermatozoa and embryo production rates. For this, 980 oocytes were obtained from slaughterhouse ovaries and 75 commercial bovine semen straws were used. The treatments were performed on the spermatozoa during the *in vitro* spermatic selection stage by the centrifugation method with 90% and 45% Percoll® discontinuous gradients according to each proposed treatment: Control (n = 182), Kp 10-5 M (n = 198), Kp 10-6 M (n = 200), Kp 10-7 M (n = 206) and 50 µM P-234 antagonist (n = 194), with the dilutions carried out on both gradients respected concentrations. The spermatozoa were analyzed before and after spermatic selection for motility and vigor, and were submitted to staining with fluorescent probes JC-1, IP and FITC-PSA, to evaluate the mitochondrial potential, plasma membrane integrity and acrosome integrity under confocal laser scanning microscope LSM 510 meta. After the treatments, spermatozoa were normally used in IVEP procedures. The production analyzes were performed on D2 and D7, based respectively on cleavage and blastocysts rates. The data were submitted to the normality and homogeneity test by guided analysis SAS, after were used the proc GLIMMIX procedure to evaluate the experimental model and later the Tukey-Kramer test for comparison of means. Differences are considered when $P < 0.05$. The results of motility, vigor and production rates were similar ($P > 0.05$) among all proposed treatments. The cleavage rate (n = 846) was higher than 82% and the percentage of blastocyst production (n = 331) higher than 29% in all treatments. The mitochondrial potential of Kp 10^{-6} and Kp 10^{-7} (92.6%) groups was higher ($P < 0.05$) to the Control (89.8%) and P-234 (83.8%) groups, the latter being lower ($P < 0.05$) than all other treatments. Plasma membrane integrity and acrosome integrity (PMI/AI) evaluations showed that treatments with Kp addition were similar, ($P > 0.05$) Kp 10^{-5} (88.9%/90.9%), Kp 10^{-6} (90.7%/91.8%) and Kp 10^{-7} (90.2%/91.4%) and the P-234 antagonist (90.5%/90.8%), respectively. The Kp 10^{-6} treatment presented a superior result ($P < 0.05$) to the Control group (87.9%/89.1%). When correlating the variables analyzed by confocal microscopy, a significant and positive correlation was observed between high mitochondrial potential and plasma membrane integrity ($r = 0.80$; $P < 0.0001$), acrosome integrity and high mitochondrial potential (r = 0, 80, $P < 0.0001$), plasma membrane integrity and acrosome integrity ($r = 0.84$, $P < 0.0001$). Finally, it can be concluded that the balanced concentration of Kp provided greater viability to bovine spermatozoa related to mitochondrial potential, acrosome integrity and plasma membrane. The P-234 antagonist presented a detrimental effect on the mitochondrial potential.

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Ozone is able to activate chronically subclinical infected mares

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Bacterial infection in the uterus can be a main cause of reduced fertility in mares. Bacteria can access the mare uterus through the cervix, during breeding or foaling or even in iatrogenic situation where there is no adequate hygienic procedures by professionals. Resistant mares are able to clear bacteria rapidly. However susceptible mares with a compromised uterine defense mechanisms infection may become chronic (Petersen et al., Veterinary Microbiology, 2015;179:119-175). In chronically infected mares, infection can be stablished deep into the stratum spongiosum of endometrium complicating the precise diagnosis and treatment. Antibiotic treatment depends upon bacterial activation and growth. In mares chronic uterine infections are resistant to antimicrobials and could be caused by biofilm production, which consists of a matrix that adhere the microcolonies preventing penetration of antibiotic. Formation of biofilm in the equine reproductive tract is theorized to be a significant cause of chronic endometritis in the mare. The objective of this study was to test if sunflower ozonized oil infused intrauterine could be able to activate bacterial growth in mares suspected of having a chronically dormant subclinical infection. A group of 24 embryo transfer recipient mares with negative swab culture and no pregnancy diagnosed in at least 3 trials were used. All mares were treated with a uterine infusion of 50 mL sunflower oil containing 66 µg of ozone. Next day, all mares were lavaged with 10-12 litters of Lactate Ringer Serum until uterine fluid was clean. As results from the 24 treated recipients, 13 (54,1%) cycled had one embryo transferred. From those, 9/13 (69,2%), got pregnant up to 30 days and 8/13 (61,5%) was still pregnant at Day 60. As conclusion, activation of bacterial growth was possibly induced and cleared with ozone sunflower oil in 13/24 (54,1%) from these recipient mares and pregnancy was stablished in 8/24 (33,3%) . This data suggests that ozone was able stimulate activation of bacterial growth in mares suspected of subclinical chronically infected mares. Further studies are ongoing in order to identify what bacteria was involved, its capacity to produce biofilm and the correlation of this biofilm production in the mares that did not respond to the activation.

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Profile of early pregnancy markers in circulating polymorphonuclear cells of Nelore heifers

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Bovine conceptus releases interferon-tau (IFNT) and stimulates several transcripts in circulating polymorphonuclear cells (PMNs) that could be used as biomarkers at the beginning of pregnancy. In the present study, we aimed to characterize the expression pattern of interferon-tau stimulated genes (ISGs) at the beginning of gestation between pregnant and non-pregnant heifers for development of an early and innovative pregnancy diagnosis in cattle. Twenty-nine Nelore heifers (18-20 months) had their estrous cycle synchronized, and were submitted to a timed artificial insemination (TAI; Day 0). Pregnancy diagnosis was made by ultrasonography on days 25 and 28 through the detection of the embryonic vesicle and heartbeats. On days 0, 10, 14, 16, 18 and 20 after TAI, 25mL of blood was collected in heparinized tubes by puncture of the jugular vein for the isolation of PMNs. The isolation was made by Ficoll®Paque Plus gradient (GE Healthcare), in an adapted method from Jiemtaweeboon et al. (1). Samples from 8 pregnant and 9 non-pregnant heifers were subjected to RNA extraction using the Direct-Zol RNA Miniprep kit (Zymo Research) according to the manufacturer's instructions. Three reference genes (GAPDH, PPIA and ACTB) were quantified by real-time polymerase chain reaction (RT-qPCR), and through the NormFinder software, the two genes with most stable expression (GAPDH and ACTB) were selected. The expression of the target genes (ISG15, OAS-1, MX1 and MX2) was normalized in relation to the two reference genes by the comparative Ct method (2). The abundance of transcripts was evaluated by analysis of variance (ANOVA) with repeated measures of time, considering the random effect of heifer and the fixed effects of group, day and group by day interaction using the PROC MIXED SAS software (Version 9.2; SAS Institute). For the ISG15 gene, a significant effect of time ($P<0.0001$) indicated an expression increase on D16 and D20, and a group effect ($P=0.04$) indicated a higher expression of this gene in pregnant heifers (1.44 ± 0.25 vs. 0.65 ± 0.06). Significant interaction of group by time ($P=0.002$) was also detected, as represented by a higher ISG15 expression in pregnant than non-pregnant heifers on D18 (1.60 ± 0.32 vs. 0.52 ± 0.11) and D20 (2.73 ± 0.86 vs. 0.39 ± 0.07). For OAS-1, a significant time effect ($P=0.01$) indicated higher expression of this gene from D18. A significant interaction of group by time ($P=0.04$) was also observed, and represented by a greater OAS-1 expression in pregnant heifers on D18 (1.77 ± 0.34 vs. 0.53 ± 0.10) and D20 (2.45 ± 0.73 vs. 0.47 ± 0.08). Although no group effect was observed, there was a trend ($P=0.06$) of greater expression of this gene in the group of pregnant heifers (1.29 ± 0.19 vs. 0.49 ± 0.03). For MX2, a time effect ($P=0.01$) reflected an increase of expression on D16, followed by a reduction on D18. A group effect ($P=0.002$) indicated higher MX2 expression in pregnant heifers along the days evaluated (0.57 ± 0.11 vs. 0.21 ± 0.03). Regarding MX1, no significant effect was detected on its expression. In conclusion, the presence of a viable conceptus can stimulate the expression of transcripts for ISG15, OAS-1 and MX2 in PMNs from D16 after TAI. ISG15 and OAS-1 are the most abundant ISGs in PMNs of pregnant heifers, especially on days 18 and 20 post-TAI, indicating that these genes are more suitable to be used as early markers of pregnancy and for development of an early pregnancy diagnosis method in cattle.

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Quantitative ultrasound assessment of the endometrium and myometrium during the physiological postpartum in ewes

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The aim of this study was to evaluate the numerical pixels of the uterine wall (endometrium and myometrium) through computadorized analysis during the uterine involution in the postpartum of healthy ewes. Twenty adult multiparous Santa Ines ewes were included in the study. Ultrasonography assessments were performed in B-mode, which was performed with the ACUSON S2000® (Siemens®, Munich, Germany) ultrasound system, equipped with convex multifrequential transducer (4C1®; 4–4.5 MHz; Siemens®, Munich, Germany), from the immediate postpartum moment (M0) and sequentially every 48 hours, during 30 days, totaling 16 experimental samples. For quantitative evaluation of the ultrasonographic characteristics, it was performed a computerized image analysis using a commercial software (Image ProPlus®). The mean numerical pixel values (NPVs), pixel heterogeneity (standard deviation of NPVs) and minimum and maximum pixel values were measured by selecting three circular regions of interest in the uterine wall (endometrium; myometrium). Statistical analyze was performed using software R® (R Foundation for Statistical Computing; Vienna, Austria). All data were analyzed to define normal residuals distribution (Shapiro– Wilk test) and homoscedastic variance (Barlett test). Actual or transformed measurements were compared between postpartum days by analysis of variance (ANOVA) in a randomized block design (animals), when differences were significant, polynomial contrast models (linear, quadratic and cubic) were tested trying to explain the sonographic parameters in the puerperium. The significance for all tests was set at 5%. The characteristics of the uterine wall did not presented significant variations in the NPVs, NPVmean, NPVmin and NPVmax, except for the endometrium NPVmean. Endometrium NPVmean, decreased gradually through the moments in postpartum uterine involution, with a remarkable descrease in the 12th day pp, and later stabilized. It was concluded that pixels numerical evaluation of uterine wall was applicable and allowed the identification of quantitative ultrasonographic characteristics. CEUA/Unesp/Protocol#12338-15.

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Sperm proteins that influence the bovine *in vitro* early embryo development

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Spermatozoa are characterized as highly differentiated and compartmentalized cells, unable to synthesize proteins. Nevertheless, it is believed that sperm proteins have a great influence on the cellular metabolism of the zygote and exert a regulatory role in early embryo development, mainly through proteins present in the sperm nuclear matrix. Therefore, it is clear the need for further study of the proteins present in the spermatozoon so that it is possible to clarify which proteins are present and how they act on the viability of the embryo and early embryo development. The objective of this study was to identify sperm proteins that in some way influence fertilization and early embryo development in cattle. Eight samples of frozen semen from different ejaculates from four bulls were used, two being highly fertile and two subfertile. All samples were used in routines of in vitro embryo production. The rate of blastocyst formation of all samples on the seventh day after the fertilization process was calculated, that is, the percentage of structures that initiated the cleavage and reached the morphologically normal blastocyst form was calculated. For the proteomic evaluation, the sperm heads were isolated by sonication and ultracentrifugation. The membranes of the sperm were degraded by baths in solution containing triton X100 and the chromatin was decondensed by buffer containing 1,4-dithiothreitol (DTT). Subsequently the material was treated with DNase and RNase. Finally, the samples passed through reduction, alkylation, enzymatic digestion with trypsin and clean up/desalting. A LTQ Orbitrap ELITE (Thermo-Finnigan) mass spectrometer coupled to a nanoflow chromatography system (LC-MS/MS) was used to obtain the total proteome of the sperm head. The Computational Proteomics Analysis System (CPAS) automatically processed the acquired data. To obtain a representative number of amount of each protein in each sample, standardization of assays was performed by transforming the number of peptides from each protein into a percentage from total peptides found in the sample. For the identification of sperm proteins that influenced the embryo development, the Pearson correlation test was performed between the amount of each protein found and the blastocyst rate. Those proteins that had a negative correlation coefficient and p-value ≤0.05 were considered to have a negative influence on early embryo development and those with a positive correlation coefficient and p-value ≤0.05 were considered to favor early embryo development. Seven hundred and sixty-six different proteins were identified in the spermatozoa from samples, eight of which had a significant negative correlation coefficient with the blastocyst rate and 14 presented a positive coefficient. 40S ribosomal protein S7 (r=-0,98, p=0,015), 60S ribosomal protein L12 (r=-0,97, p=0,035), DNA replication ATP-dependent helicase/nuclease DNA2 (r=-0,97, p=0,035), activated CDC42 kinase 1 (r=-0,97, p=0,035), ubiquinol-cytochrome-c reductase complex assembly factor 2 (r=-0,97, p=0,035), acetyl-CoA acetyltransferase (r=-0,96, p=0,037), heat shock protein beta-9 (r=-0,096, p=0,040) and limbin (r=-0,96, p=0,045) were the proteins with negative coefficient and in descending order of statistical significance. Spermatogenesis-associated protein 6 (r=0,99, p=0,0021), serine racemase (r=0,99, p=0,0066), 60S ribosomal protein L9 (r=0,99, p=0,0079), FAM71D protein (r=0,99, p=0,0091), plasma serine protease inhibitor (r=0,99, p=0,99, p=0,011), signal peptidase complex subunit 3 (r=0,99, p=0,013), 40S ribosomal protein S8 (r=0,98, p=0,016), T-complex protein 1 subunit gamma (r=0,98, p=0,017), cAMP-dependent protein kinase catalytic subunit alpha (r=0,98, p=0,028), acyl-protein thioesterase 1 (r=0,097, p=0,030), coiled-coil domain-containing protein 63 kDa (r=0,96, p=0,042), beta-nerve growth factor (r=0,96, p=0,043), succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial (r=0,95, p=0,045) and histone H2A.J (r=0,95, p=0,049) were the proteins with positive coefficient and in descending order of statistical significance. The presence of these proteins in large amount in the spermatozoa can directly interfere in the early embryo development or it can be indicative of some important sperm characteristic in this stage of the development. Further studies are needed to identify exactly how such proteins act.

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Stage-specific response to FSH source (pituitary vs. recombinant) during *in vitro* culture of isolated caprine preantral and early antral follicles

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The FSH has been widely used as a supplement for *in vitro* preantral follicle culture at different concentrations, associated to different substances, and from different sources (recombinant vs. non-recombinant, derived for example, from pituitary gland). However, studies comparing different sources of FSH under the same experimental conditions are scarce. Thus, the objective of this study was to compare three different sources of FSH (bovine recombinant - brFSH, human recombinant - hrFSH, and porcine pituitary - pFSH) during the *in vitro* culture of isolated caprine preantral and early antral follicles under the same experimental conditions. Isolated follicles were cultured for 18 days using the following treatments: basic culture medium (control); or control medium supplemented with 10, 50, and 100 mIU/mL of pFSH; or control medium with 50 mIU/mL of hrFSH; or control medium with 100 ng/mL of brFSH. At the end of the culture period, cumulus-oocyte complexes were recovered and subjected to *in vitro* maturation. The following endpoints were evaluated: follicle morphology, growth rate and antrum formation, oocyte viability, and meiotic stage. At the end of pre-antral and initial antral follicle cultures, treatments containing pFSH showed the lowest ($P < 0.05$) percentages of morphologically normal follicles, associated with a higher ($P < 0.05$) extrusion rate in these treatments. Furthermore, antrum formation did not increased from day 6 of culture, in all treatments. The diameter of preantral and early antral follicles increased progressively during *in vitro* culture regardless of the treatment ($P < 0.05$). The treatments containing pFSH at the concentrations of 10 and 100 mIU/mL decreased daily follicular growth rate in the antral follicle category, when compared to the control group, the same occurred for the category of preantral follicles when used 100 mIU/mL of pFSH ($P < 0.05$). Regarding the mean diameter of viable oocytes ≥ 110 µm, in the category of antral follicles,#the hrFSH treatment resulted in a greater diameter than brFSH, although they did not differ from the others treatments. For preantral follicles category, the FSH addition did not improve the diameter of fully developed oocytes ($P > 0.05$). The addition of brFSH in antral follicles resulted in the absence of metaphase II (MII) oocytes without difference from the control group, and the other treatments did not differ among themselves ($P > 0.05$). In the same follicular category, a percentage of efficiency of MII (% MII/meiotic resumption) was higher in the pFSH10 and hrFSH treatments comparing to the control group. For preantral follicles, the absence of MII occurred in the treatments containing 100 IU/mL of pFSH and hrFSH without difference from 10 IU/mL of pFSH10, and the other treatments did not differ among themselves ($P > 0.05$). In conclusion, during *in vitro* culture, the different follicular categories (preantral and early antral follicles) demonstrated different stage-specific response to different FSH sources. In addition, for a more desirable outcome of oocyte maturation of the early antral follicles, the use of hrFSH at 50 mIU/mL or pFSH at 10 mIU/mL is recommended. (CEUA-UECE/protocol#2422377/2016).

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Testicle development in a turtle species with temperature-dependent sex determination, *Podocnemis expansa* (Testudines: Podocnemididae)

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Podocnemis expansa known as the giant Amazon River turtle, is the largest freshwater turtle in South America and has its Temperature-dependent Sex Determination (TSD). Sex is determined during a thermosensitive period (TSP). This study aimed to characterize testicle development and differentiation in *P. expansa* in its natural environment. The study was conducted between September and December 2014, on a beach at the Center for Research and Preservation of Aquatic turtles, Uatumã river, Balbina, Amazonas (01º 54' 56.9" S, 059º 28' 18" W) (SISBIO/IBAMA 39472-4; CEUA/INPA 025/2013). Analyses by light microscopy and transmission electron microscopy were conducted throughout embryonic development, as well as determining the incubation temperature using a remote temperature sensor (Hobo data loggers, Onset™ Computer Corporation). The incubation period lasted from 58 to 64 days, with a mean nest incubation temperature of 30.3ºC. The TSP varied among the analysed nests, starting after the beginning of the second third of the incubation period and ending a little after the beginning of the last third. Gonadal development in *P. expansa* was grouped in three stages, primordial germ cells (PGCs), undifferentiated gonad establishment and gonadal differentiation. PGCs were visualized on the 5th day of incubation in the yolk sac endoderm, and on the following days migrating towards the ventromedial region of the presumptive mesonephros. On the 14th day the PGCs were established in the ventromedial region of the mesonephros and the undifferentiated gonad formation began, with two distinct regions being identified; the cortex formed by the PGCs, and the medullary region with the primitive sexual cords. The beginning of testicle differentiation occurred after the beginning of the thermosensitive period, approximately by the 35th day of incubation. Sexual cords begin to organize into tubular cords and later into the seminiferous tubules, and a thin basal lamina is observed externally supporting the cells of the seminiferous tubules. PGCs were identified by migrating and associating with the seminiferous tubules in formation. Sertoli cells are already identified in some seminiferous tubules; these cells present elongated and basal nuclei and are lighter when compared to the germ cells that present cubic nuclei. The gonadal differentiation stages of *P. expansa* in its natural environment were similar to other TSD turtles species evaluated in the laboratory. Therefore, these findings are important to support elaborating strategies for conservation TSD turtles.

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Vascular endometrial alterations after the transfer of equine embryonic fragments

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Early pregnancy in the equine has several unique features. Development of placental tissues begins only after near day 40 of gestation, therefore, the embryo development relies on histotrophic nutrition during the initial period, and the conceptus does an extensively migration in uterine lumen until day 17 when its became fixed. This initial mobility is necessary to signal the presence of the embryo at the time of maternal recognition of pregnancy. The mechanisms responsible for this recognition in equines are not fully understood. However, several studies in equines have observed changes in uterine hemodynamics between pregnant and non-pregnant mares. Vascular alterations of the arterial uterine perfusion occur after day 11 post ovulation and local uterine changes occur since the day 10 in presence of the embryo. The aim of this experiment was to evaluate vascular endometrial alterations after the transfer of equine embryonic fragments to non-pregnant mares. Ten Quarter Horse mares, with normal estrous cycles, ages ranged from 4 to 10 years, weighed 510±53 kg were used. Mares were kept in natural pastures with access to mineralized salt and water ad libitum. Mares were examined daily by transrectal palpation and ultrasonography in two consecutive cycles and ovulation was considered day 0 of cycle. In the first estrous cycle the mares (n=10) were monitored without any manipulation (Control Cycle). At the second cycle in the 5th day post ovulation embryonic fragments of a day 13 embryo recovered from another mare were transferred, and the mares (n=10) where monitored daily until day 7 post ovulation (Transfer Cycle). The analysis of hemodynamics was performed using an ultrasound equipment (SonoScape® model S8V) with transrectal linear probe in Power and Spectral Doppler mode set to 7.2 MHz, noise filter of 100Hz and 5.5 cm/s of flow detection. Images obtained were evaluated on Resistance Index (RI), Pulsatility Index (PI) and the Time-Averaged Maximum Velocity (TAMV). Images of Power Doppler mode were obtained placing transversely the transducer over uterus body and in each horn. The images were objectively evaluated by quantification of the colorized pixels and area using ImageJ v1.48 software (National Institutes of Health, USA). The data were analyzed by ANOVA and Kruskal Wallis test, with SAS 9.4 ® software. In the evaluation of the vascular perfusion of the uterine arterial, the variables RI, PI and TAMV showed no differences between treatments of the days 0 to 7 post ovulation ($P>0.05$). We observed that the colorized pixels of the blood flow of the endometrium were significantly higher on the day 6 ($P=0.03$). However, these alterations disappeared on 7th day, probably due to the limited amount of proteins, the denaturation of the proteins and/or the lack of the embryo itself or its motility. Embryonic fragments signalized changes in the endometrium vascularization and these alterations were similar to those observed in the endometrium of pregnant mares with live embryos at seven days of pregnancy.

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Assessment of body weight in neutering cats receiving estrogen replacement

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The effects of castration, currently essential, have been a matter of considerable interest in feline medicine, as it is an important risk factor for the development of obesity. Gonadal steroids influence appetite and body weight in mammals because they are important regulators of energy uptake and metabolism, a fact evidenced by the higher incidence of obesity after menopause in women, or in the case of dogs and cats, after castration. Estrogen replacement in rats, guinea pigs, and castrated rhesus monkeys decreased feed intake and body weight. The objective of the present study was to compare the weight gain among 12 healthy adult female cats, SRD, with a mean weight of three kg, divided into three groups: OSH group (cats submitted to ovariohysterectomy); group OSH + E2 (cats submitted to ovariohysterectomy with estrogenic replacement) and the SHAM group (cats submitted to non-castration celiotomy). After surgery, the animals received water and feed *ad libitum* and were kept in controlled environment (23°C with 12 light hours/day). For six months in the morning, they received 100 grams of feed in individual cages where they stayed until 6:00 p.m. Immediately after feeding, the animals of the OSH + E2 group received a subphysiological daily dose of 4 mcg of oral 17-β estradiol valerate for three months. In comparison to the mean values of weight gain between the OSH + E2 and OSH groups in relation to the SHAM group, ANOVA (software R) was performed and a statistically significant difference was observed between the groups by Tukey test. Among the OHS + E2 and OSH groups, there was no statistically significant difference ($p = 0.8439$), which led to one more quarter of treatment using a physiological oral dose of 12 mcg of 17-β estradiol valerate per animal every 24 hours. After this period, no statistical difference was observed between the groups according to the F test. These results may be related to the long duration of treatment, since for a short period of hormonal replacement it was possible to observe the decrease in food consumption, lipidemia and weight, but this effect disappeared as the treatment progressed. One of the possible mechanisms for this long-term response would be the fact that the hormonal action is largely controlled by the concentration of cellular receptors. In a self-regulatory feedback loop, estrogen induces a decline in both receptor protein and gene expression in mRNA, which limits hormone activity and ultimately the expected physiological action. Confirmation of these hypotheses requires further studies on the molecular mechanisms involved in the hormone-receptor binding, especially long-term treatment, as well as the desensitization of these receptors, making it possible to account for the physiological and metabolic responses to different estrogen replacement protocols. CEUA/UEL/Protocol#5609.2017.46.

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Assessment in equine embryo recipients about luminosity and ovarian activity in Patos/PB city

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Domestic mammals suffer direct influence by luminosity in the reproductive process. Animals from temperate regions develop circadian timing system to adapt to environment conditions, keeping inactive for period, characterized seasonal anestrous; horses breeding in tropical zones this phenomenon aren't evident. Recipients mares are a critical point in embryo transfer programs, therefore management wrong can cause failure of the technique to cause down results, being essential techniques of management of the estrous cycle. Believed that bad nutrition is the most factor on occurrence of anestrous in recipient mares of tropical regions. Through the exposed, the objective in this work was follow ovarian activity in equine embryo recipients, correlate the daily rate luminosity and anestrus in horses from Brazilian semi-arid region. The study was done in Patos city, Paraíba state**, (**latitude 7°01'13"S), was evaluated 10 mares, with breeding age, six to eight years old, were keep semi extensive breeding systems, received feed, mineral salt, native pasture and tifton 85 hay (*Cynodon spp*). The reproductive trac was evaluated by transrectal ultrasonography during the months of April to September. The ultrasound examination occurred at random intervals of 48 hours, for cyclic females and 24 hours for acyclic females in the months of June, July and August, until the gradual return of normal ovarian activities. The mean per cycle in cyclic mares was counted after ovulation and presence of corpus luteum, and on acyclic at the end of June, July and August. To compare the sizes of left and right ovarian follicles of cyclic and acyclic mares, ANOVA and Tukey's test were used. After analysis, three mares (R4, R7 and R10) showed ovarian inactivity in both the left and right ovary from June to August, totaling 30% of acyclic mares in the study.# The mean left and right ovarian follicles (LO/RO) in the cyclic recipients were: R1(31,8/19,4±3,0/3,1), R2 (24,1/30,6±6,6/7,3), R3 (22,3/29,4±7,8/4,5), R5 (24,7/24,2±9,5/4,1), R6 (18,4/20,8±0,8/6,1), R8 (23,9/25,1±4,5/8,1), R9 (25,0/23,7±9,8/3,5), with average total (24,3/24,7±3,4/2,0). In acyclics mares were: R4 (9,9/10,5±2,7/3,6), R7 (16,2/20,7±6,7/9/3), R10 (13,0/11,2±8,1/4,1), with average total (14,1/14,1±2,8/3,1). There was a difference ($p <0.05$) in the mean left and right follicle sizes between the cyclic and acyclic recipients. The females presented reduced ovarian follicles in the months of June (11h41min), July (11h43min) and August (11h50min), months of lower luminosity rate in the year 2016, indicating ovarian inactivity of animals R4, R7 and R10. In horses, for ovulations to occur, it is necessary that ovarian follicles exceed 35mm; the cyclic mares presented reduced follicles, without growth ideal for ovulation, which leads to believe in the influence of luminosity, even the animals receiving an optimal nutritional contribution. Thus, it is concluded that mares created in the tropical semi-arid region of Brazil, in the city of Patos-PB, present seasonality, regardless of nutritional support, with an impact on the availability of recipients for equine embryo transfer programs.

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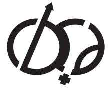

Effect of BMP-15 on morphology and activation of primordial follicles of collared peccaries (*Pecari tajacu*)

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Collared peccaries are wild animals that have a great ecological importance and economic potential. Thus, in order to increase the reproductive potential this species, biotechniques such as *in vitro* culture of preantral follicles have been studied. It is known that preantral folliculogenesis is regulated by autocrine and paracrine factors in an orchestrated mechanism that defines the course of activation and follicular growth or death. Among these factors is bone morphogenetic protein 15 (BMP-15), which acts on activation, survival and follicular development in different species. Nevertheless, the roles of Bone Morphogenetic Protein 15 (BMP-15) in the regulation of primordial follicle development in collared peccaries remains unknown. The aims of present study were to investigate the effects of BMP-15 on survival and activation of Collared peccaries primordial follicles using histological studies. To this end, fragments of collared peccaries (*Pecari tajacu*) ovarian cortex were cultured for 1 or 6 days, at 38.5 °C in an atmosphere containing 5% CO2, in TCM-199 (control medium) supplemented with different concentrations of recombinant human BMP-15 (rhBMP-15, R&D Systems, Minneapolis, MN, USA) (0, 1, 25 and 50 ng/mL). Fragments from non-cultured ovarian tissue as well as from those cultured for 1 or 6 days in a specific medium were processed for classical histology to evaluate follicular integrity and to calculate the percentages of normal follicles, and percentage of follicular activation. The results showed high percentage of viable follicles in fresh control (74 ± 3.2 %), but, after 6 days of culture, a significant reduction of follicular survival was observed in all the treatments ($P<0.05$). In addition, fragments cultured in media supplemented with BMP-15 (25 ng/mL) maintained highest percentage of viable follicles (56.6 ± 3.31 %), however, no statistical differences were observed among treatments ($P>0.05$). With regard to activation, fresh ovarian tissues predominantly contained primordial follicles, and after 1 or 6 days of culture, a significant decrease of primordial follicles and concomitant increase of growing follicles was verified in all the treatments, denoting activation follicular. However, higher concentrations of BMP-15 (25 and 50 ng/mL) increased percentage of growing follicles in relation to other treatments ($P<0.05$). In conclusion, BMP-15 did not influence follicular survival, but stimulated the *in vitro* activation of primordial follicles of collared peccaries.

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Estimating the estrus cycle phase in bitches using different diagnostic methods: preliminary results

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During the estrous cycle, the bitch undergoes behavioral and cytological changes that are physiological and coordinated by sex hormones. In this context, different methods are used to identify the phase of the estrous cycle in order to determine the ideal moment for mating or artificial insemination. However, the efficacy of the methods is varied. In this way, this work aimed to compare the efficacy of different diagnostic methods (behavioral examination, vaginal cytology, vaginal endoscopy) in the identification of the phase of the estrous cycle in bitches. For this purpose, adult, cyclic bitches (n = 10) from different breeds attended at the Metropolitan Veterinary Hospital of Caucaia, Ceará from April to June 2018 were used. The phases of the estrous cycle were evaluated through four methods: (T1) behavioral assessment, (T2) vaginal cytology, (T3) vaginal endoscopy, (CONTROL) serum progesterone assay. The behavioral response of the bitches to a male with a proven libido was evaluated. The correlation between receptivity and estrous cycle phases was measured as follows: (A) attractive + non-receptive females (proestrus), (B) attractive + receptive females (estrus), (C) unattractive + non-receptive females (metestrus/anestrous). The vaginal cytology was performed using the rapid Panoptic. A total of 200 cells was analyzed per slide. The percentage of cells for basal cells, intermediate cells, superficial (nucleated) cells, and cornified cells was analyzed. The presence of blood cells (erythrocytes or neutrophils) and bacteria in the smear were also analyzed. In the vaginal endoscopy, mucosal staining (pale or reddish), pleating (absent or present) and secretion (absent, present, serous secretion, mucous secretion or serosanguinolent secretion) were analyzed. Serum samples were submitted to chemiluminescence progesterone assay. Serum progesterone assay in bitches ranged from 0.1 to 1 ng/mL in proestrus, from 1 to 14 ng/mL in estrus and from 15 to 80 ng/mL in metestrus. In anestrus, the progesterone level declined from 80 to 0.1 ng/mL. The reliability of the methods was compared to the serum progesterone assay results. No correlation was observed between the behavioral evaluation and serum progesterone assay, so this method is unreliable to estimate the phase of the estrous cycle in bitches. The correlation between vaginal endoscopy and serum progesterone assay in the determination of estrus cycle phase in bitches was 40% (4/10). This method has proven to be the most effective, however, although it is a simple, easy to perform exam, the high cost of the device often limits its routine use. No correlation was observed between vaginal cytology and serum progesterone assay. Vaginal cytology proved ineffective in predicting the phase of the estrous cycle in bitches, probably because a single collection was performed and not a follow-up with serial collections through the estrous cycle. The vaginal endoscopy was considered the most effective method to estimate the phase of the estrous cycle in bitches.

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Morphometric and morphological characterization of ovaries, follicles and oocytes from Santa Inês ewes with *FecG^E* prolific mutation

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The mutation in the Growth Differentiation Factor-9 (*GDF-9*) gene, called Fecundity Gene-Embrapa (*FecG^E*), is responsible for an additive effect on ovulation rate (82%) and prolificacy (50%) in Santa Inês ewe. Despite this significant impact, there is still very little information on the reproductive morphology of *FecG^E* ewe that could elucidate the action mechanisms of this mutation. This work aimed to characterize the morphometry and morphology of the ovaries, follicles and oocytes of Santa Ines *FecG^E* ewes. Ewes genotyped for *FecG^E* (n = 63) as wild homozygous (WW), mutant heterozygous (EW) and, mutant homozygotes (EE), had their ovaries collected after being slaughtered, being they weighed, measured and, from fragments of their tissue, slides were prepared using hematoxylin and eosin histological protocol. The follicles visualized under an optical microscope were classified morphologically by the stage of development and as normal or degenerate. Through the captured images, follicles, oocytes, nuclei and nucleoli were measured based on the diameter (ZEN software 2011). Cumulus-Oocyte complexes (CCOs) were recovered from antral follicles and evaluated as their viability, using the fluorochromes Sybr Green and Propidium Iodide under epifluorescent microscopy. The data were analyzed considering the normality of its distribution, using the *Shapiro-Wilk* test. For the quantitative variables, we used ANOVA with *Tukey* post-test and, for qualitative, we used Chi-Square. The results were expressed by means ± standard errors considering the significance level of P <0.05. The ewes EE and EW presented lower right ovary weight than the WW (1.31 ± 0.14 g, 1.32 ± 0.11 g and 1.85 ± 0.13 g, respectively), with no difference between the genotypes for left ovary weight, and width and length of both ovaries. It was observed that the primordial and transitional follicles of the mutant genotypes are smaller than those of the wild (EE = 22.19 ± 0.65 µm and 25.40 ± 0.67 µm; EW = 20.73 ± 0.38 µm and 26.04 ± 0.7 µm and WW = 24.64 ± 0.54 µm and 29.67 ± 0.67 µm, respectively), and that the primer and antral follicles did not differ between the genotypes. Secondary follicles of the EW were larger in comparison to those of the WW (171.15 ± 7.66 µm vs. 132.50 ± 9.71 µm) and the amount of follicles at this stage in the EE was insufficient for the analysis. The oocytes contained in the primordial follicles were smaller for EW (16.03 ± 0.30 µm) compared to WW (17.46 ± 0.40 µm) and both did not differ from EE (16.94 ± 0.56 µm). Oocytes of the transitional follicles were smaller in the EE and EW (18.69 ± 0.56 µm and 19.06 ± 0.51 µm, respectively) when compared to the WW (21.70 ± 0.50 µm). There was no difference between the genotypes in the oocyte size of the primary and antral follicles. The oocytes of the secondary follicles from the EW (65.62 ± 3.38 µm) were larger than those from the WW (46.68 ± 2.52 µm) and the amount of oocytes from the EE was insufficient for analysis. The oocyte nuclei of the secondary follicles were larger in EW (27.60 ± 1.92 µm) than in WW (16.44 ± 1.10 µm), and were not visualized in EE. The nuclei of oocytes from antral follicles were not analyzed due to low frequency. There was no difference between genotypes regarding oocyte nucleolus size independent of the follicular development stage. Mutant genotypes showed a higher proportion of normal follicles in the primordial and transitional categories compared to the wild (EE = 86% and 82%, EW = 84% and 82% and WW = 76% and 74%, respectively). The viability of CCOs did not differ between genotypes. It is concluded that the *FecG^E* mutation does not interfere in the quality of mature oocytes, and that the mutant Santa Inês ewes have the right ovary with lower weight and generally present smaller follicles and oocytes as well as a higher proportion of normal follicles in the early stages of follicular development.

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Ovarian dynamics in Brazilian Warmblood mares

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The Brazilian Warmblood breed (BH) was developed in the 1970's aiming a national animal suitable for equestrian sports (1), through breeding BH stallions to BH, forming breeds (Thoroughbred, Holsteiner, Anglo Norman, Anglo Arab among others (2) or foundation mares (physique conforming to BH breed standard (1). Despite largely employed biotechniques in the breeding management of BH, there are few studies on ovarian dynamics in the breed (3, 4). This study aimed to characterize cycle length, follicular wave types, follicle numbers, mean daily growth rate (mDGR) of preovulatory follicle (POF), occurrence of double ovulation and cross sectional area and lifespan of corpus luteum (CL) in the BH mare. A total of 53 cycles in 25 cyclic non- lactating BH mares were used, mean age 12 years, mean height at withers 1.67m and mean weight 501kg, in a horse breeding farm located in Southern Brazil. The experiment included daily transrectal palpation and ultrasonic scanning during one interovulatory interval (IOI, interval between two ovulations associated with estrus (5) of each mare from October to December (reproductive season in Southern Hemisphere) during two years. Follicles anthrum were measured in mm over longitudinal and transverse axis; CL cross sectional area were measured in mm^2 using ultrasound's software. Measurements were recorded daily as to keep identity of structures. Results showed mean IOI of 22 days, with estrus lasting 5 days (during which one or more of the following were observed in presence of stallion: receptivity, rhythmic exposure of clitoris and urination); 18% of IOIs presented a secondary ovulatory wave with ovulation occurring in diestrus (three days after ovulation of the primary wave); major anovulatory waves (follicle $\geq$ 28 mm (5) undergoing athresia were observed in 12% of IOIs; minor waves (no dominant follicle) were observed in 30% of IOIs; ovulatory waves produced one (67% IOIs) or two (33% IOIs) ovulations; maximum diameter of POF was 47mm, reached 1.5 days before ovulation; mDGR of POF was 3.5 mm/day. Area of CL was 728 mm^2, reaching maximum (mean 1099 mm^2) on day 4 post ovulation. Lifespan of CL was 14.5 days. The larger diameter of POF (6, 7) was related to the daily growth rate and possibly reflects the larger ovary of the BH mare. The high rate of double ovulations may be due to the strong ancestry in forming breeds showing a high rate of double ovulation (2, 8, 9). In conclusion, the mDGR leads to a larger size of POF in BH mares compared to smaller mares and the high rate of double ovulation is similar to that found in forming breeds; length of IOI, estrus and CL are similar to mares of different body sizes. Knowledge of a larger diameter of POF in the BH mare may help in prediction of ovulation, optimizing biotechniques applied to the BH reproduction.

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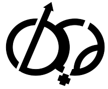

Peculiarity in estrus induction using cabergoline in Shitzu bitches

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Estrus induction in bitches has been used to improve reproductive efficiency, especially by breeders (1). However, since the mechanisms involved in the transition from anestrus to proestrus have not yet been fully elucidated in this species (2), the manipulation of the estrous cycle is challenging. Antiprolactin drugs have been shown to be promising and include dopamine agonists (cabergoline and bromocriptine) and serotonin antagonists (metergolin) (3). Cabergoline has high specificity to D2 dopaminergic receptors (4), and is considered an outstanding drugs due to its results and the fewer side effects associated to its use (5). The recommended dosage is 0.005mg/kg and may be administered for 10 to 25 days (6), however, a pilot study from our research group pointed out that female shitzus seemed to need higher dosages to reach the desired effect. Therefore, in order to confirm this observation, a total of 10 female shitzus, 2.4 ± 0.7 years of age, body weight of 4.7 ± 0.8 were included in this study. The bitches, who were in late anestrous as informed by the owners, were randomly divided into two groups: CONTROL (n = 5), when cabergoline were administrated at the dose recommended in the literature (0.005mg / kg), and CAB (n = 5), when cabergoline were administrated at higher dose (0.007mg / kg). The drug was orally ministrated, once a day, until the fifth day of the beginning of the blood vulvar discharge (the beginning of proestrus). Five days after the beginning of the treatment, vaginal smears were taken to evaluate vaginal cytology in order to identify the onset of proestrus, and then, serum progesterone was performed to monitor the estrous cycle and ovulation. None of the bitches in control group showed clinical and cytological signs compatible with proestrus, even after 20 to 25 days of continuous medication. However, in the CAB group, onset of proestrus was observed after 10 to 20 days of medication. The need of higher doses of this drug to stimulate the onset of proestrus in these bitches might be related to a higher amount of dopaminergic receptors in this breed, lower sensitivity to cabergoline or even higher secreted prolactin. Therefore, for estrus induction in shitzu bitches using cabergoline, we suggested a dose of 0.007mg/kg orally, once daily, for approximately 15 days. Thus, studies focused in the physiology and endocrinology of this breed are need in order to elucidate this finding.

