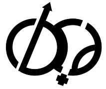

A160 Physiology of Reproduction in Male and Semen Technology

Effect of GnRH on scrotal surface temperature, testicular volume and sperm parameters of bulls with poor semen quality

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Spermatogenesis is coordinated by the hypothalamic-pituitary-gonadal axis, mainly by GnRH secretion. The testis response occurs locally but testicular dysfunctions can be caused by inefficient central stimulus which would justify the use of GnRH to increase male reproductive parameters (CONTRI et al.,Vet Quart,32:5154,2012). Therefore, the aim of the study was to evaluate the systemic treatment with GnRH on the temperature of the scrotal surface, testicular volume and seminal parameters of bulls with poor semen quality. The experiment was conducted at Embrapa, São Carlos-SP. Five Canchim bulls (37.6±6.4 months; 667.4±22.9 kg, 6.3±0.1 BCS), kept on pasture, were used. The animals underwent andrological evaluation and were characterized as low semen quality bulls, according to recommended standards (CBRA, 2013). Treatment consisted of i.v. administration of GnRH analog (gonadorelin 100µg/48 hours) for a total of 3 times. Scrotal thermograms were recorded (T300 FLIR Systems®) under controlled environmental condition during the morning in order to evaluate the mean of the scrotal surface temperature (SST, °C) after 0 (T0), 120 (T120) and 180 (T180) minutes of hormonal administration. Testicular volume (V,cm3) was calculated after measurement of testicular length and width (Bailey et al.,Theriog,49:581-94,1998) and subsequently the semen was collected by electroejaculation. Biometry and seminal quality analyzes were performed two times before hormonal treatment and six times after, every two weeks. Sperm variables evaluated were: sperm plasma membrane integrity (SPMI,%) and total morphological defects (DEF,%). The data from a completely randomized design were submitted to analysis of variance using SAS MIXED and the means were compared by Tukey test ($P < 0.05$). SST did not differ between times (T0: 34.1±0.17, T120: 34.2±0.11 and T180: 34.6±0.14°C, $P > 0.05$). There was no influence of treatment on the testicular volume (V=273.4±20.64 vs 321.3±37.34 cm3, $P > 0.05$) and on seminal quality parameters (SPMI=54.9±7.2 vs 44.1±8.1%; DEF=18.0±4.4 vs. 17.7±4.7%, $P > 0.05$) contemplating means before and after treatment, respectively. Abnormal testicular thermoregulation is one of the most important issues in the fertility of bulls, and it was not altered by hormonal administration, since the scrotal temperature remained within the range considered suitable for normal spermatogenesis in cattle (2 to 6°C below body temperature). The increase in scrotal temperature can cause testicular degeneration, decreasing the space occupied by tubular epithelium and a reducing testicular volume. An increase in abnormal sperm cells, with damaged plasma membrane and morphological defects, is also observed. In conclusion, the administration of GnRH analog maintained the functional scrotal thermoregulation system, but did not alter the testicular and sperm parameters of bulls with low semen quality.

Grants: Embrapa (BIOTEC #01.13.06.001.05.04; Adapt+ #02.12.02.008.00.03), CAPES, CNPq.

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A161 Physiology of Reproduction in Male and Semen Technology

Evaluation of scrotal skin thickness, testicular shape and vascular perfusion of 16-months Braford bulls

