A001 Male Reproductive Physiology and Semen Technology

Penis morphometry of the *Trachemys scripta elegans* (Wied, 1839) raised in Brazil

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Keywords: penis, testudines, turtle.

The penis is an organ that plays an important role in reproduction and perpetuation of a species, because it performs the introduction of semen into the female's vagina. However, information on *Trachemys scripta elegans* (*T. scripta elegans*), an underwater exotic turtle invasive to the cerrado, that when released into lakes and rivers may compromise the biological diversity (Primack; Rodrigues. Biology of the Conservation. Monograf, Londrina. 2001. 328p) is nonexistent, which may impair population control measures or comparative studies. This study aimed to describe the penis morphometry of *T. scripta elegans* raised in Brazil and correlate this parameter with body biometric data. Eleven males from the Parque Ecológico do Tietê (IBAMA Record Nr. 2491988), had the body biometrics [mass (BM), volume (BV), carapace length (CARL), carapace width (CARW), plastron length (PLAL), plastron width (PLAW) and height (HEI)] assessed and the penis dissected for morphometric analysis [mass (PM), volume (PV); length (PL), width (PW) and thickness (PT)]. Simple correlation coefficient (R) was determined among the variables (Assistat 7.6 beta). This study was approved by the Human and Animal Experimental Ethics Committee of UNIVASF (Protocol nr. 0001/160412). The BM was 587.45 ±198.37 g, BV 556.54 ± 289.75 mL, CARL 15.50 ± 2.35 cm, CARW 12.2 ± 1.39 cm, PLAL 13.72 ± 1.93 cm, PLAW 9.21 ± 1.48 cm and HEI 5.57 ± 1.34 cm. The PM was 3.55 ± 1.37 g; PV 3.36 ± 1.14 mL; PL 4.71 ± 0.78 cm; PW 1.41 ± 0.16 cm and PT 0.46 ± 0.13 cm. Positive ($P < 0.05$) linear correlation was observed between body biometric parameters among themselves and with the PM, PV and PT; and between the morphometric parameters of the penis among themselves, except with the PT. This was the first study to describe the penis morphometry of *T. scripta elegans* raised in Brazil and to correlate this parameter with body biometric data, providing information that can assist comparative studies and of population control.

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A002 Male Reproductive Physiology and Semen Technology

Does lipidic peroxidation is influenced by the addition of seminal plasma in the thawing media of swine spermatozoa?

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Keywords: boar, oxidation, semen.

Cryopreservation increases reactive oxygen species (ROS) and then lipid peroxidation (Wang et al, 1997, Urology, 49(6), 922-925). It is not well established if the addition of seminal plasma (SP) after thawing reduces lipid peroxidation of the swine spermatozoa membrane. Thus, we tested the addition of 10% SP in thawed boar semen. Four sperm-rich fractions were collected from six boars (n=24). Samples were divided in three aliquots namely no centrifuged (NC), centrifuged resuspended (CR) and centrifuged without SP (CS). After centrifugation (500xg/10min) of CR and CS, the supernatant (SP) of CS was removed and passed by another centrifugation (2500xg/30min), so SP was filtered through 0.22µm membranes and stored at -80°C for future use. CR was resuspended in its own SP. All treatments were extended in freezing extender (Botupharma®, Botucatu-SP, Brazil) to obtain a final concentration of $300x10^6$sperm/mL. The extended semen was stored in 0.5mL straws. The straws were frozen in an automatic system (TK Tecnologia em Congelação, Uberaba-MG, Brazil) using a rate of -0,5°C/min until 5°C, -20°C/min until -120°C and immersed in liquid nitrogen (-196°C). Two straws were thawed in water-bath at 37°C/30seg and extended 1:1 (semen:extender) in freezing extender. From CS added 10% SP (v:v) in freezing medium was originated treatment centrifuged added SP (CP). The samples remained in water-bath at 37°C until 120 min, and analysis occurred at 5, 60 and 120 min. An aliquot was extended in TALP to 5 x 10^6sperm/mL and stained with 2µL of Hoechst 33342, 0.5µL of C11-Bodipy and 3µL of propidium iodide for 40 min/37°C. After incubation samples were analyzed in flow cytometer (BD FACSAria-Becton Dickinson, San Jose, CA, USA). The analyses were based in fluorescent intensity (a.u.) captured in long pass 502 and band pass 530±15 nm detectors. The data were analyzed by SAS program (SAS Institute Inc., 2010) and subjected to analysis of mixed models. Treatments were evaluated using orthogonal contrast to analyze treatment effects; contrasts (centrifugation effect NCxCR; with SP effect CSxCP; without SP effect CSxNC+CP). Differences were considered significant when $P<0.05$ and all results were expressed as means±SEM. There weren't time x treatment interaction ($P > 0.05$) for variable studied. Centrifugation, addition or absence of SP had no effect in lipid peroxidation of swine sperm (241.4±8.94 a.u.; 234.28±10.22 a.u.; 233.94±7.78 a.u.; 230.32±7.22 a.u., NC, CR, CS e CP respectively). Therefore, we can conclude that seminal plasma added in frozen-thawed boar semen not reduces lipid peroxidation.

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A003 Male Reproductive Physiology and Semen Technology

Characterization of the cycle of the seminiferous epithelium stages in guinea pigs (*Cavia porcellus*)

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Keywords: seminiferous epithelium, seminiferous tubule, spermatogenesis.