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The effect of ACE inhibition on Doppler velocimetry parameters of the ovarian artery in superovulated goats

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The ovarian renin-angiotensin system is determinant in important reproductive processes, such as folliculogenesis, ovulation and formation of the corpus luteum. Previous studies also confirm the activity of angiotensin-converting enzyme (ACE) in several reproductive tissues such as uterus, oviduct, corpus luteum and blood vessels and that ACE inhibition has been shown to have positive effects during the pre- and post-ovulation period in rats due to a greater conversion of Angiotensin- (1-7) in this period. Angiotensin- (1-7) levels are higher during estrus and proestrus and have been correlated with an increase in the production of estradiol and progesterone. However, data on the use of an ACE inhibitor in goats and their effects on reproduction are incipient. The aim of this study was to evaluate the inhibition effect of angiotensin-converting enzyme (ACE), using enalapril maleate, on Doppler velocimetry parameters of the ovarian artery in superovulated goats. Anglo-Nubian cross-bred (n=9), non-lactating, pluriparous and cycling adults were selected from a farm herd and segregated into two groups: Control Group (n=4) and Enalapril Group (n=5). The ovarian status was synchronized in all females by the i.m. administration of 50 µg of a prostaglandin F2α analogue (Prolise®; ARSA S.R.L., Buenos Aires, Argentina) (Day 0) and the insertion of an intravaginal dispositive (CIDR®, InterAg®, Hamilton,New Zealand) 48 h later (Day 2). The follicular development was stimulated by the i.m. administration, at the same time, of a single dose of FSH (60 mg of Folltropin®; Bioniche, Belleville, Ontario, Canada) plus a single dose of eCG (300 UI, Novormon 5000®; Syntex, Argentina) 48 h after (Day 4) the CIDR insertion. Enalapril group received enalapril maleate (Enalapril, Lab Teuto Ltda) dissolved in saline (3 mL) subcutaneously at the following doses: 0.2 mg/kg/day for 3 days, 0.3 mg/kg/day for 3 days and 0.4 mg/kg daily from the insertion of the CIDR (Day 2). The control group received the corresponding volume of 0.9% saline solution. Goats were subjected to transrectal ovarian ultrasonography using a scanner (Mindray® Z5 VET, Shenzhen, China) equipped with a 7.5-MHz linear-array transducer. Doppler ultrasonography was performed on Day 4 (before follicular stimulation) and Day 5 (24 h after follicular stimulation). Ovarian blood flow was determined in the left and right ovarian arteries. The values of resistance index, end diastolic velocity, peak systolic velocity, and systolic/diastolic ratio were calculated by the spectral Doppler waveform and estimated by the mean value of the measurements from both ovaries. Data were submitted to analysis of variance (ANOVA) using the GLM procedures. Group (Control and Enalapril) was the main effect tested. No statistical differences were found between the groups in any of the evaluated parameters. Administration of an ACE inhibitor has been shown to be efficient in some species in modulating production of hormones (goats) and ovulation rate (rats). The manipulation of the ovarian renin-angiotensin system seems to have a potential to assist in the improvement of reproductive biotechnologies, however, new studies should be conducted to assess the feasibility of use.

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Morphological and histological evaluation of male reproductive tract of sharks *Squalus megalops*

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The *Squalus megalops* shark is one of the many species that inhabit the Brazilian coast, being a major target of the accidental fishing, due to its natural habitat to stay close to the shore. This fact, associated with low reproductive and late sexual maturation, makes studies about the reproduction of this species extremely important for conservation. Thus, this study aimed to morphologically describe the testicles and to classify the reproductive stage of sharks of the species *Squalus megalops* captured on the Brazilian Northeast coast. For this purpose, 62 males were captured, characterized morphometrically and had their reproductive stages classified according to ICES (2013). The animals were eviscerated and the testicles were weighed and measured for length and width, and the clasper was evaluated for calcification level. Fragments of the gonads were obtained, fixed, impregnated in Paraplast, sectioned for the preparation of the slides, stained with hematoxylin-eosin and analyzed microscopically. Of the 62 males captured, 5 were considered immature, 22 were in development, 21 were capable of reproduction and 14 were reproductively active. Active males and reproductive regression were not observed. In relation to the immature animals, the testicles were thin, with weights varying between 0.02 and 1.41 g, and total length (TL) between 1.9 cm and 4.3 cm. The clasper was very flexible; typical of animals in the stage of immaturity. On the other hand, from the histological point of view, these individuals presented spermatogonia and Sertoli cells, with evident germinative zone. In developing males, the testes were structured, testicular TL values ranged from 3.4 cm to 5.5 cm and the width was between 3.3 cm and 5.8 cm. Their claspers were partially calcified and had a length varying from 4.1 cm to 6.7 cm. Histologically, cysts with primary, secondary spermatocytes and more present spermatids were identified. The males capable of reproducing were those with well-developed, well-irrigated testicles, fully calcified classics and passing the pelvic fin. These animals had TL of the gonads between 4 cm and 12.5 cm. At this stage, the germinal zone was well evidenced on one side of the testicular wall and on the other side a larger number of cysts with the spermatozoa were perceptible. The spermatozoa were with the head turned towards the periphery of the cyst and the tail for the lumen. It is concluded that the studies on the maturational stages are of paramount importance to determine how the spermatogenic activity and the reproductive habits of the sharks of the species *Squalus megalops* occur.

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Morphological and histological evaluation of the female reproductive tract of sharks *Squalus megalops*

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In the Brazilian coast approximately 136 species of sharks are found. Of these, the species *Squalus megalops* stands out, being one of the most captured bycatch. However, this species presents low reproductive potential, late sexual maturation and complex ovarian cycle. It is necessary to understand the reproductive aspects as an important ecological tool for the conservation of sharks the objective of this study was to describe the morphology and histology of the ovaries and uterus of *Squalus megalops*, captured on the Brazilian Northeast coast. For this purpose, sharks of the species Squalus megalops were obtained through fishermen, in the northeast coast of Brazil. The captured animals had their total length (TL) and precaudal lenght (PCL) measured, as well as their weight. Subsequently, evisceration and recovery of the ovaries and uterus were performed, which were evaluated for length, width and weight. Fragments of these organs were fixed for 48 hours, cleaved and refixed in 24 hours, with subsequent preservation in 70% alcohol. This material was submitted to dehydration, diafinization, impregnation and inclusion in Paraplast, sectioned at 5 µm for the preparation of the slides and stained with hematoxylin-eosin for the histo-morphological analysis. At the end of the macroscopic analysis, of the total of 61 females, 11 were considered immature, 25 under development, 15 able to reproduce, 12 at the beginning of the gestational period and 10 at the middle gestation period, based on ICES (2013). By histological study the ovaries were classified as immature, developing and mature. Immature ovaries were considered those coated by simple epithelial tissue, ranging from cubic to columnar, with primordial and primary follicles. In turn, we classified as developing and mature ovaries those who had simple columnar ovarian epithelium and, near the ovarian follicles, the stratified epithelium. In the vitellogenic follicles were observed cell layer and zona pellucida glycoprotein. In relation to the uteri, three histological layers were identified, classified from the exterior to the interior as serous, with flat simple epithelium and loose connective tissue; muscular, composed of smooth muscle tissue; and mucosa, with a loose connective tissue blade and without the presence of filaments. It is concluded that histo-morphological and morphometric studies contribute to the knowledge about the formation of the reproductive tract and maturational stages of the female sharks Squalus megalops, a fact that may be crucial for the conservation of the species.

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The role of Anti-müllerian hormone signaling in medaka sex differentiation

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Anti-müllerian hormone **(**Amh) signaling is an important effector in the decision whether the bipotential gonad anlage will become a testis or ovary. Studies on the medaka mutant *hotei*, where the *amh* type II receptor (*amhrII*) is mutated, showed an over-proliferation of germ cells and a male-to-female sex reversal. Our hypothesis is that Amh signaling regulates sex differentiation in medaka. We produced a knockout line for the *amh* gene of medaka using CRISPR/Cas9 technology. To increase the probability of successful targeting and full knockout, three guide RNAs were designed, in exons 3, 6 and the 3'UTR. Mutants for *amh* was screened by PCR amplifying the mutated target site. To detect and confirm positive mutants, primers were designed which neighbor the target regions to perform the screening and sequencing to check for mutations. To identify sex reversed fish, genotyping of adult fish was done from fin clip DNA with PCR for the *dmy/dmrt1bY* gene. For determining the phenotypic sex we compared secondary sex characters - like dorsal and anal fins of mutants with the phenotype of wild-type fish. To confirm the heritability of mutation as well as to characterize and isolate the mutation, establish stable mutant lines positive G0 fish were crossed to wild-type medaka. Analysis of injected embryos revealed 96,6% efficiency where all three targets were mutated, thereby causing gene disruption. Screening results showed that 82,8% were *amh*+/- and 13,8% *amh*-/- while 3,4% were wild-type. Interestingly, only *amh*-/- underwent sex reversal into male-to-female. All *amh*-/- mutants became sexually mature at 3 months after hatching and showed fertility like wild-type medaka. Crossing of mutants F0 to wild-type was done. In the F1 fish, we retreived one type of mutation in heterozygous animals, one mutation for targets located in exon 3 and one for the 3' UTR region. Interestingly, this mutant has two remarkable phenotypic abnormalities: (1) male-to female sex reversal and (2) gonadal hypertrophy. A to male-to female sex reversal and gonadal hypertrophy was also seen in the *hotei* mutant where *amhrII* is mutated. However, in *Danio rerio*, the mutation of the *amh* gene lead to gonadal hypertrophy like occurs in medaka. In conclusion, these data confirm a crucial role of Amh signaling during sex determination and differentiation in medaka.

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Angiogenic factors involved in luteolysis of bitches

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The domestic dog has a long luteal phase, and the plasma concentrations of progesterone do not differ statistically between pregnant and non-pregnant bitches; however, the duration of cyclic diestrous is apparently longer (70/80 days) when compared to gestational diestrous (63 days) (1). In order to validate the gene expression profile of angiogenic factors present in the cyclic and gestational diestrous, the RNAseq technique was performed and the main angiogenic genes differentially expressed were selected for qRT-PCR validation. For that, canine CLs were collected subsequently ovariohysterectomy at different timepoints (10, 20, 40 and 60 days after the preovulatory LH peak) of the cyclic (n = 20) and gestational diestrus (n = 20), being that each subgroup was formed by 5 females. The monitoring of estrous cycle was conducted using vaginal cytology and serum progesterone (P4) dosages, and when the percentage of superficial cells was equal or greater than 90% in vaginal cytology and progesterone concentration was 4 ng /mL was considered day 2 (D2) after LH pre ovulatory surge or ovulation day (2). This samples obtained were stored in cryotubes at -80°C and RNA extraction was accomplished using Trizol protocol. The cDNA was synthesized using High Capacity cDNA archive kit (Life Technologies, Carlsbad, CA, USA) and RPL13 gene was used as target gene. The VEGFA, Endothelin, IGFBP5, THBS2, TGFBI and IGF2 genes were selected for validation, because presented logFC>1,5 and FC <0.05 in RNAseq analysis. The gene expression evaluation (qRT-PCR), was analyzed through statistical program GraphPadPrism 5 (GraphPad Software Inc., San Diego, California, USA) and Student t-test was applied for analysis the data obtained. The significant differences were considered when p <0.05. It was confirmed both upregulation of angiogenesis regulators (VEGFA and IGFBP5) and vasoconstrictor agents (Endothelin) at 40 days of gestation, as well as genes related to inhibition of angiogenesis (THBS2 and TGFBI) at 60 days of gestation. The results suggest that these genes are associated with the regression and lysis of CLs in pregnant bitches, indicating that luteolysis may be directly related to the inhibition of the blood supply of CLs, among other factors.

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Characterization of gene expression associated with insulin and glucocorticoid signaling pathways in the cyclic canine corpus luteum

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Animal reproduction may be impaired by high glucocorticoids (GCs) concentration acting in the organism. Besides physiological changes, GCs can affect progesterone (P4) production through molecular pathways that are still not well elucidated. In skeletal muscle and white adipose tissue GCs decrease glucose uptake and utilization by antagonizing insulin response and hampering GLUT4 translocation from cytoplasm to cell membrane. Moreover in skeletal muscle, GCs cause insulin resistance by decreasing transcription of IRS-1. In female dogs, insulin regulates luteal cells' glucose uptake, participating in the maintenance and functionality of the corpus luteum (CL); therefore if cortisol interferes in the insulin signaling pathway it may also harm P4 production. In bovines, at the early stage of luteal development, cortisol is responsible for increasing the synthesis of P4. On the other hand at the final stage of CL development cortisol reduces P4 production and accelerates luteal regression. Glucocorticoid receptor (GR) was already described in bovine ovaries but there are no studies evidencing GR gene expression or expression of other genes related to this signaling pathway in bitches. The aims of this study were to characterize the expression of genes related to GC and insulin signaling pathways in CLs of healthy bitches and to identify differential expression of these genes during early (day 20) and late (day 60) diestrus phases which correspond, respectively to luteal final growth and regression phases. For this research corpora lutea (CLs) were collected from healthy cross-bred female dogs via ovariohysterectomy (OSH) on specific days of diestrus (day 10, 20, 30, 40, 50 and 60). The CLs were utilized to create a complementary (c)DNA library and the next generation sequencing following the TruSeq RNA Sample Preparation Guide's protocol, described by Illumina. The reads were mapped against the reference genome (Canis_familiaris.CanFam3.1.75.dna.toplevel.fa) using Hisat software ('Our Galaxy' – ETH-Zurich), which generated files in BAM format (Binary Alignment/Map). Statistical analysis of this data was performed through Cuffdiff program (pipeline Cufflinks) and the relative amount of transcripts was measured in FPKM (Fragments per kilobase of exon per million fragments mapped). This work identified the expression of the following genes (codifiers of the in bracket described proteins) in canine CL: NR3C1 (GR), HSD11B1 (11-beta-hydroxysteroid-dehydrogenase 1), HSD11B2 (11-beta-hydroxysteroid-dehydrogenase 2), SLC2A4 (GLUT4), INSR (insulin receptor), IRS1 (insulin receptor substrate 1). No difference on the expression of NR3C1 ($p>0,3$; FDR>0,6) and SLC2A4 ($p>0,02$; FDR>0,1) was observed during diestrus. Nevertheless 11HSD1 ($p=0,0001$; FDR=0,0021), 11HSD2 ($p=0,013$; FDR=0,06), INSR ($p=0,002$; FDR=0,01) and IRS1 ($p=0,0006$; FDR= 0,006) presented differential gene expression in the same period. Comparing day 20 to 60 of diestrus HSD11B1, HSD11B2 and INSR showed a higher expression on day 20 (11HSD1: $\pm$ 14528 reads; 11HSD2: $\pm$ 1239 reads; INSR: $\pm$ 583 reads) compared to day 60 (11HSD1: $\pm$ 5327 reads; 11HSD2: $\pm$ 715 reads; INSR: $\pm$ 265 reads). Additionally, IRS1 showed a higher expression on day 60 ($\pm$ 1495 reads) than on day 20 ($\pm$ 775 reads). These data indicate that canine corpus luteum may also be affected by circulating cortisol, as shown already in cows, since the NR3C1 is expressed during throughout the diestrus phase and the genes codifiers of the enzymes 11βHSD1 and 11βHSD2, higher expressed in the beginning of diestrus, have a key role in the intracellular cortisol concentration. Higher expression of INSR during early luteal phase matches with the CL's growth period when there is necessity on increasing the glucose uptake. Although SLC2A4, gene responsible for the transcription of GLUT4, did not show differential expression the number of reads, qPCR data showed decrease from day 20 to day 60 of diestrus, following the INSR pattern. Moreover, the increase of IRS1 expression indicates that the bitches were not under influence of high cortisol concentration, or that cortisol concentrations were not enough to decrease IRS1 expression. These data suggest that canine CL is influenced by cortisol, as well as already demonstrated to insulin, similarly to other tissues in which cortisol may interfere in the insulin signaling pathway.

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Correlations of orbital area temperature, scrotum temperature and semen quality in young Bonsmara bulls

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The andrological evaluation of bulls is essential for selection of animals for reproduction purposes. The fertility of the male is related to the capacity of mount and the adequate quality of the semen. The testicular thermoregulation is the mechanism responsible for maintaining the temperature of the gonads below the rectal temperature to produce gametes of good quality. The temperature of the testicles can be measured by infrared thermography in a non-invasive way and aggregates information that can be analyzed along with semen characteristics. The objective was to study the correlation between infrared temperatures of the orbital area and scrotum, with the characteristics of the semen in young Bonsmara bulls, raised extensively. Were realized Infrared Digital Thermography of the scrotum and orbital area (TO) with thermal imager (FLIR E-40) using emissivity 0.98, located 1 meter from the examined areas. The scrotal thermograms were analyzed using software (Flir Tools®) for scrotal surface temperatures, right and left (D and E), neck of the scrotum (T1), thirds: proximal (T2), middle (T3) and distal (T4) of the testicles; and tails of the epididymis (T5) and were classified as to their quality as: great (1), medium (2) and poor (3). After that, the semen was collected by electroejaculation (Autojac, NEOVET) and quantitative and qualitative analysis of the ejaculates were performed by optical microscopy following the recommendations of the CBRA and the animals were classified as apt and inapt for reproduction. For statistical analysis the data were submitted to analysis of variance, Tukey's test and Pearson's correlation to 5%. The studied variables revealed the following averages: T1E 36.31 ± 1.05°C; T2E 35.08 ± 0.90°C; T3E 33.67 ± 0.83°C; T4E 32.38 ± 1.02°C; T5E 30.84 ± 1.12°C; T1D 36.23 ± 1.05°C; T2D 35.03 ± 0.95°C; T3D 33.56 ± 0.98°C; T4D 32.55 ± 1.04°C; T5D 30.48 ± 1.10°C; TO 37.92 ± 0.89°C; thermography image quality 1.77 ± 0.82; testis length 122.35 ± 8.76mm; testicular height 68.40 ± 7.4 mm; testicular width 67.42 ± 6.62 mm; scrotal circumference 35.67 ± 2.23cm; volume of the ejaculate 6.10 ± 2.98 mL; mass activity 2.60 ± 1.40; sperm motility 62.33 ± 23.73; sperm vitality 3.20 ± 1.06; major sperm defects 15.77 ± 12.36%; minor sperm defects 10.87 ± 6.31%; total sperm defects 26.65 ± 14.56%. Significant differences were observed comparing the mean values in relation to the image quality for the aspects T3E (P = 0.043), T4D (P = 0.047), sperm motility (P <0.001), sperm vitality (P = 0.032) and classification of animals as apt or inapt for reproduction (P = 0.001). Correlations (P <0.05) were observed between TO x T3D and T3E (r = 0.370 and 0.536, respectively). There was also a correlation between T1E x sperm vitality, major sperm defects, total sperm defects and evaluation as apt / inapt for reproduction (r = -0.457, 0.430, 0.445 and 0.486, respectively). The correlation also existed between T1D and T2D x major sperm defects, total sperm defects and apt / inapt evaluation (r = 0.442, 0.405, 0.397, 0.438, 0.395, 0.390, respectively). There was a correlation between thermography image quality x T3E, T3D and T4D (r = 0.437, 0.337 and 0.438, respectively). And also, correlation between image quality x gross motility, sperm motility, sperm vitality, total sperm defects and for bull evaluation as apt or inapt for reproduction (r = -0.415, -0.664, -0.619, 0.412 and 0.651 respectively). It was concluded that in young Bonsmara bulls, there is a relation between the temperatures of the different regions of the scrotum and the quality of the thermographic image with the seminal quality. In this way, the use of scrotal thermography as an auxiliary exam is recommended for andrological exam in Bonsmara bulls.

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Evaluation of post-thawing sperm quality in Chilean purebred stallions, according to age and seasonality

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Seasonality and age have been related with alterations in the post-thaw semen quality in most races stallions, mainly in sperm motility traits. Thus, yearly changes in photoperiod and hormone profile, as well as morphofunctional changes in spermatozoa of aged stallions lead to decrease efficiency of the semen criopreservation (1, 2, 3). Chilean purebred is a rustic and adaptable horse race presenting the oldest genealogy record in South America, and constitutes an interesting reproductive industry. Despite of it social, economic and cultural importance, there is not systematic studies characterizing the breed semen freezability according to age and seasonality. This is an important information because aged stallions with good genetic value are frequently used for artificial insemination around the year. The aim of the present study is to determine if age and seasonality affect sperm motility in post-thawing semen from Chilean purebred stallions. Standardized semen samples (DSO condition) were collected from ten fertile Chilean purebred stallions, 5 to 24 years old, kept in Santiago of Chile (Latitude: 33°27′S. Longitude: 70°38′ W). Two groups were conformed: young group (n=5) (5 to 12 years old) and aged group (n=5) (13 to 24 years old). Three semen samples from each stallion were obtained by means artificial vagina (Missouri model) at summer (December) and winter (July). Seminal samples were frozen with conventional method (1) using Botusemen extender and Botucrio cryoprotector (Botupharma, Botucatu, Brazil). Semen samples at a concentration of 400 x 10^6 sperm/mL were packaged into 0.5 mL straws and stored in liquid nitrogen. After post-thawign (60 seconds at 37°C) semen samples were analyzed in a CASA system (ISAS-V®, Proiser, Valencia, Spain), assessing progressive motile spermatozoa (%), mean velocity (μm/seg), and amplitude of lateral head displacement (μm) (5). Results was grouped according to age and seasonality and compared by ANOVA and Bonferroni tests (Stata 8.0 software). Statistical differences were considered when $P<0.05$. Results are presented respectively as young summer, young winter, aged summer and aged winter stallions. Different superscript letters denote statistical difference in each seminal trait. Progressive motile spermatozoa: 34.8 ± 4.7^{a}, 25.5 ± 3.8^{bc}, 28.6 ± 9.4^{acd}, $24.7 \pm 9,3^{bc}$%; Mean velocity: 45.9 ± 5.1^{a}, 38.1 ± 2.6^{bc}; 40.7 ± 6.0^{ab}; 41.1 ± 12.1^{ab} μm/seg; Amplitude of lateral head displacement: 2.7 ± 0.2^{a}, 2.3 ± 0.4^{a}, 2.7 ± 0.6^{a}, 2.6 ± 0.6^{a} μm. Only the seasonality was a variation factor, showing significantly lower values for sperm progressive motility and sperm mean velocity in the young group stallions during winter. Aged stallions showed no significant differences between seasons, but tend to show less values in both traits compared with the young group. Amplitude of lateral head displacement was not affected for age or seasonality. Sperm progressive motility and velocity are important fertility parameters (1), and in contrast with general knowledge these traits were affected by seasonality only in young stallion, so age and seasonality could be considered during evaluation and selection of Chilean purebred stallions for reproductive programs.

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Feline semen: comparative analysis of in vitro characteristics of urethral and epidydimal spermatozoa

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The feline family is composed of 37 species and the domestic cat is the only specie not threatened by extinction. The maintenance of biodiversity has intrinsic value for the genetic preservation of valuable domestic felines and extrinsic value in the maintaining of genetic lines and biodiversity of wild felids. Due to the physiological similarities, the domestic cat becomes an important experimental model for the development of reproductive biotechnologies (1; 2). The objective of this work was to evaluate the physical and morphological characteristics of spermatozoa via urethral catheretization and epididymal slicing, correlating motility with testicular surface temperature; evaluating plasma membrane integrity by the eosin-nigrosin and hypoosmotic techniques, also analyzing head morphometry and chromatin condensation of sperm cells by the toluidine blue and Diff quick techniques.Fifteen male cats were selected for semen collection by urethral catheterization and orchiectomy. After bilateral orchiectomy, the epididymis tails were dissected and semen collected for sperm analyzes (progressive motility, concentration and sperm morphology). Before the orchiectomy, the testis morphometry (length, width, thickness and mass in grams) and measurement of scrotal and ocular temperature through infrared thermography and rectal temperature by digital thermometer were performed. The eosin-nigrosin method stained spermatozoa with membrane lesions in pink. On the other hand, the hypoosmotic method classified biochemically inactive spermatozoa. The Diff Quick stain were used to assess the presence of normal pale blue spermatozoa. In the toluidine blue technique the images were obtained through the Olympus BX61 microscope, scanned and analyzed in a specific program. No significant correlation was observed between sperm motillity and rectal temperature and between sperm motillity and testicular surface temperature ($p = 0.284$). There was a significant correlation between rectal temperature, measured by digital thermometer and infrared ocular thermography ($p = 0.004$). Significant difference was observed between the average percentage of total defects ($p = 0.002$) and minor defects ($p = 0.0004$) from spermatozoa of fresh semen and the epididymis, however not with major defects ($p = 0.469$). Spermatozoa from fresh semen showed higher percentage of plasma membrane integrity by the eosin-nigrosin technique ($p = 0.005$). However, epidymal spermatozoa showed higher percentage of chromatin condensation using the toluidine blue ($p = 00.2$). There was weak to moderate correlation between the eosin-nigrosin and hypoosmotic tests. The concentration of spermatozoa in the semen collected by urethral catheterization was higher when compared to other techniques described in literature, probably due to minimal presence of seminal plasma in the ejaculate (3). The prevalence of tail abnormalities (22.5%) as reported in other studies (3; 4) probably occurs due to an osmotic influence by the dilution medium (3). A previous research observed that spermatozoa evaluated by the eosin-nigrosin technique showed higher viability in fresh semen (5) corroborating with our results. For the hypoosmotic test of epididymal spermatozoa, no specific osmolarity was developed, therefore no significant difference was observed between the two collection methods. The Diff Quick stain showed to be a valid technique for the evaluation of DNA from feline urethral and epididymal spermatozoa. The toluidine blue technique revealed that teratospermia influenced DNA stability resulting in high levels of chromatin condensation defects in fresh semen. No significant difference between the sperm head area in feline spermatozoa from urethral and epididymal semen was observed, since chromatin condensation already had finished in the epidydimal tail. The collection techniques for sperm recovery showed to be feasible, resulting in gametes of quality.

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Gene expression of MMP1 and MMP2 in the reproductive tract of stallion

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Reproductive physiology of the stallion is a complex mechanism involving hormones, cytokines, growth factors, proteases, among other regulatory mechanisms. Matrix metalloproteinases (MMPs) constitute a family of proteases characterized by playing key roles in almost all physiological processes including, cell signaling, cell migration, cell remodeling and cell-cell interactions. The objective of the study was to verify the gene expression of MMP1 and MMP2 in different regions of the stallion's reproductive tract. The complete reproductive tracts of six stallions were collected, without distinction of breed or age, with an unknown reproductive history in a commercial slaughterhouse. Tissue fragments were collected from the testis; the head, body and tail of the epididymis; of the accessory sex glands: prostate, seminal vesicle and ampulla of deferent duct. Tissue samples were stored under refrigeration in RNAlater® (Life Technologies) during transport to the laboratory, and after were frozen in a freezer at -80°C. The selected tissues were destined to the quantitative-polymerase chain reaction (qPCR) technique. Amplification was performed at 95°C for 2 minutes, followed by 40 denaturation cycles at 95°C for 3 seconds, annealing at 60°C for 30 seconds, and elongation at 60°C for 30 seconds. Relative quantification was performed, and the mRNA levels of the target genes were normalized against β-Actina (β-Actin) mRNA levels. The comparative threshold cycle (CT) ($2^{-\Delta\Delta CT}$) method was used for calculating relative mRNA expression. The gene expression of MMP1 and MMP2 was verified in all tissues evaluated. Statistical differences were observed in the gene expression of MMP1 ($p = 0.014$) in relation to the different regions of the reproductive tract of the stallion. The body and tail of the epididymis presented greater MMP1 gene expression than the testis, ampulla, prostate and seminal vesicle. Differences were observed in the MMP2 gene expression ($p = 0.012$). The testis, body and tail of the epididymis presented greater MMP2 gene expression than the prostate and the head of the epididymis. The testis and the body of the epididymis also presented greater gene expression in relation to the seminal vesicle. The body of the epididymis involves sperm maturation, and the tail stores the spermatozoa and the testis coordinates cell differentiation and sperm production. Our study demonstrated that the body and tail of the epididymis presented greater gene expression of the MMP1 than other groups. The testis, body and tail of the epididymis presented greater MMP2 gene expression than other groups, suggesting that these genes are important in the cell remodeling, cell-cell interactions and cell signaling during the development, maturation and stores of spermatozoa. In summary, MMP1 and MMP2 are probably related to spermatogenesis, transit and sperm maturation, as well as related to the accessory sex glands of the stallion, being important for the reproductive physiology of the stallion.

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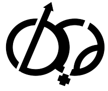

Morphometric and ultrastructural characterization of collared peccaries'sperm through scanning and transmission electron microscopy

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The collared peccary(*Pecaritajacu*Linnaeus, 1758) is awild pig of great ecological relevance.In this sense, the knowledge on its reproductive biology is determinant for the development of effective strategies for its multiplication and conservation. For the biobank formation, the knowledge about details of its sperm is required. This study aims to describe, for the first time, the morphometry and ultrastructure of peccaries' sperm at using scanning and transmission electron microscopy. For that, three adult males were submitted to electroejaculation and the ejaculates were immediately evaluated for motility, membrane integrity, membrane functionality, chromatin integrity and morphology by means of light microscopy.A sample of semen from each animal was used to form a pool, the spermatozoa were fixed in Karnovsky processed for scanning electron microscopy (SEM) andtransmission (TEM) analyzes.The spermcharacteristics of the fresh samples were within the expected pattern for the specieswith a mean volume of 2.5 ± 0.8 mL, sperm concentration of 196.7 ± 29.6 × 10^6 sperm / mL, 72.3 ± 9.9% motility, 82.7 ± 1.9% sperm with intact membranes, 91.0 ± 2.5% functional membranes, and 99.9 ± 0.1% chromatin integrity. The morphology evaluated by light microscopy showed 84.0 ± 2.5 of normal sperm, being most of the defectsthe strongly coiled tail (6.0 ± 3.0%). Regarding the morphometry, SEM revealed that peccaries' sperm presents a flattened head in a paddle format, measuring 6.07 ± 0,10µm in length and 3.84 ± 0,13. µm in width, with a vastus acrosome (4.30 ± 0,18µm)presenting the distended border, the marginal thickening in the anterior region and a clearly markedpost-acrosomal region. Normal tails measure 38.11 µm, being formed by an extensive midpiece with 15.52 µm in length, measurements like those of swine sperm whose species is the closest phylogenetically related.By means of TEM, the presence of a homogeneous nucleus was noted in the head of some fresh sperm.The presence of some acrosomal vesicles indicating a probable initiation of the acrosome reaction was also observed. This fact may have occurred by the processing for electron microscopy since the spermatozoa of peccaries is quite sensitive to the centrifugation process. In the main and mid pieces of the tail, the presence of an intact axoneme with conventional structure was identified, as well as the presence of nine outer dense fibers with drop formats around the axoneme, and an external fibrous sheath. The mitochondria were juxtaposed, thus forming the mitochondrial sheath, which was surrounded by the plasma membrane with some undulations.In conclusion, we clearly characterize, for the first time, the ultrastructure of collared peccaries' sperm.Such information maybe auxiliary to the development of assisted reproductive techniques for peccaries' multiplication and conservation.

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Study of the relationship of infrared thermograms profiles of the scrotum with the quality of fresh and frozen semen in Nelore and Girolando bulls

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The selection of bulls through the andrological examination is determinant for the improvement of the animal herd, being the influence of the genetic composition of the animals of great utility for the insemination centers. It is known that bulls of zebuine origin present better adaptability to high temperatures in relation to animals of taurine origin, being able to influence in the temperature of the scrotum and this way in seminal quality. The objective of this study was to study the relation of infrared thermograms profiles of the scrotum and the quality of the fresh and frozen semen in Nelore and Girolando bulls kept at the Artificial Insemination Center. Four bulls of the Nelore breed with ages ranging from 36 to 48 months and four Girolando bulls from 36 to 84 months were used in semen collection and freezing. The bulls were kept in individual paddocks formed by Brachiaria decumbens with access to water and mineral mixture, supplemented with 5 kg of feed and 10 kg of hay. Infrared thermography of the scrotum, ocular globe (GO) and mufla (MU) were performed twice a week at intervals of 72 to 96 hours using the FLIR E40 thermal imager, located 1m away from the animal and using emissivity 0.98, making six replicates per bull. For the scrotal thermograms, the temperatures of the spermatic cord (CED and CEE), proximal portion of the testis (PROXD and PROXE), distal portion of the testis (DISTD and DISTE) and tail of the epididymis (EPIDD and EPIDE) on the right and left sides using the Flir Tools software. At the same time, the climatic factors were measured: ambient temperature (TA), relative humidity (RH), dry bulb temperature (TG) and wet bulb temperature (WBGT). Immediately afterwards, sperm harvests were performed with artificial vagina, and analyzes of quantitative and qualitative characteristics of the fresh semen were performed shortly after the ejaculate was cryopreserved with TRIS - egg yolk - citric acid based diluent medium. After cryopreservation the semen was thawed and the thermoresistance test (TTR) was evaluated. The data were processed with analysis of variance, Tukey test and Pearson correlations, all at the 5% level. The variables studied revealed the following averages for Nelore and Girolando bulls: GO (°C) 31.83 ± 1.83; 33.5 ± 2.28, MU (°C) 25.60 ± 3.74; 26.39 ± 3.30, EEC (°C) 30.97 ± 1.91; 32.19 ± 1.75, CED (° C) 30.43 ± 2.07; 31.75 ± 2.14, PROXD (°C) 31.17 ± 1.84; 32.45 ± 1.89, PROXE (°C) 30.37 ± 2.04; 32.10 ± 2.10, DISTD (°C) 29.91 ± 1.61; 30.47 ± 2.12, DISTE (°C) 29.77 ± 1.66; 29.90 ± 2.06, EPIDD (°C) 27.99 ± 2.11; 27.88 ± 2.72, EPIDE (°C) 27.99 ± 2.11; 27.12 ± 2.63, ejaculate volume (VOL / mL) 4.70 ± 1.61; 8.60 ± 3.37, sperm concentration x 10^6 sptz / mL (EC) 1674 ± 721; 1241 ± 432.01, sperm motility (MOT /%) 55 ± 13.18; 51.25 ± 12.95, spermatic vigor (VIG) 3.8 ± 0.33; 3.54 ± 0.58, total sperm defects (DEFTOT /%) 7.2 ± 3.4; 16.70 ± 13.79, larger sperm defects (DEFMAI%) 5.1 ± 3.3; 14 ± 12.42, minor sperm defects (DEFMEN /%) 2.08 ± 2.6; 2.70 ± 5.88, post TTR motility (%) 26.25 ± 14.06; 16.04 ± 16.48 and sperm vigor after TTR 2.3 ± 1.24; 1.25 ± 1.51. Comparing the animals of the different races between the harvests, it was observed that Girolando bulls differ in volume (P <0.01), sperm concentration (P = 0.026), sperm motility (P <0.01), sperm vigor (P <0.01), sperm concentration (P <0.01), sperm motility and vigor after TTR (P <0.01), and Nellore bulls differed to ejaculate volume (P = 0.04), higher sperm defects (P = 0.049) and vigor after TTR (P = 0.015) among the 6 harvests. Comparing all the parameters obtained between the breeds, P <0.05 was observed for GO, CEE, CED, PROXD, PROXE, ejaculate volume and vigor after TTR. Strong positive correlations were observed between CEE, CED, PROXE, PROXD, DISTD, DISTE, EPIDD, EPIDD and GO (P <0.05) between the Nelore and Girolando breeds. Positive correlations were observed between smaller sperm defects and GO, CED, DISTD, DISTE, EPIDD and EPIDE (r = 0.52, 0.39, 0.49, 0.45, 0.53, 0.52, respectively). Thus, it is concluded that Nelore bulls presented higher adaptation to high temperatures than Girolando bulls, presenting superior sperm quality.

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Testicular biometry and dominance ranking of the white-lipped peccary (Mammalia, Tayassuidae)

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We aimed to describe the testicular biometry of white-lipped peccaries (*Tayassu pecari*) and to verify the relationship between these data with individual's dominance ranking. We collected data from six adult males, aging from two to six years old and weighing between 30.0 and 42.0 kg, maintained in a semi-confined system. Using the all-occurrences sampling method, three observers coded all visible agonistic encounters between group members in the 30 h of video-recorded images during feeding time. Using only the decided dyadic agonistic interactions, i.e. winners and losers clearly recognized by the three observers, we determined the linearity index (h') of the dominance hierarchy, which ranges from zero (non-linear hierarchy) to 1.0 (perfect linear hierarchy). The dominance rankings were calculated using David's scores. At the end of the observations, the peccaries were captured for biometric data collection. Spearman's rank correlation coefficients (rs) were then determined between dominance ranking with the biometric data. A strict linear hierarchy (h' = 1.0, P = 0.02) described the social structure of the males. We recorded a great variation on testicles' length (6.0 ± 1.0 cm), width (4.4 ± 1.2 cm), height (4.6 ± 1.1 cm), total scrotal width (7.8 ± 2.0 cm), and volume (67.5 ± 31.3 cm^3). There were correlations between ranking with body mass (rs = 0.99, P = 0.0003) and testicles' length (rs = 0.94, P = 0.005). There was also a trend of correlation between ranking and testicles' volume (r_S = 0.77, P = 0.07). As testicles dimensions are, in general, directly related to sperm and testosterone production, the larger dimensions of their testicles favored the reproductive performance of dominant individuals in peccary's groups. Therefore, testicular biometry of white-lipped peccaries should be taken into account for the selection of individuals more suitable for captive breeding, favoring the conservation of this species classified as vulnerable.

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Application of equine chorionic gonadotrophin subdoses in the accupoints *Bai Hui* and *Hou Hai* in goats

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Some studies have been performed in search for the minimum effective dose administrated for different routes in hormonal protocols, and the subdoses application in acupoints linked the reproductive organs, such as *Bai Hui* and *Hou Hai*, may produce satisfactory results and similar to conventional doses, as demonstrated in studies with goats, sheep and bovine. The study objective was to evaluate the effect of eCG subdoses applied in the *Bai Hui* and *Hou Hai* acupoint about gestation rate and prolificacy in goats, as well as protocols cost. The project was executed in the Fazenda Experimental da UFRB. It was used 57 Anglo Nubiana goats, previously selected by gynecological exam, with corporal condition of 2.35±0.30 and age of 2.95±0.10 old, randomly distributed in four treatments: T1- 300UI of eCG (100% of dose) IM; T2- 60UI of eCG (20% of dose) applied in the Hou Hai acupoint; T3- 60UI of eCG applied in the Bai Hui acupoint; and T4- 60UI of eCG in false acupoint (IM). The goats were initially submitted at the same hormonal protocol: on day zero (D0), received intravaginal sponges impregnated with 60mg of metroxyprogesterone acetate and 0.1mg of oxytetracycline and five days after it was applied 0.125mg of cloprostenol sodium IM. On D6 the sponges were withdrawn and at the same time eGC was applied in the doses and routes according to each treatment. It was evaluated in this study the gestation rate at the 30th day through ultrasonography exam by transrectal route; the prolificacy from de birth of the goat; and the cost of the used protocols. The data were submitted to normality analysis, followed of adequate statistic tests to each variable, considering the level of 5% significance. The gestation rate, evaluated at the 30th day, as well as the prolificacy were not affected by the used protocols in this study ($P>0.05$). It was obtained a mean of 42.2% to gestation rate, 1.4±0.7 of prolificacy and reduce of 45.40% in protocol cost to use 60UI of eCG, demonstrating the administration efficiency of eCG subdose in *Bai Hui* and *Hou Hai* acupoint in goats. (CEUA/UFRB/Protocolo#23007.003070/2015-40).