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The objectives of this study were: (1) to evaluate the skin thickness in different scrotum formats that could modify testicular thermoregulation; and (2) to evaluate vascular hemodynamics using Doppler ultrasonography in Braford bulls raised in extensive production systems. This experiment was carried out in a farm located at the municipality of Santana do Livramento, Rio Grande do Sul state, Brazil, on a single day of January (2017), from 9 am to 4 pm. A total of 106 Braford bulls, aged 16 months, were kept under extensive breeding conditions. The mean temperature of the day of measurements was 22.6 ± 2.95ºC (19.0 ~27.1ºC) and the relative humidity was 78± 9.06% (61 ~89%), used to calculated Temperature and Humidity Index (THI) and reached the value of 71 ± 3.3 (66 ~75). Evaluations of vascular hemodynamics were performed by Doppler ultrasonography to obtain the mean velocity (MV) (cm/s), end diastolic velocity (EV) (cm/s); systolic peak (SP) (cm/s), and the indexes of pulsatility (PI) and resistivity (RI). Skin thickness were measured by ultrasonography (mm), the animals were divided into two groups, thinner skin (TS – below average) and gross skin (GS – above average). The scrotal format were classified as follow: long (L), long/moderate (LM), long/oval (LO), oval/spherical (OS) and spherical (S). Tukey's test was used as the mean test and Pearson's linear correlation was established for the five variables measured (MV, EV, SP, PI, and RI). The minimum level of significance was 5% and the calculations were performed using a statistical package R. The MV was higher in the animals with LO (15.15±5.01 cm/s) compared to animals LM (13.89 ± 4.76 cm/s). The mean skin thickness of animals with LM (3.71±0.65 mm) and animals with LO (3.58±0.51 mm). The final EV in the testes of LM was lower (9.63±3.93 cm/s) compared to animals with LO (10.91±4.90 cm/s). The animals with LM testes had higher PI values (0.47±0.25) compared to the animals with LO (0.46±0.24). The results found here suggest that the skin thickness is not related to testicular format but can interfere with testicular hemodynamics. No difference was observed between the RI values in the two groups of scrotal format evaluated, with PI values being lower in LM animals compared to LO (0.46±0.24 vs. 0.47±0.25; P>0.05). The correlations found between the measured values and the calculated indices were PI and RI= 0.95, EV and RI= -0.62, and EV and PI= -0.60. The relationship between blood flow and RI and blood flow and skin thickness was negative, which represents the inverse proportionality between the characteristics. Doppler ultrasonography was effective in evaluating vascular hemodynamics in Braford bulls raised in extensive production systems.

A162 Physiology of Reproduction in Male and Semen Technology

The effect of melatonin in the cryopreservation of Crioulo Horses semen

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The cryopreservation of sperm cells, have allowed the dissemination of genetic material to different places at long distances with sanitary and health restrictions. However it success is directly related to the recovery rate of viable cells after freezing/thawing. Therefore, the identification of mechanisms to minimize losses during the cryopreservation is needed. The reduced viability of cryopreserved sperm cells has been associated with the damage caused by the oxidative stress due to the production of Reactive Oxygen Species (ROS) during the cryopreservation process. ROS can affect the plasma membrane, acrosome and the mitochondria of sperm cells, reducing its fertility. Melatonin is a potent non-enzymatic antioxidant highly protective against damage caused by ROS due to the ability to neutralize its toxic effect. Therefore, the objective of our study was to evaluate the effect of different concentrations of melatonin in cryopreserved sperm cells of Crioulo Horses. For that, 3 ejaculates from each of 5 adult stallions (total of 15 ejaculates) were diluted with a commercial extender (FG Mix Garanhão®) in the proportion of 1:1. Samples were than centrifuged (600 x g/12min), and the sperm pellet were rediluted with Equine Frozen Semen Extender (Botucrio®) to a total concentration of $100x10^6$ sptz/ml. Melatonin were added to a final concentration of 0; 1.25; 2.5 and 5 mM. The semen were cryopreseved in 0.5 cc straws and thawed 72 hours later at 37ºC for 30 secons and its motility, acrosome and plasma membrane integrity were verified using flow cytometry with probes FITC – PSA (11,7µg/ml) and PI (10µg/ml), respectively. Data were analysed using PROC MIXED (SAS, v. 9.2 for Windows) with Tukey test, comparing the LSmeans among groups. For the sperm motility the groups without melatonin (NM) (52.0% ± 2.42) and with 1.25mM of Melatonin (50.00% ± 2.18) did not presented statistically differences (P=0.0800). However the group with 2.5mM (42.6% ± 2.2) and 5mM (33.0% ± 3.00) presented lower sperm motility (P<0.0097) when compared with NM (52.0% ± 2.42) and 1.25 (50.00% ± 2.18) groups. Groups with NM (75.48% ± 3.24) and 1.25 mM (80.49% ± 1.62) did not presented difference among acrosome integrity (P=0.1895). Group 2.5 mM (82.94% ± 1.42) and 5 mM (84.90% ± 1.51) presented more damaged sperm cells when compared with group 0 (P<0.0197). When we evaluated the plasma membrane integrity groups 0, 1.25mM, 2.5mM didn’t show statistical differences 66.85% ± 3.13, 66.0% ± 2.42 and 70.07% ± 2.72, respectively P=0.2051. However group 5mM presented elevated damage levels to the plasma membrane (74.92% ± 1.98, P<0.0014). In conclusion, the use of melatonin did not presented to be effective to reduce losses caused by the cryopreservation of equine sperm cells.