The spermatogenic process occurs in cycles, called Cycles of the Seminiferous Epithelium (CSE). The characterization of stages and the definition of cellular types present in the epithelium are fundamental to understand the spermatogenesis (França; Russel. Madrid: Churchill Comm. Eur. España, 1998. p.197-219) and the physiological basis of the comparison of healthy, experimental or pathological conditions (França et al. Biol. Reprod. 49:1215-28, 1993). This study aimed to characterize the CSE stages in guinea pigs (*Cavia porcellus*) to enable their use as an experimental model. Fragments of the right testicles of thirty guinea pigs with complete spermatogenesis were collected, fixed in 10% buffered formalin for 18 hours and immersed in alcohol 70% for routine histological processing and staining by HE. The slides were examined on an Olympus BX 50 binocular microscope, equipped with digital camera and the CSE was characterized by the tubular morphology method (Courot et al. NY: Academic Press, 1970. v.1, p.339-431). This study was approved by the Human and Animal Experimental Ethics Committee of UNIVASF (protocol nr. 22041019). Eight CSE stages were observed. Stage 1 was characterized by the presence of type-A spermatogonia (AS), pre-leptotene (PL) primary spermatocytes and Sertoli Cells (SC) near the basement membrane, leptotene (LE) primary spermatocytes between the basement membrane and the pachytene (PQ) ones, and those among the PL ones and rounded spermatides (ROS), that formed between 3 and 4 layers near the lumen of seminiferous tubules. Stage 2 was characterized by the early elongation of ROS nuclei and the presence of AS, PL and LE, zygotene (ZY), PQ and diplotene (DI) primary spermatocytes and SC with irregularly elongated or pyramidal nucleus. Stage 3 was characterized by AS, ZY, DI, ROS, elongated spermatides (ELS), with nucleus intensely stained, queued with radial arrangement towards the light of the tubule and SC. Stage 4 was characterized by meiosis products, intermediary spermatogonias (INS), AS, ZY, PQ, DI, ROS organized in groups and SC. Stage 5 was characterized by AS, INS, PQ, ROS, ELS closer to the base of the epithelium and SC. Stage 6 was characterized by AS, INS, type-B spermatogonias (BS), PQ with nucleus larger than the one at the previous stage and more distant from the basement membrane, ROS, ELS organized for the lumen and SC. Stage 7 was characterized by AS, BS, PQ, ROS, ELS with nuclei placed on the luminal edge and the flagella facing the lumen of the seminiferous tubules, many residual bodies (RC) and SC. Stage 8 was characterized by ELS placed in the lumen, spermatozoa bundles in the light of the seminiferous tubules, RC, AS, BS, PL, LE, PQ and SC. It was concluded that the CSE of the guinea pig and other mammals are similar, substantiating its use as an experimental model for understanding the spermatogenesis regulation.

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A004 Male Reproductive Physiology and Semen Technology

Description of the cell types present in the seminiferous epithelium in guinea pigs (*Cavia porcellus*)

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Keywords: guinea pig, spermatogenic cells, seminiferous tubule.

Spermatogenesis is an organized and synchronous process by which a stem spermatogonia progresses, through mitotic and meiotic divisions, and undergoes a final stage of differentiation called spermiogenesis, to form the spermatozoa (França et al. Theriogenology 63:300-18, 2005). The knowledge of this kinetics has great importance for the characterization of testicular activity of a species. The use of guinea pigs as experimental model has great importance in scientific research; however works analyzing its spermatogenesis are scarce. This study aimed to describe the cell types of the seminiferous epithelium of guinea pigs (*Cavia porcellus*) with full spermatogenic activity (with 6 to 11 weeks old). The right testicle (N= 3/group) was collected, fixed in buffered formalin at 10% for 18 hours and immersed in alcohol 70% for histological processing and staining by HE. The slides were analyzed on an Olympus BX 50 binocular microscope equipped with digital camera to identify the cells of spermatogenic lineage in 200 cross-sections, always considering the most circular sections for each testicle. This study was approved by the Human and Animal Experimental Ethics Committee of UNIVASF (protocol nr. 22041019). Type-A spermatogonia were rounded, with ovoid nucleus and had granular irregularly and dark chromatin; Type-B spermatogonia were more circular and had the nucleus most prominent and with dense chromatin and coarse granules; intermediary spermatogonia had the nucleus fragmented into three parts forming small nucleoli; Sertoli cells were irregular and elongated and, sometimes, pyramidal; pre-leptotene spermatocytes were round and had the fragmented nucleus, but they were smaller than those in pachytene; leptotene spermatocytes had the nucleus most compacted and chromatin of stronger staining; zygotene spermatocytes had the compressed nucleus forming a half moon; pachytene spermatocytes had a large nucleus, circular and with dense chromatin and diplotene spermatocytes had the nucleus smaller and lighter and the chromatin was decondensed compared the previous stage. Spermatids rounded had a small, circular and dark nucleus and spermatids elongated were located near the tubular lumen. In conclusion, the cell types present in the seminiferous epithelium of guinea pigs were similar to those described in other rodents and they are essential for the characterization of the seminiferous epithelium cycle.

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A005 Male Reproductive Physiology and Semen Technology

Cryopreservation affects binding of bovine epididymis sperm to oviduct explants

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Keywords: bovine, isthmus, semen.