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Effect of age and breed differences on boar sperm quality assessed by CASA systems in tropical conditions

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The assessment of the semen quality is an essential tool to improve the reproductive indexes in swine farms. The motility is the most important parameter that used in the sperm quality evaluation because it is associate to the energy of the spermatozoon. The animals used in this study were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by Research Council of Costa Rica Institute of Technology (12/2014). The aim of this research was to determine the influence of breed composition and age on reproductive variables of sperm quality, kinetics and semen motility in boars. During 2016 (dry and rain season), 240 ejaculates were collected from 63 boars of 16 farms, with a mean age of 24.4±10.9 months. The age of animals was categorized in very young, young and adult. Six racial groups were identified: Duroc (D) (58 ejaculates), Yorkshire (Y) (27 ejaculates), Landrace (L) (31 ejaculates), F1 Pietrain*Duroc (PD) (63 ejaculates) and two genetic lines (LA and LB) (61 ejaculates). The variables: concentration, volume, total number of spermatozoa and total motility were different ($P<0.05$) of farm. The season did have differences ($P>0.05$). The interaction season*breed and age *breed was significant. The breeds with the highest ejaculate volume presented a higher total number of spermatozoa ($P <0.05$). The effect of the breed was significant ($P <0.05$) on sperm kinetics variables except for amplitude of lateral head displacement (ALH, µm). The Landrace breed presented the highest ($P<0.05$) percentage of static sperm (29.30±1.57). The most relevant differences ($P<0.05$) for total motility (MTOT) and progressive motility (MP), were presented between L and PD with values of 70.71±1.57; 77.48±1.09 and 51.80±1.97; 59.85±1.37% respectively. Adult boars (≥18 months) had higher volumes of ejaculate and total number of sperm than boars in the intermediate and young ages, however, for velocities (µm/s): curvilinear (VCL), straight line (VSL) and average path (VAP), adult boars only were different ($P<0.05$) of the boars in-between ages.

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Effect of reusing intravaginal progesterone implant on sexual behavior and follicle size in dairy Gir heifers

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Among the hormones used in reproductive protocols for estrous synchronization, progesterone devices represent the most expensive item. The reuse of progesterone implants may be an alternative to reduce costs on reproductive protocols since it does not affect FTAI rates. The aim of the study was to observe the effect of the intravaginal progesterone implant reuse on the behavior of mating receptivity and size of the dominant follicle in dairy Gir heifers. The experiment was conducted at Campo Experimental Getúlio Vargas of EPAMIG (Agricultural Research Company of Minas Gerais State, Uberaba, Brazil). A total of 57 heifers were submitted to Fixed Time Artificial Insemination (D0: 2.0 mg of estradiol benzoate (Synchrodiol ® - Ouro Fino) + slow release progesterone implant impregnated with 1g P4 + 19g of excipient (Sincrogest ® - Ouro Fino), D7: 0.52 mg de PGF2α (sodium cloprostenol, Sincrocio® – Ouro Fino); D9: Intravaginal implant withdrawal + 1 mg estradiol cypionate (EC) (SincroCp® - Ouro Fino) + 300 IU equine chorionic gonadodrophin (eCG Syncro® - Ouro Fino), D11: Artificial insemination (AI) + 2.0ml of GnRH - 10 µg im buserelin acetate), divided into two treatments. G1 (n = 28) received a first-use intravaginal progesterone implant and G2 (n = 29) received a second-use intravaginal progesterone implant. The follicular dynamics was performed every 12 hours by transrectal ultrasonography (US) after the implant withdrawal (48 hours before AI). The size of the preovulatory follicle was recorded at AI. After 12 hours of AI, transrectal US was performed to confirm ovulation. Every 10 minutes, during a period of 36 hours, the sexual behavior of the heifers was recorded regarding mating receptivity. Observations started 12 hours after implant withdrawal (D9) ending at the time of AI (D11). Transrectal US shows that all animals had ovulated. Statistical analyzes were performed using the SAS® statistical package. Among the 57 heifers observed, 46 (G1: n = 22, G2: n = 24) expressed mating receptivity behavior, however, no sexual reception behavior was observed for 11 animals (G1: n = 6, G2: n = 5). The non-expression of mating behavior was attributed to the occurrence of silent heat, which can be defined as the occurrence of ovulation with no signs of heat. According to the behavioral observation, 21 animals (G1: 7; G2: 14) were mating for the first time between 24 and 36 hours, and 25 (G1: 15; G2: 10) between 37 and 48 hours after implant removal. The variation between 24 and 48 hours in the expression of sexual behavior and in the size of the dominant follicle was attributed to the use of ECG and ECP together with the progesterone implant withdrawal, since the ECG can bind to FSH and LH receptors. Lower mean follicle dominant was observed in G1 (10.35 ± 0.27 mm) compared to G2 (12.77± 0.47 mm). The highest follicular dimensions found in G2 can be attributed to the lower concentration of the reused progesterone implant, since the exogenous progesterone inhibits the secretion of LH, which affected the growth of the dominant follicle. The follicle size and the reusing of the progesterone implants apparently did not affect the sexual behavior neither the ovulation. (CEUA/IZ/Protocol#230/16).

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Exogenous melatonin changes oviductal gene expression in pregnant undernourished ewes during the anestrous season

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The effect of exogenous melatonin on the oviductal expression of progesterone and estrogen receptors (PR, ERα) and of IGF-I and IGF-II, and its receptor (IGF-1R), was investigated in pregnant undernourished ewes during the anestrous season. Thirty-one adult, non-cycling, non-pregnant Rasa Aragonesa ewes were treated (+MEL) or not (−MEL) with a melatonin implant. After 42 days, both groups were fed to provide 1.5 (Control, C) or 0.5 (Low, L) times the daily maintenance requirements, so that experimental groups were: C−MEL, C+MEL, L−MEL and L+MEL. After estrous synchronization, ewes were mated (Day0) and on Day 5, embryos were recovered. Melatonin increased number of viable embryos both in control and undernourished ewes (1). Ewes were sacrificed and oviducts adjacent to the corpus luteum (CL) were frozen and processed to study gene expression by RT-PCR. Besides, blood samples were taken for plasma progesterone determination. Only ewes carrying embryos were included. The present protocol was approved by the Ethics Animal Research Committee of Zaragoza University. Data were analyzed by ANOVA using a mixed procedure that included the melatonin and nutritional treatments and their interactions. After 21 days of nutritional treatment, groups L−MEL and L+MEL had an average weight loss of 2.5±0.2 and 3.8±0.3 kg ($P < 0.05$). Plasma progesterone concentrations increased gradually after estrus in all groups, but no differences were observed among them. Melatonin tended to decrease oviductal PR gene expression in control ewes ($P<0.01$), and had no effect in undernourished ewes. Also, L-MEL tended to have lower PR mRNA than C-MEL ($P<0.01$). Overall, no changes of ERα expression were observed among groups. The oviductal IGF-I and IGF-II gene expression was affected in a similar manner, since undernutrition increased the expression of both IGFs ($P<0.05$). Moreover, melatonin also increased both IGFs expression in undernourished ewes ($P<0.05$). On the other hand, IGF-1R was affected by undernutrition ($P<0.01$), and no effects of melatonin or its interaction with nutrition were observed. To our knowledge, this is the first time these parameters are evaluated in the sheep oviduct during anestrus. We report here that exogenous melatonin increases IGF-I and IGF-II, but had no effect on PR and ERα mRNA expression in oviducts, mainly in undernourished ewes. Interestingly, melatonin also appeared to improve embryo quality both in control and undernourished ewes (1). Thus, it could be hypothesized that melatonin could be part of a compensatory mechanism to improve quality of embryos in undernourished ewes, via the amelioration of the oviductal milieu. In conclusion, exogenous melatonin in undernourished ewes increased IGF-I and IGF-II gene expression on the oviduct during early embryo development in the anestrous season.

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Hibiscus sabdariffa extract for use in stallions semen refrigeration

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Equine semen presents inferior results when compared to other animal species regarding the use of refrigerated semen in artificial insemination (AI). This reduction affects the growth of the horse production chain, which actively participates in the Brazilian agribusiness. It is known that the low resistance of the equine spermatozoa to temperature variations and their susceptibility to oxidative stress conditions this reduction in fertility. Due to its antioxidant properties, *Hibiscus sabdariffa* is a vegetal alternative for the cryopreservation process, because it is rich in anthocyanins, vitamin C, lycopene, beta-carotene and polyphenols, it has antioxidant activity in the sequestration of free radicals, in order to help in these conditions. The objective of this study was to test hibiscus extraction techniques, since extraction is one of the most used processes for the isolation of active products present in a medicinal plant, to study sperm cells of stallions. This study was approved by the Committee on Ethics in the Use of Animals/UFPB (Protocol n.124/2016). In the preparation of the hibiscus extract for addition in the equine semen diluent for cooling, 22g of *H. sabdariffa* dry flower was added in 300 mL of methanol for three days and in the dark for extraction. The extract was filtered and evaporated to extract the pigment of interest. It was then diluted in the concentration of 2.5 mg/mL distilled water and tested on sperm cells of horses (n= 5), collected by artificial vaginal and kept under refrigeration (5°C). Concentrations from this dilution were made (1, 5, 10%) to be evaluated in equine spermatozoon. From motility, two evaluators analyzed the spermatozoon under the microscope and a mean was made from their values. For eosin-nigrosin staining, an aliquot of 20 µL of semen was deposited on a slide, mixed with 20 µL of the eosin-nigrosine dye, and homogenized, and the smear was performed after the procedure. The hyposmotic test was performed an aliquot of 10 µL of thawed semen was added to 100 µL of hypo-osmotic solution adapted to the equine spermatozoon preheated to 37°C. The sample was then incubated for 30 minutes and then saline formaldehyde was added. One drop of 10 µL of each sample was analyzed in slide and coverslip, being visualized in phase contrast microscope, with magnification of 400x. Two hundred cells were counted in each technique to quantify the spermatozoa with an integral plasma membrane at 0, 24 and 48 hours. The results showed that in the 10% concentration by hibiscus extract, membrane motility and integrity were better ($P<0.05$) than the control analyzed up to 24 hours, according to ANOVA. This leads to the belief that the property of the hibiscus, in view of the antioxidant action, occurred and that with the evaluation of the super motile activated the equine sperm cell in the first hours and up to 24 hours. Current studies follow to visualize the action of the hibiscus in equine semen.

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Immunolocalization of androgen receptor during gonadal development in *Podocnemis expansa*

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Chelonians are animals that have temperature-dependent sex (TSD), and consequently, they directly suffer the impacts of the environment on the gonadal development. In these species, there is a thermosensitive period, approximately in the middle third of the incubation time, in which the temperature is responsible for the definition of the proportion between males and females since the undifferentiated gonad is sensitive to temperature suggestions and hormonal responses. There are still few studies regarding the morphogenesis of the reproductive organs during the embryonic period, its hormonal control and relation with the incubation temperature. This research aimed to observe the expression of the androgen receptor during the gonadal development of *Podocnemis expansa* (CEUA/INPA nº025/2013). Fifty-five embryos from five different nests were collected from the 22nd to the 64th day of incubation on a beach of Eletrobrás Center for Research and Preservation of Aquatic Chelonia (CPPQA), located near the Balbina Hydroelectric Power Plant on the Uatumã River - Amazonas, Brazil (0154-556.9 "S, 059o28'18" W) (SISBIO / IBAMA 39472-4) were used in the present research. The incubation temperature in the nests was measured using a remote temperature sensor, Hobo data loggers (Onset ™ Computer Corporation). After eggs collecting, the embryos were euthanized using an overdose of thiopental (100 $mg.kg^{-1}$) in combination with lidocaine (4 $mg.kg^{-1}$) intraperitoneally. They were dissected and the gonad-mesonephros complex removed for histological processing and subsequent immunohistochemistry for androgen receptor (AR) using rabbit polyclonal antibody against androgen receptor (AB74272, ABCAM) and secondary using goat anti-rabbit IgG H&L conjugated (AB205718, ABCAM). Gonads were analyzed at three different stages of development: undifferentiated or bipotential (between 22nd and 29th days), in differentiation (30th to 36th day) and differentiated gonad (37th and 64th days). The mean incubation temperature of the nests was 30.3 °C. In the bipotential phase, there was weak marking in the germinal epithelium of the ovary. During in differentiation period, the germinal epithelium present moderate marking, in contrast to the weak reaction in the ovarian medulla. It was also possible to observe the labeled primordial germ cells (PGCs). It is important to note that the distal and proximal contiguous tubules of the mesonephros had a strong reaction, whereas the cells in the transitional region with the gonad presented a moderate reaction, evidencing that these tissues are responsive to androgens, and that this hormone participates in the differentiation of the mesonephro in epididymis. In the differentiated phase, the ovaries showed a strong marking in the germinal epithelium, in the PGCs and medullary region, suggesting the involvement of androgens in the ovarian medulla reduction during sexual differentiation, whereas this intense reaction in the germinal epithelium and the CGP indicates the proliferative effect of this hormone for expansion of the cortex. In the tests, there was a strong marking in the cells of the seminiferous tubules, certainly linked to the tissue differentiation and to the development of the germline cells.

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In vivo and *in vitro* evaluation of gelatin-based scaffolds in burn wound treatment for regenerative medicine applications.

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Wound repair is one of the most complex biological process in human life. The optimal three-dimensional restoration of injured tissues required templates to guide the growth of cells. These artificial templates are known as scaffolds and their improvement is a major task in the field of regenerative medicine. Fibroblasts and mesenchymal stem cells (MSC) are an excellent tool for studying the behavior of this type of biomaterials and their ability to guide the growth and repair of tissues. For production of porous scaffolds, gelatin (G), chitosan (CH), aloe vera (A) and snail mucus (S) suspensions was prepared. Briefly, the scaffold forming suspensions (SFS) were poured into a glass petri dish and maintained for 1 h at −80ºC, prior to freeze-drying, at −58ºC, for 18h. The resulting sponge-like material was placed inside desiccators containing glutaraldehyde (10%), during 2 h, to allow crosslinking between polymers. The sponge was washed and freeze-drying again. For wound healing in vivo studies, eight sixteen-week-old female mice (Winstar) were housed at room temperature and humidity (25ºC) and 50-60%, and were used to evaluate the biodegradability and biocompatibility of gelatin based scaffolds subcutaneously implanted until 4 weeks. Mice were anesthetized with intra-peritoneal injections of ketamine/xylazine (30 mg/kg and 15 mg/kg respectively). After anesthesia, 5 wound were inflicted on dorsal by contact with an incandescent metallic bar with size of 10.0 mm^2 for 5s. The animals received analgesics (tramadol 10mg/kg) for 7 consecutive days after the burn was performed. The scaffold of G, CH, A and AS was carefully cut and implanted in each wound made, a bare control (with serum) was used as negative control for wound healing. Reductions in the wound surface area were assessed until 28 days by taking images with a digital camera at the same magnification for each sample. The areas of wounds were analyzed using Image J software. Wound closure was expressed as percentage closure of the original wound. Histological evaluation was performed after eutanasia. In parallel, to evaluate the adhesion and proliferation capacity of cells, fibroblasts and MSC were seeded on the scaffolds during a 28-day incubation period in a 24 well plate at 37ºC. SEM images were taken to evaluate the microstructure, growth and adhesion in the scaffolds. The scaffold was observed to be biocompatible on subcutaneous implantation which was supported by scaffold integration with tissue and presence of blood vessels in histological studies thereof. Further, application of scaffolds wound in mice model lead to accelerate healing remarkably, which was evidenced by histological studies.

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Perineal laceration in a Texel breed ewe

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The perineal laceration is one of the most common injuries related to the parturition, mostly in the equine species. This laceration is classified in first, second and third-degree according its tissue injury extension. The first-degree laceration involves only the mucosa of vestibule and skin of the dorsal commissure of the vulva. Second degree includes both the mucosa and submucosa of the dorsal vulva, and some of the musculature of the perineal body, in particular the constrictor vulvae muscle. There is no damage to rectal mucosa. The third degree results in tearing of the vestibular and sometimes vaginal wall and disruption of the perineal body, anal sphincter and rectal wall. As a consequence there is a common opening between the rectum and the vestibule. The concern is that loss of functional vulvar and vestibular conformation of the vaginal vault eventually lead to infertility through uterine infection. Thus, reconstruction of perineal laceration is necessary to return the animal to breeding. This disease is uncommon in ovine, being more frequent in mares, therefore justifying the importance this case report. The present work aimed to present a case report of a third-degree laceration in a Texel breed ewe. Where received at the Veterinary Clinics School of the UNICENTRO (Paraná - Brazil) a Texel ewe, primiparous, 1.5 year of age, with historic of laceration during the birth of an only male lamb with 4,5kg. During the physical examination, all parameters were normal, but the laceration involved the dorsal commissure of the vulva, anal sphincter and a small portion of the vestibule. The preconized treatment was the immediate surgical intervention, once the animal was recent lambed and still not yet developed an intense local edema. The ewe was sedated with xilazine (0.05mg/kg, IM), followed by water and neutral detergent lavage, antisepsis of the perineal region with PVPI. Due the lesion at rectum and vestibule, were performed an epidural and infiltrative local anesthesia with lidocaine 2%. The mucosae suture pattern was continuous Cushing, using an absorbable thread n.0 (poliglactina 910). To the vulva reconstruction was necessary the resection of a segment of the mucous-cutaneous junction of each vulvar lip with less than 3mm width, followed by a single point suture, using a non-absorbable thread n.2-0 (nylon monofilament). At the postoperative were administered sulfa + trimetoprim (15mg/kg, IM, SID) during seven days, and flunixin meglumine (1.1mg/kg, IM, SID) for three days. Daily wound cleaning was performed using PVPI diluted in NaCl 0.9% solution, finishing with a fly repellent around the perineal region. The suture was removed after 10 days, with the complete wound healing. Despite the successful surgery treatment outcome, the owner decided to discard the ewe after the lamb weaning.

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Proinflammatory gene expression relative to the collection technique of endometrial samples from cows with and without subclinical endometritis

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Uterine inflammation has a negative effect on reproductive performance and is considered as an important cause of infertility and subfertility in dairy cows. Several studies have investigated the utilization of gene expression to evaluate the inflammatory response of the uterus of cows by endometrial sample collected by biopsy or cytology. This study investigated the differential expression profile of proinflammatory cytokines (CCL5, CXCL8, IL6 and IL1B) between the caruncular and intercaruncular endometrium and the percentage of polymorphonuclear cells (PMNs) in subclinical endometritis. Additionally, these results were then used to compare the method of endometrial tissue collection by biopsy and cytology. The reproductive tracts were collected from a slaughterhouse. Subsequently, the endometrial tissues (punch biopsy) and cells (cytobrush technique) were harvested for analysis of mRNA genes expression by qPCR. After the PMNs count by endometrial cytology, 20 uteri with ovarian at Stage I (days 1-4 of oestrous cycle) were categorized into three groups. Uteri with 0% PMN (*n=10*) were assigned to group Zero, uteri with >5-15% PMNs (n=5) to group Medium (12.2±1.6 % PMNs), and uteri >15% PMNs (n=5) to group High (53.8±32.87 % PMNs). The experiment was done using a 3x3 factorial design, and the data were analysed with Two-way ANOVA with Bonferroni Multiple Comparison post-test (P<0.05) from GraphPad Prism. The results from gene transcripts demonstrated that the location of the endometrial biopsy had no influence on any of the degrees of inflammatory reaction observed (P>0.05). However, the gene expressions were more elevated in the endometrium of cows with high inflammatory reaction when compared to the endometrium without (CCL5, CXCL8, IL6, IL1B) and medium (CCL5, IL6) inflammation. There was no difference in the expression of the genes evaluated between the endometrium without and with medium inflammation (P>0.05). However, differences in the uterine inflammation classified as high inflammation was observed in which all genes were comparatively more expressed in samples collected by cytology relative to those derived from biopsies of both anatomical regions (P<0.05). In conclusion, there was no difference in gene expression between the caruncular and intercaruncular regions evaluated, and this response was dependent on the degree of inflammation. The method of endometrial tissue collection technique was directly related to gene expression only in cases of high endometrial inflammation.

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Scroto-testicular thermographic parameters and semen analysis of sheep with and without bipartite scrotum raised in the semiarid region of Paraiba, Brazil

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Studies on goats have shown that scrotal bipartition is a desirable feature in animals living in climates with high environmental temperatures, acting as an adaptation to this condition and improving the reproductive physiology of males of this species. In contrast, studies demonstrating the appearance or even the usefulness of this trait in the reproductive physiology of sheep are still scanty. The aim of this study was to evaluate the influence of scrotal morphology on the scroto-testicular thermoregulation and semen quality of mixed breed (MB) sheep, with a predominance of the Santa Inês breed, from the semi-arid region of northeastern Brazil. The study involved 14 MB rams, 12 to 18 months old, handled in the same way at Fazenda Águas da Tamanduá, in Sousa, Paraíba, Brazil (Ethics Committee for Animal Use/UFCG protocol Nº 277-2015). The animals were divided into two groups, G1 consisting of 7 rams with bipartite scrotum and G2 of 7 rams with normal scrotum. Images of the caudal part of the scrotum were recorded with a Fluke thermal imager (Ti25®). The images were analyzed using thermal imaging software (Fluke SmartView™), by tracing lines on the proximal, middle and distal thirds of the testis and tail of the epididymis. Two samples of semen were collected for analysis at an eight-day interval, using an electroejaculator for sheep (Boijektor-2001), and the macroscopic (volume) and microscopic parameters (sperm motility and vigor) of the semen were analyzed. The procedures of semen collection and analysis followed the standards of the Andrology Manual of the Brazilian College of Animal Reproduction (CBRA, 2013). The surface temperatures of the proximal, mid and distal regions of the testicle and epididymis tail did not show significant statistical difference ($P>0.05$) between the groups. Although no significant statistical difference was observed, the bipartate animals had larger measurements for TSC (Body Surface Temperature) showing more efficiency in heat dissipation and indicating that these animals used to a greater extent peripheral vasodilation to eliminate excess heat and thus have a smaller energy expenditure, the G1 showed a great ability to regulate the temperature in the tail region of the epididymis ($P=0.062$), location of the bipartition, and the difference in temperature between the body surface and the epididymis tail was 0.54ºC much lower than the G2. The semen parameters studied, in both groups, were within the desirable values for the species, but there was no significant difference ($P>0.05$) between the groups. It is concluded that scrotum bipartite in rams was shown to be an evolutionary indicator showing that animals with this characteristic dissipate heat more efficiently. However, as the rams with scrotum bipartite presented division of less than 50% of the scrotum length, this degree did not influence the scrotum surface temperature and semen quality, as has been observed in goats with the same characteristic.

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Uterus morphometry from prepubertal Murrah buffaloes heifers

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Puberty in the female can be defined as the period in which the first ovulation is established, followed by a normal corpus luteum. In literature, there are several reports that the buffalo female has a late puberty establishment when compared to *Bos taurus* breeds. However, puberty should not be interpreted as an isolated event, being characterized as the final step of innumerable physiological and morphological changes that culminate in the ability to conceive and maintain gestation. Thus, a very employed definition for puberty is the one that considers the first fertile ovulation accompanied by a luteal phase of normal duration. Several factors are related to the beginning of the reproductive life of a female; among them is the uterine development, which is important for future pregnancies. Thus, the objective of this study was to measure the uterine diameter of prepubertal buffalo females and uterine development up to 24 months of age. The experiment was carried out in the Animal Science Department of the Federal Rural University of Pernambuco (DZ-UFRPE), located in Recife, Pernambuco. From November 2017 to July 2018, twenty three prepubescent Murrah females were ultrasonographically monitored from 15 months and until 24 months of age. The animals were kept in the barn in individual stalls, with access to feed and water. All buffaloes were submitted to gynecological examination by transrectal ultrasonography, animals were kept standing and, after the digital removal of the feces, carboxymethylcellulose gel was applied directly to the rectum, in order to facilitate contact and eliminate air between the transducer and the rectal wall. As the transducer was introduced into the rectum it was observed, the bladder, the cranial portion of the vagina and the cervix could be visualized in a longitudinal plane. After visualization of the cervix, a caudal portion of the uterus and its horns were observed, where the measurement was made to determine the diameter of the uterine body. Measurements occurred at six different moments, at 15, 16, 17, 20, 22 and 24 months of age, with a mean of 1.24cm ± 0.19cm; 1.37cm ± 0.16cm; 1.40cm ± 0.12cm; 1.48cm ± 0.19cm; 1.50cm ± 0.16cm and 1.63cm ± 0.26cm of uterine diameter, respectively. Up to 17 months of age in ultrasonographic visualization, little ovarian activity was observed with follicles smaller than 0.5 mm. At 24 months, the animals showed larger follicles (> 0.8 mm), showing a higher ovarian activity. According to the literature as puberty approaches, there is a uterine development influenced by the action of steroid hormones. It is concluded that before the onset of puberty there is no expressive development of the uterus. Research approved by the Ethics Committee on the Use of Animals, registration nº 079/2016-UFRPE.

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Acute inflammation stimulates the function of stem cells: an *in vivo* and *in vitro* evaluation

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The study of stem cells started 20 years ago and today this type of cell are used to aid in a big number of treatments [1] and in the last years studies related stem cells and the effect in reproduction started to appear [2]. Due to this scenario, the knowledge about the factors that affect the stem cell's quality is fundamental for the success of the cellular studies and future therapies [3]. For this reason, the aim of this study was to analyse if the acute inflammatory state from the donor interferes in the quality of the adipose-derived stem cells (ASC) - a important niche of multipotent stem cells of the body [1]. And compare the benefits of autologous versus allogeneic therapy. We choose the horse as animal model because the cellular therapy is widely used in this specie and its reproduction has a ruge value. There was two groups in the experiment: the healthy animals (HA, n=5) and the sick animals (SA, n=5). The SA were equines which undergone to colic surgery, which also presented a clinical sign of endotoxemia. The equine adipose-derived stem cells (eASCs) were collected and submitted to cell primary culture, growth curve, colony forming unit assay (CFU), post-thaw viability test and the differentiation assays. The areas of extracellular matrix differentiated were analysed using Image J software [4]. The assays were made in order to research the applicable characteristics of the cells such as the time to establish the culture, multiplicity, viability, clonogenic and multipotent potential. There was no statically difference between the time to establish the primary culture, viability and multipotent potential exposed as differentiation assays. Although the SA showed a higher number of colonies in the CFU assay than the cells from HA, demonstrating that the SA presented a better clonogenic potential then HA. The acute inflammatory environment from the SA group did not affect negatively the stem cells. Actually, the acute inflammatory state of patient activated the cloning potential of the stem cells and this effect continued *in vitro* during the passages. This discovery together with the facts that: autologous transplantation causes less rejection response to tissue [5] and the immunomodulatory function of cells is stimulated by inflammatory environment *in vitro* [6], provide abasement to conclude that autologous stem cells (from patients with a acute inflammatory state) is more advantageous than using the allogeneic cells.

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Artificial insemination with frozen-thawed semen in dogs: a retrospective study

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The pregnancy rates of artificial insemination (AI) in dogs with frozen-thawed semen are significantly lower when compared with fresh and chilled semen (1). This is most likely due to the effect of freezing on the sperm causing damage and reducing the sperm longevity (2). The frozen-thawed semen should be deposited in the uterus to improve fertility. The methods used (endoscopy or surgery) do not seem to affect the fertility rate (3), leading to an ethical debate about the use of surgery to breed a bitch (4). Another aspect that seems to affect pregnancy rate is the moment of insemination in relation to the ovulation time. To contribute with the information about the time of AI with frozen-thawed semen in relation to the ovulation time a retrospective study was done using the records of breeding data of the Centre d'Etude en Reproduction des Carnivores (CERCA) of the École nationale vétérinaire d'Alfort. The data of all bitches inseminated with frozen semen from 2007 up to 2018 were examined. The data of AI in relation to the ovulation time estimated by progesterone levels and ovarian ultrasonography, the number of inseminations, the pregnancy diagnosis (around 25 days post-ovulation) and the litter size were evaluated. All AIs were performed by experienced personnel with trans-cervical endoscopy or with the Norwegian catheter. No selection was done for the population of bitches although maiden bitches were advised to be avoided. A total of 243 bitches inseminated with frozen- thawed semen were evaluated. The first insemination was done 2.93±0.4 days from ovulation. Two inseminations were performed in 56.6% (111/196) of the bitches and in this case, the second AI was scheduled around 12 hours later. At the owner's request, one bitch was inseminated three times at 10 hour interval. Pregnancy rate was 62.6% (127/203) and prolificity 4.9±3.1 puppies per litter. The decision to perform the 1st AI around 3 days post-ovulation was made taken into consideration the time of oocyte maturation. Fully matured MII oocytes appear approximately 48–54 hours after ovulation when they can be fertilized and stay viable up to 92 hours based on *in vivo* studies (5). Therefore, insemination with frozen-thawed semen should be optimal around 72 hours post ovulation. The pregnancy rate of 62,6%, although not optimal, is in accordance with the results presented in the literature.

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Biological effect of purified recombinant hPDGF-B expressed in mammalian cells on feline fibroblasts used for mesenchymal stem cell induction

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Obtaining mesenchymal stem cells (MSC) might be troublesome for some species, particularly the feline. Further, in patients with complex pathologies or trauma like wild cats, when biopsy sampling is an issue, it can be even more complicated. To help overcome this, we thought to reprogram otherwise terminally differentiated skin fibroblast to a MSC-like status named induced MSC (iMSC). Platelet-derived growth factors (PDGFs) are important for tissue growth and maintenance and have been previously used to produce human iMSC. The objective of this study was to produce h-PDGF-B by stable transfection of human SiHa cells for future use in conjunction with epigenetic reprogramming agents in a cocktail to produce feline iMSC. The hPDGF-B cDNA was synthesized in vitro with a histidine 6X tag (GenScript; Hong Kong), and cloned into the PCI-Neo expression vector. For stable transfection, SiHa cells were plated in 60 mm culture dishes. At 50% confluency, cultures were transfected with PCI-Neo-hPDGF-B using polyethilenimine. SiHa cells were passaged at 48 h after transfection, and further selected in medium containing G418 (800 µg/mL) for approximately 28 days. Colonies were selected, amplified and hPDGF-B was purified from the supernatant of fully grown cultures using anti-histidine affinity chromatography and its expression tested by dot blot with His Tag Monoclonal Antibody HRP (ThermoFisher). Skin feline fibroblasts were isolated from ventral region of female cat and cultured from explants. At passage 3 they were used to study the effect of hPDGF-B on the proliferation. For this, fibroblasts were cultured to 60% confluence and placed in DMEM medium with 0.5% FCS for 48 hours. Subsequently the medium was changed to DMEM plus: 1) 0.5% FCS; 2) 0.5% FCS+ 100 ng / ml of hPDGF-B; 3) 10% SFB + 100 ng / ml of hPDGF-B for another 48 hours. Cell proliferation and cell cycle were analyzed by propidium flow cytometry. For iMSC cells were cultured either in medium containing five reprogramming and three grow factors together (8) for six days (CHIR99021, PD0325901, 5-AZA, ALK, A8301+ LIF, hPDGF-B, bFGF). After, was evaluated the expression of Oct4, Nanog, E-Cadherin, Snail, CD44 and CD105 genes. Also colony formation assay (at seeding densities: 250, 500, 1000, 2000, 4000 and 8000 cells/cm^2) and mesodermal differentiation were performed. Feline fibroblasts presented a high rate of proliferation in media containing hPDGF-B. Cells kept only with 0.5% FBS had lower percentage (4 and 1%) of cells in S; G2-M phases compared with cells treated with h-PDGF-B (11 and 8%). Former cells detached from the dish as a consequence of senescence. The expression of Oct4, Nanog and E-Cadherin decreased on day 6 of the treatment ($p < 0.05$) compared to fibroblasts without treatment. Expression of Snail and CD44 was similar, but CD105 increased at day 6 ($p=0.006$). Induction of multipotency probably requires longer treatments than in our experiments, thus low activation of Oct4 and Nanog might be a result of this. Induced cells seeded at low density (250 and 500 cells/cm^2) did not form colonies, while control uninduced did. Nevertheless, colonies formed by the induced cells had a more rounded morphology, smaller size and more homogeneous organization. This is coincident with the characteristics of PDGF-induced colonies, with round and smaller morphology due to actin reorganization. Cells induced to differentiate with the cocktail 8x showed differential pattern of mesodermal differentiation; with discrete percentage of cells going towards adipogenic and chondrogenic lineages. However, this protocol requires further refinement in order to achieve a greater reprogramming success of fibroblasts towards iMSC. Here we show that terminally differentiated feline skin fibroblasts can be induced to an iMSC status. This can be of importance for regenerative medicine therapies and potentially for more efficient nucleus transfer in Felidae. This work was supported partially by the Animal Biotechnology Laboratory Department of Animal Science, Universidad de Concepción, Chillan, Chile and by grant CONICYT-Chile National Doctorate Scholarship 63140147 to DME.

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Caffeine addition to equine thawed semen improves the sperm recovery after swim- up

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There are differences among stallions on sperm motility after frozen-thawing semen, which can influence the sperm fertilization ability. Thus, some reproductive techniques have been used to improve the *in vitro* and *in vivo* fertilization of animals with low fertility. One of them is the sperm selection with the swim-up technique. Moreover, the addition of substances which stimulate sperm metabolism could be useful to improve its fertilization capability. Since the sperm recovery after the swim-up can be used as an indirect way to evaluate the sperm motility, the aim of the present work was to test the effect of different caffeine concentrations on the recovery rate of stallion sperm submitted to the swim-up after thawing. One ejaculate of five stallions was frozen with the INRA82 frozen extender. After thawing, different caffeine concentrations were added to the semen samples according to the treatments: T1) control INRA82 (without caffeine addition), T2) T1+3mM caffeine, T3) T1+5mM caffeine and T4) T1+7.5mM caffeine. To the swim-up, one aliquot of each semen sample was deposited on the bottom of each 1.5 ml conical tube containing Medium 199 with Hank's salt and 10% fetal bovine serum in a proportion of 1:3 (semen: medium). The tubes were disposed in 30° in water bath at 37°C during different incubation time: 20, 40, 60 e 80 min. After incubation, approximately 1/3 of the total tube volume was taken from the supernatant and the sperm concentration/ml was calculated with a hemocytometer and bright field microscopy. The statistical analysis was performed with variance analysis (ANOVA) and the Duncan test. Although, there were no differences on the sperm recovery among the period evaluated ($P>0.05$), the mean value of 5mM caffeine led to the highest sperm recovery (7.9×10^6esp/ml ± 1.7×10^6esp/ml) compared to the control group (3.4×10^6esp/ml ± 0.7×10^6esp/ml, $P<0.05$). The 3mM (5.7×10^6esp/ml ± 1.3×10^6esp/ml) and 7.5mM caffeine (5.3×10^6esp/ml ± 1.1×10^6esp/ml) did not differ from the control and the 5 mM caffeine treatment ($P>0.05$). In conclusion, the addition of 5 mM caffeine increased the sperm recovery after swim- up right after 20 min of incubation, and thus, could be suggested to improve the *in vitro* e *in vivo* fertility of equine sperm after thawing. However, further experiments are needed to test whether 5 mM caffeine treatment could be used to improve the fertilization rate of frozen-thawed equine semen.

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Characterization by gene expression of mesenchymal stem cells derived from amniotic fluid of goats after *in vitro* culture

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In the last decade, there has been an increase in the presence of mesenchymal stem cells (MSC) in fetal attachments such as amniotic fluid (AF), due to the strong potential of cells for the use of medical devices in cellular therapies because they have characteristics such as an absence and the presence of anti-inflammatory and antifibrotic activities, presence of immunomodulatory properties and strong inhibition of T-lymphocyte proliferation. However, there are still limited information on the effect of non-phenotypic artificial culture and characteristics of MSC of AF differentiation and plasticity in goats. Therefore, the aim of this study was to evaluate the expression of CD73, CD90, CD105, CD34 and CD45 genes related to the pluripotency and differentiation of MSCs isolated and cultured *in vitro*, derived from AF of goat fetuses. For this, 10 mL of 4 goat fetuses AF were collected at 3 months of gestational age by laparotomy and aspiration with a syringe and sterile needles containing 10 mL of PBS and 10% Bovine Fetal Serum (BFS). The suspensions were centrifuged at 342 *g* for 10 min and the pellets containing MSC were resuspended in 1 mL DMEM + 10% BFS + 2% penicillin/streptomycin and subjected to culture *in vitro* at 38.5 °C, saturated humidity and 5% CO_2. After reaching 80% confluence, the cells after 2 passages (P2) on day 17 of *in vitro* culture were trypsinized and evaluated for characterization by RT-PCR (Supermix Invitrigen PCR) by the level of gene expression of antigens CD73 (XM_005684823.3), CD90 (XM_013969538.2) and CD105 (XM_018055826.1) and differentiation CD34 (XM_018060790.1) and CD45 (XM_005691063.3). GAPDH gen was used as endogenous control. The relative abundance analysis of target gene transcripts evaluated showed that under standard *in vitro* culture conditions the MSCs AF-derived were CD73-, CD90- and CD105-positive, cell surface markers found in mesenchymal lineage, and negative for CD34 and CD45, related to hematopoietic lineage. These data demonstrate the mesenchymal properties of the selected cell population and therefore agree with the findings on pluripotency of MSC derived from AF and on pluripotent AF cell characterization. The possibility of using pluripotent and multipotent MSC derived from AF was considered satisfactory due to the relative ease and safe procedure required to recover the cells from their source. Thus, isolation, expansion and characterization of MSC derived from AF in goats are efficient alternatives and presents a simple and rapid protocol for obtaining stem cells.

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Comparison in the cryopreservation of spermatic cells between E*quus Caballus* and *Equus Asinus*

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Equidae belong to the taxonomic family of horses *(Equus Caballus),* donkeys *(Equus Asinus),* zebras *(Equus grevyi)* and many other species known only from fossils. All extant species are in the genus *Equus.* Today, horses, donkeys and their hybrids are widely used for sport, work, entertainment and culture. It is important to preserve the animals of the genre Equus, because some breeds of donkeys were declared at risk of extinction, and some breeds of horses have low genetic variability due to strong selection. Many studies determined differences in the physical, morphological, and biochemical aspects of seminal plasma, and the spermatic cells between 2 species. The aim of this study was to evaluate and compare the use of the modified INRA 82 with dimethylformamide for spermatic cryopreservation of *Equus Asinus* and *Equus Caballus.* Donkeys (n=5) and horses (n=5) clinically healthy with proven fertility of the breed Colombian Creole, aged between 5 to 12 years, were used as semen donors. The animals were located in Chiquinquirá, Boyacá, Colombia and were kept under controlled feeding (cut grass, concentrate, water and mineral salt ad libitum) and housing conditions. Sperm samples were collected with a Missouri type artificial vagina without the gel fraction. Initially, sperm samples were collected to eliminate the extragonadal sperm reserves, until the sperm parameters stabilized. Samples were processed when the following conditions were reached: progressive motility≥60%, ≥ concentration $60X10^6$/mL and≤30% of total sperm morphology abnormality per ejaculate. Seminal material was diluted 1:1 in Kenney solution. After centrifugation (600 × g for 10 min), the pellet was resuspended to 150×10^6 sperm / mL in modified INRA 82 extender (skim milk-egg yolk and 5 sugars) with Dimethylformamide (DFM) at 5% and packaged into 0.5 mL straws. The straws were cooled at 5 ° C for 80 minutes, then kept 4 cm above liquid nitrogen for 10 minutes, then plunged in liquid nitrogen. The sperm samples were thawed in a water bath at 37°C for 30 seconds. After thawing, the motile evaluation was performed by a computer-assisted sperm analysis (CASA) (Hamilton Thorne IVOS II, v 14.0). Motility parameters of 25 different fields were analyzed. The variables considered in this study were total motility (TM, %), progressive motility (PM, %), average path velocity (VAP, m/s), straight line velocity (VSL, m/s) and curvilinear velocity (VCL, m/s). Sperm morphology (SM) was evaluated using smears on slides with aqueous solution of Malachite Green. Sperm chromatin integrity (SCI) was assessed with acridine orange epifluorescence. Functional integrity of the spermatic plasma membrane was made by Hypo-osmotic swelling test (HOST). Thermoresistance testing (TR) was performed, maintaining an incubation at 37°C during various times. Sperm motility and vigor were evaluated after 30, 60, 90 and 120 minutes. Statistical analysis was perform by ANOVA and Kruskal Wallis with SAS 9.4 ® software. Post-thaw semen parameters were: donkeys: TM = 47±14%, PM = 17±7%, VAP= 64±7 m/s, VSL= 47±7 m/s, VCL = 122±1 m/s, SM = 75±9%, SCI = 57±1%, HOST = 31±1% and horses: TM = 61,0±9%, PM = 27,4±1%, VAP = 64±7 m/s, VSL = 47±7m/s, VCL = 122±18 m/s, SM = 75±9%, SCI = 57±1%, HOST = 31±1%. The variables VSL (P=0.030), VAP (P=0.020), sperm morphology (P=0.030) and HOST (P=0.003) showed significant statistical difference. The results of the thermoresistance test showed differences in 60, 90, 120 and 180 minutes, where the motiliy and vigor of the seminal samples of donkeys did not exceed 120 minutes and the horses did not exceed 180 minutes. Based on these results, we hypothesized that this cryopreservation protocol of spermatic cells for donkeys does not confer adequate protection in comparison with the horses. These results are likely associated with a major susceptibility of the donkey's spermatic membrane in comparison with the horse's spermatozoid.