A163 Physiology of Reproduction in Male and Semen Technology

Influence of transport temperature on the production of reactive oxygen species of ovarian cattle

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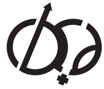
Oxygen is essential for the oxidation of organic compounds and energy production for cellular metabolism. However, part of this oxygen is reduced, producing several highly reactive chemicals. These substances are called reactive oxygen species (ROS), which can cause tissue damage and, at high concentrations, damage cellular organelles, nucleic acids, lipids and proteins. The objective of the present study was to determine the effect of the ovarian transport temperature of *Bos taurus indicus* females on ROS production. Ovarian cortex fragments (n = 18) were obtained with sterile punch (5 mm) and transported (40 min) individually in Minimal Essential Medium (MEM) solution from ovaries (n = 12) collected at local slaughterhouse in one of three temperatures: i) -196°C; ii) 4°C; and iii) 27°C. For the evaluation of ROS, the measurement of the production of superoxide anions in tissue homogenates (10 mg/mL in 1.15% KCl) with a modified nitroblue tetrazolium (NBT) assay was used to quantify the free oxygen production in the tissue homogenate. The reduction of NBT was measured at 600 nm (Multiskan GO, Thermo Scientific) and the mass of the tissue was used for normalization of the data. The averages were compared by the Tukey's test ($p \leq 0.05$). It was observed that samples transported at -196°C showed similar values of superoxide anion reduction (16.2 OD/mg protein ± 1.29) with samples transported at 4°C (15.8 OD/mg protein ± 1.03, p± 0.91; P>0.05). Our results showed an effect of the transport temperature for 40 minutes on the production of ROS in bovine ovaries. In this way, we conclude that the higher transport temperatures differentially affect the production of reactive oxygen species (ROS) of ovaries of *Bos taurus indicus* females.



A164 Physiology of Reproduction in Male and Semen Technology

Identification of seminal parameters predictive of conception rates in *Bos indicus* cows submitted to timed-artificial insemination- partial results

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Ability to predict male fertility is highly desirable for bulls used in timed-artificial insemination (TAI) to achieve better conception rates, consequently reducing reproductive program costs. Our goal was to correlate different methods of post-thaw semen evaluation with the pregnancy (P/AI) of Nelore (zebu) cows subjected to TAI to identify candidate predictors of sire conception rate. The P/AI data from 43231 Nelore cows, inseminated in TAI with basic protocols with application of Estradiol Benzoate on D0 (2 mg im) and use of the intravaginal device with 1 g of P4 for 8 days, followed by application of 1 mg of estradiol cypionate, 150µg of d-cloprostenol and 300 IU eCG on D8. The TAI was performed 40-56 h after, with frozen-thawed semen from 21 Nelore and 50 Angus bulls (P/AIfrom experimental datas and fertility index (IFERT®-Lagoa da Serra). Three samples were evaluated from each bull, with semen batches analyzed for physical, functional and morphological aspects, including subjective means [gross motility, thermal resistance test (TRT), morphology, sperm concentration per ml (total and viable)], sperm tail mitochondrial sheath (MS) length stained with aggresome probe; Computer Assisted Semen Analysis [CASA- total motility, progressive motility, VAP, VSL, linearity, STR, ALH and VCL], hyposmotic swelling test (HOST), and image-based flow cytometry: mitochondrial membrane potential (JC-1), and over 1,300 image-based calculations from nuclear stain DAPI, acrosome status/integrity-detecting lectin PNA (*Arachis hypogaea*/peanut agglutinin) aggresome-detecting probe (AGG), bright field, and side scatter. Data was analyzed using ANOVA (GLIMMIX), Partial Least Squares (PLS) regression with use of Wolds criterion to explore the relative importance of individual sperm variables related to fertility (P/AI). The differences in P/AI were found between bulls (P<0.001), and between breeds – Nelore: 54.44%, and Angus: 49.23% (P<0.001).The following *in vitro* sperm variables were determined to be important predictors of P/AI with negative coefficient: total and tail defects, AGG minor axis intensity standard deviation (SD); AGG width SD; AGG minor axis intensity median absolute deviation (MAD); AGG width mean; PNA H entropy mean; PNA H energy mean; PNA H variance SD. Predictors with positive coefficient included: MS length, polarized (JC-1), gross motility, vigor-TRT, CASA variables (progressive motility, VAP, VSL and VCL), viable sperm concentration, DAPI Elongatedness Head MAD; side scatter H contrast mean; PNA H Variance Mean; PNA H Entropy MAD; PNA H Entropy median; AGG Width MAD; AGG H Entropy SD; PNA +++ Gated; AGG Grad MAX Mean; AGG H Contrast Median; AGG H Contrast Mean. In conclusion, Angus and Nelore bulls differ in P/AI when mated to *Bos indicus* cows. Such multiplex studies correlating sperm parameters and differences in fertility rates observed in TAI are under way and provide an advancement in better understanding sperm fertility potential.