The use of epididymal sperm is an important tool for the propagation of genetic material in cases of the unexpected death or acquired reproductive incapability of high genetic value sires. A better physiological behavior understanding of those sperm is needed to optimize their use either *in vivo* or *in vitro*. One of the features required for *in vivo* fertilization is the formation of a reservoir on the female oviduct. Studies in our laboratory have shown that epididymal sperm have the same ability to bind *in vitro* to those cells as the ejaculated sperm. Considering that cryopreservation can induce sperm plasma membrane changes, we hypothesized that those changes may directly influence the sperm binding to the female reproductive tract. Thus, the aim of this study was to evaluate the effect of cryopreservation on the ability of epididymal sperm to bind to oviduct cells. Testis from four *Bos Indicus* sires, were used. The sperm was recovered from the epididymis of each animal and were separated into two fractions. One was used just after their recovery and was referred as fresh sperm (FS), and the other fraction was used after being cryopreserved and was denoted as cryopreserved sperm (CS). Sperm from each group were co-incubated for 30 minutes, 6 and 24 hours with 60 oviductal cells explant (OCE) per group/moment. Images of each OCE after co-incubation were captured using Zeiss Axiophot software, coupled to a phase contrast microscope. The perimeter of each OCE and the number of sperm bound to the periphery of each explant were evaluated. Then, the number of sperm bound per mm of the explant was calculated for each aggregate. One evaluation was done for each bull/group, being the bull considered as biological replica. Data were analyzed by the software Sigma Stat 3.11 (Systat Software, Inc., Richmond, California, USA), compared by Mann Whitney test (mean ± SD, $P \leq 0.05$). The FS group had a higher ($P=0.05$) number of bound cells after 30 minutes of incubation (130.92±39.54) than the CS (88.04±28.06) group. At 6 hours of incubation there was no difference between FS (47.33±17.54) and CS (54.42±43.0) group. However, at 24 hours FS group showed higher ($P=0.008$) number of sperm bound to OCE (131.74±92.18) than the CS (20.38±20.15) group. It is important to note that the amount of bound sperm on FS group at 6 hours was lower than that found at 24 hours. However, on the CS group, the decrease on the number of sperm bound to OCE was constant throughout the entire culture period. It can be concluded that cryopreservation of epididymal sperm affected their binding to oviductal cells, causing a drastic reduction on the sperm bound number at 24 hours post-thawed.

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A006 Male Reproductive Physiology and Semen Technology

Influence of plasma testosterone and total protein of seminal plasma on the sperm parameters from Nellore bulls with semen of high or low freezability

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Keywords: sperm analysis, testosterone, total protein.

The beneficial effect of testosterone (T) and some protein fractions of seminal plasma have been reported for bovine fertility. However, researches aiming to correlate plasma T or total protein of seminal plasma (TP) and seminal parameters are scarce. Thus, the goal of this study was to verify the influence of T and TP on the sperm parameters from Nellore bulls with semen of high or low freezability. Ejaculates (n=40) from eight bulls, housed at Center of Semen Collection and Processing (CSCP), were obtained through artificial vagina, in the months of June and July. Animals were selected from data of semen freezability over a period of two years, based on sperm analysis of motility (M) and vigor (V). Therefore, bulls were split in two groups, as follows: semen of high (H; M≥50%; V≥4; n=4) and low (L; M=≤40%; V=3; n=4) freezability. Seminal samples were evaluated for motility, vigor, concentration and sperm abnormality (% of major defect, minor and normal), before freezing process. The seminal plasma was obtained by centrifugation (700 x g/10 min). Blood samples (five per animal) were obtained just after semen collection through vein puncture. The T and TP were measured using commercial kits (DPC- Diagnostic Products Co®, EUA; Pierce, EUA, respectively), according to manufacturer's instructions, being kept at -86º C until use. The coefficient of Spearman correlation was utilized to verify possible correlations between spermatic variables with T and TP. For comparison between the groups, mean values were analyzed through use of Tukey's test. $P < 0.05$ was considered as significant. Higher mean values ($P<0.05$) for motility, vigor and normal sperm, as well as lower values for major defect and minor sperm defect were observed for bulls in group H than for L. However, no difference was found between the groups for sperm concentration. Significant correlation ($P<0.05$) was found only between TP and major sperm defect ($r = -0.45$) for group H. Nevertheless, for bulls in group L, significant correlations ($P<0.05$) were detected for TP and concentration ($r = 0.52$) and sperm motility ($r = -0.54$). As for T, significant difference ($P<0.05$) just was found for minor sperm defect ($r = 0.50$) in group H. No difference was observed between H (639.6±277.7 ng/dL) and L (729.3±410.2 ng/dL) groups for T. However, higher mean value ($P<0.05$) for TP was found in group H (41.7±9.4 mg/mL) than in L (31.9±10.3 mg/mL) group. In conclusion, the results suggest that TP can be used as an indicator for sperm quality in bulls maintained in CSCP. However, the mechanism through which TP affects such sperm parameters needs to be elucidated.

A007 Male Reproductive Physiology and Semen Technology

Sperm quality separation through density gradient in dogs

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Keywords: dog, percoll, spermatozoa.