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Comparison of gene co-expression networks in oocytes and blastocysts in vitro produced of buffalo and bovine using RNA-seq data

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In vitro embryo production (IVP) protocols of buffalo are based on bovine protocols, however, there are morphological and molecular differences between the species. In this study, we sought to find differences between the transcriptomes of mature oocytes and blastocysts in vitro produced from buffalo and cattle by comparing their gene co-expression relations. For that, the Weighted correlation network analysis (WGCNA) were used to describe clusters of co-expressed genes in oocytes and blastocysts from buffalos and cattle, with coexpression network and module preservation analysis using RNA-seq data. Buffalo RNA-seq data was obtained by Ion Proton™ sequencing and bovine RNA-seq data was obtained from the literature (Graf et al 2014. PNAS 111:4139-4144). First, the WGCNA package in R program was used to build independent co-expression networks for buffalo and bovine RNA-seq data. Second, the co-expression networks were analyzed for the correlation of the eingengenes modules and the stage of development, thus the modules that presented correlation greater than 0.9 and p-value < 0.05 were considered specific stages. Third, using the *modulePreservation* function of the WGCNA package was performed the preservation of buffalo co-expression modules compared to bovine. Finally, genes that characterize the module co-expression profile (hub genes) were compared between buffalo and bovine networks. As a result, 7 co-expression modules were identified in buffalo and 4 were strongly preserved (Zsummary > 10) in bovine, such as exosome component, steroid metabolism, cellular proliferation and morphogenesis. Cell cycle and transmembrane amino acid transport modules were poorly preserved between buffalo and bovine (Zsummary < 2). The poor modules preservation suggests that the related biological functions might be differently regulated in buffalo and cattle, therefore, it may be important to investigate their roles in buffalo embryo development. Next, one specific module was significantly correlated to embryos ($r = 0.97$, $p < 0.05$) in both buffalo and cattle, which the gene ontology was exosome component, thus the co-expression interactions within this specific module were investigated. As a result, 61% (1,451 hub genes) of the hub genes in the embryo specific modules were different between buffalo and cattle, indicating that the co-expression relations within the modules were different. We conclude that the transcriptomes of matured oocytes and blastocysts produced in vitro from buffalo and bovine showed strong evidence of preservation, except in terms of development kinetics and amino acid transport. However, the expression profiles within the blastocyst modules showed greater dissimilarity, showing that even within conserved modules there are important expression differences between buffalo and bovine, which justify the elaboration of specific IVP protocols for buffalos.

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COX-2 immunohistochemical characterization of inflammatory and proliferative uterine abnormalities in dogs

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Uterine proliferative and inflammatory abnormalities may present subclinically, becoming causes of reproductive failure in bitches. The objective of this study was to determine immunohistochemical patterns in the uterus of prepubertal and pubertal bitches with or without uterine abnormalities. Forty bitches, aged between 5 months and 15 years, weighing between 5 and 29 kg, were used. All animals were submitted to elective ovariohysterectomy. A fragment of the middle portion of each uterine horn and both ovaries were collected. Tissues were fixed in buffered formaldehyde solution at 10% and kept in 70% ethanol. Subsequently, they were submitted to dehydration, diaphanization, and inclusion in paraffin. Three replicate slides were assembled: one slide for histomorphology (hematoxylin and eosin stained) and two others for immunohistochemistry. Vaginal cytology (Giemsa stain) and ovarian histology were used to identify the phase of the estrous cycle. Uterine histomorphology was performed for the diagnosis of alterations, such as cystic endometrial hyperplasia (HEC), pyometra, and endometritis. Immunohistochemistry was performed using primary anti-COX-2 antibody (clone CX-294, 1: 100 dilution, Dako®), according to the manufacturer's instructions and an antibody diluent was used as a negative control. Slides were counterstained with hematoxylin. For evaluations, the endometrium was divided into three cell groups: luminal epithelium, glandular epithelium, and inflammatory cells present in the endometrial stroma. The first two cell groups were evaluated throughout the uterine extension, using a light microscope with a 20x objective, receiving two scores: extension from 0 to 3 (0 = absent, 1 = focal, 2 = multifocal, 3 = diffuse) and intensity from 0 to 3 (0 = negative, 1 = weak, 2 = moderate, 3 = intense). For the third cell group, the marked cells were counted in the endometrium at the superficial and basal layers. The intermediate layer was divided between the two layers mentioned above. For each cut, the number of cells was counted in four randomly selected fields in each layer, totaling 8 fields per animal, using a light microscope with a 40x objective. The total area measured per animal was 1272 mm² (159.1 mm² / field). The cell count was estimated by the sum of each cell type per endometrial area evaluated. Evaluation of the intensity was subjective, with a scale ranging from 0 to 3 (0 = negative, 1 = weak, 2 = moderate, 3 = intense), for each field evaluated. Bitches with uterine alterations were grouped into three groups: HEC, endometritis, and HEC/inflammation. The results of endometrial immunomarked cells were compared to those of healthy pubertal (16/40) and prepubertal (5/40) bitches. In the second analysis, the results were compared to the diestrus and anestrous (9/40) and proestrus and estrus (7/40) of healthy bitches. Statistical tests were performed by IBM SPSS Statistics for Windows, version 20.0 / 2011 (Armonk, NY: IBM Corp.), with a significance level of 5%. Normality was verified using the Shapiro-Wilks test. The bilateral Mann-Whitney (two categories) or Kruskal-Wallis test, followed by the bilateral Mann-Whitney test (3 or more categories) were used for non-parametric variables. Based on the histomorphological evaluation, 47.5% (19/40) of the bitches were diagnosed with a uterine alteration: HEC (4/40), endometritis (11/40), and HEC with inflammation (4/40). Only 5% (2/40) of the bitches presented moderate and multifocal immunostaining in the luminal epithelium, one being a carrier of acute endometritis and one of HEC and chronic endometritis. One animal (2.5%) presented pronounced and diffuse immunostaining in the luminal epithelium, and accentuated and multifocal in the glandular epithelium, being affected by pyometra and HEC. With regard to marked immune cell count, no statistical differences were observed ($p>0.05$) between the groups, although the HEC with inflammation group was demonstrated to be 2 (healthy prepubertal) to 17 (HEC) times higher than the other groups. The most pronounced and extensive COX-2 immunostaining occurred in the endometrium glandular and luminal epithelium cells of bitches with intense infiltration of neutrophils in the superficial layer of the endometrium, accompanied by edema and congestion. It was concluded that evaluations of COX-2 immunostaining in the canine endometrium may be useful in the identification of uterine alterations and differentiation between inflammatory and non-inflammatory alterations.

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Cryotolerance of porcine oocyte matured with IGF-I and vitrified by Cryotop method in the presence of reduced glutathione

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Although oocyte cryopreservation is an important tool for the exchange of genetic material and the *in vitro* production of embryos, in pigs this biotechnology is still little practiced due to the low resistance of the oocytes to this process. However, studies have shown that insulin-like growth factor I (IGF-I) can regulate protein synthesis during cellular response to cryopreservation, whereas reduced glutathione (GSH) can maintain the stability of the nucleoprotein structure and increase the viability of cryopreserved swine gametes. Therefore, the aim of this study was to evaluate the effect of adding IGF-I in the *in vitro* maturation medium and reduced glutathione (GSH) in the vitrification and warming solutions on quality of *in vitro* maturated porcine oocytes (percentage of maturation, cellular viability and DNA fragmentation). For this, fresh oocytes were *in vitro* matured in the presence or absence of 100 ng/ml IGF-I, and after 44 hours of maturation these cells were vitrified/warmed in the presence or absence of 2.0 mM GSH using the Cryotop method. Before the vitrification, nuclear maturation rate was evaluated using Orcein/Dapi staining. Oocyte viability (evaluated by ethidium homodimer-1 staining) and DNA fragmentation (terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling - TUNEL) were evaluated before and after vitrification/warming process. All variables were checked for normality (Shapiro-Wilk) and homogeneity of variances (Levene tests). The effects of IGF-I in *in vitro* maturation were determined with a *t*-test for independent samples and the effects IGF-I combined or not with GSH after vitrification/warming were determined using a two-way ANOVA followed by Bonferroni post-hoc test for multiple comparisons. Before vitrification/warming, control and IGF-I groups showed similar rates ($P>0.05$) of maturation rate, cellular viability, and DNA fragmentation. After warming, the percentage of live oocytes with intact DNA is higher ($P<0.05$) in oocytes matured in the presence of IGF-I and vitrified/warmed with GSH. Compared to control group, the isolate use of IGF-I in the maturation media or the GSH in the vitrification/warming medium improves cell viability and DNA integrity. In conclusion, the addition of IGF-I in maturation media and GSH in vitrification-warming media improve the cryotolerance of cryopreserved porcine oocytes.

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Effect of ACP-103® and ACP-116® extenders on kinetics parameters sperm from white-lipped peccary (*Tayassu pecari*)

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Coconut water-based extenders have been used to conserve semen of several domestic and wild species. There is no report, however, on its use for white-lipped peccary semen (*Tayassu pecari*). Therefore, we aimed here to evaluate the effect of ACP-103® and ACP-116® seminal extenders on the white-lipped peccary sperm movement characteristics. Despite we tried to collect semen from 15 adult males; we used ejaculates of just five adult white-lipped peccaries, which reach at least a volume of 0.2 mL, a minimum concentration of 365 x 10^6/mL, and total motility above 60%. We used a net to capture the animals, which were submitted to the anesthetic protocol composed of an association of acepromazine (0.2 mg/kg intramuscularly - IM) and ketamine (5 mg/kg IM) after feeding fast for 12 hours and water for 6 hours. After being anesthetized, 5 IU of oxytocin were administered intravenously five minutes prior to semen collection through the use of an electro-ejaculator (Eletrogen® model). After collection, semen samples were placed in dry bath at 37°C for evaluation of macro and microscopic parameters of fresh semen, such as volume, color, odor, appearance, concentration, and sperm movement. Half of the samples was then diluted in ACP-103® and the other half in ACP-116® to achieve the minimum concentration of 35 million sperm/mL. Sperm Class Analyzer (SCA®; Microptics SL, v.5.2, Barcelona, Spain) was used to evaluate the sperm movement parameters using the recommendations of the software to evaluate the sperm swine, as follows: 25 images/second with 25 Hz; size of the captured particle between 10 and 80 μm/m^2; spermatozoa considered to be <10 μm / s, slow <25 μm / s, mean> 25 μm / s and <45 μm / s and rapid> 45 μm / s. Thereafter, 5 μL of semen were placed between the leaf and cover sheet preheated on the heating plate at 37°C and five different fields were digitized. The following parameters were evaluated: total motility (MT; %), progressive motility (MP; %), velocity straight line (VSL; μm/s), average path velocity, VAP (μm/s), curvilinear velocity (VCL; μm/s), amplitude of lateral head (ALH; μm), beat cross frequency (BCF; Hz), linearity (LIN;%), and straightness (STR; %). Using randomized block design, we compared macro and microscopic parameters of the diluted semen with ACP-103® and ACP-116® by one-way ANOVA. The fresh semen showed white color, sui generis odor, milky appearance with the following characteristics (average ± standard deviation) - volume: 0.34 (± 0.2) mL; sperm concentration in the ejaculate: 947.3 (± 651.9) x 10^6/mL; total motility: 85.7 (± 9.3) %; progressive motility: 46.7 (± 13.7) %, VCL: 51.2 (± 13.8) μm/s, VSL: 19.8 (± 6.1) μm/s, VAP: 34.2 (± 12.8) μm/s. The LIN and STR were 38.8 (± 8.1)% and 59.4 (± 8.6) %, respectively. The ALH was 2.3 (± 0.4) μm, while the BCF was 6.6 (± 0.9) Hz. After stabilization, the macroscopic and microscopic characteristics did not differ (P>0.05) between the dilution with ACP-103® or ACP-116® with the following respective means: total motility 72.7 and 70.2%; progressive motility 33.3 and 32.8%; VCL 39.1 and 38.8 μm/s, VSL 13.4 and 14.2 μm/s; VAP 22.9 and 23.8 μm/s; LIN 33.8 and 34.7%; STR 57.4 and 57.9%; ALH 2.2 and 2.2 μm; BCF of 6.2 and 5.9 Hz. Therefore, both ACP-103® and ACP-116® can be used to dilute the semen of *Tayassu pecari*.

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Effect of adding oxytocin on the protocols for pharmacologically-induced ejaculation in stallions

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Traumatic injuries, neoplasias, ejaculatory disorders, behavioral disturbances and several diseases can prematurely end the breeding careers of many stallions. Pharmacologically-induced ejaculation has been shown to be an important tool to obtain ejaculates from disable stallions. It has been suggested that oxytocin is involved in epididymal motility and sperm transport through the male genital tract in domestic animals. Oxytocin also plays a role in the ejaculatory process, stimulating sperm release from the epididymal storage. Thus, the aim of the study was to evaluated the success rates in inducing ex copula ejaculation in stallions when oxytocin was added to pharmacological protocols. We evaluated ex copula ejaculatory response to the combination protocols of the antidepressive tryciclic imipramine hydrochloride (3mg/kg/v.o), followed 90 minutes later by the alpha-adrenergic agonist detomidine hydrochloride (0.01mg/kg/i.v) (Protocol 1) and the combination of imipramine hydrochloride (3mg/kg/v.o), followed 90 minutes later by detomidine hydrochloride (0.01mg/kg/i.v) associated with oxytocin hormone (20UI/ i.v) (Protocol 2). Fourteen sexually-experienced stallions (3 to 25 years old; 380 to 500 kg) were each underwent 3 treatment trials conducted at 4-day intervals without sexual prestimulation. The trials were conducted in the animals' stalls where they were observed for 90 minutes following treatments. Induced ejaculates were collected into a plastic bag positioned over the prepuce by a girth strap, as described by McDonnell and Love (1991), for the comparison of semen characteristics with ejaculates collected using Botucatu® artificial vagina model (Botupharma Ltda., Botucatu, SP, Brazil). No ejaculation was observed in Protocol 1and ejaculation occurred in 6 of 14 stallions (42.8%) when oxytocin was added to the protocol (Protocol 2). Ten of 28 trials (35,7%) resulted in ejaculation. The mean ejaculation occurred within 5 minutes of injection. Erection occurred in 5 stallions while masturbation was observed in 3 of them. Only 1 ejaculation occurred during masturbation. There were no correlation ($P>0.05$) between erection and masturbation with ejaculation success.The induced ejaculates had significantly ($P<00.5$) lower total volume (3-15ml), lower gel volume, higher concentration (1-8 x 10^9 per ml) and higher total numbers of spermatozoa (7,5-25 x 10^9) than the ejaculated collected by artificial vagina. The semen characteristics suggests decreased accessory glands fluids, probably resulting from imipramine treatment and increased epydidimes tail contraction, probably resulting from oxytocin and alfa-agonist effects. Thus, oxytocin do not stimulate accessory glands contraction.

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Effect of anethole during in vitro maturation on bovine embryo development

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During in vitro maturation, there is the higher production of reactive oxygen species (ROS) from the high oxygen tension at which the oocytes are exposed in vitro, which may affect the oocytes quality and capacity to develop the embryo. Because of this, several substances with antioxidant characteristics, have been added to in vitro maturation media to improve oocyte development, allowing gametes to the resumption of meiosis and to generate embryos. This study aimed to investigate the effect of anethole in the in vitro maturation (IVM) of oocyte bovine in three different concentrations (30, 300, and 2000 mg/mL) on the resumption of meiosis and embryo development. The cumulus oocyte complexes (COCs) were collected from follicles (4-8 mm) and *in vitro* matured in TCM-199 medium (TCM-199), supplemented with 10% FCS, 0.2 mM sodium pyruvate, 2 mM L-Glutamine, 50 µg/mL gentamicin, 10 IU equine chorionic gonadotrophin (eCG) mL and 10 IU human chorionic gonadotrophin (hCG) mL this medium was referred to as TCM 199^{+}. For IVM, COCs were randomly allocated into four groups as follows: TCM-199^{+} (control group) or TCM-199^{+} supplemented with anethole at 30 (AN30), 300 (AN300), or 2000 µg/mL (AN2000). Groups of 50 COCs were cultured in 500 mL of IVM medium for 24 h. After maturation, the COCs were *in vitro* fertilized (IVF) for 18 h and, after IVF, the presumptive zygotes were transferred for the cultured embryos medium for 7 days. After in vitro maturation all, oocytes resumed meiosis. The percentage of MII oocytes were similar ($P> 0.05$) among the treatments and ranged from 77.3 to 95.7 %. Anethole at 300 µg/mL was the only treatment that yielded higher ($P < 0.05$) cleavage rate and embryo development (morula plus blastocyst) production compared to the control treatment. Interestingly, AN300 treatment increased the average number of total cells in blastocysts in relation to the control and AN30 treatments ($P < 0.05$). In conclusion, our results demonstrate that supplementing the MIV medium of bovine oocytes with 300 µg / mL anethole improves the quantity and quality (evaluated by the number of total cells in the blatocysts) of embryos produced in vitro.

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Effect of physiological stage and season on thermograms in dairy cows

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The objective of this study was to investigate the influence of season and pregnancy stage on the temperature of body areas of Holstein cows using digital infrared thermography, an effective and non-invasive technique. Temperature was recorded at several areas of the body surface to determine the most reliable body area for measurement of rectal temperature in pregnant and non-pregnant animals. Holstein cows (n = 24) were divided into groups according to their physiological stage. The experimental period was 365 days, containing a dry (April–September) and rainy (October–March) season, with parameters measured every 28 days. Thermographic data of body areas, rectal thermometry, ultrasonography and climatic data were collected between 7:00 and 9:00. Thermogram-recorded temperatures significantly differed ($P < 0.05$) between seasons and reproductive phases. Moreover, significant differences were noted between the temperatures of the flank, lateral udder, and perineal areas across seasons ($P < 0.05$). The udder, perineal, and rectal temperatures differed according to the reproductive phase ($P < 0.05$). Significant correlations ($P < 0.01$) were observed between reproductive phase and rectal temperature, ocular globe, snout, flank, and perineum. The body areas examined by thermographic imaging presented different temperatures, showing physiological variations. Season and physiological stage influenced the temperature of body areas of milk cows. (CEUA/UNOESTE/Protocol#2918/2015).

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Effect of subdose application of human chorionic gonadotrophin in the acupoint *Hou Hai* about the luteal development and serum progesterone in jennies

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The corpus luteum (CL) formed in metaestrous of the estral cycle is responsible for the biosynthesis and progesterone secretion (P4), fundamental for the gestation maintenance, witch levels are increased after this structure's formation. The hCG synchronize the estrus and the ovulation and may promote increase in plasmatic concentration of P4, however, the used protocols in jennies are the same indicate for mares, as well as the application routes, which justified the execution of studies turned for the asinine species, using reduced doses, that can be applied by alternative routes, such as acupoint Hou Hai, that for being linked to the reproductive organs, can potentialize the subdose effect and promote satisfactory results. This way, the study objective was to evaluate the effect of hCG subdose application in the Hou Hai acupoint about the luteal development and P4 serum in jennies. For such, it was used in three consecutive periods 11 jennies of Pêga race in reproductive activity, healthy, with corporal condition between 4 and 6, distributed in randomized block design, being the used period as a blocking factor, in three treatments: T1- 1500UI of hCG applied IV in jugular; T2- 450UI of hCG/IV and jugular and T3 - 450UI of hCG applied in Hou Hai acupoint, totalizing 11 repetitions per treatments. The mean diameter evaluated of CL with ultrasonography mode-B in D0, D2, D4 and D8 after ovulation, as well as P4 serum, whose blood collection was performed in D8. The data were submitted to normality analysis, followed by ANOVA and Turkey test, 5% significance. The hCG dose reduceapplied both by IV and Hou Hai acupoint did not promote any effect (P>0.05) about the mean CL diameter in moments D0 (23.0±0.6mm); D2 (27.7±1.9mm); D8 (28.2±0.8mm), and serum concentration of P4 (10.50±2.99 ng.mL-1). However, the application of 450UI of hCG in the Hou Hai acupoint resulted in bigger diameter of CL on D4 (30.7±5.1mm) regarding the application of 1500UI IV (25.8±4.4mm), being both similar to the application of 450UI IV (27.0±4.5mm) (P=0.04). This results show the use of hCG subdose applied in the Hou Hai acupoint is effective in maintain the CL development and consequently the P4 synthesis, indicating that the doses used can be overestimated. (CEUA/EMVZ/UFBA/Protocolo#67/2016).

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Effect of subdose of hCG applied in the acupoint *Hou Hai* about pre-ovulatory follicle vascularization and corpus lutheum in mares

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To obtain better results with reproductive biotechnology in equideoculture, the use of inductors is essential tool to anticipate ovulation, being the hCG the most used hormone. However, it does not exist consensus on the effective dose, besides that, repetitive use and excessive doses may stimulate antibodies production and reduces its efficacy. Alternatively to the traditionally used routes, studies have demonstrated the efficacy to administrate drugs in acupoints linked to the reproductive system, such as *Hou Hai*, making possible the use of reduced doses and similar results. This way, the objective study was to evaluate the effect of hCG in the use of the Hai acupoint on the follicular and luteal vascularization, and the correlation between them and the P4 serum of seas. It was used 15 mares in reproductive activity, healthy, body condition between 4 and 6, in three consecutive periods, distributed in the randomized block design, considering the period as blocking factor. The treatments consisted of two hCG doses applied in different routes, being: T1- 1500UI of hCG applied IV in jugular; T2-450UI of hCG / IV in jugular and T3-450UI of hCG applied in *Hou Hai* acupoint, totalizing 15 repetitions per treatment. The ovulation induction with hCG occurred when the follicle reached ≥35mm diameter and uterine edema grade 3. From then on, the evaluations were performed by Doppler ultrasonography and through numeric value of pixels (NPV) mean, ovulatory follicle vascularization and CL in moments D0, D2, D4 and D8, and then it preceded to evaluate the correlations between the NPV, CL diameter and P4 serum. The data were submitted to normality analysis, followed by ANOVA and Turkey test, 5% significance. The correlations were verified by Pearson. The use of 1500UI of hCG IV applied increased vascularization of pre-ovulatory follicle, evaluated by NPV minimum (40.33 ± 2.57) in relation to the use of 450UI IV (36.84 ± 2.24) and similar to the group that received 450UI in *Hou Hai* acupoint (39.31 ± 3.36) (P <0.05) and the same way, it was obtained to moderate positive correlation between the corpus lutheum diameter and P4 concentration on D8 for the mare group that received 450UI of hCG IV. However, the values of numeric pixel mean, maximum and heterogeneity were similar between treatments, both to pre-ovulatory follicle and CL (P> 0.05). The use of 450 IU of hCG by IV route in the acupoint *Hou Hai* was efficient in promoting the follicular and luteal adequate vascularization in mares. (CEUA / EMVZ / UFBA / Protocol # 67/2016).

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Effect of the addition of phytic acid antioxidant to refrigerated boar semen diluents

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Boar semen has high sensitivity to cryopreservation, probably due to the high content of polyunsaturated fatty acids present in the sperm membrane, which are susceptible to oxidative stress. Thus, the addition of antioxidants to the diluent is aimed at delaying or preventing oxidation, protecting the plasma membrane. The objective of this study was to evaluate the effect of the addition of phytic acid to BTS extender on the kinetics, morphology, and membrane integrity of the refrigerated spermatozoa at 17°C for up to 96 hours. The ejaculates of eight boars from the Landrace, Pietrain, Large White, and Duroc breeds, with an average age of 2 years, were harvested by the gloved technique. Still on the farm, the semen was divided into two aliquots of 30 to 50 mL, which were diluted 1:1, one with BTS extender and the other with BTS plus 0.5 mM of phytic acid (BTS + AF). After determination of the concentration, 50x10 6 spermatozoa/mL were maintained as final concentration in the groups (BTS and BTS + AF). The sperm characteristics were evaluated at five moments: fresh (D0), after cooling at 17 ° C for 24h (D1), 48h (D2), 72h (D3), and 96h (D4). Sperm kinetics were evaluated by the CASA system and membrane integrity and spermatic morphology by eosin-nigrosin staining. Immediately after the sperm evaluation, the samples were frozen at -20°C and after 30 days the lipid peroxidation was evaluated by the thiobarbituric acid reactive species (TBARS) technique. For statistical analysis, the t-test and Mann-Whitney test were used, considering a significance level of 5%. The results of the BTS versus BTS + AF groups for total motile were: D0 (67.9 versus 71.9), D1 (46.1 versus 53.5), D2 (36.6 versus 42.3), D3 (30.5 versus 37.0), and D4 (26.1 versus 37.3), and for progressive motility (%) were: D0 (31.9 versus 43.8), D1 (21.6 versus 25.6), D2 (13.9 versus 14.9), D3 (10.9 versus 15.1), and D4 (9.6 versus 17.6). Membrane integrity values were higher for the BTS + AF group than for the BTS at moments D3 (93.7 versus 89.4) and D4 (93.6 versus 89.2). There was a difference at D0 between BTS versus BTS + AF for HLA (6.1 versus 5.4), STR (75.0 versus 79.9), and LIN (39.6 versus 46.0). There were no differences in lipid peroxidation, demonstrating that the addition of 0.5 mM of phytic acid was not efficient in reducing peroxidation reactions, however, there was greater protection of the sperm membrane after 72 hours of cooling. Since this is the first study using phytic acid in porcine semen, new studies with different concentrations are required.

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Effect of the alcoholic extract of *Hibiscus sabdariffa* on caprine spermatozoa after cryopreservation

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The hibiscus (*Hibiscus sabdariffa*) is an annual, shallow-branched shrub with red-cupped flowers that has both antioxidant and antibiotic potential. The cryopreservation process causes many deleterious effects to the cell due to the large production of reactive oxygen species, making the hibiscus extract a potential ally, with vegetable origin, on cryopreservation process. Commercial semen diluents are made from animal raw material, which makes possible to spread infectious-contagious diseases. In this work, different concentrations of hibiscus extract were tested in thawed sperm cells from goat to verify the action of this substance on the efficiency of these cells. To achieve this goal, cell kinetic parameters analysis was performed through CASA (computer assisted sperm analysis). Plasma and acrosomal membrane integrity tests were also performed, as well as the observation of oxidative stress generation during the process, using different probes for each parameter by flow cytometry. The experimental groups tested in this study were: Control Group (CG): semen without addition of the extract (180µL semen + 20µL of PBS); Group 1 (G1): semen + 1% of hibiscus extract (180 µL semen + 18 µL PBS + 2 µL extract); Group 2 (G2): semen with 5% extract (180 µL semen + 10 µL extract + 10 µL PBS); Group 3 (G3): semen solution with 10% extract (180 µL semen + 20 µL extract). The analyzes were performed at 0 h and 2 h after thawing. The data were evaluated by the Kolmogorov-Smirnov test with 5% significance. T test was used for comparisons between the times of the same treatment and ANOVA was used for comparison between treatments, followed by Tukey's post-test ($p \leq 0.05$). A significant difference was observed in the amplitude of lateral head (ALH) values, when the control group (CG) was compared to the group with the highest extract concentration (10%, G3), the G3 group presented higher values in the shortest incubation time. The GC group and the 1% (G1) extract group presented differences between the incubation times in the analysis of the ALH, presenting higher values at 2h of incubation. In the analysis of beat cross frequency, a difference in GC was observed depending on the incubation time, higher values were observed in 2h of incubation when compared to the immediate analysis of the semen; these results may be related to a state of spermatozoa hyperactivation due to the presence of calcium in the hibiscus extract. No differences ($p > 0,05$) were observed in the other analyzed values: total motility (MT), progressive motility (MP), curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), linearity LIN), wobble (WOB), oxidative stress and plasma and acrosomal membrane integrity. Incubation of thawed goat semen with hibiscus alcoholic extract induces a dose-dependent increase in kinematic values. Thus, the hibiscus extract may influence the hyperactivation of goat sperm cells and can bring benefits to the immediate fertilization.

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Efficiency of SlimCASA for bovine sperm analysis from image obtained from smartphone

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In the context of animal reproduction, the use of the artificial insemination technique has been increasing, strengthening the market for bovine semen and spreading the access and multiplication of superior animals, observing a market in full expansion, due to the small amount of females that are currently inseminated in the country. In the field, it is necessary to have a routine of sperm analysis to ascertain the quality of the semen used in insemination. In this way, the objective was to test the efficiency of SlimCASA software with images obtained from smartphone, as it is a method of image capture of low cost and practicality of use, in order to make possible the use of the developed software and to standardize the spermatic analyzes in the field. The motility and sperm concentration of 100 samples of thawed commercial bovine semen were analyzed. Each sample of 0.5 mL was removed from the liquid nitrogen canister and placed in a water bath at 37°C for 30 s to thaw. A 10μL drop of the sample was placed simultaneously on a microscopy slide for SlimCASA analysis and motility estimation by the technician, another in a Neubauer chamber for counting by the technician, and a third on the Hamilton Thorne CASA apparatus. The slide with the sample was taken under a microscope, and a smartphone camera was positioned in the microscope eyepiece by means of a generic holder. Videos of each sample were produced and, in a later stage, converted to .tiff format, through a free program provided online. After this, they were analyzed with the software SlimCASA that is based on the cellular analysis functions of the existing open source program ImageJ. The concentration and sperm motility data of the samples were submitted to analysis of variance, and their means were compared by the Tukey test, at a significance level of 5%. Only 96 of the 100 samples were valid, two were discarded because it was not possible to convert to .tiff format because the size of the file was larger than the one supported and two due to an error occurred at the moment of video capture. The files obtained by the smartphone camera contained on average the size of 56.42 ± 12.27 MB and average duration of 28.30 ± 5.90 seconds. The mean results obtained for sperm concentration were 62.35 ± 63.06a million/mL for analysis by SlimCASA, 75.07 ± 25.38a million/mL by the technician, and 197.56 ± 58.43b million/mL by the CASA equipment. There was no difference between the analyzes performed by the software and the technician, both differing from the analyzes of the home equipment ($p > 0.05$). For motility, it was 73.57 ± 12.29a% for analysis by SlimCASA, 65.39 ± 17.65a% by technician and 65.89 ± 20.59a% by CASA ($p<0.05$). There was no difference between the different types of analysis. The results reveal the potential use of SlimCASA software coupled with a smartphone camera. The SlimCASA software took on average 125.42 ± 11.46 seconds to perform the analyzes of concentration and sperm motility. The mean number of spermatozoa counted by the software in each sample was 29.69 ± 30.03 cells, being 0.89 ± 1.79 cells considered inert. It is concluded that the combination of use of SlimCASA software with image capture by the smartphone is a viable and efficient option for bovine semen analysis.

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Equine semen cooled in extender containing docosahexaenoic acid (DHA) and Trolox®

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The use of cooled semen in the equine industry is greater than frozen semen. However, many stallions have poor fertility results and this can be attributed to the type of protocol or extenders used. The objective of this study was to evaluate the effect of docosahexaenoic acid (DHA), associated or not with Trolox®, in extenders for cooling semen from Mangalarga Marchador stallions. Ten ejaculates were cooled in the following extenders: D1) BotuSemen® (BS; control); D2) BS + 30 ngmL^{-1} DHA (BS30DHA); D3) BS30DHA + 40 µM Trolox® (BS30DHA40T); D4) BS + 50 ngmL^{-1} DHA (BS50DHA); D5) BS50DHA + 40 µM Trolox® (BS50DHA40T). The diluted semen from each stallion were conditioned in 15 mL conical centrifuge tubes in a BotuFLEX® (Botupharma, Botucatu, SP, Brazil) storage and transport system for a period of 48 hours. The final storage temperature of the refrigerated semen used in the isothermal system was 5 °C after a cooling rate of approximately 0.05 °C / min. The sperm movement, structural and functional integrity of the plasma membrane and sperm longevity were evaluated after 48 hours of cooling in BotuFlex®. All extenders tested preserved motility, linearity, straightness of trajectory, amplitude of lateral head displacement, beat cross frequency, hyperactive, the structural and functional integrity of the sperm membrane similarly ($P>0.05$). The BS50DHA extender was superior to BS30DHA40T in preserving VCL and VSL and was superior to BS30DHA40T and BS50DHA40T in preserving VAP and oscillation index ($P<0.05$). It was possible to identify that of the ten stallions used in the experiment, eight of them were considered "bad coolers", since they presented motility less than 30% after 48 hours of cooling. For this category of stallions, it was possible to observe that VCL was better preserved in DHA extenders without Trolox® ($P<0.05$) and VSL and VAP were better preserved in the control and the DHA extenders without Trolox® ($P<0.05$). The motile parameters, LIN, STR, ALH, BCF and structural and functional integrity of the membranes were similarly preserved in all extenders used in the cooling process for these stallions ($P>0.05$). It was concluded that the use of Trolox® in extenders for cooling stallions sperm containing DHA at the proposed concentrations is not indicated by altering sperm movement parameters considered important for fertility.

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Evaluation of sheep sperm viability after cryopreservation in extender with or without green tea extract

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Artificial insemination (AI) and semen cryopreservation are responsible by genetic beneficial, without distance and time limitations to semen use, crucial factor to the international livestock (1). However, the sperm are susceptible to cryoinjuries (2), and for this reason, studies have been realized to search agents with a protective effect. Green tea (GT) compounds present great antioxidant functions to prevent oxidation damages, caused by reactive oxygen species (ROS) (3, 1). Whereas, was aimed determine the ram sperm kinetics after refrigeration in egg yolk based extender without or with GT extract at 0.25%. For this, three semen *pools*, from two male sheep, were divided in two fractions, diluted according experimental groups (G1=egg yolk based extender without GT; G2= egg yolk based extender with GT extract at 0.25%), submitted to cryopreservation (5ºC) and analyzed to kinematics at 0 hour and 16 hours after refrigeration. The parameters of computerized system (CASA) considered were: total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), average path velocity (VAP, μm/s), linearity (LIN, %), straightness (STR, %), wobble (WOB, %), amplitude of lateral head displacement (ALH, μM) and beat cross frequency (BCF, Hz). One-way ANOVA and Tukey-Kramer were used for statistical analysis, considering 5% significance. No statistical differences (P>0.05) were observed between the both groups concerning TM (0h: G1=55.50±3.90, G2=51.40±6.85; 16h: G1=53.93±0.96, G2=51.13±6.91), PM (0h: G1=79.00±12.85, G2=88.63±3.91; 16h: G1=89.43±1.05, G2=89.27±1.44), VCL (0h: G1=103.80±13.53, G2=94.57±9.84; 16h G1=89.33±4.11, G2=96.30±8.63), VSL (0h: G1=106.10±92.10, G2=84.50±75.97; 16h: G1=68.10±73.80, G2=84.50±76.63), VAP (0h: G1=82.10±16.93, G2=64.13±12.49; 16h: G1=61.80±4.91, G2=64.17±10.03), LIN (0h: G1=78.57±7.78, G2=67.37±6.77; 16h: G1=69.10±2.25, G2=66.47±6.14), STR (0h: G1=89.03±2.76, G2=84.07±3.15; 16h: G1=83.73±0.38, G2=83.70±3.55), WOB (0h: G1=88.20±6.70, G2=80.03±5.16; 16h: G1=82.53±2.97, G2=79.33±5.05) and BCF (0h: G1=9.88±2.27, G2=11.80±0.75; 16h: G1=11.33±1.46, G2=11.87±1.08). Although this, ALH was higher (P<0.05) in G2 at zero (2.87±0.12) and 16 hours (2.93±0.15) than in G1 at the same times (2.37±029 and 2.47±0.06), respectively), which can be explained by the presence of caffeine in GT, that stimulate thermogenic response and anticipate spermatozoa capacitation (4). Is possible that lower concentrations present better results. In conclusion, the addition of 0.25% GT extract to sheep semen extender increase ALH after refrigeration, without more cinematics modifications.

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Evaluation of vitrified goat ovarian tissue after xenotransplantation

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Ovarian tissue cryopreservation and xenotransplantation is an alternative approach for studying mechanisms of follicular development in different animal species and for improving cryopreservation and grafting protocols. Nonetheless, it is currently unknown whether xenotransplantation of fresh or cryopreserved goat ovarian tissue into immunodeficient mice can allow follicle survival and development. Thus, the aim of the present study was to evaluate the caprine preantral follicles enclosed on vitrified ovarian cortex grafted to nude BALB/ mice during one month. The ovarian cortex from goats (n = 8) was fragmented (3 × 3 × 0.5 mm) and divided into four groups: fresh control, vitrified control, fresh transplant and vitrified transplant. Follicular morphology, development and density, fibrosis as well as apoptosis, and tissue revascularization were evaluated. After one month of xenotransplantation, it was observed a significant decrease of morphologically normal preantral follicles (primordial, transition, primary and secondary) in both vitrified control and vitrified transplant treatments when compared with both fresh control and fresh transplant. In addition, the follicular density was lower in both fresh and vitrified transplant treatments when compared to fresh control. On the other hand, fresh control and fresh transplant exhibited a similar percentage of developing follicles. Additionally, vitrified control showed a significant increase of developing follicles in comparison with both fresh control and fresh transplant. We also observed high fibrosis in both fresh and vitrified transplant, since a high area of collagen type III was observed in these treatments and collagen type I was predominantly only in fresh control. Furthermore, the mRNA expression of caspase 3 was lower in both fresh and vitrified transplant in comparison with vitrified control. In conclusion, xenotransplantation is an excellent strategy to maintain normal preantral follicles morphology after vitrification/warming of goat ovarian tissue. However, in order to ensure the survival and development of these follicles, it is essential to improve the revascularization of the graft. (CEUA-UECE/protocol#2917497/2015).

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Gene Expression of ATP-Binding Cassete (ABC) transporters in preantral follicles after vitrification and in vitro culture of ovarian tissue from sheep

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The ATP-Binding Cassete (ABC) transporters have various roles across multiple reproductive tissues. They play a major role in biodistribution of many physiological factors involved in different reproductive processes, as the efflux of clinically relevant drugs and environmental toxins across cell plasma membrane. During the ovarian tissue vitrification, the plasma membrane plays a crucial role in the influx or efflux of water and substances such as cryoprotectants and the channel proteins are required in this process. Thus, the aim of this study was to verify the relative expression of ABC transporters ABCB1, ABCG2 and MRP2 after vitrification and in vitro culture (IVC) of ovine ovarian tissue. Ovarian cortex fragments from sheep (n=4) were divided into four groups: fresh control, vitrified control, fresh culture and vitrified culture. The IVC of non-vitrified and vitrified ovarian fragments was performed at 39°C in 5% CO_2 in a humidified incubator during 2 days. Thereafter, in order to evaluate the gene expression of ABCB1, ABCG2 and MRP2 transporters in the ovarian cortex, the qPCR was performed in all treatments. The follicular morphology (survival and development) was evaluated by histological evaluation. The expression levels of ABCB1 and ABCG2 mRNA were similar in the vitrified control compared with fresh control. However, there was a down-regulation of ABCB1 and ABCG2 mRNA ($P<0.05$) in the fresh culture and vitrified culture treatments compared with fresh control. With respect to the follicular morphology, there was no significant difference between the fresh control and vitrified control in the rate of morphologically normal follicles. On the other hand, after two days of IVC, the percentages of morphologically normal follicles from tissues of fresh culture and vitrified culture groups significantly decreased when compared to the fresh control ($P<0.05$). In addition, the percentage of developing follicles was significantly higher in the cultured treatments (fresh culture and vitrified culture) when compared to fresh control and vitrified control ($P<0.05$). A relationship between degenerated follicles and mRNA expression levels of ABCB1 and ABCG2 was performed by regression analysis. Overall, there was a negative association ($P<0.001$) of degenerated follicles with ABCB1 and ABCG2 gene expression. There were no detectable levels of the MRP2 gene expression in this study. In conclusion, our data revealed for the first time, the gene expression for ABCB1, and ABCG2 in ovine ovarian cortex. In addition, the altered expression of these proteins channels may indicate a deleterious effect of the osmotic stress on the follicular survival during the vitrification procedure. Furthermore, only after IVC these effects were detected. Nonetheless, further studies are required to investigate the role of ABC transporter in ovine folliculogenesis, specially after in vitro culture of ovarian tissue.