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Effect of Coenzyme Q-10 on sperm motility, plasma membrane integrity, acrosomal integrity and mitochondrial membrane potential of stallions cryopreserved spermatozoa

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Coenzyme Q-10 plays a crucial role in cellular bioenergetics acting as a cofactor in the electron transport chain in the mitochondria (respiratory chain), and is therefore essential for the production of energy in ATP form. In addition to acting as a carrier for electrons and protons in the mitochondria, its reduced form plays a potent lipophilic antioxidant role and is able to "recycle" and "regenerate" other antioxidants such as tocopherol and ascorbate. Therefore, the objective of this study was to analyze the effect of CoQ-10 on sperm motility and plasma membrane and acrossomal integrity, and mitochondrial membrane potential of stallion's cryopreserved spermatozoa. Five ejaculates from five stallions (n=25) were used. Each ejaculate was separated into three treatments: a) control freezing extender (BotuCrio®- Botupharma, Botucatu, SP, Brazil); b) CoQ-10 1 mM and c) CoQ-10 50 µM (added at the respective concentrations to the same extender used in the control treatment). Then, the semen was cryopreserved in automated TK 3000® (Uberaba, MG, Brazil) system and analyzed post-thawing. The analyzed variables were total motility (MOT), progressive motility (MOTPR) and percentage of rapid cells (RAP), which were assessed using the CASA system (HTM-IVOS, version 12.3, Hamilton Thorn Research, Beverly, Massachusetts, USA). In addition, plasma membrane integrity (propidium iodide and *Hoechst* 33342), acrosomal (FITC-PSA) and mitochondrial membrane potential (JC-1) were assessed using fluorescent probes. Cells were classified as the percentage of completely intact cells, I.E., those having intact plasma membrane, intact acrosome and high mitochondrial membrane potential (PIAIH); percentage of cells with intact plasma membrane (IPM); percentage of cells with intact acrosome (IA); and percentage of cells with high mitochondrial membrane potential (HMMP). Data were tested for the normality of residues, and comparisons of the treatments were performed by the MIXED procedure of the SAS program (version 9.3), the differences between the treatments were given by the Tukey test. A significant difference was considered when $P \leq 0.05$. The results of the characteristics of MOT, MOTPR and RAP were similar between treatments. Regarding membranes integrity, the CoQ-10 1 mM addition showed higher ($P<0.05$) percentage of PIAIH cells (31.86 ± 2.00), IPM (32.64 ± 2.05) and HMMP (32.12 ± 1.97) when compared to the control group (27.88 ± 2.35; 29.00 ± 2.49; 28.26 ± 2.39, respectively). Therefore, it is concluded that the addition of CoQ-10 at 1 mM concentration into the freezing extender is better to the membranes integrity preservation of equine cryopreserved spermatozoa.

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A166 Physiology of Reproduction in Male and Semen Technology

Biochemical profile of the Collared Peccaries' seminal plasma obtained during dry period under a semiarid climate