The sperm separation by density gradient is a technique used to remove the extender from cryopreserved samples and select sperm with high motility for its use in reproductive biotechnologies. The 45% and 90% Percoll® gradient separate the semen into two distinct fractions: low sperm motility in the interface between the two Percoll® layers; and high motility sperm in the sediment. However, studies in dogs are still required to confirm the quality of spermatozoa selected by Percoll®, in both fresh and cryopreserved samples. The aim of this study was to verify the quality of sperm samples separated by Percoll® from fresh and cryopreserved-thawed canine semen. Nine dogs were used to obtain semen samples, which were divided into two aliquots: one designed for immediate evaluation and the other was cryopreserved by standard protocol and submitted to post-thaw evaluation. Semen samples were layered over two-steps pads of Percoll® (1 ml of 90% Percoll® and 1 ml 45% Percoll®). The tubes were centrifuged for 20 min at 300 xg . After separation, fresh and post-thaw samples from the interface and sediment were analyzed by computerized assessment of sperm motility; integrity of plasma and acrosomal membrane by eosin/nigrosin and Fast-Green/Rosa-Bengala stains, respectively; and mitochondrial activity by 3,3 '-diaminobenzidine (DAB) stain. We used Student's t-test and Wilcoxon test. The level of significance adopted was $P<0.05$. Both fresh and post-thaw semen had higher percentage of sperm with intact acrosome in the sediment (thawed: 81.8% ± 14.5; fresh: 93.4% ± 14.1) compared to the interface (thawed: 53.3% ± 7.7; fresh: 78.8% ± 19.7). We observed differences on the percentage of spermatozoa with high mitochondrial activity in the interface (thawed: 23.9% ± 18.4; fresh: 56.3% ± 29.6) and sediment (thawed: 65.8%± 26.2; fresh: 76.8% ± 17.6). High sperm motile spermatozoa was selected in the post-thaw semen sediment (interface: 16.2% ± 16.6; sediment: 31.7% ± 48.4) and fresh semen sediment (interface: 4.5% ±8.6; sediment: 35.3% ± 35.9). No difference on plasma membrane integrity among samples from sediment and interface were verified. However, post-thaw sediment samples (26.4% ± 18.2) had lower percentage of sperm with intact plasma membrane compared to fresh samples (65.2% ± 27.5). In conclusion, Percoll® gradient selects the best quality sperm in fresh and post-thaw samples. In addition, cryopreserved cells have greater sensitivity during passage through the gradient, resulting in plasma membrane injury.

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A008 Male Reproductive Physiology and Semen Technology

Comparison between the spermatic parameters of boars during the months of Summer and Winter

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Keywords: ejaculate, season, swine.

The present study aimed to evaluate the influence of the season of the year (temperature and humidity) comparing the spermatic parameters between summer and winter. Five boars were used aging from two to three years old, with weight of 250 to 300kg, from hybrid breed without physiologic alterations and with satisfactory reproductive performance. The ejaculate was collected by digital manipulation once a week of each boar. The evaluated spermatic parameters were the spermatic concentration using a sperm densimeter and Neubauer chamber, and the spermatic morphology. The weather temperature and humidity were measured during the summer and winter months of 2012 and 2013. After obtained the data those were subject of comparison between summer and winter using Student`s T test, at the significance level of 5%. The boars in this experiment represented the control of themselves, since they were used in both seasons (summer and winter) and years (2012 and 2013). The maximum and minimum temperatures during the summer were 29.3°C and 21.7°C, respectively, and for the winter were 28.1°C and 18°C, the mean temperature was 24.86±2.28°C at summer and 24.18±3.41°C ($p=0.495$).The humidity (%) in the summer was 82.25±10.76 and 81.66±0.75 at winter. Beside the numeric difference of temperature between the seasons analyzed were not found statistical difference ($p>0.05$). The spermatic concentration estimated with the sperm densimeter was statistically different from the calculated using the Neubauer chamber during winter (256.5±44.54a and 170.9±44.98b, respectively; $p<0.001$), probably due to a higher level of cellularity in the ejaculate during this season, leading it to a higher opacity. However at summer, no difference was found between the two techniques to achieve the spermatic concentration (243±57.98 by sperm densimeter and 237.9±56.25 by Neubauer chamber; $p=0.844$). That result reveals the importance of the manual evaluation because any change at the opacity of the ejaculate can be a factor to overestimate the spermatic concentration. The mean ejaculate volume were lower at summer than at winter (261.5±50.22b and 348.5±52.02a mL, respectivelly; $p<0.001$). The heat stress can cause a hormonal imbalance that could affect the seminal plasma production by the accessory glands (Scheid, Suínos & Cia, v.1, p.25-28, 2002). The major defects were found in higher incidence ($p=0.009$) at summer (20.1±15, 8%a) than winter (5.25±2.3%b), being higher than acceptable for the specie at summer (Hafez, Reprodução Animal, Ed. Manole, 2004). It was concluded, using these partial results, that the temperature elevation at summer had a negative influence in the sperm quality of the studied boars, showing a lower ejaculate volume, a lower sperm concentration and a higher presence of major defects during the summer season, also the importance of the Neubauer chamber to evaluate the sperm concentration.

A009 Male Reproductive Physiology and Semen Technology

Fatty acid profile of spermatozoa and epididymal fluid during sperm maturation in dogs

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Keywords: dog, epididymal maturation, fatty acid.