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Growth of ovine preantral follicles in situ cultured with Insulin-Like Growth Factor 1(IGF-1) and Epidermal Growth Factor (EGF)

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The ovary is an organ that provides a suitable environment for the production of various substances, such as growth factors and hormones. The manipulation and culture of oocytes included in ovarian preantral follicles is one of the main tools currently used for studies of the initial folliculogenesis and rescue of primary oocytes, to cultivate them *in vitro* until maturation. Therefore, this study aimed to verify the effect of EGF-associated factor IGF-1 on the survival, activation and growth in culture of preantral follicles enclosed in ovarian tissue of sheep. The ovaries were obtained from ovine abattoirs (n: 60), and the ovarian cortex was fragmented. The control group consisted of fresh fragments immediately preserved for later analysis and the remaining fragments were cultured in vitro for 8 days. The cultures were incubated at 39 ° C and 5% CO^2 in 24-well plates, each well containing 1mL Minimum Essential Medium added supplements (α-MEM +) supplemented or not with IGF-1 (50 ng / mL) and EGF (50 ng / mL) , or the association of IGF-1, EGF and pFSH (50ng / mL). After culture, all fragments were fixed and submitted to usual histology technics, and stained with hematoxylin-eosin. Follicles were classified as primordial, developing (primary and secondary) as well as normal or atresic. In addition, the follicular diameters were evaluated. The evaluation was carried out in triplicates and compared to cultured and fresh control groups. Data were analyzed by ANOVA. The results showed that α-MEM + treatment plus IGF-1 and EGF, and α-MEM + treatment plus IGF-1 and EGF and FSH resulted in a greater follicular development, higher normal follicular index and greater diameter of developing follicles than α-MEM + treatment. Thus, the concentration of 50 ng / ml of IGF-1 associated with 50 ng / ml of EGF, or 50 ng / ml IGF-1, 50 ng / ml EGF and 50 ng / ml pFSH increase the diameter of follicles developing and follicular development in preantral follicles. Further studies are now being performed, including immunohistochemistry for evaluation of folicular apoptosis after culture. (CEUA-UFRPE-Process N 23082.016487 / 2012-27).

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In vitro culture of bovine ovarian tissue fragments in the three-dimensional magnetic levitation system associated with nanoparticles

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The use of the three-dimensional in vitro (3D) culture system is an attempt to represent the in vivo system and recreate a similar environment to the extracellular matrix of the ovary. This system improves the modulation of cellular behavior, growth, response and stimulus evaluations, and the communication with surrounding cells. The aim of this work was to evaluate the development of bovine ovarian tissue fragments three-dimensional in vitro culture using nanoparticles (gold, iron oxide and poly-L-lysine) associated with magnetic levitation at different concentrations and compare to traditional culture system (2D). After 07 days, the cell viability of the fragments was evaluated, verifying the production of reactive oxygen species (ROS), apoptosis and cell degeneration by confocal microscopy. The ovaries were collected from a local slaughterhouse and fragmented at the laboratory (3x3x5 mm). The sample were distributed in the 24-well plates with the following treatments: two-dimensional (2D), three-dimensional (3D) culture with 300µL / mL and three-dimensional (3D) concentration of 400µL/mL. The confocal analyzes were performed immediately after collection (D0), 24 hours after cultivation (D1) and at the end of culture period (D7). It was evaluated the viability, apoptosis and oxidation of ovarian tissue. The wavelength for each dye was pre-programmed and the sections performed at 8-micron intervals averaging 8 to 12 Z-Stack sections. Statistical analyzes were performed using Sigma Plot 11.0 software. Data were presented as mean (± standard error of the mean) and percentage and the results considered significant when $P < 0.05$. In general, the three-dimensional culture treatment with 400 µL / ml of nanoparticles presented the lowest ($P < 0.05$) rate of cellular degeneration detected by propidium iodide. After 24h (D1) of culture, all treatments had lower rates of cellular degeneration ($P < 0.05$) compared to fresh control. At the end of the 7 days of cultivation (D7) the treatment of 400 µL / ml presented the lowest rates of cellular degeneration ($P < 0.05$). The level of cell apoptosis on the first day of culture was lower ($P < 0.05$) for t of 300 µL / ml and 400 µL / ml treatments compared to the fresh control group. Two-dimensional (2D) treatment presented apoptosis rate similar to fresh control ($P > 0.05$). Moreover, all treatments presented similar apoptosis rates ($P > 0.05$) and lower than the fresh control ($P < 0.05$). In addition, the apoptosis rate was generally similar between the three-dimensional and two-dimensional culture groups ($P > 0.05$). On the first day of in vitro culture, the emission of ROS was lower ($P < 0.05$) in the three-dimensional culture groups compared to fresh control and two-dimensional (2D) culture. After the end of the culture, ROS emission reduction was observed for all the cultured groups compared to fresh control ($P < 0.05$). Additionally, the three-dimensional culture groups the ROS emission remained constant between the first and the last day of in vitro culture ($P > 0.05$). The cell degeneration index has a positive correlation with ROS yields and rates of apoptosis. Finally, we observed a beneficial effect of the three-dimensional magnetic levitation system on the viability of ovarian fragments.

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Influence of the use of GnRH according to the diameter of the follicle at the time of insemination on the conception rate of Nelore cattle in FTAI programs

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Failure to detect estrus and postpartum anestrus are factors that contribute to low herd fertility. Fixed-time artificial insemination (FTAI) is a technique to reduce these problems by synchronizing ovulation with the use of drugs and establishing the correct timing of insemination. There are several protocols for FTAI, consisting of hormonal combinations, according to the type or the animal category in question. Among the most commonly used hormones, there are GnRH analogs that induce the pre-ovulatory peak of luteinizing hormone (LH) and consequent ovulation or luteinization of the follicle inducing a new wave of follicular growth. These drugs can be used strategically within a synchronization protocol, improving the follicular characteristics, ovulatory capacity and the quality of the corpus luteum. Therefore, the objective of this study was to evaluate the influence of the use of GnRH according to the diameter of the follicle at the time of insemination on the design rate in FTAI programs. For this study, 184 lactating Nelore bovine females submitted to the same FTAI protocol were used, differing only in the application or not of the GnRH analog at the time of AI. On the random day of the estrous cycle, day 0 (D0), cows received 2mg of estradiol benzoate (Gonadiol®, Zoetis, São Paulo, Brazil) intramuscularly (IM), associated with an intravaginal progesterone device (DIB®, Zoetis , São Paulo Brazil). On day 8 (D8), the device was removed and 500µg of Cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) IM, 1mg of estradiol cypionate (ECP®, Zoetis, São Paulo, Brazil), Equine chorionic gonadotropin IU (Novormon, Zoetis, São Paulo, Brazil) IM. On day 10 (D10) all animals were examined by transrectal ultrasonography to measure the diameter of the ovulatory follicle (DFOL) immediately after they were inseminated. At that time, the females were categorized into two groups according to the diameter of the largest follicle and randomly received the application of 10 µg of buserelin acetate (Sincroforte®; Ouro Fino, São Paulo, Brazil) IM. The gestation diagnosis was performed by ultrasonographic evaluation 30 days after AI. The Statistical Package for the Social Sciences (SPSS) version 13.0 was used for statistical analysis and the variables were analyzed using the chi-square test, with a significance level of 5%. The overall conception rate was 46.2% (85/184); no statistically significant effects of GnRH at conception rate were observed between the groups with GnRH 44.2% (34/77) and no GnRH 47.7% (51/107), (P = 0.065). As for the DFOL groups (≤13 mm), with 33.3% (18/54) without GnRH and 29.3% (12/41) with GnRH, (P = 0.273), and the groups DFOL (> 13 mm) without GnRH rate of 69.1% (29/42) compared to GnRH with 65.6% (21/32), (P = 0.258). Thus, it can be concluded that the administration of the GnRH analog at the time of AI, in general, did not influence the gestation rate of the animals submitted to the FTAI protocol.

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Investigation of the initial damage after transplantation of fresh and cryopreserved ovarian tissue in domestic cats

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Ovarian tissue transplantation has been an effective strategy to preserve fertility in humans and could be considered for wild felines conservation. Previous studies of our group have confirmed the success of the heterotopic transplant of fresh ovarian tissue in domestic cats, with development of antral follicles from 28 to 63 days post-transplantation. In contrast, other studies with cryopreserved ovarian tissue, have demonstrated a marked reduction in the number of follicles as soon as day 7 after transplantation. The present study aimed to evaluate the follicular morphology and vascularization in fresh and cryopreserved ovarian tissue of domestic cats in the first 6 days after transplantation. Ovaries of five cats were divided into eight fragments, one of which was immediately fixed (D0) and three were immediately transplanted (fresh group) into the subcutaneous tissue of the dorsal neck. The other four fragments were cryopreserved (slow freezing with 1.5M DMSO) and kept in liquid nitrogen for 2 days. After thawing, one fragment was fixed, (D0) and the three remaining fragments were transplanted (cryo group) to the region. Both fresh and cryopreserved ovarian tissue fragments were recovered on days 2 (D2), 4 (D4) and 6 (D6) post-transplantation and processed for histology. Sections were stained with hematoxylin and eosin and all preantral follicles were counted and classified as morphologically normal or degenerated. Smooth muscle actin antibody was used to identify vascularization in the ovarian tissue samples, and the percentage of tissue area occupied by vessels was calculated. The percentage of morphologically normal follicles (MNF) was analyzed by the chi-square test and the area of vascularization by ANOVA and Tukey test. Data were considered significant when $p<0.05$. The mean percentages of morphologically normal follicles of fresh ovarian tissue before transplantation (D0) and on D2, D4 and D6 post-transplantation were, respectively, 82.8±12.2, 33.3±20.3, 46.9±28.4 and 55.3±36.7. In the cryopreserved material, the mean percentages of morphologically normal follicles were 57.5±20, 34.1±47.2, 22.2±44.4 and 0±0, respectively for D0 (before transplantation) and D2, D4 and D6 post-transplantation. The percentage of MNF in fresh ovarian tissue was significantly higher ($P<0.05$) than in cryopreserved tissue on D0, D4 and D6, but not on D2. Within the cryo group, the percentage of MNF decreased gradually and significantly from D0 to D6 ($P<0.05$), while within the fresh group there was no difference ($P>0.05$) between D4 and D6. Concerning vascularization, there was no significant difference ($P> 0.05$) between fresh and cryopreserved tissue. However, in both cases, the area occupied by blood vessels was significantly larger ($P <0.05$) on D4 and D6 compared to D0, and there was no difference from D2 to any other day analyzed. In conclusion, although there was no difference in the vascularization of fresh and cryopreserved tissue after transplantation, the loss of MNF was more accentuated in cryopreserved tissue than in fresh tissue. Cryopreserved tissue showed a gradual decrease in the percentage of MNF in the first 6 days after transplantation, reaching zero on D6. Therefore, it is still necessary to develop efficient alternatives to promote the survival of follicles in cryopreserved ovarian tissue.

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Isolation and analysis of stem cells MUSE (multilineage-differentiating stress-enduring) derived from bovine fetuses

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SKPs are multipotent cells found in the dermis region that have the differentiation potential in several cells. Recent studies have reported that it is possible to obtain skin cells with differentiation ability in three germline and specific markers such as SSEA-3 and CD105, these cells can be separated by trypsin stress, called MUSE. These cells are extremely rare in the skin but have the advantage of being stress tolerant, showing more than 20-fold expression of the SSEA-3 marker after injury.Thus, the objective in this work is to establish MUSE cells and to generate SKPs from fetal bovine fibroblasts, aiming at future application in reproductive biotechnologies. Three skin fragments of female fetuses of bovine animals aged 3 to 5 months were used, originating from an slaughterhouse of the city of Castanhal. To obtain the fibroblasts, the biopsy fragments were washed with phosphate-saline buffer solution (PBS), cut with scalpel aid into smaller fragments and plated in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and penicillin / streptomycin (DMEM-10%) and incubated in culture oven at 37 ° C and 5% CO2. For the stress protocol, bovine fetal fibroblasts were subjected to stress for 3 hours at 37ºC, followed by 18 hours at 4ºC and cultured in DMEM / F12 medium plus growth factors and B27 for 3 and 6 days. For the SKPS evaluations from the MUSE cells the quantification of the number of spheres formed, alkaline phosphatase assay and immobilocytochemistry were performed. The results showed that MUSE cells were established and generated beads from bovine fibroblast labeled positive multipotent cells. However, they were negative for alkaline phosphatase which is a marker of pluripotency, besides being of lower quality when compared to the control group, indicating that they are not indicated for the formation of SKPs.

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Mangalarga Marchador stallion scrotal thermography: correlation with spermatic movement

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Intratesticular temperature control is one of the requirement for efficient spermatogenesis. In domestic mammals a temperature from 2 to 6°C below body temperature is essencial for the production of fertilizing spermatozoa. Forty-three Mangalarga Machador stallions (range: 4-18 years old) were used. Data was collected from breeding farms in Coaraci, Itabuna, Itajuípe and Itapé cities, located in the southern state of Bahia, Brazil. The thermograms were performed with FLIR E60bx® Thermovisor and the images analyzed by FLIR Tools® software (version 5.12.17023.2001). The thermovisor focus (e=0.98) was directed one meter apart to the scrotal surface and oriented perpendicularly to measure seven points of the of both testicles as follows: 2 points in the proximal zone, 3 points in the middle zone and 2 points in the distal zone. After obtaining the temperatures of each zone per testis, the mean temperature of both testicles was determined.. The animals' rectal temperature was also measured by digital clinical thermometer. Semen collections were performed using an appropriate artificial vagina, Botucatu® model, previously filled with warm water, in order to guarantee an internal temperature of 42º C. A dummy were used for semen collection. The ejaculateswere evaluated for macroscopic and microscopic parameters according to the Brazilian College of Animal Reproduction. The data from temperatures in the proximal, middle and distal areas of the testicles as well as the total surface temperature were evaluated for normality by the Shapiro-Wilk test. ANOVA was performed to compare spermatic parameters and Pearson correlations between zone temperatures and total surface temperature of the testes with the seminal parameters of spermatic movement. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS). The thermal behavior of the testicles of all stallions studied was 33.9 ± 0.8°C for the proximal region (PR), 33.9 ± 0.9°C for the middle region (MR), 34.0 ± 1, 0°C for the distal region (DR) and 33.9 ± 0.9°C for the total testis surface (TSTT). All testicular zones evaluated, as well as the total superficial temperature of the testicles presented lower mean temperature in relation to the rectal temperature (37.8 ± 0.4°C). The, difference of 3.9°C for the PR; 3.8°C for MR; 3.9°C for DR and 3.9°C for TSTT, create a favorable condition for spermatogenesis. Sperm movement characteristics were: 65.6 ± 14.7 (%) of total motility, 59.2 ± 15.2 (%) of progressive motility and 3.3 ± 0.4 of vigor. There was no significant correlation ($P > 0.05$) between the testicular zone temperatures (proximal, middle and distal) and the total superficial temperature of the testis with sperm movement parameters. Mangalarga Marchador stallions from southern Bahia had testicular temperature around 34ºC in the study period, within the ideal conditions for spermatogenesis, indicating a good thermoregulation condition. Seminal quality changes occur when a transient or permanent increase on intratesticular temperature above the physiological limit is verified.

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Membrane integrity of canine testicular sperm retrieved after tissue vitrification

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Vitrification is an ultra-rapid freezing method that can prevent ice-crystal formation, presenting excellent studies and results about gonadal tissue cryopreservation. In Brazil, there are 28 species of terrestrial carnivores, according to ICMBio, four are endangered, in the vulnerable category. The aim of this study was vitrifying testicular tissue from post-pubertal mongrel dogs (n=20) from the Center of Environmental Control, Garanhuns, Pernambuco, Brazil, using Dimethyl sulphoxide (DMSO) and Ethylene glycol (EG) as cryoprotectant agents in two different immersion times (15 and 30 minutes). The testis-epididymis complexes were washed with a saline solution containing 1% gentamycin, dissected from epididymis and fragmented. After exposure to equilibration (modified TCM 199 plus 20% FCS, 7,5% DMSO and 7,5% EG)/Room temperature/10 minutes) and freezing media (modified TCM 199 plus 20% FCS, 15% DMSO, 15% EG and 0,5M sucrose/4°C/15 or 30 min), testicular tissue fragments were subjected to vitrification by laying them on an aluminum surface over the liquid nitrogen and stored (-196ºC). After one week, all samples were thawed at room temperature for 30 seconds and transferred to the thawing medium (modified TCM 199 plus 20% FCS and 1M sucrose) at room temperature for 10 minutes and then moved to a retrieval medium (modified TCM 100 plus 10% FCS) for sperm recovery. After 10 minutes, sperm membrane integrity was assessed by fluorescent microscopy, using the combination of two stains, SYBR 14 and Propidium Iodide (Live/Dead® Sperm Viability Kit L - 7011, Molecular Probes). Spermatozoa were classified as intact/live (bright green color) or lesioned/dead (red color). Data were subjected to normality test by Shapiro-Wilk and Wilcoxon-Test (R 3.2.3) to compare treatments ($P < 0.05$) (R3.2.3, Package PWR). Treatments were all different from control group ($P<0.05$), however, immersion time did not significantly influence the testicular spermatozoa membrane integrity on both times ($P>0.05$). Membrane integrity in control, 15 and 30 minutes immersion were 79.30% ± 21.23, 14.06% ± 8.73, 13.74% ± 12.63, respectively. In summary, vitrification is an excellent and practical method able to contribute to germoplasm conservation. Nevertheless, more studies must be performed for protocol adaptation.

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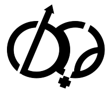

Morphological viability of preantral follicles included in bovine ovarian tissue after xenotransplantation

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Currently the number of patients surviving after cancer treatment has increased. However it is known that some patients may have limited ovarian function, since the methods used to seek the cure of cancer, mainly chemotherapy and radiotherapy techniques, are toxic to the reserve of ovarian follicles, being responsible for infertility in women. Ovarian tissue transplantation is a promising technique to preserve endocrine function and fertility after oncologic treatment. The aim was evaluate the morphological viability and follicular development of bovine ovarian tissue after a xenotransplantation procedure in female mice of the Balb C line. Seven pairs of bovine ovaries, collected from a local slaughterhouse, were divided into three replicates. After collection, the ovarian cortex was fragmented (3 x 3 x 1 mm) and randomly divided into fresh control and fresh xenotransplantation groups. In each mice, after anesthesia, five fragments of ovarian tissue were transplanted and fixed to the peritoneum in the abdominal cavity. Graft recovery was done seven days after transplantation and all fragments were analyzed by classical histology. The data were compiled and analyzed with the aid of the Sigma Plot program. The percentage of morphologically normal ovarian follicles found in the group of fragments submitted to xenotransplantation, 69.9% (519/742), was superior (P <0.05) to the fresh control group, 63.8% (424/664). Regarding the distribution of follicles in the development stage, the group of fragments submitted to fresh xenotransplantation presented a reduced percentage (P <0.05) of normal primordial follicles, 45.1% (234/519), compared to the fresh control group, 58.7% (249/424). On the other hand, the proportion of normal transitional follicles was higher (P <0.05) in the xenotransplant group, 41.2% (214/519). In addition, the percentage of normal primary follicles was similar (P> 0.05) between the groups analyzed. Surprisingly, the proportion of normal secondary follicles of the fresh xenotransplantation group, 6.7% (35/519), was higher (P <0.05) than the fresh control group, 2.8% (12/424). The follicular activation rate was higher (P <0.05) in the fresh xenotransplantation group, which consequently presented a lower percentage (P <0.05) of normal primordial follicles. The seven-day ovarian tissue xenotransplantation procedure stimulated moderate follicular activation, suggesting that xenotransplantation provided a viable environment for the maintenance of the reserve of primordial follicles. Probably the removal of ovarian environment leads to the absence of inhibiting factors of follicular activation process and may stimulate follicular activation mechanisms. The environment in which the ovarian fragments were implanted provided enough conditions for the maintenance of the follicular viability even of those in more advanced stages. It is being performed further studies, including analysis of immunohistochemistry, PCR, confocal and cytogenetics to complement the research of tissue and follicular viability after xenotransplantation. (CEUA/UFU/Protocol 006/17).

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Morphology and membrane integrity of spermatozoa from canine cryopreserved testicular tissue

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Creating a germplasm bank is an important tool for germ cell conservation for animals with high zootechnical value as well as endangered species. Although there is no report on cryopreservation of canine testicular fragments, the domestic dog presents a good experimental model for endangered canids. An ideal protocol for testis cryopreservation should preserve DNA integrity and sperm membrane since these aspects are important for future ICSI or xenotransplantation. Thus, the aim of this study was to verify the possibility to cryopreserve fragments of canine testicular tissue from post-pubertal mongrel dogs (n=20) from the Center of Environmental Control, Garanhuns, Pernambuco, Brazil, using two-step freezing and ethylene glicol (EG) and dimethyl sulfoxide (DMSO) as cryoprotectants. After orchiectomy, testicles were washed using a saline solution (1% gentamycin), dissected from epididymis, fragmented and submitted to equilibrium (modified TCM 199 plus 20% FCS, 7,5% DMSO and 7,5% EG)/Room temperature/10 minutes) and freezing media (modified TCM 199 plus 20% FCS, 15% DMSO, 15% EG and 0,5M sucrose/4°C/15 or 30 min), two-step freezing and stored (-196ºC). After one week, all samples were thawed. Testicular sperm membrane integrity was assessed by fluorescent microscopy, using the combination of two stains, SYBR 14 and Propidium Iodide. Spermatozoa (200 cells) were classified as intact/live (bright green color) or lesioned/dead (red color). Testicular fragment tissue was also evaluated about histology. The epithelium was assessed by the detachment of cells from the basement membrane, scored as zero if absent, one if mild, two if partial, and three if total or observed on 75% of the circumference. Nuclear condensation was scored as zero if absent or present in only one nucleus, as one if, 40% of nuclei were condensed and as two if 40% were pyknotic. Data were submitted to normality test by Shapiro-Wilk and Wilcoxon-Test (R 3.2.3) to compare treatments (P <0,05). Concerning membrane integrity, there was no difference between DMSO and EG (38.84%±16.22 vs 37.65%±13.01) and both were different from control group (79.30%±21.2). The two cryoprotectant agents were similar regarding nuclear condensation and about 60% of sample presented score 2 (40% pyknotic nuclei) but were similar to control. It was observed a significative detachment on DMSO and EG (compared to control), and the first one made a better conservation, prevented more efficiently nuclear condensation, observing 65% of the samples with a score 0 or 1 (condensation absent or present in only one nucleus). The two-step freezing process is a simple technique for the cryopreservation of canine testicular tissue with promising results using dimethyl sulfoxide or ethylene glycol as cryoprotectants.

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Noninvasive analysis of oocyte quality by near infrared after *in vitro* maturation in cattle

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The improvement of competent oocyte selection criteria aims to improve the embryo development potential and consequently the success of biotechnologies of *in vitro* embryo production. The main techniques used for assessing viability and oocyte selection involve subjective analyzes of cumulus cell morphology and cytoplasmic integrity that culminate with variable results on *in vitro* production of embryos. The objective of the present study was to verify the ability of near infrared (NIR) to identify the quality of *in vitro* matured oocytes by absorbance spectra. For this, bovine ovaries were obtained from local slaughterhouses and transported to the laboratory in 35°C thermos box containing saline solution (0.9% NaCl). In the laboratory, follicles above 2 mm diameter were aspirated with syringe attached to 21G needle to collect the cumulus-oocyte complexes (COCs), which were handled in TCM199-Hepes® medium containing sodium pyruvate, 1% antibiotic and 2% fetal bovine serum (FBS). The COCs were selected and divided into two groups according to morphological characteristics of the cytoplasm and cumulus cells in viable (V) and non-viable (NV): V – at least 3 layers of cumulus and homogeneous oocyte cytoplasm; NV - oocyte with abnormal, denuded and/or heterogeneous cytoplasm or incompact or expanded cumulus. For *in vitro* maturation (IVM), a group of 20 COCs per 100 µL drop were incubated in TCM199® medium supplemented with LH, FSH, cysteamine, EGF, estradiol and 10% FBS in petri dishes under mineral oil, at temperature of 38.5°C, 5% CO_2 and saturated humidity for 22 h. After the IVM, the culture medium of each group was collected and stored in freezer cryotubes at -80°C for NIR analysis. A total of 50 µL of culture media from each group (V and NV) were analyzed by NIR (DA 7200 Perten®), in excipient itself at temperature of 25°C. The spectra in NIR were obtained in duplicates, with 4 minutes of exposure at the wavelength between 955 and 1650 nm. The spectra were evaluated by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) using the mixOmics package in R programming environment. The first and second major components retained 97% and 3% of the variance total of data. By evaluating the scores of the main components of the experimental units, we can observe a separation between the two experimental groups, in which the observations of group V presented negative scores for the second main component, while the group NV showed positive values. The first and second major components retained 97% and 3% of total variance of data. By evaluating the scores of the main components of the experimental units, we can observe a separation between the two experimental groups, in which the observations of group V presented negative scores for the second main component, while the group NV showed positive values. The wave length 1145 had the highest loadings for the first component. For the second component, the wavelengths with the highest loads were 1,590 and 1,595, that is, the different feasibility groups produced different spectra. In visual evaluation of spectra, we observed an overlap of the curves of the groups V and NV, with a significant variation between the repetitions within the groups. The use of partial least squares for discriminant analysis (PLS-DA) presented a cross validation error rate for the groups with three main components of 0.049. We performed 100 simulation routines using different training and test groups, totaling 617 predictions, of which 611 observations had their group correctly predicted (V or NV). The sensitivity, specificity and accuracy values were 100% (only one wrong prediction), 95.52% and 99%, respectively. In this way, we can conclude that it is possible to successfully characterize bovine oocytes of different quality from spectra of culture medium obtained during the IVM process.

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Orthotopic Autotransplantation of Ovarian Cortex in Domestic Cats

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The aim of this study was to develop and attest the efficacy of a technique for orthotopic autotransplantation of ovarian cortex in domestic cats. Adult female cats (N=5) were used and the technique tested was based on techniques used for orthotopic autotransplantation in humans. Since primordial follicles were on a 200 µm maximum depth from the surface of the ovary (data from a preliminary study), 1mm thick slices were cut until the ovarian cortex was completely removed from both ovaries. Afterwards, cortex slices from one ovary were grafted on the remainder medulla of the contralateral ovary, while the other ovary was kept with only the medullar region as a control. Both ovaries were weekly scanned by ultrasound for 6 weeks. By the end of this period, both ovaries were surgically removed and processed for histology. Antral follicles were observed by ultrasound on both ovaries of all the cats. Adherences were also observed. Under light microscopy, both ovaries still presented primordial follicles after 6 weeks, although lower numbers were observed on ovary that did not receive cortex grafts. Thus, a second experiment was performed to compare the orthotopic implant directly on the medullar region with implants on peritoneal pockets (also considered orthotopic). Follicular development was observed both on medullar and peritoneal grafts. However, antral follicles development still occurred on the medullar region that did not received grafted tissue. The number of primordial and growing follicles (per 5mm3) on samples grafted to the peritoneal pocket (2655±1322 and 1854±1703) were statistically similar ($P>0.05$) to those found on samples grafted to the ovarian medulla (2432±1681 and 1296±1834). The percentage of morphologically normal primordial follicles was significantly lower on peritoneal grafts (32±14) than on medullar grafts (46±23), however the percentage of morphologically normal growing follicles was similar (56±11 x 57±13, respectively). Since the main objective of stablishing the technique of ovarian tissue transplantation in cats is the future possibility to use the domestic cat as the surrogate for wild felines' ovarian tissue, it is essential that the development of follicles are exclusively from the graft. In summary, with this work we concluded that removing 1 mm thick slices of ovarian cortex is enough to recover most primordial follicles, but not enough to render the queen infertile; grafting the cortex slices on the remainder medullar tissue does not guarantee that developing follicles are from the grafted tissue, and the implant on peritoneal pockets allow good conditions for follicular development in cats. This way, for now, the peritoneum is the best local for the orthotopic transplant of ovarian tissue, and may be a suitable site for the xenotransplantation of wild felines' ovarian tissue to domestic cats.

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SlimCASA software efficiency for analysing concentration of equine sperm: preliminary results

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This study aimed to develop a software for equine sperm evaluation, from images taken under a microscopy. This program should be compatible with notebooks and PCs for home use, easy to use, in the Portuguese language and free. It is intended, with this software, standardize the sperm concentration analysis carried out by veterinarians during andrological examinations at field, cheapening the cost of acquisition of specific equipment. Twenty-eight images of equine semen were made by microscopy from thawed commercial semen doses. The 0.5 mL reed were thawed for 30 seconds in a water bath at 37°C. Semen drops were deposited on Hamilton Thorne (Spectrun), slides and Neubauer chamber both covered with cover slip for microscopy. The images were obtained from phase contrast microscope (Jenamed2) with 1.3 MP camera attached (Coleman). The software was developed from resources already available in an open source Java solution called ImageJ. The approximate count of the sperm contained in the image was possible through particle analysis capabilities. Initially, the video images were converted into frames and subjected to some treatments, using only 8-bit color and segmenting grayscale so that the software could do the analysis of the image particles. The 28 semen samples were analyzed by the technician in Neubauer chamber, by Hamilton Thorne CASA and by the software SlimCASA for determination of spermatozoa concentration (from microscope image video). For statistical analysis, the results of the sperm concentration were subjected to analysis of variance (SAS, 2012) at a significance level of 5%. The average values of the sperm concentration were 182.90 ± 71.51a by the Slim CASA software, 241.56 ± 131.34b by the Hamilton Thorne CASA and 184.79 ± 69.54a by the technician ($p < 0.05$). The software was highly efficient for sperm concentration, being a convenient and easy to use solution. The CASA instruments have shown high levels of accuracy and reliability using different methodologies of classification that provide a great tool to improve our knowledge and ability to analyze sperm, making it essential to research, personnel training and standardization between laboratories. Regardless of the manufacturer, the different instruments are based on similar principles, but differ in terms of optics and software used to identify the sperm and the construction of the track, respectively. Our differential is the gratuity and ease of use, since it is a specific software for sperm analysis and available in Portuguese. In conclusion, the SlimCASA software showed the same efficiency as the concentration carried out by the technician and was better than Hamilton Thorne CASA.

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Sperm kinematics of sorting goat semen in continuous density gradients

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The possibility to pre-determine the sex of progeny by sperm sexing is a permanent desire of the livestock industry. In goats, the use of sexed semen, in combination with other biotechnologies of reproduction, is considered a means of increasing reproductive and productive efficiency of these animals. Allied to this, the big difference between the DNA content in goat spermatozoa, carrying the X and Y chromosomes (4.4%), makes the sexing technique in Percoll density gradients attractive for use in this species with lower cost; despite its lower acuity. Thus, the aim of this study was to evaluate the sperm kinetics before and after separation in Percoll density gradients. Ejaculates from five mature male goats were collected at 48 h intervals by artificial vagina, totalizing six *pools*. The Percoll density gradients was prepared by diluting (9:1, v:v) of commercial Percoll® (PercollTM; GE Healthcare Bio-Sciences AB, Rapsgatan, Uppsala, Sweden) in DMEM solution 10X concentrated (135 g DMEM, 0.3% BSA, 10 mg/L antibiotic, 6 mM HEPES, 1 L water Milli-Q q.s.ad). The 90% Percoll solution was used as a basis to form Percoll solutions at 85 and 80%, which was made possible by new dilutions in medium 1X DMEM concentrated (13.5 g DMEM, 0.3% BSA, 10 mg/L antibiotics, 6 mM HEPES, 1 L water Milli-Q q.s.ad). Each one of the six semen *pools* (n=6) were diluted in 1X DMEM (pH 6.8) to the end concentration of 800 x 10^6 sperm/mL. After, the semen samples were homogenized and aliquots (500 µL) were deposited on the density gradients. The tubes containing the gradient were centrifuged at 2000 rpm in a horizontal rotor (Baby®I, Model 206 BL, Fanem® Ltda., SP, Brazil.) for 20 min at room temperature (24 °C). Subsequently, the supernatants were removed using an automatic pipettor. Aliquots of fresh semen selected or not selected, were diluted in 1X DMEM to an approximate concentration of 50 x 10^6 sperm/ml. The parameters evaluated using the computerized system (CASA) were: total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, µm/s), straight line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN, %), straightness (STR, %), wobble (WOB, %), amplitude of lateral head displacement (ALH, µM) and beat cross frequency (BCF, Hz). For statistical analysis, one-way ANOVA and Tukey-Kramer were used, with 5% significance. Regarding to sperm kinematics, goat semen samples selected in Percoll® continuous density gradients showed higher ($P<0.05$) progressive motility (63.8±11.7), LIN (88.0±4.3), STR (77.3±2.3) and WOB (95.1±1.8) than samples not selected (41.1±4.6, 65.8±4.9, 77.3±2.3, 85.1±3.9, respectively); parameters positively correlated with fertility rate. The ALH of the not selected (2.8±0.2) group was higher ($P<0.05$) than of the selected group (1.8±0.2); parameter which is associated with sperm hyper activation. Thus, centrifugation of semen on Percoll® continuous density gradients showed to be a viable technique to sorting goat sperm, once that select gametes with greater competence to move. Furthermore, continuous gradient can be stored, which facilitates the commercial use of this technique. However, in the future is necessary determine the accuracy in separate X and Y goat sperm populations by this methodology, which is a determinant factor for its use in sperm sexing.

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Spermatozoa morphometry of the jaguar (*Panthera onca*)

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Sperm morphometry allows to describe subtle characteristics of the structure of the spermatozoa, as well as to generate reference data of the measurement of the different regions of the gamete. In addition, morphometric analysis has been studied in several species with the purpose of giving subsidy to computerized seminal evaluation systems, or even to differentiate peculiarities of the reproductive biology of each species and subspecies. In this context, the jaguar does not present studies referring to the morphometric description of its sperm cells, being fundamental the increment of this study for the understanding of the sperm characteristics of this feline. The aim of this study was to describe the morphometry of spermatozoa from the fresh semen of jaguars. For that, seminal collection by electroejaculation of five male jaguars (*Panthera onca*), aged between 4 and 17 years, healthy was performed. The animals belonged to the institutions: Dois Irmãos Park (Recife - PE); Bica Zoo (João Pessoa - PB); Ecopoint (Fortaleza - CE); São Francisco Zoo (Canindé - CE) and Zoological Park of Teresina (Teresina -PI). For adequate sedation and analgesia, the combination of dexmedetomidine (0.04 mg/kg) and ketamine (5 mg/kg) injected with a blowpipe was used. The animals had their penis exposed, sanitized and approached to a polypropylene tube previously heated at 37 °C. Prior to collection, they also had the bladder emptied and washed with 0.9% sodium chloride solution to avoid possible contamination of the semen with urine. The electroejaculation protocol consisted in the application of three series, totaling 10 increasing electrical stimuli (1st series: 5 V, 6 V and 7 V, 2nd series: 6 V, 7 V and 8 V, 3rd series: 8 V and 9 V), with duration of 2s for each stimulus. From the obtained semen, a 10 µL aliquot was used to make the smear with Rose Bengal. Afterwards, 200 sperm cells of normal morphology were collected in random fields for each seminal sample. Images were evaluated using an optical microscope (1000 X) connected to the computer and ImageJ software to measure the spermatic structure of the head (width and length), intermediate piece (length) and tail (length), as well as the total size of the cell. Data were expressed as mean ± standard deviation. The measurements of the spermatic head were 3.6 ± 0.8 µm and 4.9 ± 0.5 µm for width and length, respectively. The intermediate piece had 9.7 ± 1 µm in length, while in the tail region it was 54.5 ± 4.4 µm. The total length of the sperm cell was 59.4 ± 4.4 µm. In conclusion, data from the present study provide information on the morphometric pattern for the jaguar species. These results may be used in future research on germplasm conservation of endangered wild felines, as well as assist in the standardization of calibration and devices for computerized analysis.

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Spermfilter® an alternative for seminal plasma separation in boars?

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The presence of seminal plasma during cryopreservation may or may not exert beneficial functions. To concentrate the sperm cells, seminal plasma removal should be performed. Although centrifugation is the technique currently used for seminal plasma separation, it has been related to sperm cell damage, thus, ejaculate filtration has emerged as an alternative to minimize damage caused by centrifugation. The aim of this study was to test the efficiency of Sperm-filter® for seminal plasma separation by evaluating sperm viability, sperm kinetics, the occurrence of hyperactivation, and lipid peroxidation reactions, for up to 96 hours of refrigeration. Eight adult boars of the Landrace, Pietrain, Large White, and Duroc breeds were used, with an average age of 2 years. Ejaculate collection was performed by means of the gloved hand technique. Immediately after collection a sample of 30 to 50 mL was diluted in BTS in a ratio of 1: 1. In the laboratory, the samples were divided into three equal parts: total semen + BTS (TOTAL), centrifuged at 600g for 10 minutes + BTS (CEN), and filtered using Sperm Filter® + BTS (FIL). A final concentration of 50 x 10^6 spermatozoa/ml was stipulated for all groups. The sperm characteristics were evaluated at three moments: fresh (D0), 48h (D2), and 96h (D4) after refrigeration at 17 ° C. Sperm kinetics were evaluated by computerized analysis (CASA system). Hyperactivation was considered when the sample presented ALH> 7µm, VCL> 80µm/s, and LIN≤ 65%, simultaneously. After the kinetic evaluation, the samples were frozen at 4ºC for 30 days until the oxidative stress measurement by the TBARS technique (thiobarbituric acid reactive substances). Statistical analysis was performed using the Tukey test and Kruskal-Wallis test, p <0.05. The results showed better total motility values (%) for the TOTAL group at D0 (67.9, p=0.001), D2 (36.6, p=0.004), and D4 (26.1, p=0.003). Hyperactivity was observed in the TOTAL group at D4 (ALH = 7.2 µm, VCL = 141.2 µm, LIN = 36.8%). In addition, TBARS showed higher levels of peroxidation for the TOTAL group at D0 (8.1 mM MDA / mL, p = 0.01), D2 (7.4 mM MDA / mL, p = 0.02), and D4 (6.41mMol MDA/mL, p = 0.008) when compared to the FIL and CEN groups. The TBARS reaction showed a gradual decrease in the MDA formation over the cooling time in the CEN group, at D0 (3.9 mMol of MDA/mL), D2 (2.4 mMol of MDA/mL), and D4 (1.7 mMol of MDA/mL), p = 0.03. In the FIL group, the TBARS technique did not identify a difference in peroxidation in the refrigeration periods evaluated (D0: 2.9 mMol MDA/mL, D2 2.7 mMol MDA/mL, D4: 3.8 mMol MDA/mL). The study results indicated that seminal plasma separation is necessary due to sperm hyperactivation induction in TOTAL semen samples after 96 hours of refrigeration, and the increase in ROS production. Based on the results obtained, it can be concluded that the filtration technique with Sperm Filter ® proved to be an efficient alternative for boar seminal plasma removal.