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Collared peccaries *(Pecari tajacu* Linnaeus, 1758) are widely distributed and well adapted to captivity. They have been bred for commercial purposes and used as experimental models for endangered close-related species as the *Tayassu pecari* and the *Catagonus wagneri*. In this sense, knowledge about their reproductive aspects is of extreme importance for the development of assisted reproductive techniques aiming their conservation and multiplication. Therefore, we aimed to characterize the biochemical profile of seminal plasma and to verify the existence of relations among biochemical compounds and semen characteristics of collared peccaries raised under semiarid conditions. The study was conducted during dry period, from September to November 2017. Sixteen adult males were restrained using a net and anesthetized with Propofol (5 mg/kg) in their intravenous bolus. One ejaculate per individual was obtained by electroejaculation and evaluated for volume, pH, sperm motility, vigor, concentration, membrane integrity and functionality, morphology and chromatin condensation. Samples were centrifuged at 385 ×g for 10 min and the supernatant was stored at -20°C. Subsequently, the samples were warmed at 25°C for 2 min and evaluated for biochemistry at using commercial kits. The absorbance was measured in a spectrophotometer according to the wavelengths indicated for each analysis. The results were expressed as mean ± standard error and the existence of correlation among biochemical parameters and semen characteristics was checked by Spearman correlations' test ($P<0.05$). Ejaculates presented 2.0 ± 0.3 mL, pH 7.8 ± 0.2 and 208.8 ± 41.7 x 10^6 sperm/mL, with 84.1 ± 5.3% motile sperm, 84.3 ± 1.9% membrane integrity, 78.2 ± 4.4% functional membrane,76.6 ± 4.7% morphologically normal sperm and 93.7 ± 5.9% condensed chromatin. By biochemistry analysis, the following components were found: citric acid (170.5 ± 43.4 mg/dL), fructose (97.2 ± 31.8 mg/dL), fructosamine (519.9 ± 122.5 µmol/L), calcium (16.1 ± 3.7 mg/dL), cholesterol (137.4 ± 27.9 mg/dL), total proteins (6.5 ± 1.8 g/dL), triglycerides (1427 ± 782.3 mg/dL), magnesium (5.8 ± 0.4 mg/dL), chlorides (415.1 ± 153.9 mEq/L), albumin (34.2 ± 23.3 g/dL) and phosphorus (249.2 ± 237.1 mg/dL). A positive correlation was identified between sperm membrane integrity and total proteins ($r = 0.66$; $P<0.0174$); on the other hand, sperm membrane integrity was negatively correlated with magnesium ($r = –0.59$; $P<0.028$). In conclusion, this is the first description of the biochemical profile of seminal plasma in collared peccaries and we clearly demonstrate that some biochemical compounds (total protein and magnesium) can directly influence the integrity of their sperm membrane.

A167 Physiology of Reproduction in Male and Semen Technology

Effect of exogenous cAMP pathway modulators on capacitation-related events in equine sperm

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In vitro fertilization is inefficient in the horse either using *in vivo* or *in vitro* matured oocytes. However, the transfer of these oocytes to the oviduct of inseminated mares generates fertilization rates comparable to those obtained by natural mating, suggesting that low fertilization rates observed *in vitro* are due to the inability of stallion sperm to penetrate the oocytes as a consequence of the inability to capacitate *in vitro*. Sperm capacitation has been defined as a series of biochemical transformations and plasma membrane rearrangements that allow the spermatozoon to acquire the ability to fertilize the female gamete. Although spermatozoa of different mammalian species can be capacitated *in vitro*, optimal conditions for efficient equine sperm capacitation have not yet been achieved. Only recently, a few studies have demonstrated an increase in protein tyrosine phosphorylation, however, embryonic development to the blastocysts stage has yet to be demonstrated. The present study evaluated the effect of three exogenous cAMP pathway modulators on capacitation-related events in equine sperm. For this, fresh semen was collected from 4 Chilote breed stallions, diluted to $10x10^6$ sperm/mL in no capacitating and capacitating conditions and incubated with different combinations of the inductors for 0 and 4 h at 38°C in air atmosphere using Whitten's medium (McPartlin et al., 2008 Theriogenology, 69, 639-650). Evaluation of sperm capacitation parameters included membrane fluidity (MC540+) and intracellular calcium levels (FLUO 3-AM) as early markers of capacitation, while tyrosine phosphorylation events (PY mAb) and the spermatozoa's ability to perform acrosomal exocytosis (PNA/FITC) were used as late markers. All evaluations were carried out by flow cytometry using a FACS CANTO II flow cytometer (Becton Dickinson, Mountain View, USA). For statistical analyses, proportional data were transformed to arcsine, treatment effects were analyzed by ANOVA and means were compared using Tukey´s test with GraphPad Software (La Jolla, California, USA). The results obtained confirm that capacitation inductors increase the intracellular calcium level and membrane fluidity compared to the non-capacitating control conditions, being significantly higher when 2 or 3 inductors are used simultaneously. Similarly, the inclusion of 2 or 3 inductors significantly increased tyrosine phosphorylation levels. Additionally, sperm incubated under these conditions showed a high percentage of acrosomal exocytosis after exposure to progesterone (58%) compared to non-capacitated spermatozoa (13%). In conclusion, high levels of tyrosine phosphorylation, intracellular Ca^{2+}, membrane fluidity together with the significant rates of acrosomal exocytosis reported confirm that sperm incubated under these conditions underwent molecular and membrane changes consistent with sperm capacitation.