Fatty acids are the functional unit of the sperm plasmatic membrane, which undergo changes in their chemical composition during sperm maturation in the epididymis, enabling the transport, storage and the fertilizing ability of spermatozoa. However, elucidations of such changes in the canine species are important to improve canine reproductive biotechnologies. Therefore, the purpose of this study was to determine the fatty acid profile of the sperm membrane and epididymal fluid during sperm maturation in the epididymides. The fatty acid analysis was conducted in the sperm samples of twenty dogs submitted to bilateral orchiectomy. Epididymis were stored at 5°C for up to 24 hours and then the epididymal samples were collected through incisions (<1 mm) individually in the caput (CAP), corpus (COR) and tail (TAIL). Samples were deposited in 300 µl of PBS, centrifuged and then separated into samples of sperm (pellet) and fluid (supernatant). The determination of the concentration of fatty acids was performed using the transesterification method, followed by injection into the gas chromatograph. Data were compared by ANOVA and LSD test ($p \leq 0.05$). For the epididymal fluid, the samples from TAIL showed higher concentration of total fatty acids (4363.1±1933.7 mg/dL) compared with the CAB (632.7±171.5 mg/dL). However, in the sperm samples, the lipid composition of sperm varied during epididymal migration. We observed the presence of undecanoic and nervonic fatty acids only in the CAB and COR and the absence of tridecanoic and palmitic fatty acids in these same segments, but presents in the TAIL. Also, the concentration of saturated, monounsaturated and polyunsaturated fatty acid were higher in TAIL, especially the stearic and caprylic fatty acids (saturated) and heptadecanoic (monounsaturated) and docosahexaenoic acid (DHA - polyunsaturated). Such modifications during epididymal transit denote the necessary modulations of the fatty acids profile in the plasmatic membrane, which are considered essential for chemical and structural remodeling of the spermatozoa. Additionally, the epididymal fluid was considered a marker or regulator of lipid secretion. Fatty acids secreted by epididymal cells from CAB are incorporated to sperm. Thus, it is expected a higher concentration of fatty acids in the region of the TAIL epididymis. In conclusion, the fatty acid profile in spermatozoa and epididymal fluid varies according to the epididymal transit, reflecting the modulation of lipid profile. Such events should result in normal sperm motility and high mitochondrial activity and, ultimately, ability to fertilization.

A010 Male Reproductive Physiology and Semen Technology

Use of different types of egg yolk and insulin concentrations in cryopreservation of ram sperm

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Keywords: chicken, duck, insulin.

The objective of this study was to evaluate the viability of ram sperm, submitted to the diluting and cryopreservation process, using two different types of egg yolks diluents and three insulin concentrations. One ejaculate from six Santa Ines rams, was divided into treatments T1 (Lactose yolk chicken x Insulin NPH - 0µIU/mL, 100µIU/mL and 200µIU/mL) and T2 (Lactose yolk duck x Insulin NPH - 0µIU/mL, 100µIU/mL and 200µIU/mL), previously evaluated according to CBRA 1998 parameters (Manual for breeding soundness examination and evaluation of animal semen. Belo Horizonte, 1996. 65p. Prepared according to the CBRA/MA n. 017/1998 agreement). Subsequently, the semen was packaged and then frozen using an automated system for cryopreservation (TK - 3000® model), through equal stability times. After thawing, the semen was evaluated for motility, vigor, rapid thermoresistance test (RTT), hypoosmotic swelling (HOST) and Trypan Blue/Giemsa staining tests. It was a randomized block design with a 2x3 factorial (two types of yolk x three concentrations of insulin). For parametric analysis, the Tukey test was used, while for the non-parametric analysis the Freidman test was done ($P<0.05$), using BioStat 5.0. For the RTT, there was no significant difference ($P>0.05$) between the periods within extenders and insulin concentrations, and being grouped with mean T1: 35 and 3.61% and T2: 28 and 4.44% for times 0 and 30 minutes, respectively. The same was found in HOST, with average values of 7.83% and 6.33% in T1 and T2, respectively. In the evaluation with Trypan blue/Giemsa stain, the integrity of the membrane was similar ($P>0.05$) for insulin concentrations and types of egg yolk used. The rate of acrosome reaction evaluated by Giemsa, showed no significant difference ($P>0.05$) for both alive with and without acrosome for insulin concentrations and types of egg yolk used. However, the dead sperm cells with and without acrosome showed significant difference ($P<0.05$) when 100µIU/mL of insulin was used in both extenders. Thus, it was observed that among the dead sperm, those with higher amount of intact acrosomes were from the duck egg yolk extender supplemented with 100 µIU/mL of insulin, obtaining 77.83% of sperm with acrosome compared to 44.83% of sperm acrosome with chicken egg yolk extender treatment. These results indicate that the duck egg yolk can be used in cryopreservation of ram semen and the supplementation of 100µIU/mL of insulin in duck egg yolk extender can promote fewer acrosome reactions during cryopreservation.

A011 Male Reproductive Physiology and Semen Technology

Evaluation of thermal comfort, physiological and seminal parameters of buffaloes kept in artificial insemination center under tropical climate

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Keywords: animal welfare, *Bubalus bubalis*, semen quality.