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Study on the viability of Babosa (*Aloe vera*) as a cryoprotectant for sperm cells cryopreservation

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During cryopreservation process sperm cells goes through temperature variations that induces to cryogenic injuries and decreases its viability making it necessary to use animal compounds to minimize these deleterious effects. However, these compounds are difficult to standardize due to variance in its production and risk of contamination. Several studies are developed to produce a vegetable source extender that assimilate to animal source extender commonly used. *Aloe vera* present several active principles and polysaccharides that confers viscous consistency and widely use in the food, pharmaceutical, cosmetic and herbal medicine industries. In view of the above, the aim of this work was to evaluate different concentrations of *Aloe vera* (AV), comparing to an egg yolk extender. Plants of *Aloe barbadensis Miller* were used without regular irrigation to concentrate their active principles in order to obtain the crude extract and this extract was submitted to the test of determination of reducing sugars. Then, four experimental groups were performed: GC= Control Group (20% egg yolk + TRIS + antibiotic); G5= Group 5% (5% AV + TRIS); G10= Group 10% (10% of AV + TRIS); and G20= Group 20% (20% of AV + TRIS). The groups were submitted to hydrogenation potential tests, rheological tests at 5 °C and 37 °C and microbiological growth test with extenders refrigerated at 5 °C for 48h and 72h. The data were evaluated by the Kolmogorov-Smirnov test with 5% significance. For comparisons between the times of the same group, the T test was chosen, and ANOVA was used for comparison between groups, followed by Tukey's post-test ($P \leq 0.05$). In the reducing sugars determination test it was observed that the extract of the AV presents approximately 0.205 g/L of fructose and glucose. For the hydrogenation potential, pH 6.5 was observed in all groups with extract and GC. Difference was observed ($P<0.05$) to viscosity at 5 °C between GC and groups with lower AV concentrations compared to G20 (GC= 3.00±0.33 *vs* G5= 3.64±0.14 *vs* G10= 2.57±0.57 *vs* G20= 5.54±1.96). At 37 °C, difference was observed ($P<0.05$) in GC and G20 compared to G5 and G10 (GC= 1.67±0.20 *vs* G5= 0.78±0.16 *vs* G10= 0.91±0.11 *vs* G20= 1.08±0.18). For the test of microbiological growth, it was observed that the G20, after 72h of refrigeration, presented antimicrobial effect (GC: T48= 160, T72= 40 *vs* G5: T48= 60, T72= 400 *vs* G10: T48= 60; T72= 60 *vs* G20: T48= 140, T72= 0). One of the components present in the extenders is the sugar that provides energetic support and buffer in maintaining the pH close to neutrality. Although the AV extract presents a low concentration of sugars in its composition, this condition may be reversed adding sugar in the extender production process. Regarding the extenders, the hydrogen ionic potential of the AV and control groups remained close to neutrality, which was the optimal pH for spermatozoa. One of the most important characteristics associated with the fertilizing capacity of semen is motility, as it is essential for the transport of spermatozoa in the female reproductive tract and fertilization. Regardless of the concentration of the extract, the viscosity of the AV groups maintained the same rheological behavior of the control group, showing that the viscosity will not interfere on the movement of the spermatozoa in the medium at 37ºC. Another important factor in the extender is the presence of antibiotics that reduces the risk of contamination, which could lead to the loss of the material. The AV groups even without antibiotic addition achieved similar effects to the control group, since the plant has a broad antimicrobial spectrum, acting on fungi and bacteria. Based on the above, it is concluded that *Aloe vera* is a viable alternative as cryoprotectant, has microbiological protection without antibiotic addition. Besides maintaining the pH close to neutrality in the presence of the buffer and presents rheological behavior similar as observed on egg yolk.

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Swim-up with or without caffeine addition after semen thawing increases the percentage of equine sperm with normal morphology

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Post- thaw sperm motility and viability can be poor and thus, partially responsible for the low fertility results after artificial insemination. Therefore, the swim-up technique can be used to select better quality sperm after thawing. Although the recovery rate is usually low, it is important that the recovered sperm have normal morphology. Thus, the aim of the present work was to test the additive effect of different caffeine concentrations after semen thawing on the percentage of equine sperm with normal morphology after swim-up sperm selection. One ejaculate of five stallions was frozen with the INRA82 frozen extender. After thawing (Treatment 1: T1, control without swim-up), swim-up procedures and different caffeine concentrations were added to the semen samples according to the treatments: T2) swim-up (without caffeine addition), T3) T2+3mM caffeine, T4) T2+5mM caffeine, and T5) T2+7.5mM caffeine. To the swim-up, one aliquot of each semen sample was deposited on the bottom of each 1.5 ml conical tube containing Medium 199 with Hank's salt and 10% fetal bovine serum in a proportion of 1:3. The tubes were disposed in 30° in water bath at 37°C during different incubation time: 20, 40, 60 e 80 min. After incubation, approximately 1/3 of the total tube volume was taken from the supernatant. Then, the sperm were preserved in a buffered formal saline solution and the morphology was evaluated under phase contrast microscopy (1000x). The statistical analysis was performed with variance analysis (ANOVA) and Kruskal Wallis. There was an increase of sperm with normal morphology in all treatments after swim-up with or without caffeine (80%) compared to the control group (70%, $P<0.05$). However, the percentage of sperm treated with caffeine (80.4%) did not differ from the swim-up group without caffeine (79%, $P>0.05$). Although the swim-up (T2) did not select the sperm with middle piece defect (8.6%) compared to the control group (T1: 7.2%, $P>0.05$), the 5 and 7.5mM caffeine treatments (10.5 and 9.4%) increased the percent of middle piece defect compared to the swim-up (T2) and 3mM caffeine groups (6.6% and 8.1%). Moreover, all swim-up treatments decreased the percentage of tail defect (1%) compared to the control (9%, $P<0.05$), while there was no selection of sperm with head defect (4.7%), proximal (3.4%) and distal cytoplasmic droplet (4.0%) compared to the control group (5.4%, 4.4% and 2.4%, respectively, $P>0.05$). In conclusion, the swim-up with or without caffeine addition increased the percentage of sperm with normal morphology and decreased the sperm with tail defect of thawed equine sperm.

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The effect of erythropoietin on the development of follicles of cryopreserved cat ovarian tissue xenografted to peripheral site: a pilot study

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The conservation of germplasm is an excellent alternative for preserving wild feline species that suffer with threat of extinction. The best alternative to reestablish the ovarian function and recover female gametes is grafting cryopreserved ovarian tissue. Therefore, there is a massive follicular loss in the first days after transplantation due the period of ischemia, which occurs prior to the revascularization of the tissue. In this sense, this study aimed to evaluate the efficacy of administration of erythropoietin (EPO) on the development of follicles of frozen-thawed cat ovarian tissue xenografted to a peripheral site (CEUA protocol 11/2018). Four cat ovaries originated from elective ovariohysterectomy (veterinary clinic) were transported to the laboratory in saline solution (0.9% at 37°C) and were fragmented (1x1x5mm) and cryopreserved by slow freezing (1.5M DMSO). One fragment was immediately fixed for histology (fresh control). After one week, they were thawed and one ungrafted sample (cryopreservation control - CC) was also fixed for histology. The fragments were randomly distributed to be autografted to the peripheral site to six nude mice immediately after thawing and ovariohysterectomy (4 fragments per animal). Three of them (EPO group) were treated with EPO (500 IU/kg/day via intraperitoneal) for four days (one day before, on the same day and two days after the ovariohysterectomy) and the other three mice (control group) received saline injection in the same way. The grafts were removed after 7 days and 14 days and fixed for histological evaluation. The percentages of morphologically normal (MN) primordial and growing follicles on fresh control were 95% and 84% and for the CC were 96% and 94%, respectively. After 7 days of grafting, the percentages of MN follicles was 100% in the EPO group for MN primordial follicles and 75% for the saline group. For the growing follicles the percentage was basically the same proportion in saline and EPO group (94% and 93% respectively). Also, for the EPO group after 7 days of transplantation there were 5 antral follicles and 2 corpus luteum and in the saline group there were 3 antral follicles. After 14 days, the proportions of MN primordial and growing follicles were 90% and 85% for the EPO group and 60% and 75% for the saline group, respectively. There was no statistically difference between the groups both in the 7 and 14 days. In conclusion, it seems that EPO did not show effect on development of follicles in this pilot study, but the results are encouraging further studies to improve the method.

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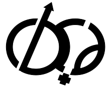

The role of Kisspeptin in the process of *in vitro* fertilization of bovine embryos

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The aim of this work was to evaluate the different concentrations of Kisspeptin (Kp) and it's role in the process of *in vitro* fertilization of bovine embryos about the quality and production rates. In Experiment 1 the minimum concentration of Kp was determined to be used at FIV, starting from the treatments: control (n=240); Kp 10^{-5} M (n=223); Kp 10^{-6} M (n=225) and Kp 10^{-7} M (n=245). In Experiment 2, the maturated oocytes were distributed in four treatments: control (n=362); Kp 10^{-1} M (n=425), Kp 10^{-7} M (n=304), and P234 $4x10^{-6}$ M (n=424). The sperms were enabled by Percoll® discontinuous gradient, and coincubated with the oocytes from 16 to 18 hours in stove culture at 38.5°C with 5% of CO_2. After this period, the zygotes were incubated medium SOF during 7 days. The rate of cleavage was evaluated 48 hours after fertilization and the rate of blastocysts and development degree/quality on the seventh day. A numerical score was adopted from 1 to 4 to evaluate the blastocyst development degree, as 1 corresponds to initial blastocyst, 2 blastocyst, 3 expanded blastocyst and 4 hatched blastocyst. The blastocyst quality was evaluated by 3D confocal microscopy, and the analyzed data by PROC GLIMMIX/SAS and Kruskal-Wallis test. In Experiment 1, there wasn't difference between the treatments rates of cleavage and blastocysts respectively: control (82,0%/34,2%); Kp 10^{-5} M (81,6%/26,9%); Kp 10^{-6} M (80,8%/29,3%) and Kp 10^{-7} M (80,8%/29,4%) ($P > 0,05$). In Experiment 2, the averages rates of cleavage and blastocysts were similar among treatments: control (85,1%/38,1%), Kp 10^{-1} M (82,6%/33,6%), Kp 10^{-7} M (83,6%/34,9%) and P234 (81,4%/31,6%) ($P > 0,05$). A total of 514 embryos were produced, with the following averages of development score: control 2,89; Kp 10^{-1} M 3,04; Kp 10^{-7} M 2,79 and P234 2,63, without statistical difference among them ($P > 0,05$). About the quality, 360 embryos were evaluated, n=30/treatment. The parameters were: production of reactive oxygen species (ROS), necrosis, apoptosis and mitochondrial potential. The Kp 10^{-1} M showed the lower proportion of ROS emission: control 3,76%; Kp 10^{-1} M 0,25% ($P < 0,05$); Kp 10^{-7} M 2,95% and P234 0,91%. No influence of KP was observed on the percentage of cellular degeneration: control 1,51%; Kp 10^{-1} M 1,73%; Kp 10^{-7} M 4,71% and P234 1,98% ($P > 0,05$). The average apoptosis levels were similar between the treatments: control 2,79%; Kp 10^{-7} M 1,67% and P234 6,11% ($P > 0,05$). The mitochondrial potential was superior in the group P234: control 0,51%; Kp 10^{-1} M 0,26%; Kp 10^{-7} M 0,22% and P234 1,43% ($P < 0,05$). The addition of kp-10 on fertilization didn't interfere in the rates of cleavage and blastocyst, as well as in the embryonic development degree. On the other hand, the Kp decreased the intracellular levels of ROS, suggesting it's involvement in the antioxidant control.

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The SlimCASA software is efficient for buffalo's sperm motility evaluation

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The aim of this study was to built up a software to evaluate buffalo sperm, from images taken under a microscopy. The program should be free and user-friendly, it also has to be compatible with laptop and PCs. It is expected, with this software, to standardize the sperm motility analysis carried out by veterinarians during the andrological examinations at field, and also to lower the price of acquisition of specific equipment. Twenty images of buffalo semen were made by microscopy from thawed semen doses. The 0.5 mL reed were thawed for 30 seconds in a water bath at 37°C. Semen drops were deposited on Hamilton Thorne (Spectrun) and slides both covered with cover slip for microscopy. The images were obtained from phase contrast microscope (Jenamed2) with 1.3 MP camera attached (Coleman). The software was developed from resources already available in an open source Java solution called ImageJ. The approximate count of the sperm contained in the image was possible through particle analysis capabilities. Initially, the video images were converted into frames and subjected to some treatments, using only 8-bit color and segmenting grayscale so that the software could do the analysis of the image particles. The 20 semen samples were analyzed by the technician, by Hamilton Thorne CASA and by the software SlimCASA for determination of sperm motility (from microscope image video). For statistical analysis, the results of the sperm motility were subjected to analysis of variance (SAS, 2012) at a significance level of 5%. The average values of the sperm motility were 18.94 ± 16.60a % by the SlimCASA software, 17.15 ± 10.94a % by the Hamilton Thorne CASA and 14.80 ± 10.47b % by the technician ($p < 0.05$). The SlimCASA performance was similar to the well known Hamilton Thorne CASA. Showing more credibility in the results than the technician. In conclusion the SlimCASA software had shown to be efficient for buffalo's sperm motility evaluation.

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The use of Doppler in neovascularization evaluation of the pre-ovulatory follicle and corpus luteum of jennies submitted to hCG subdose protocol applied in the *Hou Hai* acupoint

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The corpus luteum (CL) diameter and the serum progesterone (P4) level may be an indicative of CL functionality, however the association of echogenicity characteristics and blood perfusion of CL, and plasmatic concentration of P4, represent more reliable results as to this structure's functionality. The hCG, as well as in the equine species, is used in jennies as ovulation inductor, however, mistakenly, the doses used in jennies are the same as for mares. Studies with other species using hormonal subdoses associated to pharmacopuncture have reported satisfactory results. In this way, the objective was to evaluate the blood perfusion of pre-ovulatory follicle, CL and the correlation between them and the serum P4 of jennies submitted to protocols with hCG subdoses applied in the *Hou Hai* acupoint. A total of 11 jennies in reproductive activity, healthy, with corporal condition between 4 and 6 were used in three consecutive periods, distributed in the randomized block design, considering the period as blocking factor, in three treatments: T1-1500UI of hCG applied IV in jugular; T2-450UI of hCG/IV in jugular and T3 450UI of hCG applied in acupoint *Hou Hai*, totalizing 11 repetitions per treatment. It was used the Doppler ultrasonography to evaluate the vascularization of the ovarian structures, preovulatory follicle and CL in moments D0, D2, D4 and D8, through the numeric value of pixels (NPV) average, maximum and minimum, NPV heterogeneity, and then preceded to evaluation of the correlation between NPV, CL diameter and P4 serum. The data were submitted to normality analysis, followed by ANOVA and Turkey test, at 5% significance. The correlations were verified by Pearson. The administration of 1500UI of hCG IV resulted in increased CL blood perfusion in D8, evaluated by NPV minimum (41.91±1.17) in relation to the application of 450UI IV (37.63±6.27) and in the *Hou Hai* acupoint (37.89±4.28) (P=0.04), however, the pixel numeric values average, maximum and heterogeneity were similar between treatments, both to the preovulatory follicle and to CL in moments D0, D2, D4 and D8 (P>0.05). It was obtained a positive correlation (P<0.05) between the average NPV and the P4 concentration to the groups that received 450UI of hCG IV or in the *Hou Hai* acupoint. The results demonstrate that the reduction of the hCG dose and its application both in IV and in the *Hou Hai* acupoint does not harms the vascularization of ovarian structures, being able to be a viable alternative in the protocols to induce ovulation. (CEUA/EMVZ/UFBA/Protocolo#67/2016).

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Treatment of prepubertal sheep oocytes with meiosis inhibitor roscovitine

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It is known the *cumulus*-oocytes complexes from prepubertal animals are less competent to support the *in vitro* maturation because they have not yet suffered all ultra-structural and molecular changes necessary for developmental competence (1). Besides, the proportion of oocytes from prepubertal animals that reach the blastocyst stage is low and the rates of foetal loss and malformations after transfer to recipients are high compared to oocytes from adult donors (2). So, this study proposed to evaluate the efficiency of roscovitine, a selective inhibitor of cyclin-dependent kinases, to inhibit the meiosis in lamb oocytes *in vitro* cultured . For this, COCs from juvenile prepubertal sheep (30–40 days of age, body weight 6–12 kg) were recovered in HEPES buffered TCM-199 with antibiotics and 0.1% (w/v) polyvinyl alcohol without heparin at 30ºC. Only COCs showing several intact *cumulus* cell layers and cytoplasm with homogenously distributed lipid droplets were selected and transferred to basic maturation medium consisting of TCM 199, 10% heat-treated oestrus sheep serum, 100µM cysteamine, 0.36 mM piruvate (Control) supplemented with 75µM roscovitine (Rosco). In this condition, about twenty-five COCs were cultured, for 6h, in 5% CO_2 in air at 38.5ºC in four-well Petri dishes with 450µL of medium without mineral oil coverage. For reversion of meiotic inhibition, COCs from each treatment (Control and Rosco) were washed several times in HEPES buffered TCM 199 and *in vitro* matured, for a further 18h, in basic maturation medium supplemented with 0.1 IU/ml FSH and 0.1 IU/mL LH, under the same conditions described above, but layered with 300 uL mineral oil. An additional sample of COCs (Standard) was culture for 24h without interruption, in basic maturation medium supplemented with 0.1 IU/ml FSH and 0.1 IU/mL LH and layered with mineral oil. After 0, 6 and 24 h of *in vitro* culture, samples of oocytes were stripped from their *cumulus* cells by repeated pipetting and transferred to droplets of Hoechst 33342 dissolved with glycerol (10µg/mL) on a glass slide covered with a coverslip. Oocytes were examined under a fluorescence microscope and classified according to the stage of nuclear maturation as: germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), anaphase, telophase, metaphase II (MII), and degenerate. Data were subjected to analysis of variance and means were compared by the Tukey's test at 5% probability using Rstudio software. The quantity of oocytes kept at GV (95.4%) from Rosco tretatment was significantly higher than the Control (44.5%), which indicates that roscovitine 75µM was efficient to prevent the meiosis resumption in oocytes of prepubertal lamb during the 6h of culture. In contrast, proportion significantly higher of oocytes from Control reached the GVBD (24.6%) and MI stages (30.9%) while only 4.6% and 0% of oocytes from roscovitine were at these same stages, respectively ($P<0.05$).The rate of oocytes at MII after *in vitro* maturation for 18h in was significantly lower in Rosco treatment (34.6%) compared to Standard (92.2%) and Control (68.9%). In Rosco treatment, the most oocytes were at MI (45.9%), which differed significantly the MI rate observed in Standard (2.9%) and Control (11.7%). Besides this, the significant rate of oocytes from Rosco treatment at anaphase (9.1%) and telophase (7.6%) demonstrates the meiosis was in progression but the time of culture was not enough to reach the MII. The proportion significantly different of oocytes from Control at MII (68.9%) in comparison to Standard (92.2%) as well as the rate of 11.7% at MI in Control suggest the oocytes of prepubertal lambs require more time to complete the nuclear maturation after the previous culture for 6h without gonadotropins (CETEA/UFMG/Protocol# 13/2016).

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Ultra structural evaluation of in vitro maturation bovine oocytes associated to nanoparticle and magnetic levitation

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Research on reproduction biotechnologies, such as in vitro embryo production (IVEP), have sought to meet the needs of women facing infertility as well as the zootechnical development of Brazilian and world herds. Research and development of news methodologies to in vitro maturation (IVM) are necessary to get better the results of IVEP. This study had as goal to evaluate, by using electronic transmission microscope, IVM of bovine oocytes in tridimensional system (3D) with levitation magnetic associated to nanoparticles related with IVEP, using three different concentration (0µL/mL; 50µL/mL and 75µL/mL) and two qualities of oocyte (good and poor). Seven routines of IVEP were performed, totalizing 686 oocytes and production of 252 blastocysts. Ultrastructural analysis of oocytes was performed and compared to the rate of cleavage and formation of blastocysts. The results demonstrated that oocytes matured without nanoparticles (concentration of 0µL/mL) presented better rates of cleavage and blastocysts than the other groups with nanoparticles; there was an increase in cytoplasmic area occupied by vacuoles and a decrease in cytoplasmic area occupied by mitochondria with increase nanoparticle concentration; there was no difference between all groups related to cortical granules. In this way, we conclude that used of nanoparticles in in vitro maturation bovine oocytes does not disturbs fecundation, but is not advantageous to maturation and does not contribute to embryonic development.

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Ultrasonography of cryopreserved cat ovarian tissue autografted to the subcutaneous tissue with or without administration of erythropoietin

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Cryopreservation of ovarian tissue is an important alternative for the conservation of female gametes within preantral follicles. Cryopreserved tissue may be transplanted after thawing to allow those follicles to develop to antral stages. However, in the process of transplantation there is an initial period of ischemia, which affects the survival and development of the follicles, which is especially deleterious for cryopreserved tissues. The aim of the present study was to evaluate the effect of erythropoietin (EPO) on the development of antral follicles in cat ovarian tissue cryopreserved and autografted to a peripheral site. Six adult cats (n=6) were used. Four of them were treated with EPO (500 UI/Kg/day, Sc) during seven days (three days before, on the same day and three days after the ovariohysterectomy), and the other two cats received no medication. All cats were submitted to ovariohysterectomy and small pieces (1x1x5mm) of ovarian tissue were taken and cryopreserved (slow freezing with 1.5M DMSO). After 2 days, the ovarian tissue pieces were thawed and transplanted to the subcutaneous tissue of the dorsal neck of the same cat (autografting). Grafts were monitored weekly for nine weeks by ultrasound and follicles >1mm were recorded. The 4 cats treated with EPO developed total of 24 antral follicle > 1mm. Cat #1 presented antral follicles every week from week 4 to 9, in a total of 8 follicles. On Cat #2 a total of 7 antral follicles were seen on weeks 3, 4, 7, 8 and 9. Cat #3 presented 6 antral follicles on weeks 3, 7 and 8, and Cat #4 presented 3 antral follicles on weeks 4 and 7. The biggest follicle observed in animals treated with EPO measured 2.7mm. Non-treated animals developed a total of 7 antral follicles. One of the non-treated cats showed 1 follicle on week 8, and the other showed a total of 6 follicles on weeks 4, 5, 6, 7 and 8. The biggest follicle observed on non-treated animals measured 1.7mm. These results demonstrated that the administration of EPO enhances the development of antral follicles on cryopreserved ovarian tissue transplanted to a peripheral site.

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Use of bioinformatics for the screening of new proteins associated with bovine sperm resistance to cryopreservation

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Chicken egg yolk in bovine semen extenders are used for more than 60 years but only recently the mechanism by which its components protect spermatozoa from cryodamage was elucidated. After ejaculation, several cholesterol molecules of the sperm plasma membrane are removed by Binder of Sperm proteins (BSP) present in the seminal plasma. This cholesterol efflux makes the cell membrane more fragile and less resistant to freezing. However the addition of egg yolk, a rich source of cholesterol, reduces the membrane cholesterol efflux by binding to BSP, preserving the cell during the freezing process (1). Applying bioinformatic tools, the aim of this study was to investigate if other seminal plasma proteins could bind to cholesterol as BSP, contributing to membrane cholesterol efflux. The approach used was to first identify proteins that could dock to cholesterol using PharmMapper (2). Secondly, a list of bovine seminal plasma proteins obtained from proteomic studies (3,4) was compared with the docking targets obtained previously. Three proteins were identified as potential targets for cholesterol binding, as follows (with rank and z'-score): serum albumin (4; 0.634748), calmodulin (49; 1.23132) and gluthatione-S-transferase P (62; 1.22254). To verify if these potential targets have possible binding sites, molecular docking was performed using the SwissDock server (5). For this, PDF files of the target proteins were selected based in model resolution and sequence similarity, available at the RSBC PDB Database. PDF file of cholesterol was obtained at PubChem. The numbers of docking clusters for cholesterol found in serum albumin, calmodulin and gluthatione-S-transferase P were 27, 29 and 33, respectively. Using the ViewDock plugin of UCSF Chimera software, clusters were ranked according to Fullfitness score and most favorable energy. The predicted binding modes had the following Fullfitness and Energy: serum albumin: -3969.68 and -7.68, calmodulin: -1367.34 and -7.10 and gluthatione-S-transferase P: -5153.02 and -7.18. Based on these results, bovine seminal plasma proteins serum albumin, calmodulin and gluthatione-S-transferase are potential candidates as important players in sperm cryoresistance due to possible cholesterol binding properties. To confirm the above-mentioned binding models, clusters will be evaluated using AutoDock4 software and the plugin Vina. Final validation of the role of serum albumin, calmodulin and gluthatione-S-transferase in sperm protection during semen freezing must be performed experimentally.

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Use of HSPA5 protein during oocyte *in vitro* maturation improves *in vitro* embryo production in cattle

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The oviduct provides important structural, environmental and nutritional support for fertilization and early embryonic development. During the ovulation, cumulus-oocyte-complex (COC) is released to infundibulum (initial region of tube). In the bovine oviduct, the COC is exposed to oviductal fluid (OF) during last four hours of its maturation. Even the OF is not totally defined, it is known the presence of HSPA5 protein. The HSPA5 is a member of the heat shock 70 class protein, also known as GRP78, and seems to have an important role in fertilization and embryo development. Thus, the present study aimed to investigate the effect of HSPA5 during the last four hours of *in vitro* maturation of COC on further impacts on in vitro embryo production. For this, COCs were recovered from ovary of slaughtered cows and in vitro matured in TCM199 medium plus pyruvate, amicacin, BSA (bovine serum albumin) and humidified atmosphere at 5% CO_2 for 20h (n=4 replicates with 20 COCs/group). After, during last four hours of maturation, 100 ng/mL of HSPA5 protein was added (G100 group) or not (control group). After, total 24 hours, the matured COCs was fertilized and cultured in humidified controlled atmosphere at 5% CO_2 and O_2 until 7,5 days after fertilization. The culture medium was partially replaced at day 3 and 5. The statistical analysis was performed transforming the blastocyst yield in arcsine then the means were compared by T-test using JMP (JMP software, SAS Institute Cary, NC). Significant differences were considered when $p \leq 0.05$. The blastocyst yield from G100 group was higher than control group (42.50±1.19 and 30.75±4.04 respectively; p= 0.0340). Taken together these results show that HSPA5 at 100 ng/ml concentration during last four hours of maturation, seems to have a positive effect on COC during in vitro maturation and improves the blastocyst yield.

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Use of near-infrared spectroscopy in the non-invasive assessment of the viability of amniotic fluid stem cells from goat fetuses after *in vitro* culture and cryopreservation

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Near-infrared spectroscopy (NIR) technology has emerged as an automated, high throughput analytical methodology for rapid, non-invasive investigation capable of predicting the quality of cell structures when cultured *in vitro*. We highlight the use of stem cells that, although it is a promising technology with high potential for use in cell therapies, there is no data regarding quality prediction when cultured *in vitro* and cryopreserved. Therefore, we aimed to develop a predictive model through a multivariate technique to differentiate culture medium from stem cells cultured *in vitro* and cryopreserved according to the absorbance profiles obtained by NIR. Mesenchymal stem cells (MSCs) from amniotic fluid (AF) were obtained from 9 goat fetuses with 3 months of gestational age by laparotomy. After isolation, the MSCs of AF were cultured *in vitro* in 1 mL of culture medium containing DMEM + 10% FBS + 2% penicillin/streptomycin in petri dishes, at 38.5°C in a humidified atmosphere of 5% CO_2 in the air, and the medium was changed every two days. The CTMs monitoring was performed daily to observe the morphology and confluence, which, when reaching 80%, the culture medium (CONTROL) was collected and stored in cryotubes at -80°C for NIR analysis, and the MSCs were trypsinized, quantified and resuspended in cryopreservation medium containing 45% DMEM + 45% SFB + 10% DMSO in the concentration of 20,000 cells packaged in 0.25 mL straws and vitrified in liquid nitrogen. After 30 days the samples were thawed in a water bath at 37°C, 2 mL of culture medium added for washing and withdrawal of the cryoprotectant by two cycles of centrifugation at 342 g for 10 min. The centrifuged pellet was resuspended and cultured in 1 mL of medium for 5 days according to the CONTROL group, in which the medium derived from culture of the cryopreserved MSCs (CRIO) was stored and then submitted to the NIR. A total of 500 µL of the culture medium from each animal of the CONTROL and CRIO groups were analyzed by NIR (DA 7200 Perten®), in an adequate recipient at a temperature of 25 °C. The spectra in the NIR were obtained in duplicates, with 4 minutes of exposure at the wavelength between 955 and 1650 nm. The spectra were evaluated by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), using the mixOmics package in the R programming environment. PC1 and PC2 retained 63% and 30% of the total variance of the respectively. Furthermore, through the evaluation of the PC1 and PC2 scores of the experimental units, it was possible to observe a separation between the two experimental groups, in which the CONTROL group presented positive scores for PC1, while the CRIO group presented negative values. We performed 100 simulation routines using different training and test groups, totaling 524 predictions, of which 523 observations had their group correctly predicted as CONTROL or CRIO. The calibration model was able to predict with sensitivity, specificity and accuracy of 99.5%, 100% and 99.8%, respectively. Thus, the use of NIR as a tool for chemical analysis associated with PLS-DA was possible to estimate with high accuracy the cryopreservation process used in the samples, generating a fingerprint of the culture medium *in vitro*.

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Visual evaluation of fetal bovine ovarian tissue fragments submitted to xenotransplantation

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In order to allow the preservation of preantral follicles (FOPA) present in ovarian tissue, the xenotransplantation of this tissue in immunosuppressed animals may be an option to promote follicular development and maturation. Based on the principle of animal breeding, the descendent are expected to possess genetic value superior to previous generations. So, the conservation of genetic material of fetuses and their subsequent *in vitro* maturation decreases the generations interval and successively increases the genetic gain (1). The conservation of bovine fetal ovarian tissue contributes to maintenance of evolutionary intention, even if this fetus is not compatible with life. In addition, FOPA from tissue of bovine fetuses suffered less environmental interference, and during the fetal phase, the animal has a greater reserve of primordial (quiescent) follicles, which is more tolerant to cryopreservation, due to its small size and relatively unspecific morphology, in addition to the low metabolism (2). In this regard, scores of morphology, hemorrhage and adherence of fetal bovine ovarian tissue fragments submitted to xenotransplantation for 7 or 14 days were evaluated. Ten pairs of fetal bovine ovaries collected in local slaughterhouse, fragmented (3 x 3 x 1 mm) and fresh xenotransplants were used in 20 female mice of the Balb C line, so that each ovarian pair provided 5 fragments for xenotransplantation for 7 days and 5 fragments for xenotransplantation for 14 days, therefore each mice received 5 fragments. After recovery the fragments were evaluated in scores from 1 to 5 according to morphology, hemorrhage and adhesion characteristics, in which note 1 represented the worst and note 5 the best evaluation. The data were compiled and analyzed with the aid of the Sigma Plot program. The fragments recovered after 14 days of xenotransplantation received the best average scores for the morphology, adhesion and hemorrhage characteristics (4.2 ± 0.2, 4.4 ± 0.2, 4.0 ± 0.3, respectively) in relation to those recovered after 7 days (3.4 ± 0.1, 3.2 ± 0.1, 2.7 ± 0.2, respectively) ($p < 0.05$). The time elapsed after transplantation determines the period necessary for body adaptation to the fragment, showing that the longer period analyzed, 14 days, was more efficient for tissue ambiance than 7 days. Adherence and hemorrhage showed a strong and positive correlation (0.62, $p < 0.01$), demonstrating that the perfusion of blood vessels to the transplanted fragment influences its adhesion, which are fundamental factors for tissue viability after surgery. It was concluded that xenotransplanted fetal bovine ovarian tissue fragments had better responsiveness of adherence, hemorrhage and morphology when recovered after 14 days. Further studies are now being performed, including analysis of histology, immunohistochemistry, PCR, confocal and cytogenetics to complement the study of tissue and follicular viability after xenotransplantation. (CEUA/UFU/Protocol 006/17).

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Characterization of hyperactive boar spermatozoa through its evaluation by computer-assisted sperm analysis

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Hyperactivation of spermatozoa corresponds to changes in their motility pattern, defined by asymmetric and high amplitude flagellar beats. This new pattern has been associated with important events prior to fertilization such as detachment of spermatozoa allocated in spermatic reservoir, passage of them by viscoelastic substances such as the mucus secreted by the oviduct epithelium and the cumulus oophorus matrix, as well as the zona pellucida penetration. A detailed investigation of hyperactivated boar spermatozoa by computer assisted sperm analyzer (CASA) already was performed. Therefore, studies report differences between results obtained from analyzes performed in different computerized systems, which does not allow standardization of reading on all CASA platforms. By this way, the current study was performed to identify and characterize the motility pattern showed by hyperactivated boar sperm using a commercial system Sperm Class Analyzer (SCA® Ver. 5.0; Microptics S.L., Barcelona, Spain). Thus, nine ejaculates (3 ejaculates per boar) were used. Boar semen was collected by the gloved-hand method and only sperm-rich fraction was obtained. After raw semen analysis, the sperm-rich fraction was extended 1:1 (v:v) in BTS (Beltsville Thawing Solution) and kept at 17 ºC for 24 h. Then, samples were centrifuged at 500 × *g* for 10 min and the supernatant was discarded. Spermatozoa were resuspended in TALP-HEPES medium with minor modifications - $CaCl_2$ (final concentration: 50 μM) and bovine serum albumin (BSA; final concentration: 0.6 %). A final concentration was adjusted to 30 × 10^6 of spermatozoa/mL. The sample was split into two aliquots: one was used as a control treatment (CT; final volume: 500 μL), and the other (final volume: 499 μL) was added with 1 μL calcium ionophore A23187 (2.500 μmol/L) to hyperactivate spermatozoa (HI). Samples were subsequently incubated at 38 ºC and 5% CO_2 for 30 min and after this, analyzed through SCA® system. For each treatment, a total of five fields were captured and, at least, 150 sperm cells were analyzed. The following individual kinetic parameters were examined: curvilinear velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s), linearity (LIN, %), straightness (STR, %), oscillation index (WOB, %), amplitude of lateral head displacement (ALH, μm) and beat cross frequency (BCF, Hz). After a significant treatment effect ($p < 0.01$) was found by Student's t-test for these characteristics, four of them showing smaller coefficient of variation were selected: LIN (-12.64), STR (-17.12), ALH (14.36) and WOB (-10.35). The limit values for each of the characteristics that together could characterize the hyperactivate boar sperm were defined considering the addition or subtraction of three standard deviations in the mean values (previously obtained from all the cells analyzed for each treatment, CT and HI). Thus, new mean values were established for each characteristic for both treatments (LIN – CT: 45.76 / HI: 41.45; STR – CT: 58.15 / HI: 79.57; ALH – CT: 2.97 / HI: 3.16; WOB – CT: 73.88 / HI: 53.89). Finally, sperm presenting values for LIN < 41%, STR < 60%, ALH > 3μm and WOB < 54% simultaneously, were characterized and considered as hyperactivate. In view of the multivariate analysis developed in this work, with specific parameters and values capable of identifying the population of boar hyperactivated spermatozoa in the Sperm Class Analyzer (SCA®), this study may help in future works developed in the same platform.

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Correlation of Doppler velocimetry of the testicular artery with kinetics and sperm morphology in dogs

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Sperm quality can be influenced by reduction in the testicular blood flow and the impact of this alteration in the testicle can be measured by means of Doppler ultrasonography. The aim of this study was to correlate the Doppler velocimetric evaluation of the testicular artery with the kinetics of epididymal spermatozoa of dogs. Thirty-four testicles from 22 dogs were used, evaluated by Doppler ultrasonography in five regions of the testicular artery, according to a technique described by Trautwein et al. (1). Immediately after the orchiectomy, the epididymal spermatozoa were recovered in physiological solution, with the technique of compression of the tail of the epididymis and vas deferens, and analyzed for kinetics in an automated system (CASA system), morphology (modified Karras), and spermatic membrane integrity by eosin-nigrosin staining. The variables were analyzed by the Pearson correlation with a significance level of 5%. The mean total motility was 69.00%, progressive motility 43.75%, average path velocity (VAP) 126.96 µm/s, curvilinear velocity (VCL) 221.04 µm/s, and velocity index (SVI) 389.89. There was a positive correlation between the peak systolic velocity (PSV) in the proximal supratesticular region and the SVI (r = 0.529), VCL (r = 0.555), and VAP (r = 0.473); and negative correlation with the percentage of slow spermatozoa (r = -0.463). To the authors' knowledge, this is the first study to correlate the sperm kinetics with the Doppler evaluation of the testicular artery of dogs. It is concluded that the velocity of blood flow in the testicular artery can positively influence the speed of movement of the spermatozoa evaluated.
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Correlation of seminal plasma proteins and the semen freezability in dogs

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The ability of sperm to survive cryopreservation may be related to the presence of seminal plasma proteins that interact with the sperm membrane. These proteins can be used as fertility biomarkers, as demonstrated in other species. The aim of this study was to evaluate the correlation between seminal plasma proteins and the freezing rate of semen of dogs. Ten dogs, aged between 2 and 6 years, were used. The semen was harvested/obtained/collected by digital manipulation and the seminal plasma was separated by centrifugation (10000 xg for 60 minutes at 5°C) and the sperm cells were subjected to cryopreservation. Proteomic analysis of seminal plasma was performed by mass spectrometry, with shotgun approach. The multivariate statistics analysis for clusters was made by online software MetaboAnalyst 2.0 (Xia e Wishart, 2016) and the main component (PCA) was used to describe the variation of samples. In total, 38 proteins were found, and the cryopreserved samples were separated by clusters according to post-thawed quality, being divided into low; medium; and high cluster. Ten proteins were identified only in the high group. Among these proteins, was found that Alkaline phosphatase can be used as a marker of fertility and azoospermia. Matrix metalloproteinase 9, are proteins related to sperm maturation, fertility, motility and morphologically normal cells, and may be a potential molecular marker of sperm quality in dogs. Glutathione peroxidase, another protein found in the high cluster, acts in the concentrations of reactive oxygen species, and can act in the process of cryopreservation by reducing oxidative stress of the sperm cell. In the medium cluster were found 5 proteins exclusive to this group as Calpain 2 catalytic subunit, which is associated with the cellular fusion process during oocyte penetration. To our knowledge this is the first report, but the other proteins have not been described in sperm cells. Five proteins were identified exclusively to the low cluster and none of them were described before in the sperm cell, such as Cyclin dependet kinase 12; glycoxalase domain containing protein 5; Neurobeachin like protein; Source recognition complex subunit 1; Roundabout homolog 2. The clusterin was absent only in the low cluster and it is mediated by the chaperone protein. This protein has already been identified in several tissues, and is involved in cell proliferation and adhesion related to apoptotic profactors. The arginine esterase, produced by prostate epithelial cells, belonging to the kallikrein family, was the main protein identified in all clusters, the most abundant seminal plasma protein of dogs. The proteins found in all clusters were Albumin, arginine stearase, EGF LAG seven pass G, epididymal secretory protein, fer 1 like protein 5, glutamate rich protein 6B, hemoglobin subunit alpha, polyhomeotic like protein 3, serum albumin and trypsin. It was concluded that there is influence of the seminal plasma proteins with spermatic freezing.