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A168 Physiology of Reproduction in Male and Semen Technology

Influence of centrifugation with a single layer of equine colloidal silica on canine semen freezing

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Canine semen freezing is used to facilitate the management of dog colonies, but also to preserve genetic material of males with high zootechnical and /or affective values. Our aim was to evaluate the influence of a layer of equine colloidal silica-Androcoll-e® (Minitube, Germany) on canine semen freezing. For that, five dogs of different breeds with satisfactory seminal characteristics (CBRA, Manual for andrological examination and evaluation of animal semen, 104, 2013) were used. Seven ejaculates/animal were collected, subdivided in: group a – samples were centrifuged at 300g for 10 minutes using androcoll-e®; and group e- samples were centrifuged at 700g for 10 minutes using caniplus enhance® medium (Minitube). Supernatant was discarded and pellet was resuspended in caniplus enhance® (1ml of medium /100.10^6 spermatozoa), followed by packing (25.10^6 mobile sperm/vane), refrigeration (2 to 6 °c for 2 hours), freezing (20 minutes in n2 at -196 ° c), and thawing (37 ° c for 60 seconds). After that, the samples were resubmitted to centrifugation, forming 4 subgroups (16 straws/animal/subgroup): AA (androcoll-e® in the pre- and post-thawing steps), AE (androcoll-e® in pre-freezing and caniplus enhance® after thawing), EA (caniplus enhance® in pre-freezing and androcoll-e® after thawing) and EE (caniplus enhance® in the pre- and post- thawing steps). The concentration, morphology, vigor, membrane integrity, and subjective and computerized motility were analysed. Test of variance, tukey and kruskall Wallis were used for data analyses and differences considered significant when $P<0.05$. There was no difference between the groups on sperm recovery, subjective motility, vigor and membrane integrity. In computerized analyses, best results were obtained on average of trajectory velocity (VAP) and curvilinear velocity (VCL) in EE group, while crossed flagellar beating (BCF) and rectilinearity (STR) occurred in EA group, and linearity in a group. Regarding sperm morphology, EA group presented the highest percentage of normal cells (95%), whereas in AE group, minor defects (23%) and higher were greater than acceptable (12%). There was no evident improvement of all seminal parameters as previously reported by Morrel et al (Animal Reproduction, 3: 340-345, 2016) using silica specific for dogs. However, the use of colloidal silica equine proved to be a good alternative on canine seminal freezing process since its use after thawing (EA group) was effective in selecting a higher percentage of morphologically normal spermatozoa.

A169 Physiology of Reproduction in Male and Semen Technology

Association between sperm hyperactivation and ovulatory capacity on fertility in timed AI postpartum cows

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The objective of this study was to characterize the degree of hyperactivation in frozen semen of bulls and to evaluate the relationship between hyperactivated motility and ovulatory capacity in timed AI postpartum beef cows. Data from 46 batches of semen from 20 bulls were acquired from a semen-processing center (Seleon Biotechnology, Itatinga, SP, Brazil). The following variables were analyzed by CASA: total motility (TM), progressive motility (PM), average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), straightness (STR), and linearity (LIN). Sperm characteristics of VCL, ALH and LIN were used to classify batches as Hyperactivated (H+; LIN<53%, ALH>7.17 μm and VCL>164.28 μm/s), and Non-Hyperactivated (H-; LIN>53%, ALH<7.17 μm and VCL<164.28 μm/s) (adapted from Mortimer and Mortimer, Journal of Andrology, 11:195-203, 1990). From 20 bulls evaluated in this study, only batches from 2 bulls were selected for field study because they presented batches characterized as H+ and H- within bull. Multiparous lactating Nelore cows (*Bos taurus indicus*; n = 244) from two comercial beef farms from Rondônia - Brazil were used. All cows were subjected to a TAI protocol (D0: 2 mg of BE (IM) + CIDR-in; D8: 150 μg of D-Cloprostenol (IM) + 300 UI of eCG (IM) + 1 mg of CE (IM) + CIDR-out; and D10: TAI). On the morning of Day 10 (07:00 am), the diameter of the preovulatory follicle (POF) was assessed in all cows by ultrasonography. According to our previous data (Pfeifer *et al*., Animal Reproduction Science, 163:89-96, 2015) cows were categorized according to the diameter of the POF in Early ovulation group (EO group, n=126) in which cows were with POF ≥ 13 mm; and Late ovulation group (LO group, n=118), in which cows were with POF<13 mm. H+ and H- semen from each of the chosen bulls were distributed homogeneously into the ovulatory group of cows (EO and LO). Thus, after distribution, the groups were: EOH+ (n=65), EOH- (n=61), LOH+ (n=75), and LOH- (n=43). Pregnancy per AI (P/AI) was higher for EOH+ (69.2%, 45/65), EOH- (63.9%, 39/61), and LOH- (67.4%, 29/43) groups than for LOH+ group (48%, 36/75). These results demonstrate that the use of VCL, AHL, and LIN parameters to characterize sperm hyperactivation contributes to predict fertilizing potential of semen according to the capacity of ovulation of cows in TAI programs. Moreover, this study demonstrates that cows with smaller POF are less likely to become pregnant when they are inseminated with straws considered as hyperactivated (H+). In contrast, when inseminations are performed with straws considered as non-hyperactivated (H-), late and early ovulated cows provided adequate and similar P/AI.