The study evaluated the variation of thermal comfort indices, physiological parameters and seminal buffalo bulls kept in place of tropical humid climate (Koppen of Afi) in Belém-PA. Ten bulls (55.0 ± 8.9 months; 701.4 ± 82.8 kg) were kept in collective cages in Central Biotechnology of Animal Reproduction (UFPA) from April to August 2013, with free access to the trough and automatic drinking fountain. Inside the sheds three data loggers were installed to monitor the temperature (Ta) and relative humidity (RH). The temperature and humidity index (THI) within the bays (average of the three dataloggers 06h00 to 09h00 12h00 to 15h00) was calculated according to the formula: THI=(0,8Ta)+(RH/100)[(Ta-14,4)+46,4]. Every 25 days with respiratory rate (RR, mov / min), heart rate (HR, beats / min), rectal temperature (RT, ° C) and calculated the index of thermal comfort Benezra (ICB) were measured according to the formula: during the morning (6:00 a.m. to 9:00 a.m.) and afternoon (12:00 to 15:00). Semen was collected weekly and ejaculates subjected to physical and morphological evaluation. Data were analyzed using the general linear model of SAS software, version 9.3, with mean comparisons performed by Tukey test and correlations calculated the Pearson test. The level of significance was 5%. Ta and RH were averaged 31.5 ± 0.8°C and 81.3 ± 3.8%, respectively. The average value of THI within bays ranged between 75.9 and 83.4, above recommended for buffaloes. During the months of July and August, the values of RR, HR and ICB showed a significant increase, with 28.9 ± 3.3 and 27.6 ± 3.4 mov / min, 66.9 ± 2.6 and 65.6 ± 2.4 beats / min and 2.25 ± 0.15 and 2.19 ± 0.15 respectively, while the RT was reduced (38.1 ± 0.5 and 37.8 ± 0.7 ° C, respectively). Regardless of the month, values of RR, HR, RT and ICB showed elevation of the morning to late, but always within the physiological range (Garcia et al., 2011. Brazilian Agricultural Research, v.46, p.1409-1414). The positive correlation between the average THI within the bays and RT (0.63, P <0.001) showed the rise of animal internal temperature due to the increase of Ta. The seminal parameters showed mean 2.2 ± 1.4 mL for volume, 1322.0 ± 501.9 x106sptz/mL for sperm concentration, 68.7 ± 10.2% for progressive motility, 67.8 ± 9.7% for integrity of the plasma membrane, 5.7 ± 4.2% for minor defects, 13.9 ± 7.0% for major defects and 19.7 ± 8.7% for total defects (P> 0.05). The average THI within the cages correlated with plasma membrane integrity (-0.17, P < 0.05), indicating that Ta can have negative impacts on semen. Therefore, despite variations in thermal comfort, the thermoregulatory system enabled quickly offset the caloric gains, saving homeothermy without manifestation of heat stress and maintenance of sperm quality.

A012 Male Reproductive Physiology and Semen Technology

Comparison of kinetic characteristics of frozen-thawed sperm from fertile *Bos taurus* and *Bos primigenius* bulls in Peru

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Keywords: bull, kinetic, spermatozoa.

The breed "Lidia" (*Bos primigenius*) is a very important cattle in many areas of Central and South America, due to resistance and adaptabitliy in the area since 1540. Artificial insemination is little applied in these animals, due to the agressive and dangerous behaviour of the bull. Semen collection is possible only with restraint and electroejaculator. There are few proven bulls for artificial insemination programs. Sperm motility becomes critical at the time of fertilization and its evaluation depends on the experience of the operator, the desire for precision and repeatability, and the availability of equipment. Computer-assisted sperm analysis (CASA) basic concepts for identifying sperm and their motion patterns have little changed and to make the assessment of semen quality more objective and detailed and several specific motility parameters describing the movements of spermatozoa in a more detailed manner can be obtained. The current work has as main objective the investigation of sperm kinetic characteristics of fertile *Bos Taurus* (Holstein and Brown swiss) and *Bos primigenius* (Lidia Bull) bulls living in Peru by using CASA. Ten smears of *B. taurus* semen and four smears of *B. primigenius* semen have been evaluated. A total of 29,349 spermatozoa were quantified in terms of the following parameters: Curvilinear velocity (VCL), Average path velocity (VAP), Straight line velocity (VSL), Beat cross frequency (BCF), Amplitude of lateral head displacement (ALH), Linearity of track (LIN) and Straightness of track (STR). Differences between species were analyzed in a completely randomized design with two treatments with significance defined at $P < 0.05$. Statistical evaluations were carried out using the SAS Software, PROC GLM. We obtained higher ($P < 0.05$) VCL (66.6 vs. 50.4 um/s), VAP (37.5 vs. 29.6 um/s) and BCF (7.1 vs. 5.8 Hz) for *B. taurus* than for *B. primigenius*. However, there were no differences ($P > 0.05$) between *B. taurus* vs. *B. primigenius* for VSL (22.9 vs. 17.8 um/s), ALH (3.4 vs.x 2.9 um), LIN (34.9 vs. 35.4%) and STR (61.1 vs. 60.4%). These differences may indicate that the kinetic characterization of sperm cells of bulls may have peculiarities that are specific for each subspecies, requiring further investigation.

A013 Male Reproductive Physiology and Semen Technology

Biometrics characterization of accessory sex glands and vascular indexes of testicular and dorsal arteries in Dorper rams

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Keywords: bulbourethral, prostate, vesicular glands.