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Cytoplasmic droplet translocation during epididymal sperm maturation in horses

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During spermatogenesis, most germ cell cytoplasm is phagocytozed by the Sertoli cells and a remnant residue remains attached at the neck region of the testicular spermatozoa, called cytoplasmic droplet (CD) [1]. The migration of CD along the midpiece from neck to annulus is related to sperm maturation and fertility [2]. In most species, the mechanism of transport has not been elucidated as well as the specific region of droplet translocation. As such, the present study investigated the migration of droplet migration during sperm maturation in equine epididymis. Ten epididymides from five adult (3-4 yr old) stallions were subdivided into 10 regions (E0–E1–E2, proximal caput; E3–E4, distal caput; E5–E6–E7, corpus; E8–E9, cauda).Epididymal sperm were obtained by microperfusion with sterile phosphate buffered saline, pH 7.2. Samples were evaluated for the presence of CD (proximal, middle piece, distal) by differential interference phase contrast (DIC) microscopy equipped with a video camera (Leica Microsystems), counting 100 cells per region.Statistical analyses were performed using One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with a significance level of $P \leq 0.05$. Data are presented as means ± standard deviation (SD). A higher percentage of proximal droplet was observed inproximal caput (65.1±21.4%) in comparison to distal caput (25.7±12.2%), corpus (13.7±6.2%) and cauda segment(7.4 ± 2.9%) ($P < 0.05$). The amount of middle piece droplet was significantly higher in distal caput and tail bendingwas observed in most spermatozoa.No differences were observed in the amount of middle piece droplet in corpus and cauda sperm ($P > 0.05$). On the other hand, caudasperm (38 ± 11.7%) presented higher percentage of distal droplet compared to proximal caput (3.3 ± 5.1%), distal caput (14.5 ± 7.5%) and corpus sperm (16.7 ± 7.5%) ($P < 0.05$). After leaving the testis, spermatozoa undergo a series of functional and morphologicalmodifications in epididymis and the most common morphological change during maturation is the migration of the cytoplasmic droplet fromthe neck region to the distal end of the midpiece [3]. The droplet migration variesfrom species to species, but it generally occurs in the caputepididymidis [1], as observed in equine sperm. Tail bending in distal caput seems to be related to CD translocation and, whenpassing along the sperm, the tail is re-straightened since the number of bent tails is reduced and no tail pathologies were observed in the next epididymal segment. In different species, normal CD morphology and translocation are associated with normal motility development duringepididymal maturation of spermatozoa [4] and some sperm pathologies are linked to a failure in droplet migration, which may lead to decreases in fertility[5].Therefore, understanding the site of CD translocation may help to elucidate the mechanisms involved in the process of equine epididymal sperm maturation. In conclusion, the distal caput is the site of droplet translocation from the proximal to the middle piece region, characterized by tail bending; and in the corpus segment, CD migrates from the middle piece to the distal regionduring equine sperm maturation.

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Effect of the Addition of Green Tea Extract over the Integrity of the Plasma Membrane of Refrigerated Ovine Spermatozoon

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The demands of the production systems for genetically improved animals stimulate the biotechnologies of reproduction to advance (1), with emphasis on sperm cryopreservation and on artificial insemination, which allows the conservation and dissemination of valuable genetic material (2). However, the gametes are susceptible cryoinjuries (3), fact that is leading to the search of alternatives that enable a better maintenance of those cells, such as antioxidant therapies. Taking in consideration the fact that green tea is rich in antioxidants (4), the goal was to evaluate it is effects over the plasma membrane integrity of ram semen refrigerated. For such, three semen *pools* (n=3) were used, collected from two ovine breeding males by artificial vagina. These were fractioned and diluted, following the experimental groups (G1= egg yolk based extender, G2 = egg yolk based extender added of 0.25% green tea, G3 = egg yolk based extender added of 0.5% green tea, G4 = egg yolk based extender added of 0.75% green tea, G5 = egg yolk based extender added of 1% green tea). Afterward, the samples were refrigerated at 5°C, and evaluated to plasma membrane integrity on the time 0 and 16 hours of refrigeration, using the method of double coloration with Carboxyfluorescein Diacetate (CFDA) and Propidium Iodide (HPLC) in epifluorescence microscope (5). The obtained data was subjected to the statistic test for analysis of variance (ANOVA) and Tukey for the comparison between the groups, considering the level of significance of 5% (P<0,05%) and the results obtained as mesures and standard deviations. There were no significant diferences identified (P>0,05%) for the integrity of the membrane between the studied groups, despite the amount of the cooling time (hour zero: G1 = 82.5±3.34, G2 = 78.5±1.55, G3 = 73±7.19, G4 = 69±4.3, G5 = 62.5±4.3; 16 hours: G1 = 77±4.24, G2 = 83±14.04, G3 = 84.5±2.66, G4 = 81±4.19, G5 = 63.5±9.83). Thus, despite the antioxidant's protection over the oxidative damege on the gametes avoiding the lipoperioxidantion of the plasma membrane (6), and the know antioxidant and protective potential of green tea extract (7), it was not effective on the maintenance of the plasma membrane of cooled ovine spermatozoon. However, it is important to highlight that the catechins, main antioxidants of the green tea, have great affinity for proteins, so the substract utilized of egg yolk could have contributed for the reduction of the antioxidant concentration available to protect the sperm cell (8). It was concluded that of different concentrations (0.25%, 0.5%, 0.75% and 1%) of green tea extract, on diluent based on egg yolk, does not enhance the preservation of the plasma membrane of refrigerated ovine spermatozoon, however, more studies are necesary.

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Influence of extender, seminal plasma and follicular fluid on stallion epididymal sperm motility

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The “ready to go” sperm cells are kept into the stallion epididymal tail, still immotile, high concentrated and waiting for the ejaculation time, when will be added to secretions produced by accessory glands to become, this mixture, the ejaculate itself. Structural membrane modifications and ionic exchanges occur in sperm, leaded by these secretions to stimulate its motility, one of many fundamental characteristics of fertility. On equine practice, several reasons implicate in male castration, but the produced sperm stored in the epididymal tail can still be used for reproduction if necessary. However, these cells do not have contact with homologues motility stimulators, a technical challenge when looking for fresh insemination or cooled shipping. Based on that, it is necessary to evaluate the influences of extender main compounds, pre-ovulatory follicular fluid and heterologous seminal plasma in order to lead future steps on this searching toward a better handling and higher insemination performance. The aim of this study was to evaluate the effects of egg yolk and milk-based extenders added with pre-ovulatory follicular fluid (FF) or heterologous seminal plasma (SP) on epidydimal sperm progressive motility. Four sexually mature and healthy stallions were surgically castrated after fifteen days of sexual rest and the eight epididymal tails were harvested after dissection, followed by bipartition. Each half-side tail was sliced to allow the sperm to flow through the extender in two different major experimental groups based on cooling media formula, denominated as shown: EP (EP; EquiPlus®; Caseinate; Minitube GmbH, Tiefenbach, Germany) and G (G; GENT®; Egg yolk; Minitube GmbH, Tiefenbach, Germany). After 30min of extender immersion in a Petri dish on a 37°C warming plate, each group sample was filtered to remove tissue debris and the sperm sample concentration was adjusted to $200x10^6$ total sperm/mL. Each diluted cell sample was split in triplicate to create six treatments (n=8) according to biological fluid addition (v/v), as described: T1 (Only EquiPlus® extender), T2 (Only GENT® extender), T3 (EP + 10% FF), T4 (G + 10% FF), T5 (EP + 10% SP) and T6 (G + 10% SP). The SP was obtained by centrifugation of a fertile stallion ejaculate and the FF was collected by transrectal follicle aspiration of preovulatory follicles. Thirty minutes after SP and FF addition, the progressive motility of the 37°C warm samples were measured by CASA system (AndroVision®; Minitube GmbH, Tiefenbach, Germany). The data are described as means±SEM and were compared using Fisher test <0.05. The general groups EP and G were different (n=24) and the treatments (n=8) comparison is demonstrated by letters, as follow: $T1=37.3\pm9.8^b$, $T2=8.2\pm2.5^c$, $T3=45.0\pm6.8^{ab}$, $T4=13.5\pm3.7^c$, $T5=50.7\pm8.7^a$ and $T6=13.8\pm3.4^c$. It was observed higher motility in all EP treatments when compared to G. Perhaps the higher membrane stabilization due the GENT® formulation did not allow properly the ionic changes, a crucial event for epidydimal sperm motility. Furthermore, within EP group, the treatment with SP (T5) had a motility enhancement considering T1, demonstrating its motility stimulation proprieties in addition to the high Ca^{+2} sample concentration due caseinates. The positive motility effect of follicular fluid, attributed in part to the progesterone into it, could not be statistically supported by this trial. In conclusion, more attempts must be done to list all the stimulatory substances into EP and SP or to find out the inhibitory reason of G. However, considering the motility role in fertility when inseminating with stallion semen after castration, the protocols with EP demonstrated to be more indicated for epididymal sperm.

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Kinematic analysis of sperm from Angus bulls with different fertility after FTAI

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In beef cattle industry, bull fertility is five times more relevant than other productivity factors, since one sire can service many cows in a breeding season or be a semen-donor bull in artificial insemination (AI) programs[1,2]. Sperm motility is commonly used as an indicator of bull fertility, and objective analysis of sperm kinematics (CASA) could be an *in vitro* test able to predict *in vivo* fertility. Due to different techniques adopted to determine *in vivo* fertility and the growing of fixed time artificial insemination (FTAI) in Brazil, the goal of the present study was to evaluate the kinematic sperm parameters of bulls with different fertility after FTAI. Cryopreserved semen samples from eight Angus bulls classified as having excellent (EF, n=4) or normal fertility (NF, n=4) were used on the study. Fertility score was determined by an Artificial Insemination Center, based on data of thousands of inseminations in FTAI programs. Three different batches from each bull were evaluated using CASA system (SCATM, Microptics, S.L, Barcelona, Spain) and the variables assessed were: total motility (TM, %), progressive motility (PM, %), linearity (LIN, %), straightness (STR, %), wobble (WOB, %), curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), average path velocity (VAP, μm/s), amplitude of lateral head displacement (ALH, μm), and beat cross frequency (BCF, Hz). After thawing (37 °C, 30 sec), semen samples were loaded into microtubes, and kinematic data were determined immediately (0 h) and following 2 h of incubation in water bath (37 °C), with semen kept in the same freezing medium. All data were tested for normality using Shapiro-Wilk test and differences between means were compared using ANOVA followed by Tukey's test. Data are presented as means ± SE and differences were considered significant if $P < 0.05$. There were no differences ($P > 0,05$) on kinematic parameters evaluated between EF and NF bulls, both immediately and after 2h incubation. Incubation time has no influence ($P>0.05$) on STR, ALH and BCF. However, while there was no difference in VCL between 0 h and 2 h of incubation in NF bulls (59.32 ± 3.16; 48.11 ± 2.61 μm/s, respectively), semen samples from EF bulls had similar LIN between 0 h and 2 h of incubation (53.36 ± 1.47; 45.27 ± 2.88 %, respectively). Therefore, in spite of no differences in kinematic parameters between EF and NF bulls within same evaluation time, challenging sperm can reveal differences on sperm resistance during incubation, which could be related to sub-lethal damages and/or sperm cryotolerance.

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Methodology for purification of the *ram seminal vesicle protein* (RSVP22) from seminal plasma

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RSVPs (Ram Seminal Vesicle Proteins) are secreted by the ovine seminal vesicles and bind to the sperm plasma membrane. Amino acid sequences along with disulfide bond assignment, confirm the structural similarity of RSVPs to BSPs (Binder Sperm of Proteins). The RSVP22 isoform is among the most abundant proteins in the seminal plasma of rams, binding to the sperm plasma membrane ejaculation. The objective of this study was to develop a methodology to purify RSVP22. Semen was collected by electroejaculation from 16 adult and reproductively healthy Morada Nova rams. Seminal plasma was then obtained by centrifugation (700 g, 4°C, 15 min; 5000 g, 4°C, 60 min) and stored at -20°C. To precipitate proteins, concentrations of ammonium persulfate were added according to the volume of the sample. These fractions were desalted by dialysis followed by lyophilization for 48 hours at -50°C and minimum pressure of 0.035 mBar. Seminal plasma proteins were then subjected to affinity liquid chromatography using a gelatin-Sepharose matrix coupled to an automated chromatographic system. The proteins were eluted at three chromatographic peaks, in which the first two peaks represented the NGBP fraction, with no affinity to gelatin. The third peak represented the GBP fraction, comprising proteins with fibronectin II domains with high affinity to gelatin. The percentage of gelatin binding proteins (GBPs) was estimaded based on integration peaks, using PrimeView Evaluation Software. GBPs corresponded to 69.95% of the ovine seminal plasma proteins. Fractions containing GBPs and NGBPs were mixed, desalted and centrifuged using Amicon MWCO 10 kDa filters and 20 μg of proteins from each fraction were subjected to the SDS-PAGE (12.5%). Gels were then analyzed by Quantity One software (Bio Rad, USA). The GBP fraction was subjected to a second chromatographic step (HiTrap ™ Heparin HP column, GE, USA). Two chromatographic peaks were formed and well defined. The first fraction (NHBP) contained only proteins with low or no affinity to heparin and the second fraction (HBP) contained proteins with high affinity to heparin, representing 30.04% of all seminal plasma proteins. The proteins of both NHBP and HBP peaks were subjected to SDS-PAGE, with detection of RSVP22 in the two fractions. However, RSVP22 appeared isolated by itself in the HBP fraction. Thus, affinity chromatography is an efficient technique for purifying RSVP22 from seminal plasma. News experiments are being conducted to confirm this protocol and evaluate functional attributes of RSVPs.

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Morphological changes between cauda epididymal and ejaculated sperm in stallions

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Epididymal cauda primarily serves as a reservoir for functionally mature spermatozoa and during ejaculation epididymal and vas deferens spermatozoa interact with accessory gland secretions within seconds, exposing these cells to a new environment [1]. As such, the aim of this study was to investigate the morphology abnormalities between cauda epididymal and ejaculated sperm in horses. Eleven stallions between 3 and 4 years of age were submitted to semen collection and then to bilateral orchiectomy. Epididymal sperm were obtained by retrograde flushing with sterile phosphate buffered saline, pH 7.2. For sperm morphology, samples obtained from cauda epididymis and ejaculated were fixed in 1mL formol-saline and evaluated under a differential interference phase contrast (DIC) microscopy equipped with a video camera (Leica Microsystems), counting 100 cells per sample. Sperm abnormalities were classified as major and minor sperm defects as defined by Blom [2]. Statistical analyses were performed using One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with a significance level of $P \leq 0.05$. Data are presented as means ± standard deviation (SD). A trend toward significance was observed between cauda epididymal (39.7±14.2) and ejaculated sperm (28.4±16.5) for the percentage of major defects (P=0.07), in which a higher percentage of tail bending following droplet translocation and a tendency for proximal cytoplasmic droplets (PCD) were found in cauda epididymal sperm (P=0.09). A higher percentage of minor defects was found in cauda epididymal sperm (30.7±7.7) in comparison to ejaculated sperm (8.4±7.9), due to the higher percentage of distal cytoplasmic droplets (DCD) observed in epididymal sperm ($P<0.05$). No significant differences were found in tail morphology between groups. The amount of PCD found in ejaculated samples may be related to the age of animals used in this study, since semen quality (gel-free volume, sperm concentration, total sperm count, sperm abnormalities) has been reported to be lower in stallions under 3 years old [3]. The fact that very few ejaculated spermatozoa have DCP suggests that they are removed around the time of ejaculation. However, frozen epididymal and ejaculated sperm have been proved to be equally fertile and viable [4], indicating that distal droplets do not influence the fertility process in equine species. In conclusion, cauda epididymal sperm has a greater percentage of minor defects compared to ejaculated sperm and the loss of the distal cytoplasmic droplets happen close to or after ejaculation.

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Plasma membrane integrity on sheep sperm cryopreserved in soy milk or egg yolk based extender

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Sperm cryopreservation in egg yolk base extender, added of Tris buffer solution (Tris egg yolk), is one of the most used extender to sperm cryopreservation in many species (1). However, negative effects are now known, such as the risk of microbial contamination, which reduces the fertilizing potential of the sperm (2). To improve this aspect, alternative extenders have been tested, such as soy milk based extender, that demonstrate good preservation of sperm quality (2). Thereby, this study aimed to determine the capacity of soy milk based extender to maintain plasma membrane integrity on sheep spermatozoon cryopreserved compared to egg yolk. For this purpose, two breeding sheep were used, subjected to three seminal harvests each, of which formed three *pools* (n = 3). The seminal *pools* were divided and diluted according to the experimental groups (G1: egg yolk, G2: 10% soy milk, G3: 20% soy milk), cooled at 5 °C and analyzed immediately after and 16 hours of cooling. To check the plasma membrane integrity was used fluorescent probes carboxyfluorescein diacetate and propidium iodide in a epifluorescence microscopy. A total of 200 cells were analyzed and classified as integrated or injured (3). The values obtained were subjected to the analysis of variance test (ANOVA), and the results were expressed in the form of mean and standard deviation (mean ± SD). No significant differences were observed ($P > 0.05$) between the experimental groups and evaluation times (time zero hour: G1 = 81.00 ± 6.56, G2 = 74.25 ± 2.65 and G3 = 55.50 ± 19.65; time 16 hour: G1=84.00 ± 5.72, G2 = 52.5 ± 33.37 and G3 = 86.50 ± 33.30). Thus, soy milk based extender has a promissing protective effect on sheep sperm submitted to cryopreservation, and proving to be a viable alternative to sperm extenders free of animal products and it's sanitary risks. Therefore, it could be concluded that the dilutive based of soy milk, as well as of egg yolk, preserves the plasma membrane integrity on sheep sperm subjected to refrigeration.

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Proteomic analysis of sperm cells from the Brazilian Amazon squirrel monkey (*Saimiri collinsi* Osgood, 1916)

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The *Saimiri collinsi*, popularly named “squirrel monkeys”, is a Neotropical primate endemic from Amazon-Brazil, with a strict reproductive seasonality. Therefore, in this study we aimed to describe the sperm proteome in *S. collinsi*. Semen samples were collected by electroejaculation in four adults males provided by the National Primate Center (CENP, Ananindeua, PA, Brazil) during 12 months. The characterization of the protein profile was performed in Norwegian University of Life Sciences (Ås, Norway). To separate the viable sperm, the semen diluted in ACP®-118 (powdered coconut water; ACP Biotecnologia, Fortaleza, Ceará, Brazil) was performed a 45/90% percoll gradient and the samples were washed in TRIS/NaCl medium,and stored in a liquid nitrogen until the lyophilization. The freeze-drying sperm was resuspended in 50 µL of lysis buffer, centrifuged and the supernatant was reserved for bottom-up proteomic analysis. The extracted peptides were analyzed by an Ultimate3000 RSLCnano/QExactive system (Thermo Fisher Scientific, Bremen, Germany), set up with a Nanospray Flex ion source. The tryptic peptides were separated in a 120 minutes gradient of 12-45% acetonitrile and MS/MS data were recorded using a standard data dependent acquisition method. Thermo’s Xcalibur software (v3.1) was used to evaluate raw data and Mascot Server searches were performed on an in-house server against a genus *Saimiri* database. Protein categorization was analyzed using the GoFeat and STRAP software. The 2.342 proteins were identified in purified sperm samples. The cellular component categories of squirrel monkeys sperm proteins were mainly defined as cytoplasm (12.4%), cytoskeleton (9.4%), nucleus (8.9%), extracellular (8%), macromolecular complex (7.9%), mitochondria (6.8%) and others (46.6%). The biological processes were mainly described as cellular process (41.9%), regulation (17.6%), metabolic process (11.5%) and others (29%), whereas the most prominent molecular functions were catalytic activity (42.9%), binding (42.9%) and others (14.2%). Most of sperm proteins identified have been reported in other primates, such as A-Kinase Anchor Protein 4-Like, Izumo Sperm-Egg Fusion Protein, Dihydrolipoyl Dehydrogenase and Sperm Acrosome Membrane-Associated Protein 3, which plays important role in the sperm hyperactivation, capacitation, acrosome reaction and sperm-egg fusion. Other proteins of the *S. collinsi* sperm were identified as Serpin B3 and B12, Annexin A2, A6 and A11, Calmodulin, Binder Of Sperm Protein Homolog 1, Dynein Heavy Chain 1, 5, 8 and 9, Phosphoglycerate Kinase 2, Tektin-5, Lactotransferrin, Clusterin, Superoxide Dismutase, Catalase and Apolipoprotein D. Such molecules are associated with sperm protection, motility, ion and lipid transport. Proteomic strategies have been widely used to explore the biological and biomedical significance at the molecular level in the field of male reproduction. In conclusion, our study represents a unique description of the sperm proteome of *S. collinsi*, in order to better understand the mechanisms that involve the sperm cell and thus the reproductive physiology. The *S. collinsi* has been proposed as an experimental model for reproductive biotechnology in other species of the genus *Saimiri* listed as vulnerable (*S. oerstedii* and *S. vanzollini*) and threatened (*S. ustus*) to extinction.

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Purification of BSP1 from bovine seminal vesicle fluid by affinity chromatography

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In recent decades, special attention has been given to the proteins present in seminal vesicle fluid and about their role in sperm function. BSP proteins are known for their interaction with sperm membrane, their role in sperm capacitation, molecular chaperones, markers of cell death and interaction between sperm and oocytes. The present study was conducted to purify Binder of SPerm 1, a 15-kDa protein present in the seminal vesicle fluid of Bos indicus bulls. We used BSP's heparin and gelatin-binding property to isolate them by affinity chromatography. The seminal fluid was extracted from seminal vesicle glands, obtained in a slaughterhouse. Immediately after extraction of seminal vesicle fluid, a protease inhibitor cocktail was added to the sample. Then, the fluid was centrifuged and the supernatant, stored. To precipitate proteins, we added concentrations of ammonium persulfate according to the volume of each sample. These fractions were desalted by dialysis followed by lyophilization for 48h at -50ºC (minimum pressure of 0.035 mBar). Seminal vesicle fluid proteins were subjected to heparin affinity chromatography (Hitrap Heparin HP; GE, USA), followed by gelatin affinity chromatography. Fractions of heparin-binding proteins (HBPs) were pooled, desalted and centrifuged using Amicon MWCO 10 kDa filters. A peak integration of the PrimeView Evaluation Software was used to estimate the percentage of HBPs in seminal vesicle fluid. HBPs were separated by gelatin affinity chromatography (Gelatin Sepharose 4B), followed by dialysis and lyophilization of the samples. To verify the process of purification and to confirm the presence of BSP1, SDS-PAGE and Western blotting with BSP1-specific antibody were used. Based on the chromatographic profiles, HBPs represented 51.5% of seminal vesicle fluid proteins from Bos indicus bulls. Further separation by gelatin affinity chromatography showed that 42.7% of HBPs interacted with gelatin. Western blot with anti-BSP1 antibody purified from rabbit antiserum confirmed the presence of BSP1 in seminal vesicle fluid, heparin and gelatin-binding fractions. Thus, affinity chromatography is an efficient technique to isolate BSP1 from seminal vesicle fluid. Therefore, studies with purification and functional tests are necessary in order to fully understand the mechanisms by which BSP's modulate sperm capacitation.

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Response of hydroxyethyl starch supplementation in commercial diluents on the viability of cryopreserved bovine spermatozoa

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In order to improve sperm viability rates and consequently artificial insemination results, companies and researchers were looking for better freezing protocols, cryoprotection and dilution media. The study objective was to evaluate hydroxyethyl starch (HEA) effectin commercial diluents medium, seeking for greater sperm sample efficiency after hawing. Three ejaculates of 10 nelore bulls (n = 30) wereused. The ejaculates were separated intos even freezing treatments: control group (Tryladil®diluentonly) and 0.6%, 1.5%, 2%, 3%, 5% and 10%ofhydroxyethylstarchinclusions, in the same diluent. All the seven treatments were evaluatedfor microscopic parameters as motility, vigor, membraneintegrity (Eosin), membranefunctionality (HOST) after cryogenic process. Fresh semen were used for internal cryogenic control. Data wereexpresses as meanand standard deviation. T test were used to compare fresh semen versus controlgroup after thawing to evaluate cryogenic protocols. Variance Analysis, KrussKal-Wallis non-parametrict and ANOVA parametric test with Dunnet's post-test were performers to compare the seven treatment into microscopic parameters. Differenceswereconsideredsignificantwhen $p < 0.05$.Fresh semen versus unfrozen control group were statistically significant in all parameters evaluated as motility (65.8±4.6 vs 47.5±7.2); vigor (4.1vs 3.8); integrity (87.2% vs 67.8%) and functionality (70.4% vs 41.3%). After unfreezing protocols, control group (47.5±7.2 and 67.8±9.3) comparing 0.6%(42.5±6.5 and 66.4±9.8), 1.5% (35.7 ±8.9and 64.1±10.1) and 2% (41.3±6.7 and 66.4±10.6) of HEA concentrations did not showed difference in motility and membrane integrity, respectively. However, differences were observed in 3% (18.8±10.6 and 52.9±8.2), 5% (25.2±8.4 and 56.9±9.1) and 10%(14.3±6.8 and 46.8±11.1).The HEA addition into Tryladil® did not improve thawed semen parameters and concentrations above 2%becomesdeleterious for frozen semen.

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Seasonal variations in the seminal plasma composition of male goats

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Despite the importance of seminal plasma in fertilization, few studies highlight the presence and possible functions of the components of seminal plasma of goats during the reproductive and non-reproductive seasons. These elements, mainly proteins, could be identified as markers of the reproductive capacity of males. This study evaluated the monthly changes in the seminal plasma protein and biochemical profiles of Alpine goats that were bred in a tropical climate of high altitude. The assessment of the progressive motility of spermatozoa was performed on fresh and thawed semen, while the evaluation of the functionality of the membrane (hypoosmotic test) was performed after thawing. After evaluating the collected semen, the seminal plasma was centrifuged and frozen. At the end of the experimental period (12 months), the seminal plasma was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 10 and 14%, which paralleled the analysis of the biochemical parameters (calcium, phosphorus and cholesterol). In a 10% polyacrylamide gel, 22 protein bands with molecular weights of 25-181 kDa were identified, while in the 14% gel, 16 protein bands with molecular weights of 5.7 to 165 kDa were identified. The protein fractions of 5.7 and 34.3 to 34.5 kDa showed a profile that varied with the reproductive seasonality of goats, with increased production occurring during the breeding season. In the analysis of the biochemical parameters, the concentrations of calcium, phosphorus, and cholesterol also showed seasonal variation during the experimental period. However, the analysis of the progressive motility of fresh and thawed semen, as well as the hypoosmotic test of thawed semen showed no changes during the experimental period ($P > 0.05$). These results indicate that changes in the protein and biochemical profiles of seminal plasma during the year did not alter the quality of fresh and thawed semen of Alpine goats that were raised in a tropical climate of high altitude.

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Sperm proteins profile of four canine breeds

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The proteomic of the spermatozoa have been studied in some species as bovine, human and horse, but there is no study focused on dogs, otherwise concerning isolated proteins. Studies are done combining different canine breeds in the same experiment disregarding the difference between them. Inbreeding is carry out in dogs to fix desirable phonotypic or behavioral characteristics and may influence semen characteristics compromising reproductive biotechnologies results. Then, this study aimed to evaluate sperm proteins profile from four different canine breeds. The ejaculate of thirteen healthy dogs with ages between one and five years were collected by manual stimulation of the penis in the presence of a bitch on heat when possible. The animals were divided according to breeds: Golden Retriever (n=3), Great Dane (n=3), Bernese Mountain Dog (n=4) and Maremmano-Abruzzese Sheepdog (n=3). The semen was evaluated micro and macroscopically and the seminal plasma were removed from sperm cells by centrifugation at 800g for 10 min. Spermatozoa were washed three times with a buffer (50 mM TRIS, pH 7.2) containing protease inhibitors (0.8 mM EDTA, 1.0 µg/mL aprotinin, 1.0 µg/mL leupeptin and 35.0 µg/mL phenylmethylsulfonil fluoride) by centrifugation at 800 g for 10 min. A protein solubilization buffer (150 mM NaCL, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) was added at a final concentration of 200 x 10^6 spermatozoa/mL. The sperm cells were sonicated with a 3.0 mm probe and 20% amplitude for 30 s, in ice bath, in 10 series, with 1 min arrest between series. After sonication, all the samples were centrifuged at 10,000 g at 4º C for 30 min. Total proteins concentration was determined in duplicate by A280 method in spectrophotometer (NanoDrop A280, Thermo Scientific NanoDrop One Microvolume UV-VIS Spectrophotometers, Waltham, Massachusetts, EUA). The SDS-PAGE was done to remove solubilization buffer components which may compromise the mass nanochromatography column. A total of 50 µg protein was used in 12% separation gel at a vertical mini-cube (Hoefer MiniVE Vertical Electrophoresis System, GE HealthCare, São Paulo, SP, Brazil). The proteins were analyzed by mass spectrometry operated in a top three mode in which a mass spectrum is acquired followed by MS/MS of the three most intense peaks detected. The spectra were acquired using the MassLynx™ software v.4.1 (Waters Corporation, Milford, MA, USA) and the raw data files were converted to a peak list format (.mgf, mascot generic format) without adding the scans and searched against UniprotSProt_012015 (http://www.uniprot.org/) *Mammalia* taxonomy database, using the Mascot tool 2.3.02 *version* and Mascot Distiller MDRO 2.4.0.0 *version* (Matrix Science Inc, Boston, MA, USA). The relative quantification of each protein in the mixture was determined by exponentially modified protein abundance index (emPAI), obtained by Mascot Distiller software. A total of 53 sperm proteins were identified. Seven proteins were common to all breeds. Individual proteins for each breed were expressed, Golden Retriever showed 17 (32.10%), Great Dane 7 (13.2%), Bernese Mountain Dog 11 (20.8%) and Maremmano-Abruzzese Sheepdog 2 (3.85%). As seen for other species, sperm proteins profile seems to be different between canine breeds.

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The Effect of Diluents on the Viability of Bovine Spermatozoa

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This study aim to verify integrity and functionality of bovine sperm plasma membrane, post-dilution and thawing before the addition of three different diluents: Tryladil®, Botu-Bov® and OptiXcell®. For that, two ejaculates of eight Nelore bulls were collected by artificial vagina method and analyzed. In vitro, diluted and thawed samples were evaluated for total motility, vigor, membrane functionality (HOST) and membrane integrity (Eosin) using light field microscopy. Data were expressed as mean and standard deviation (two replicates of every eight animals). Data comparison between *in natura* versus diluted and thawed groups were performed by Variance analysis. *In natura* results presented, in average, concentration of 1886.8 x 10^6 (esp / ml); motility 71.6%, membrane activity by functionality test 77% and by the integrity test 84.4%, viable cells. After dilution it was observed that results for Tryladil®, Botu-Bov® and OptiXcell® diluents were: Membrane functionality 64.9 (%) vs 62.6 (%) vs 67.3 (%); Functionality 47.8 (%) vs 38.8 (%) vs 42.8 (%), 83.0% vs. 83.0 (%) vs 79.0 (%), after freezing; Integrity 65.3 (%) vs 54.3 (%) vs 52.3 (%) respectively. Although the cryogenics deleterious effects were minimized with the diluent use, it reconsiders that this result may have an intrinsic relation with the cooling and freezing curve.

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The role of the antioxidant astaxanthin on the biochemical profile of heat-shocked sperm

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The deleterious effects of heat stress on spermatogenesis and fertility has been well characterized. However,the direct effect of heat stress on mature spermatozoa has not been fully understood. Insemination of cows under heat stress exposes the mature spermatozoa to adverse temperature in the female reproductive tract. Moreover, direct exposure of the mature sperm cell to heat shock increases sperm reactive oxygen species (ROS) leading to DNA damage. Therefore, the objective of this study was to determine the effect of the carotenoid antioxidant astaxanthin on the biochemical profile of bovine sperm exposed to heat shock. Frozen-thawed sperm (2 bulls/replicate) was purified by Percoll gradient. Sperm biochemical profile was evaluated immediately after Percoll purification (0 h control group). Sperm cells (1.5 x 10^6 motile sperm/ml) were then incubated in SP-TALP and SP-TALP-Ethanol (astaxanthin vehicle control: 1.5% ethanol) at 38.5 and 41°C for 4 h, or incubated at 200 nM astaxanthin in SP-TALP-Ethanol at 41°C for 4 h. Sperm cells were centrifuged twice in 10 mM phosphate buffer saline (PBS) and subjected to Raman microscopy (micro-Raman spectroscopy) using a 633 nm laser (Raman Renishaw InVia) for evaluation of sperm head biochemical profile. Spectral acquisition was set up for 10 accumulations of 20 seconds each and center of 1200 cm^{-1}. Raman spectra were analyzed using GRAMS/AI software. This experiment was replicated 5 times. Data were subjected to least-squares analysis of variance using the General Linear Models procedure of SAS. Raman spectroscopy is a non-invasive approach that examines the inelastic scattering of photons by molecular bonds as these scattered photons either lose or gain part of their energy. In the current study micro-Raman spectroscopy from individual sperm cells generated a biochemical fingerprint mostly characterized by the presence of DNA nitrogenous bases adenine (A), guanine (G), cytosine (C) and thymine (T), amide I-III, amino acids, proteins, lipids, hydrocarbons, methylene and methyl groups. Interestingly, there was a temperature x astaxanthin interaction ($P< 0.05$) for most of these vibrational markers of sperm integrity. Sperm exposure to heat shock reduced ($P< 0.01$) Raman peak height intensity for all DNA nitrogenous bases A, T, C, and G as well as amide I-II, proteins and lipids. However, 200 mM astaxanthin reverted these deleterious effects of temperature on sperm biochemical profile to levels similar to control. In conclusion, the antioxidant activity of astaxanthin counteracted the deleterious effect of heat shock on sperm DNA backbone, peptide bonds, proteins and lipids. Therefore astaxanthin protected the sperm cell from the indirect effect of heat-induced ROS damage. Micro-Raman spectroscopy has the potential to assess DNA status on a living sperm.

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Effect of the presence of shade and genetic group on the quantity and quality of oocytes and number of embryos from dairy cows on tropical pasture

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Thermal stress compromises ovarian follicular dynamics and the ability to determine the dominance by the larger follicle, which may compromise oocyte viability. Zebu cattle breeds from Asia are more resistant to heat stress than those from Europe, however, there is evidence that reproductive functions can be compromised by heat. The objective of the study was to evaluate the quantity, quality and recovery rate of oocytes, as well as the embryos rate from zebu dairy cows kept on pastures with shade and without shade. The natural shade occupied 8% of the area and was provided by eucalyptus trees arranged in rows distant each 24 m and 1,5 m between plants. A total of 34 cows were divided into two experimental groups: 17 cows grazing under sun (control group) and 17 cows grazing under areas with shade. Each group consisted of 9 ½ crossbred Gyr/Holstein cows and 8 Gyr cows. All cows were submitted to ovum pick-up (OPU) sessions in the interval between 15 and 21 days. Ten OPU sessions were performed for each group. In vitro fertilization was performed using semen from a bull with known fertility in the laboratory and embryo rate production was evaluated. This study was approved by the Embrapa Cerrados Animal Ethics Committee (CEUA/Cerrados), nº. 533-2541-1 / 2017. The average of black globe humidity index (BGHI) was 79 under the shade and 83 under the sun. The experiment was completely randomized. Data were submitted to the Hartley test and subsequently to a 5% T-test. The variables without normal distribution after transformation were analyzed by non-parametric method and the results were expressed in average per treatment. Effects of environment and of genetic group were tested. The total number of oocytes retrieved was higher for ½ crossbreed cows under sun (22.3) than under shade (14.6) and superior than cows Gyr under sun (15.3). The amount of grade III oocytes was higher in ½ crossbreed cows group than Gyr cows group (8.8 x 5.7) under sunny environment. The number of grade IV oocytes for ½ crossbreed cows was higher in the environment under sun than in shade (12.3 x 6.9). The amount of grade IV oocytes in Gyr cows group (9.7) was higher than in ½ crossbreed group (6.9) under the environment with shade. The rate of oocyte recovery was higher for ½ crossbreed cows on pasture under sun than shade (54% x 42%). The number of follicles from 3 to 8 mm was higher for ½ crossbreed cows under sun than under shade (34 x 26). The amount of viable oocytes were higher for ½ crossbreed cows (10) than Gyr (6) cows under sun. Gyr cows group under sun produced 28 blastocysts in average and under shade 30 ($p > 0.05$). In environment with shade, ½ crossbreed cows group produced 18 blastocysts in average and 15 under sun ($p > 0.05$). The blastocysts rate in Gyr cows group under sun and shade was equal (17%) and in ½ crossbreed cows group was 25% under sun and 30% under shade ($p > 0.05$). The main differences in oocyte characteristics were associated with genetic clustering and not with the influence of the environment. The environment did not affect the amount of embryos among groups. The results indicate that the environment under full sun increases the degeneration of oocytes in ½ crossbreed Gyr/Holstein cows.

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Protein testicular profile in quail (*Coturnix coturnix coturnix*) fed diets containing anacardic acid

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Many studies demonstrate the harmful effects of reactive oxygen species (ROS) in the reproductive and hepatic systems of poultry. Anacardic acid, a phenolic compound found in different parts of the cashew tree (*Anacardium occidentale L.*) and, in a higher proportion, in cashew nuts, has been studied for its antioxidant effects. It is known that the group of anacardic acids have a great antioxidant capacity, inhibiting mainly the production of superoxides and acting in the inactivation of hydroxyl radicals, not propagating their oxidation reactions. Thus, this study investigated the testicular proteome of European quail (*Cortunix cortunix cortunix*) fed with different concentrations of anacardic acid. For this, an experimental plan was performed with four different concentrations of anacardic acid (0%, 0.25%, 0.50% and 0.75%, m: m DM), with six biological replicates in each treatment. The animals started treatment at 21 weeks of age, with an experimental time of 350 days. After slaughter, the testicles were excised, measured, weighed and frozen in liquid N_2. The tissue samples were freeze-dried and the proteins extracted in Triton X-100. The proteins were then quantified and analyzed on polyacrylamide gel (12.5%; SDS-PAGE). The gels images were analyzed by Quantity One v. 4.6.3 (Bio-Rad - USA), using densitometric data for analysis of variance and Tukey's test ($P <0.05$, software R (see 3.3.1). Weight, length, diameter and testicular volume did not show differences between treatments. The electrophoretic profile showed 35 bands of testicular proteins, of which 11 bands were less expressed in animals fed with the supplementation of 0.75% of anacardic acid in the diet. In conclusion, there is no physical difference in the testicular development of quails when fed with the supplementation of up to 0.75% of anacardic acid in the diet, although it presents a difference when it comes to protein expression when supplemented with 0.75% of this acid. Therefore, the protein alterations resulting from anacardic acid supplementation should be better evaluated in order to make it feasible to use quails aiming to maximize the performance and health of these animals, at a cost compatible to consumers. (CEUA/UFC/ 2552250718/2018)

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Deleterious effects of testicular heat shock on seminal quality of wistar rats

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The greatest extension of the Brazilian territory is located in the tropics, whose high temperatures have been related to the changes in the physiological and behavioral parameters of the animals, including the reproductive potential of the males. Exposure of the testicles to heat can initiate the degeneration of the gonad, with impairment of spermatogenesis, fact for which it was objectified to evaluate the quality and quantity of spermatozoa of Wistar rats exposed to testicular heat shock. For this, 10 animals with 120 days of age were divided in two experimental groups (G1: not exposed to heat shock and G2: exposed to heat shock). In this sense, after being anesthetized, the G2 individuals had the testicles immersed in a water bath at 43° C for 15 min. After 30 days of heat shock, the animals were euthanized and the epididymides recovered by dissection. For the recovery of the spermatozoa, the technique of flotation of the tail of the epididymis in Tris-buffer solution was used, with subsequent evaluation of total motility (MT) (0-100%), vigor (1-5) and concentration (10^6). Motility and vigor were analyzed subjectively under optical microscopy (Leica DM500, 100x), while the concentration was determined in Neubauer's chamber under a phase contrast microscope (Olympus, 400x). The data obtained were submitted to the statistical analysis of variance (ANOVA) for comparison between groups, followed by the Tukey test. The percentage data were transformed by the sine arc, a level of significance of 5% ($P < 0.05$) was considered and the results expressed as mean and standard deviations (mean ± SD). In the group not exposed to heat shock (G1) the MT (86.00 ± 4.18), the vigor (2.80 ± 0.67) and the sperm concentration (11.55 ± 6.94 x106) were greater ($P < 0.05$) than those in the group exposed to heat shock (G2, MT: 22.00 ± 16.05, vigor: 2.00 ± 0.0 and spermatic concentration: 1.75±0.35 x10^6). The results showed that the elevation of the testicular temperature was prejudicial to the maintenance of the quality of the gametes collected from the tail of the epididymis after 30 days of thermal insult. This fact can be justified based on the average time required for complete sperm formation in the rat, which is approximately 30 days, associated with the known role of heat in initiating the production of reactive oxygen species (ROS), which are responsible for structural and functional damage to gonads and gametes. Among the injuries caused by ROS, lipid peroxidation, characterized by the destruction of the fatty acids present in the membranes of the spermatozoa and consequent cell death, are highlighted. Based on the findings, it was observed that the high temperatures affect the formation of the gametes, as well as the cells that are in transit along the epididymis, altering both the quality and quantity of gametes. (CEUA/UFRPE/Protocol#059/2015).