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Selenium supplementation influence on the biochemical profile of ram`s ejaculate

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Selenium is an essential trace element that plays an important role in the reproductive activity, primarily acting spermatogenesis and testicular development (Hefnawy and Tortora-Perez, Small Ruminant Res. 89(3), 185-192, 2010). The aim of the present work was to study the selenium supplementation, in mineral mix supplement, effect on the levels of citric acid, potassium, sodium, calcium, selenium, zinc, manganese, sulfur and lead of ovine semen. Were used 30 rams, with reproductive age, similar weight, fed with hay and ration in an intensive system. The animals were divided in five experimental groups (n=6/group) supplemented with different selenium concentrations: 0 mg; 10 mg; 15 mg e 20 mg of Se/kg of mineral mix. Group control (GC; 0 mg/Se), group 1 (G1; 5 mg/Se); group 2 (G2; 10 mg/Se); group 3 (G3; 15 mg), group 4 (G4; 20 mg/Se). Each group passed through every treatment, every 56 days the treatment were changed randomly. The semen samples were obtained at the end of every treatment period, using electroejaculation in order to perform the fructose concentration (Mann, Journal of Agricultural Science, 38(3):323-331, 1948), citric acid (Saffran and Densted, Journal of Biological Chemistry, 175(2):849-855, 1948), potassium, sodium, calcium (technique of selective ions), zinc, manganese, sulfur (Atomic Absorbance in Flame), selenium and lead (Atomic Absorbance in Graphite Oven). The project was conducted in a latin square distribution 5x5, with the means difference verified using the Tukey test with 5% of probability. There was no difference observed among the treatments for the mean values of none of the analyzed parameters. The mean values with the standard mean error of the maximum value and minimum value of the analyzed parameters in the different groups were: fructose (mg/dL) GC=33.15±2.29 and G1=25.85±2.34; citric acid (mg/dL) G1=6.26±0.85 and G2=5.49±0.83; potassium (mmol/L) G2=10.90±0.89 and GC=9.30±0.85; sodium (mmol/L) G1=136.98±1.68 and G2=133.46±1.64; calcium (mmol/L) GC=0.52±0.021 and G3=0.49±0.022; selenium (µg/dL) G2=0.038±0.004 and G1=0.035±0.005; zinc (mg/L) GC=0.32±0.018, and G2=0.27±0.019; manganese (mg/L) G2=0.39±0.03 and G1=0.28±0.03; sulfur (mg/L) G2=18.12±1.47 and G1=13.98±1.25 (Tukey; P>0.05). The mean concentrations of lead were below the detectable levels of the performed test ($1x10^{-3}$±0 mg/L). The different levels of selenium supplementation evaluated at the present study did not increase, in a statistically mean, the biochemical concentration at the ram`s ejaculate. Independently of the selenium supplementation used, this did not alter the bioavailability of the parameters analyzed. It is probable that the absence of difference of the seminal contents should be related to the mechanisms of their absorption and excretion. During the experimental period, no ovine presented clinical signs of Se poisoning.