The study aimed to evaluate in rams of Dorper breed the measures of male accessory glands (B-mode ultrasound) and theirs correlations with the testicular biometry. Doppler mode was used to evaluate the vascular parameters of testicular and dorsal arteries. Thirty four healthy and sexually adult males were used, between 10.38±3.01 months old and weighing around 65.06±13.23 Kg. The transrectal ultrasonography exams were performed with linear probe using the frequency of 7,5MHz in MyLab 30Vet (ESAOTE, Holland). The glands measures were obtained with specific software from the ultrasound device, the organs visualization were accessed in cuts obtaining their maximum diameter (MTD = mean of two dimensions: vertical and horizontal). The testicular length and scrotum perimeter were obtained with a tape measure. For all measures from pair structures (left and right) a mean were calculated, except for the prostate gland wherein three portions (caudal, medial e proximal) were measured. More else from testicular and dorsal arteries were obtained the vascular index (peak systolic speed [PSS], final diastolic speed [FDS], pulsatility and resistance index [PRI, VRI]). From the data of testicular and male accessory glands measures were performed a Pearson correlation test and descriptive analyses on SAS. The values of vesicular, prostatic and bulbourethral glands were 25.09±3.25, 12.81±1.24 and 13.65±1.23 mm, respectively. Testicular length (dorsum-ventral) and scrotum perimeter were 9.13 ± 1.10 and 32.09±2.17 cm, respectively. Testicular width (lateral-medial), longitudinal approach, and testicular diameter, transversal approach, were 47.36±6.43 and 4.86±0.39 mm respectively. Testicular volume was 339.10 ± 70.60 mm3. The vascular indexes of the testicular artery were 0.16±0.03 (PSS), 0.05±0.02 (FDS), 0.65±0.19 (VRI) and 1.40±0.83 (PRI). The values from dorsal artery were 0.15±0.04 (PSS), 0.03±0.01 (FDS), 0.78±0.09 (VRI) and 2.07±0.63 (PRI). A significant positive correlation ($P \leq 0.05$) were obtained from the measures of the testicular length with MTD of the vesicle glands (r=0.76), scrotum perimeter with MTDs of the vesicle (r=0.80) and prostatic glands (r=0.90), and from testicular volume with MTD of the prostatic gland (r=0.90). In conclusion, the ultrasonography access of the male accessory glands measures will provide news tools for breeding soundness evaluation in rams. The correlations with testicular biometry can be used as an auxiliary tool (normality reference) to diagnose pathologies in rams.

A014 Male Reproductive Physiology and Semen Technology

In vitro production of bovine embryos using frozen semen with or without the presence of seminal plasma

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Keywords: centrifugation, filtration, Nellore.

Seminal plasma, that mixes with sperm in the ejaculate, and serves as a means of transportation through the female genital tract, has been described as beneficial and harmful to the spermatozoa. There are reports of negative influence of seminal plasma on the storage of semen, due to components harmful to sperm viability. An alternative to reduce the concentrations of seminal plasma of the ejaculate is its semen centrifugation or filtration. However, several studies have reported apparent injury to bovine sperm damaging fertilization by the method of centrifugation. The objective of this study was to evaluate rate of bovine embryos produced in vitro (IVP) using frozen semen with or without the presence of seminal plasma. Semen used for IVP was obtained from 21 Nellore bulls collected by electroejaculation. The semen sample was divided into three equal aliquots and the treatments performed as Campanholi et al. (VII Congresso Interinstitucional de Iniciação Científica (CIIC), Campinas, São Paulo, Brasil, 2013, p.1-8). Treatment 1 (Traditional) constitutes the dilution of semen in the traditional freezing to the final concentration of $60x10^6$ spz/mL with extender BotuBov®. Treatment T2 (Centrifuged) involved the initial dilution of the semen in a 1:4 ratio with extender BotuSemen® and centrifugation for 10 minutes at 600xg (2200 rpm) for removal of seminal plasma. Treatment T3 (Filter) was performed by the Sperm Filter® (filtration device). After centrifugation and filtration of semen, spermatozoa were resuspended with extender BotuBov® at the same concentration of T1. After treatments, semen was packaged at room temperature in 0.5 mL straws and frozen using the machine TK 4000®. Bovine oocytes for IVP were obtained from follicular aspiration from slaughterhouse ovaries. The statistical analyzes were performed in SAS PROC GLM, using 5% significance. . No differences among treatments were detected in cleavage rate (T1 = 82.8 ± 1.5%, 2540/3067, T2 = 81.2 ± 1.5%, 2455/3024, and T3 = 84.9 ± 1.50%, 2583/3043). However, the rate of embryos evaluated on D7 and D9 (hatched blastocyst) were higher in treatment that used SpermFilter® (T3) than T1 and T2 treatments. Evaluation in D7, T1 = 28.4 ± 1.56% (870/3067), T2 = 23.7 ± 1.56% (717/3024) and T3 = 29.8 ± 1.56% (908/3043), P <0.001. Hatched blastocyst rate in D9; T1 = 21.7 ± 1.43% (665/3067), T2 = 20.2 ± 1.43% (612/3024) and T3 = 26.4 ± 143% (803/3043), P<0.001. Seminal plasma removal using SpermFilter® improves the rate of embryos on days D7 and D9.

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A015 Male Reproductive Physiology and Semen Technology

Effects of freezing boar semen stored at different concentrations in 0.5 mL straws

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Keywords: packaging, spermatozoa, swine.