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Epigenetic changes during male embryonic development and the spermatogenic process of the porcine germline cells *in vivo*

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Primordial germ cells (PGCs) are the precursors of gametes, these cells approach the epiblast region and migrate to the mesenchymal gonads, forming the genital ridges. PGCs undergo several essential epigenetic, genetic and morphological changes during embryonic development. For this reason, these cells have been studied in several species, including pigs, in order to identify, correct and understand the changes they undergo during the process of forming a new individual and to improve their development and reproductive performance. The objective of the present study is to analyze the dynamics of epigenetic markers and the factors associated to the development of Primordial Germline Cells (PGCs) in male porcine, from the beginning of embryonic development until the spermatogenic process. Different gestational age (24, 26, 29, 35 e 40 days) porcine embryos were collected from pregnant sows artificially inseminated, furthermore, testis tissues were obtained from neonate and adult pigs. Sex identification was performed through Polymerase Chain Reaction (PCR) and electrophoresis in agarose gel using the Sry and Zfy-Zfx genes. The samples identified as male were collected and analyzed for the expression of epigenetic, pluripotent and germline markers (POU5F1, DDX4, DAZL, STRA8, 5mC, 5hC, H3K9me2 and H3K27me3) by microscopy and immunofluorescence techniques. These results showed, there are evident morphological differences among the pig embryonic ages, occurring the initiation of the genital ridge development about day 24 after fertilization (AF) until day 29 AF, when differentiation of the primitive gonads takes place and the sexual dimorphism is noticed by 35 days AF. 40 days AF seminiferous cords are formed and give rise to the seminiferous tubules after birth. Between day 24 – 29 the PCGs arrive to the genital ridge and a quick loss of the histone methylation (H9K3me2/me3, H3K27me3) occurs, although this event is followed by a global methylation and demethylation. After birth a new epigenetic modification occurs in the testicles with the activation of histone H3K9me2, a repressor marker with high stability. Later on, in the adult testis H3K9me2 repression is detected while the histone H3K27me3, a repressive marker with apparent plasticity, increases. Based histological analysis and immunofluorescence of the pig embryos at different gestational ages analysis in this study, it was observed that although the majority of migration patterns of CGPs in mice are found in pigs, there are some differences about the embryonic development, emphasizing the importance of further studies on the particularities of each specie so it will be possible to improve the cell culture techniques and to enable the prevention and treatment of reproductive problems and support studies involving gametogenesis of those species *in vitro*.

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Is Protein Disulfide Isomerase (PDIA1) in stallion seminal plasma associated with fertility?

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Infertility is an important aspect on human and animal reproduction, but in most cases the cause remains unknown. From the comparisons between the protein profile of semen from fertile, subfertile and infertile males of different species, it has been suggested several seminal plasma and spermatozoa proteins as possible markers for fertility and seminal traits. These results point out the need for proper protein folding and function in order to execute their biological roles. Thus, the presence of chaperones in the seminal plasma, like the members of the Protein Disulfide Isomerase (PDI) family, is important for protein quality control. PDIA1 is expressed in several tissues and is involved in estrogen modulation, play a role in cellular events that are dependent on this hormone. The aim of this work was to verify the presence of PDIA1 in equine seminal plasma and sperm, and compare its expression on seminal plasma from fertile and subfertile stallions. Twelve adult stallions with at least two breeding seasons were used. For the study, four semen collections of each animal were performed. Immediately after collection, analysis of motility and sperm morphology were assessed. Then, stallions were divided in two groups, according to the semen analysis and previous breeding history: Group 1: sperm motility greater than 70% and previous history of pregnancy rate higher than 80%; Group 2: sperm motility up to 30% and pregnancy rate under 35%. After the analysis, samples were processed for separation of seminal plasma from spermatozoa. Ejaculates for first centrifuged at 800g for 5 min; the supernatant was centrifuged as 11.000 g for 1h at 4°C to remove cell debris. To the supernatant, protease inhibitor was added and samples were stored at -80°C until analysis. Relative quantification of PDIA1 in seminal plasma was assessed by Western blotting, using anti-PDIA1 antibody and anti-beta-acting. Bands were detected by enriched chemiluminescence method and protein relative abundance was calculated using ImageJ software. PDIA1 was detected in seminal plasma of all ejaculates. There was no difference in PDIA1 in seminal plasma between both groups. According to these findings, it is not possible to consider PDI as a fertility marker in stallions. Despite the present results and considering the presence of PDIA1 in seminal plasma and its the role on estrogen activity, more research on the topic could verify an associatio of this chaperone with sperm traits.

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Relationship between the onset of puberty and the chaperone PDIA1 in cauda epididymis in the colt

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Puberty in the equine species may be defined by the appearance of mature spermatozoa in young animals' ejaculates, as well as endocrine function maturation. Spermatozoa leave testicles with no fertility potential in and need to undergo the epididymal maturation process. During epididymal transit, several proteins are secreted by ether epididymal epithelial cells, interacting with the spermatozoa, providing important, such as: ability to recognize specific sites on oocyte surface during fertilization; acrosome matrix formation; and sperm motility. Since epididymal maturation is driven by proteins, the presence of chaperones is important to guarantee their correct folding and function. Protein Disulfide Isomerase A1 is a chaperone that has a special feature of modulates the availability of oestrogen in a give milieu. This hormone is fundamental for epididymal proper function, thus suggesting that PDIA1 might have key role sperm maturation in the epididymis, in the equine, this chaperone was not yet studied. Thus, objective of this study was to identify PDIA1 in equine cauda epididymis (CE) during the onset of puberty, and quantify it in both epididymal sperm and fluid. Epididymus from 22 healthy Crioulo colts was obtained after routine castration. Samples were classified in three groups according to their age: G1: up to 24 months-old; G2: from 24-36 months-old and G3: older than 36 months-old. Immediately after castration, testicles were measured, weighed, and the epididymis was dissected for epididymal fluid collection, which was put into tubes and centrifuged at 800 g for 10 minutes to separate epididymal fluid from sperm. Supernatant was removed, and cryopreserved at -196º C. Sperm were re-suspended in PBS, lysed, recentrifuged at 11.000 g for 1h at 4°C, and supernatant stored at -196º C. Protein dosing of samples was performed with BCA Kit and relative abundance of PDIA1 was accessed by western blotting using anti-PDIA1 and anti-beta-actin antibodies. PDIA1 was detected in all samples of the three groups both in epididymal fluid and spermatozoa. A higher abundance ($P < 0.05$) of the protein was detected in CE fluid of colts older than 24 months. Sperm PDIA1, however, had no difference between groups. This is the first report of the presence of PDIA1 in equine epididymis and its association with the onset of puberty.

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Steroidogenic enzyme expression is altered throughout development in the testis of iNOS knockout mice

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Nitric oxide (NO) is an important mediator of intra- and extra-cellular processes and is produced by enzymes called nitric oxide synthases (NOS). Several studies in rodents have shown that NO can inhibit testosterone production *in vitro* and *in vivo*, whereas NOS inhibitors can up-regulate steroidogenesis. The inducible nitric oxide synthase (iNOS) enzyme is constitutively expressed in the adult testis in Leydig (LC), Sertoli (SC) and germ cells (GC), which suggests that this enzyme may play a regulatory role in both spermatogenesis and steroidogenesis. Recent studies from our group showed that adult iNOS knockout mice (iNOS$^{-/-}$) exhibit lower GC apoptosis, an 80% increase in SC and LC numbers, and higher sperm production, which are probably a consequence of the higher proliferation index of SC and LC during the perinatal period in these mice. In the present study, our aims were to characterize and evaluate, by immunohistochemistry and qPCR, the expression of important steroidogenic markers in wild type (WT) and iNOS$^{-/-}$ mice, during perinatal testis development and in adulthood. For this purpose, WT and iNOS$^{-/-}$ mice were evaluated on e18.5 (embryonic day), and on Pnd1, Pnd5, Pnd10, Pnd15, Pnd20 and Pnd70 (postnatal days). Blood samples were collected at all ages after euthanasia, centrifuged and plasma stored at -80ºC for testosterone measurement. The testes were either frozen for later quantitative PCR or fixed in Bouin's solution and routinely prepared for immunohistochemistry analysis. The qPCR data were analyzed by taking an average of four duplicates, normalized to β-Actin expression and corrected for the volume density of the cell type in which the marker gene was expressed. The significance level considered was $p<0.05$. Our results show that the anogenital index (an important marker of androgen exposure during fetal life) was significantly increased in iNOS$^{-/-}$ mice at all ages evaluated, except at Pnd5 and Pnd10. The qPCR analyses showed that steroidogenesis was crucially affected by iNOS knockout during the perinatal period (i.e. at e18.5, Pnd1 and Pnd5). Thus, in comparison to the WT, in this period the mRNA expression for luteinizing hormone receptor (LHR), steroidogenic acute regulatory protein (StAR), 3β-HSD, cholesterol side-chain cleavage enzyme (Cyp11a1), 17β-HSD, 5α-reductase and aromatase were all significantly reduced in iNOS knockout mice. Androgen (AR) and estrogen (ER) receptor gene expression were also reduced at e18.5 and Pnd1. In an opposite way, at Pnd15, Cyp11a1, 17β-HSD, 5α-reductase and AR mRNA expression were all significantly increased in the iNOS$^{-/-}$ testis in comparison to WT. In adulthood, the expression of the receptors AR, ERα and LHR was increased in iNOS knockout mice. Furthermore, immunohistochemistry analysis revealed that AR expression was present in LC and peritubular myoid cells (PTM) at all ages evaluated and in SC from Pnd5 onwards in both groups, whereas aromatase and 3β-HSD protein expression were observed in Leydig cells of WT and iNOS$^{-/-}$ mice at all ages. The AR staining pixel intensity was reduced in the LC and PTM of iNOS$^{-/-}$ mice immediately after birth (Pnd1 and Pnd5), but were increased at Pnd10. Regarding SC, AR expression intensity was significantly increased in iNOS$^{-/-}$ mice at Pnd20 (coinciding with the end of SC proliferation) and in adulthood, in all stages of the seminiferous epithelium cycle. Our preliminary plasma testosterone measurements showed no difference between WT and iNOS$^{-/-}$ mice at e18.5 or in adulthood; however, more analyses are necessary to draw conclusions regarding hormone levels in these mice. Taken together, these data demonstrate high variation in the expression of steroidogenesis-related genes during development, as well as in their differential expression in wild type and iNOS$^{-/-}$ mice. Therefore, as expression of most evaluated genes was reduced during the perinatal period, it is suggested that reduced NO levels may negatively affect fetal LC steroid production. However, anogenital distance is increased in iNOS$^{-/-}$ mice and this parameter is considered a marker of androgen exposure during fetal masculinization. The increased expression of some genes observed at Pnd15 could signify that adult LC (which population arises around this time point) is differently affected by iNOS deficiency. In order to better characterize the mechanisms involved in iNOS/NO regulation of steroidogenesis and Leydig cell development, further studies are currently ongoing in our laboratory.

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Testicular development and serum testosterone concentrations in young Brahman males

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The present study had the objective of evaluating the physical and testicular development, and the serum testosterone concentration of 8 to 18 months old male Brahman cattle on grazing weight gaining performance tests. Bovine cattle (n=40), aged 259.76 ± 26.15 days and weighing 239.71 ± 33,94kg had the following characteristics evaluated every 56 days, corresponding to 8, 10, 12, 14, 16, and 18 months of age (six different data collections): body weight (BW), scrotal circumference (SC), girth (G), height at the withers (WH), body length (BL), body mass index (BMI), right and left testicular length (RTL and LTL, respectively), right and left testicular height (RTH and LTH, respectively), average daily weight gain (ADG), testicular volume (TV), and serum testosterone (ST). Analysis of variance and Tukey's test at 5% probability were used. Correlations between variables were assessed using Pearson's method at 5% confidence. There was significant difference ($P < 0.05$) from 12 months of age onwards for ADG and ST. There were differences ($P < 0.05$) for: BW, SC, G, WH, BL, BMI, RTL, LTL, RTH, and TV from 14 months of age. There was a positive correlation between: ST x G (r=0.38; $P < 0.01$); ST x WH (r=0.38; $P < 0.01$); ST x RTH (r=0.23; $P < 0.05$); ST x LTH (r=0.21; $P < 0.01$); ST x TV (r=0.22; $P < 0.008$); TV x weight (r=0.70; $P < 0.01$); TV x SC (r=0.90; $P < 0.01$); and TV x BMI (r=0.93; $P < 0.01$). A rapid increase in serum testosterone concentration occurred between 12 and 14 months of age, followed by rapid body and testicular growth. An elevation in testosterone levels is an indirect indicator that an acceleration inphysical and testicular growth is approaching. It is recommended to calculate both BMI and TV to follow cattle growth due to the high correlation between these variables. (CEUA/UNOESTE/Protocol#1634/2012).

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The expression of pluripotency genes is affected by the exposure to hypoxic conditions during the generation of equine induced pluripotent stem cells

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The objective of this study was to determine whether hypoxic (5% O_2) culture conditions could improve the efficiency of derivation and maintenance of induced pluripotent stem (iPS) cell lines from equine fetal fibroblasts (50-day fetus). A *piggyBac* system was used, as described previously (1) to induce pluripotency. Briefly, plasmids PB-TET-MKOS, PB-CAG-rtTA, PB-CAG-GFP and pCyL43 (2) were used to transfect cells using an electroporation system (Neon Transfection System, ThermoFischer Scientific). After transfection, cells were transferred to an incubator at 37°C and 5% CO_2 either in 5 % O_2 (in a chamber filled with a gas mixture of 90%N_2, 5%CO_2 and 5% O_2) or 20% O_2 (atmospheric oxygen). Cell culture media was composed of DMEM/F12 Knockout (ThermoFisher Scientific #12660-012 Waltham, USA), 20% Knockout serum replacement (ThermoFisher Scientific #10828010 Waltham, USA), 2mM GlutaMax (#35050061, ThermoFisher Scientific Waltham, USA) 0,1mM Non-essential amino acids (ThermoFisher Scientific #11140050 Waltham, USA), 0,1mM β-mercaptoetanol (SigmaAldrich # M6250 St. Louis, USA), 1% Penicilin/Streptomicyn (ThermoFisher Scientific #15140163 Waltham, USA) 1000U/ml LIF (Millipore #ESGRO Burlington, USA), 10ng/ml bFGF (Peprotech #100-18B Rocky Hill, USA), 1,5 µg/ml doxycycline (SigmaAldrich #D9891 St. Louis, USA), 3µM GSK inhibitor (StemGent #CHIR99021, Cambridge, USA), 0.5µM MEK inhibitor (StemGent #PD0325901 Cambridge, USA), 2.5µM ALK/TGF inhibitor (StemGent #A83-01 Cambridge, USA) and 1 µM Thiazovivin (StemGent #Thiazovivin Cambridge, USA). After formation of the colonies an alkaline phosphatase staining was performed (Sigma Aldrich #86R-1Kt St. Louis, USA), followed by the assessment of *OCT4*, *SOX2*, *NANOG* and *REX*-1 endogenous gene expression using equine-specific RT-PCR primers. The statistical significance between two groups was determined using the unpaired t-test (GraphPad Prism). Differences were considered significant when $P<0.05$. iPS cells were tested for embryoid body formation in adhesion-free culture conditions. Both groups cultured in 5% and 20% O_2 formed iPS-like cell colonies (24 and 29 colonies in 5% and 20% O_2, respectively, being the reprogramming efficiency 0,0232% in high oxygen and 0,0192% in low oxygen). Both colonies from 5% and 20% O_2 were positive for alkaline phosphatase staining at passage 5. Expression of endogenous *OCT4* was higher in colonies cultured in 5% O_2 (1,079 ± 0,06235, n=9) when compared to the 20% O_2 group (0,92 ± 0,03629, n=9) (P=0,0426). SOX2 expression, however, was lower in 5% O_2 (1,087 ± 0,1059, n=9) than 20% O_2 (1,76 ± 0,1249, n=9) (p=0,0008). No difference was observed for *NANOG* or *REX-1* expression. iPS colonies derived at both 5% and 20% O_2 were equally capable of forming embryoid bodies. Therefore, hypoxic culture conditions seem to have a positive effect on increasing the expression levels of *OCT4*, a key pluripotency marker. However, more studies are necessary to examine the epigenetic mechanisms by which low oxygen levels affect the expression of pluripotency genes.

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Anti- Müllerian hormone concentration in female gray wolf (*Canis lupus*) – preliminary results

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Anti-Müllerian hormone (AMH) is considered an attractive a biomarker to assess fertility potential, gonadal function and follicular reserve due to the constant expression by ovarian granulosa cells (1). For domestic carnivores, AMH can be used to as a potential predictive marker of fertility (2) and to predict the success of in vitro maturation (3). Data regarding AMH in exotic carnivores is limited, especially for canid species. In cheetahs, AMH concentrations are lower in older females and in females treated with contraceptives (4); no data for wolves are available. The objective of this study was to measure AMH concentrations in female gray wolves. Blood was collected from seven female gray wolves during breeding (winter) and nonbreeding (summer) seasons for two consecutive years. A total of four samples were collected from each female. Five of those females were under contraceptive treatment (deslorelin acetate) in 2016, however in 2017 all of them gave birth to healthy cubs, indicating that in 2017 breeding season they were no longer under deslorelin effect. AMH concentrations in the serum were measured using a canine AMH ELISA (AnshLabs LLC, Texas, USA). AMH mean ± SD was 1,27 ± 1,34 ng/mL and 2,31 ± 0,08 ng/mL, during winter of 2016 and 2017, and 0,44 ± 0,60 ng/mL and 0,43 ± 0,24 ng/mL during summer of 2016 and 2017, respectively. Although AMH concentration was higher during breeding season, no differences were observed between summer and 2016 winter. Since most females were under contraception in 2016, we believe that those low AMH levels are due to deslorelin effect. Similar results were described in cheetahs under contraceptive treatment (4). In 2017, an AMH rise was detected in the contracepted females who had low AMH in 2016, indicating that AMH may be used to assess successful contraceptive reversibility. Furthermore, our data reveal that AMH production in wolves is modulated by seasonality, as was also observed in Siberian hamsters (5) and beluga wales (6). So far, these results indicate that, in female gray wolves, AMH production is lower during the nonbreeding season and in females are under deslorelin acetate contraceptive treatment. More samples will be collected in other to confirm these results.

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Combinations of different cryoprotectants for the vitrification of testicular tissues in collared peccaries (*Pecari tajacu*) – Preliminary results

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Development of biobanking strategies are critical for the management and conservation of collared peccary's populations. In addition to the cryopreservation of spermatozoa, oocytes and ovarian tissues, freezing testicular tissues is another potential source of germplasms from genetically valuable individuals. The objective of our study was to develop a vitrification protocol for peccary's testicular tissue using combinations of different cryoprotectants. Testes from two mature individuals were excised after euthanasia, washed in alcohol 70% and dissected into small fragments (3 mm^3). Three fragments constituting the fresh control group were immediately evaluated for: 1) viability by vital fluorescent probes (Hoechst 33358 and propidium iodide); 2) presence of Sertoli cells by immunohistochemistry using vimentin labelling and scored from 0 (all Sertoli cells lost from the tubule) to 4 (all Sertoli cells present); and 3) histo-morphology after fixation and staining (eosin/hematoxylin) before observations of 30 seminiferous tubules evaluated according to separation of the basal membrane, vacuolization, integrity, swelling, cell loss and rupture, and then scored from 1 (good preservation) to 3 (bad preservation). Other tissue biopsies were immersed in a MEM-based vitrification solution with 10% fetal bovine serum supplemented with 0.25M sucrose. Samples were exposed to different cryoprotectant combinations (three fragments per combination): 1.5 M dimethyl-sulfoxide (DMSO) + 1.5 M ethylene glycol (EG); 1.5 M DMSO + 1.5 M glycerol (G); or 1.5 EG + 1.5 G. After 5 min equilibration, tissues were plunged and stored in liquid nitrogen. After two weeks, samples were warmed at 37°C, washed step-wise in decreasing concentrations of sucrose for cryoprotectants removal and evaluated as described for fresh controls. Data were expressed as means ± standard error. Viability was compared using Fisher's PLSD and morphology by Mann Whitney test. Fresh samples contained 86 ± 6% viable cells and similar values ($P > 0.05$) were found for samples vitrified in DMSO + G (64 ± 3%) and EG + G (69 ± 6%). However, viability decreased ($P < 0.05$) with DMSO + EG (48 ± 16%). Regarding morphology, control samples had a score of 1.21 ± 0.02 for seminiferous tubules and 3.30 ± 0.10 for Sertoli cells. After warming, scores for seminiferous tubules (DMSO + EG: 1.97 ± 0.04; DMSO + G: 2.02 ± 0.03; EG + G: 1.84 ± 0.03) and Sertoli cells (DMSO + EG: 2.10 ± 0.20; DMSO + G: 2.00 ± 0.10; EG + G: 2.30 ± 0.10) were lower ($P < 0.05$) than fresh controls but no significant difference was found among treatments. Preliminary results suggest that combinations of DMSO + G or EG + G preserve testicular tissue viability after vitrification, but that morphology is not affected by the combination of cryoprotectants.

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Corporal and Testicular biometry and seminal characteristics association in wild live cats

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Most of the species included in the *Felidae* family in Brazil are considered endangered and reproductive information for conservation intent is limited. In this context, assisted reproduction techniques come as potential instruments to face such challenges and compile important information for species conservation. Corporal and testicular measurements have been studied for the establishment of correlations with sperm characteristics. The present study corporal and testicular measurements were collected and correlated with seminal parameters from ejaculated obtained from uretral catheterization in wild felids. This project was approved by ethics committee on the use of animals (CEUA n° 10/2016) and ICMBio (SISBIO n° 47822). Three male captive adults of the species *Leopardus wiedii, Puma yagouroundi* and *Panthera onca* were included in the study. The animals were anesthetized with the administration of (1mg/ml Vetoquinol Orion Pharma, Group Orion, Italia.) and 5 mg/kg of ketamine (10% Syntec, Tecnologia Farmacêutica Aplicada a Medicina Veterinária, São Paulo, Brazil) IM. The sedation took place in the animal's recint and then they were transferred to the semen collection site, where they were monitored for respiratory and heart rate and blood pressure each five minutes. The corporal measurements were accessed with metric tape and the testicles were measured with a digital caliper. The prepuce and penis were washed with sterile saline solution and a sterile urethral tomcat catheter (13mm x 1mm, with front opening only, Provar Ltda, São Paulo, Brazil) was introduced in the penile urethra until 7 cm. The sample was aspired with the assistance of a 1mL syringe an then transferred to a micro tube (1,5mL) containing 50-200µL of an extender based on TRIS-egg yolk, pre-heated to 37ºC. Seminal parameters were evaluated for total motility (TM), progressive motility (PM), spermatic vigor (VIG), sperm concentration, percentage of spermatozoa with plasmatic membrane structural (EOS) and functional integrity (HOST), and percentage of morphological alterations. Statistical analysis was performed by the SPSS v.13 software using the Pearson's linear correlation to establish correlations among the variables. A high and positive correlation was observed between full length, muzzle length to tail, thoracic diameter, tail length, head circumference and length (0,9, $P \leq 0,05$). Important and sigficative correlations between corporal and testicular biometry. The variables VIG, PM and TM were positivity correlated ($P \leq 0,05$). High and positive correlations ($r = 0,99$, $P \leq 0,05$) between the parameter EOS and and body weight and head circumference, such as testicular volume, weight and area ($r = 0,99$, $P \leq 0,05$). The results obtained showed that the correlation between corporal and testicular biometrics and seminal characteristics could be predictive criteria for seminal quality of wild felids. In addition, testicle size showed an important correlation with sperm concentration and plasmatic membrane structural integrity. Therefore, the obtained results contribute to a better understanding of the wild male felids.

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Evaluation of epidermal growth factor on *in vitro* maturation of collared peccaries' (*Pecari tajacu* Linnaeus, 1758) oocytes

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Oocyte *in vitro* maturation (IVM) is the first step of the *in vitro* reproductive technologies that enables matured oocytes to be used for embryo production. In this sense, the establishment of culture environment, as medium composition, is essential for the success of the IVM. In some domestic mammals, the supplementation of IVM medium with 10 ng/mL of epidermal growth factor (EGF) increased the IVM rates, and we hypothesized that this effect could also be observed for collared peccary oocytes. Therefore, the aim was to evaluate the EGF on the IVM rates of collared peccary oocytes, wild mammals of great commercial and ecological interest. Thus, eight adult collared peccaries (two females per session) were ovarian stimulated with 600 IU of PG600® (400 IU eCG and 200 IU hCG, MSD Saúde Animal, São Paulo, Brazil). Four days after hormone administration, ovaries were recovered, and all visible follicles with a 3–6 mm diameter were aspirated for the oocyte recovery using a 21 G needle attached to a 5.0 mL syringe containing oocyte recovery medium. Oocytes were classified with a stereomicroscope and only oocytes with more than one layer of *cumulus* cells and homogeneous cytoplasm were used for IVM. Thus, oocytes were matured in TCM199 contained 20 µg/mL of FSH-LH, 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 divided randomly in both groups and matured *in vitro* for 44 h at 38.5°C in a humidified atmosphere with 5% CO_2. Immediately after the IVM, oocytes were evaluated for expansion of *cumulus* cells using a stereomicroscope. After, oocytes were denuded and assessed for the presence of first polar body using a stereomicroscope. Finally, to evaluate the nuclear stage, denuded oocytes were fixed in 4% paraformaldehyde in PBS for 30 min. Then, oocytes were stained with Hoechst 33342 (10 µg/mL) for 15 min and visualized with a fluorescent microscope for identification of nuclear status in second metaphase. All data were expressed as mean ± standard error and analysed by the chi-square test ($P < 0.05$). A total of 172 follicles were aspirated after four sessions of ovarian stimulation. The oocyte recovery rate was 69.8% ± 8.3 (120/172) with an average of 7.5 ± 2.4 oocytes per ovary, and 5.3 ± 1.7 viable oocytes per ovary. 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 (97.6% ± 1.2 *vs.* 100% ± 0.0), presence of first polar body (65.9% ± 1.2 *vs.* 70.5% ± 1.8) and nuclear status in second metaphase (62.5% ± 11.6 *vs.* 68.4% ± 4.9), respectively. Probably, as occurred in swine oocytes, domestic mammals phylogenetically closely to the collared peccaries, the presence of EGF during the IVM can act in the protein synthesis and this effect could be observed during the embryonic development. In conclusion, according to the meiotic potential observed in collared peccary oocytes, EGF has not improved the IVM rates in this species.

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Immunolocalization of leptin receptor of Spix's Yellow-toothed Cavy ovary

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Leptin is a cytokine produced by adipose tissue. Several studies report the role of leptin in maintenance of normal reproductive function, regulating folliculogenesis, oogenesis and estrous cycle. The aim of this study was to investigate immunolocalization of leptin receptors (Ob-R) in the ovary of *galea spixii*. Ovaries from 20 adults, non-pregnant, healthy females were collected. The samples were fixed in 4% phosphate buffered paraformaldehyde, embedded in paraffin and sectioned for immunohistochemistry using antibody against leptin receptor (OB-R H-300 Rabbit IgG, sc-8325, Santa Cruz). The sections were photomicrographed and color intensity of the reaction was measured. In order to quantify the intensity of the positive reactions of each ovary structure, three sections were selected for each structure and analyzed by three independent observers, following the criteria: absence (1), weak (2), moderate (3), strong (4) and very strong (5). Strong immunoreaction was observed in oocyte and theca cells, moderate in ovarian stromal cells and large luteal cells and weak stained in granulosa, endothelial, perivascular and small luteal cells. When compared to receptor expression along follicular development it was observed that the oocyte and the theca cells remained with expression at the same intensity. However, the granulosa cells showed strong stained in the preantral stages, whereas in the antral follicles it expressed low intensity. Thus, the presence of leptin receptor in the main structures of ovary suggest that this hormone acts fundamental role in the reproduction of this species.

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Morphological characterization of the female and male reproductive system of the ocelot (*Leopardus pardalis*): Preliminary data

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The ocelot (*Leopardus pardalis*) is the largest species of this genus and although it has a wide distribution in the American continent and is included in the main lists of species threatened with extinction. This specie is widely studied as to its conservation, but it lacks studies on its morphology. Thus, this study aimed to describe the morphology of male and female reproductive systems of ocelot in order to obtain more information, which may be useful for the reproductive management of this species. Ten specimens (n = 10) were used, of which five were males and five females. All animals g were collected after being victimized by trampling on highways next to Alta Floresta city - Mato Grosso - MT, Brazil (Ethics Committee CEP/FZEA 14.1.1465.74.6; SISBIO 6202874). The study of the reproductive systems was performed through the morphological description, conformation and positioning of the organs and histological analysis through Hematoxylin – Eosin (HE) and Masson Trichrome techniques. In the macroscopic description of the ocelot females, the findings showed that the ovaries are pairs and are located dorsally in the sub lumbar region and caudally to the kidneys. When longitudinally sectioned, we can observe the functional structures of the ovaries, composed of the medullary region, stromal rich in blood vessels and external parenchymal region, surrounded by tunica albuginea. The uterine tubes, which are also pairs, perform the communication between the ovaries and the uterus. The uterus is bicornuated, in which the uterine horns extend into the abdominal cavity reaching the ovary sac and being suspended by the suspending ligament of the uterus. The uterine body has small dimensions and is followed by the cervix, which is composed of a thick wall of smooth musculature. The uterine cervix performs the communication between the uterus and the vagina which is composed of circular thick muscle fibers that make up the longitudinal folds. The vulva presents stern lips in thick conformation and evidenced clitoris. In males, the scrotum is located in the perineal region as an extension of the abdominal epithelium, in the form of a membranous pouch divided by a median septum, which separates the two testicles that are rounded ovoid, with concave lateral border and another medial straight. They are covered by a fibrous outer membrane of whitish pink coloration. Covered by a thin serous membrane and located at the middle edges of the testicles are the epididymis, extending from the cranial end to the caudal, formed by head, body and tail. The head of the epididymis, the most prominent portion of the structure, is located in the cranial portion of the gonad, firmly attached to the vaginal tunica. The prostate is composed of a large, irregularly shaped, compact mass located caudally to the urinary vesicle and cranially to the bulbourethral gland, which presents in two pairs with rounded conformation, located dorsolaterally to the pelvic urethra and caudally to the prostate. The foreskin is thick and covered by hairs, with its ventrally turned orifice, through which urine and semen are ejected. The penis is divided in body, tail and gland with the presence of spicules. From a cross section can be observed their cavernous bodies that are surrounded by tunica albuginea, erectile tissue and deep artery. The microscopic findings of the organs composing the male and female reproductive systems of ocelot are being analyzed and will be presented later.

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Profile of the ejaculate of a Jaguarundi through the pharmacological ejaculation and urethral catheterization

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Electroejaculation is the most used method to obtain semen in felines but is a technique that requires special equipment and trained operator, besides stimulating the animal, requiring larger anesthetic doses. Samples from these protocols are highly diluted and often contaminate with urine. Pharmacological ejaculation with urethral catheterization is a safe alternative with no effect on sperm quality. This study evaluate the ejaculate profile of a Jaguarundi (*Puma yagouaroundi*) obtained through pharmacological ejaculation. This project was approved by the ethics committee on the use of animals (CEUA nº 10/2016) as well as by ICMBio (SISBIO nº 47822). The specimen was kept in captivity at the Zoo botanical Park Getulio Vargas (Salvador, Bahia), weighed 6,3 kg, was already in sexual maturity and received a diet based on beef and chicken, presenting no malnutrition or health problems. The animal was chemically restrained with a combination of 0,1mg/kg of medetomidine hydrochloride (1mg/ml Vetoquinol Orion Pharma, Group Orion, Italy.) and 5 mg/kg of ketamine (10% Syntec, Tecnologia Farmacêutica Aplicada à Medicina Veterinária, São Paulo, Brazil). The prepuce and penis were washed with sterile saline solution and a sterile urethral tomcat catheter (13cm x 1mm, Provar Ltda, São Paulo, Brazil) was introduced in the penile urethra until 7 cm, 20 after the pharmacological induction. Before the procedure, the prostate was digitally stimulated through the rectum and a 1 mL syringe was connected to the catheter and negative pressure was applied to increase suction. After the procedure, the anesthesia was reverted by administration of 0,25 mg/kg antipamezole (4,28 mg/ml, Vetoquinol Orion Pharma, Group Orion, Italy). The volume obtained of semen was measured then diluted in 50 µL of a maintenance extender based on TRIS egg yolk in a polypropylene tube (1,5 mL) and kept at water bath at 37ºC. To obtain the kinetics parameters, 5µL of diluted semen were evaluated under a microscope (100x magnification). The percentage of total motility (TM) and progressive motility (PM) and classification (0-5) of spermatic vigor (VIG) were evaluated subjectively. For the supra-vital test (SV), 5µL of the diluted semen were colored with 5 µL of eosin, a non-penetrant stain, and the smear was confectioned and evaluated microscopically to access the spermatozoa membrane integrity. The sperm concentration was accessed by counting the spermatozoa in a Neubauer chamber. The functional integrity of the plasma membrane was determined by the hypoosmotic swelling test (HOST). The semen (5µL) was added in a 1:10 proportion solution of distilled deionized water and incubated at 37ºC for 5 min. This solution was evaluated under a microscope and the reactive spermatozoa was calculated by counting the percentage of ones that whose flagella swelled and curled and diminishing by the percentage of the sperms that presented curled tails in the morphological test. The diluted semen was fixed in buffered saline formal and the stained smear was evaluated for its morphology under phase-contrast microscopy (1000x magnification). One hundred cells were counted and classified individually as normal, major defects or minor defects. The semen presented 20 µL of volume with $11,4 x 10^6$ total cells and the parameters observed were 60% TM, 50% PM, VIG 3, SV 63%, HOST 81%, 5% major defects and 26% minor defects. The urethral catheterization after medetomidine and ketamine administration is an efficient inducer of ejaculation in Jaguarundi; the sample obtained had no quality change and was not contamined with urine.

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Semen collection in a captive margay (*Leopardus wiedii*) by urethral catheterization after pharmacological induction

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Pharmacological induction for semen collection comes as an alternative to electroejaculation due to its simpler and effective protocol. This method does not require highly specialized equipment or trained operator and reduces the risk of urine contamination in comparison to the electroejaculation. Studies contemplating this method for the margay (*Leopardus wiedii*) were not found in the literature. This study was designed to evaluate the efficiency of pharmacological semen collection in an exemplar of *Leopardus wiedii* from the Zoo Botanical Park Getúlio Vargas in Salvador - BA through urethral catheterization post medetomidine anesthesia. This project was approved by the ethics committee on the use of animals (CEUA n° 10/2016) as well as by ICMBio (SISBIO n° 47822). The species exemplar was a male captive adult who was a sexual mature animal and showed no malnutrition or health issues signs. It weighed 3,95kg and received a diet based on bovine and chicken meat. The animal was chemically restrained with a combination of 0,1mg/kg of medetomidine hydrochloride (1mg/ml Vetoquinol Orion Pharma, Group Orion, Italia.) and 5 mg/kg of ketamine (10% Syntec, Tecnologia Farmacêutica Aplicada a Medicina Veterinária, São Paulo, Brazil). Semen was collected 20-40 min after induction by urethral catheterization. The prepuce and penis were washed with sterile saline solution and a sterile urethral tomcat catheter (13mm x 1mm, with front opening only, Provar Ltda, São Paulo, Brazil) was introduced in the penile urethra until 7 cm. A 1 mL syringe was connected to the catheter and negative pressure was applied to increase suction. In addition, the prostate was digitally stimulated through the rectum. After the procedure, the anesthesia was reverted by administration of 0,25 mg/kg antipamezole (4,28 mg/ml, Vetoquinol Orion Pharma, Group Orion, Italia). The collected semen was pipetted to obtain its volume and then diluted in 50 µL of a maintenance extender based on TRIS egg yolk in a polypropylene tube (1,5 mL) and kept at water bath at 37ºC. The sample showed no signs of urine contamination. To obtain the parameters of sample kinetics, 5µL of diluted semen were evaluated under a microscope (100x magnification). The percentage of total motility (TM) and progressive motility (PM) and classification (0-5) of spermatic vigor (VIG) were evaluated subjectively. For the supra-vital test (SV), 5µL of the diluted semen were stained with 5 µL of eosin, a non-penetrant stain, and the smear was confectioned and evaluated microscopically to access the spermatozoa membrane integrity. The sperm concentration was accessed by counting the spermatozoa in a Neubauer chamber. The functional integrity of the plasma membrane was determined by the hypo osmotic swelling test (HOST). The semen (5µL) was added in a 1:10 proportion solution of distilled deionized water and incubated at 37ºC for 5 min. An aliquot of this solution was evaluated under a microscope and the reactive spermatozoa was calculated by counting the percentage of ones that whose flagella swelled and curled and diminishing by the percentage of the spermatozoa that presented curled tails in the morphological test. The diluted semen was fixed in buffered saline formal and the stained smear was evaluated for its morphology under phase-contrast microscopy (1000x magnification). One hundred cells were counted and classified individually as normal, major defects or minor defects. The animal was successfully anesthetized and through this technique, it was possible to collect the sperm sample with adequate quality, therefore this methodology did not affect it. The semen presented 20 µL of volume with 2,4 x 10^{6} cells and the parameters of 90% TM, 80% PM, VIG 4, SV 63%, HOST 81%, no major defects and 7% of minor defects. Therefore, the urethral catheterization post anesthesia induction with medetomidine and ketamine combined is a practical and effective protocol for sperm collection that can be used in a Leopardus wiedii as it has been used with others wild felines.

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Vaginal microbiota at the different phases of the estrus cycle in *Dasyprocta prymnolopha* Wagler, 1831

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The agouti is a rodent found in the tropical zone of the Americas that has easy reproduction in captivity, presenting a continuous proestrous sexual cycle[5]. During the estrus cycle, several populations of microorganisms present enzymes that allow them to prepare for survival and multiplication in the vaginal environment. The increase in glycogen that occurs at some phases of the reproductive cycle favors the predominance of acidophil organisms among the heterogeneous group that forms the normal vaginal flora[6]. Knowledge of the diseases that affect the reproductive system of wild animals can contribute to control of reproductive disorders and help in the implementation of reproductive management programs, by selecting fertile animals for mating, gamete freeze drying and artificial insemination[14]. There is very little information on health management of the most frequent diseases, that can damage health in captivity[8] therefore knowing the microbiological dynamic in healthy situations may help in the identification, treatment and control of such reproductive pathologies, potentializing the productivity of these captive animals. The objective of the present study was to identify and characterize the prevalence of bacteria existing in the vagina of agouti (*Dasyprocta prymnolopha*) in the different phases of the estrus cycle. Two complete estrus cycles were followed in a total of 12 healthy females of the species, where, by cytological collections with sterile swabs, samples were replicated in culture media and then the colonies were identified by carbohydrate battery. A high variety of colonies was obtained, with bacteria with considerable pathogenic potential among some bacteria common to various mammal species. The bacteria of the genera *Staphylococcus* sp., *Escherichia coli*, *Bacillus* sp. and *Streptococcus* sp. were present in all the phases of the estrus cycle, especially the genera *Staphylococcus* sp. that was the most prevalent in all the phases of the agouti estrus cycle. Among the phases of the estrus cycle analyzed, only in the material collected in the metaestrus were was not verified absence of growth of bacteria colonies of the females analyzed. The Estrus and proestrus presented the highest percentage of absence of growth of bacteria colonies of the collections made. The genera *Enterobacter* sp. and *Klebsiella* sp. were identified only in the estrus and proestrus phases, respectively, whereas strains of *Citrobacter amalonatus* were found in both the phases but the genus *Proteus* sp. was found only in the proestrous phase. It was concluded that knowledge of the microbiota in the reproductive system of wild animals can favor early diagnosis of reproductive pathologies, potentializing all the productive and conservationist process of the species, as for example the agouti.

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