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Gene expression of insulin-like growth factor 1 in testes of Wistar rats under different kinds of physical training

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Physical exercises in general cause anabolic changes in body tissues. This effect is the result of the stress caused by the exercises, but the effects depends on the modality of the exercises. The present study (ethics committee number 3400) evaluated the effect of the training (aerobic and anaerobic) in the gene expression of *Igf1* on the testes. For this, the testes were collected from rats were divided into 5 groups (n=7): Control (CT); Aerobic Training in Swimming (TAN); Treadmill Aerobic Training (TAE); Resistive Climbing Training (TRE) and Water Resistant Training (TRA). The animals of the TAN and TAE groups were evaluated for their aerobic capacity by the minimum lactate test (Lan), to determine the training load, the TRE and TRA groups performed maximum strength test for the same purpose. After 4 weeks of training the animals were euthanized and the testes were collected and stored in the freezer at -80°C. The stored testes were analyzed by RT-qPCR for quantitative *Igf1* gene expression. Statistical analysis of the relative gene expression data was ANOVA followed by Tukey, differences were considered for $P<0.05$. There was a significant difference between the groups in the relative *Igf1* gene expression in the testes, CT (0.44 ± 0.16) had lower expression than TAE (2.16 ± 0.44, $P<0.05$) or TRA 3.04 ± 0.28, $P<0.001$). TAN (1.23 ± 0.36) and TRE (1.45 ± 0.40) groups also exhibited lower *Igf1* gene expression than TRA group ($P<0.05$). It is concluded that the physical training modality interferes in the gene expression of *Igf1* testicular, and the resistance training in aquatic environment induces a greater relative abundance of mRNA for *Igf1*. Support by: UNOESTE (protocol number: 3400).

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miR-216b abundance level is different between embryos produced from high and low fertility bulls

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The aim of this study was to investigate miRNAs profile in sperm cells of bulls that present high and low field fertility. For that, six semen commercial batches provided by Alta Genetics® (Uberaba, Brazil) were used. The semen batches were obtained from six Aberdeen Angus bulls (*Bos taurus*) classified as high fertility (HF, n=3, 54.3±1.0%) and low fertility bulls (LF, n=3, 41.5±2.3%) based on field pregnancy rates (P=0.007). Two straws from each batch were thawed (37°C/30s) and analyzed regarding morphological and functional sperm features: motility characteristics using Sperm Class Analyzer (SCA, Microptics, Barcelona, Spain) software; sperm abnormalities by differential interference contrast microscope (Nikon 80i, Tokyo, Japan); and sperm plasma and acrosome membranes integrity and high mitochondrial membrane potential by fluorescent microscopy (Nikon 80i, Tokyo, Japan). The sperm profile of 380 bovine mature miRNAs was analyzed using real time PCR with 100ng of total RNA from each sperm sample using miScript PCR® (Qiagen) according to manufacturer's instructions. Two miRNAs consistently detected among the samples (bta-miR-99b and -425-5p) were used to evaluate the relative abundance levels of the miRNAs. Only miRNAs with Ct value lower than 38 were considered for analysis. Afterwards, sperm miRNAs with P<0.1 in the relative abundance level between high and low fertility bulls were analyzed in mature oocytes (n=6) and in zygotes (n=18) fertilized *in vitro* using sperm from the same batches previously evaluated. Data were analyzed by ANOVA using Mixed procedure of SAS® and means compared with Tukey test when necessary. Except to sperm miRNAs analyses, statistical difference was considered when P<0.05. Sperm samples from high and low fertility bulls were similar (P>0.05) in all morphological and functional features evaluated. Sperm miRNAs analyses detected 14 miRNAs with different abundance levels (P<0.1) between high and low fertility bulls. Among the evaluated miRNAs, bta-miR-216b presented different (P=0.01) abundance level between zygotes derived from high fertility sperm samples, zygotes from low fertility sperm samples and oocytes. The relative abundance level of this miRNA was high in zygotes from LF and low in zygotes from HF and oocytes. In sperm cells, bta-miR-216b levels were higher (P=0.08) in LF than in HF. Bioinformatics analyses performed using DAVID Functional Annotation Tool (DAVID Bioinformatics Resources 6.7) predicted the regulation of important signaling pathways including Wnt, MAPK and focal adhesion. These findings suggest that miR-216b is a potential biomarker for bull fertility that is important for initial embryo development. Our group has been engaged to describe the role of miR-216b on embryo development.

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