The freezing of boar semen allows the dissemination and preservation of insemination doses of high genetic value. This presents as an alternative to situations in which the movement of these animals and semen is restricted, in addition to controlling the spread of sexually transmitted diseases. For these reasons the reach of successful cryopreservation may represent an important advance in the swine production system (BAILEY et al., 2008, Theriogenology, 70, 1251-1259). It is desired that a straws has enough sperm concentration to inseminate a female. Nonexistent in literature studies with an optimal concentration for freezing boar semen in 0.5 mL straws. Therefore, this experiment was to evaluate sperm characteristics post-thaw motility related to boar sperm frozen at different concentrations in 0.5 mL straws. It was only collected the rich fraction from five ejaculates from five adult boars by the gloved hand method. After initials analysis semen (in natura - total motility) was diluted in Botusui® (Botupharma) in order to obtain five different concentrations: C1) $100x10^6$, C2) $200x10^6$, C3) $300x10^6$, C4) $600x10^6$ and C5) $800x10^6$ spermatozoa/mL. The diluted semen was packaged in 0.5 mL straws (IMV International, St. Paul, Minnesota, USA) and then subjected to freezing. Cryopreservation of semen was performed using an automated system (TK 3000®, TK Freezing Technology Ltd., Uberaba, Minas Gerais, Brazil). After thawing, the semen was analyzed for patterns of motility by computerized semen analysis (CASA-MICROPTIC®, MICROPTIC SL, Barcelona, Spain). Data were subjected to analysis of variance (PROC GLM) and Tukey's test, using the SAS program (1998). The results for total and progressive (%) motility were, respectively, at the following concentrations: C1) 20.31 ± 2.16 and 13.45 ± 1.80 a, C2) 19.87 ± 1.94 and 12.69 ± 1.73 a, C3) 20.7 ± 1.84 and 12.40 ± 1.44 a, C4) 14.60 ± 1.55 b and 7.86 ± 1.02 b, C5) 9.90 ± 1.16 b and 4.42 ± 0.72 b. It was observed that increasing the concentration from 100 to $300x10^6$ sperm / mL in the straw causes no loss in total and progressive motility characteristic. From $600x10^6$ sperm / mL there is a significant reduction in total and progressive motility. Therefore, one can conclude that storage of cryopreserved porcine semen can be carried out up to a concentration of 300 x 10^6 sperm per mL in 0.5 mL straws without any negative effects on total and progressive motility.

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A016 Male Reproductive Physiology and Semen Technology

Penis morphology of the *Trachemys scripta elegans* (Wied, 1839) raised in Brazil

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Keywords: copulatory function, turtle, testudines.

The reptiles developed special copulatory organs (Storer et al. São Paulo: Cia Ed. Nacional. 2000. p.642-654). Lizards and snakes have a pair structure in the cloaca, the hemipenis; and turtles and crocodiles a structure which may be homologous to the mammalian penis (Carvalho et al. Pesq Vet Bras 30:289-294, 2010). In Brazil, *Trachemys scripta elegans* is the most marked turtle, however, it is an exotic and invasive species, uncontrolled in terms of population by authorities (Connor. Tortuga Gazette 28:1-3, 1992). Although the penis is much discussed in the order of reptiles and other vertebrates, information on part of this species are nonexistent. This study aimed to describe the penis morphology of *Trachemys scripta elegans*, by anatomical and histological studies, to assist population control measures or comparative studies. Eleven males originating from the Parque Ecológico do Tietê, São Paulo-SP, Brasil (IBAMA Record Nr. 2491988) were anesthetized with 2% xylazine (40 mg/kg/IM) and 1% ketamine hydrochloride (60 mg/kg/ IM), euthanized by administration of 2.5% thiopental sodium (60 mg/kg/EV); frozen and sent to the Laboratory of Anatomy of Domestic and Wild Animals, UNIVASF, Petrolina-PE, Brazil. After thawing, the cadavers were opened by removal of the plastron and the penis was dissected, fixed in 10% buffered formalin for 18 h and immersed in 70% ethanol for histological processing and staining by EH. This study was approved by the Human and Animal Experimental Ethics Committee of UNIVASF (Protocol nr. 0001/160412). The penis was a muscle organ, odd, with dark pigmentation and positioned longitudinally from the ventral wall of the cloaca. It had the root, which included bulbar and initial portion of the organ; the body composed of corpora cavernosa interspersed by a longitudinal spermatic groove and the glans that was terminal portion of the organ. It was retractile and had only copulatory function, because the ureters were short and opened directly into the cloaca, whereas the deferent ducts lead into a groove at the base of the penis. Microscopically had a cylindrical shape formed by the corpora cavernosa, which contained spaces covered by endothelium interlaced with collagen, fibroelastic connective tissue and smooth muscle and the presence of a central groove. The wall of the erectile body contained collagen fibres arranged in alternating layers, and in each layer fibres were either parallel to the long axis of the penis or perpendicular to it. This was the first study to describe the penis of *Trachemys scripta elegans*, which has homologous structures to the mammalian penis, except for the absence of the corpora spongiosum and penile urethra.

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A017 Male Reproductive Physiology and Semen Technology

Dilution and temperature alter the pH and viability of cooled equine spermatozoa

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Keywords: cooling, extender, semen.

This research evaluated the effect of temperature (5° or 15°C) and dilution of semen (1+1, 1+2 and 1+3) cooled for 48 hours on the viability of spermatozoa of Brazilian ponies. Nine ponies, aged 7-12 years, underwent semen collection (two ejaculates/pony) in January to June/2013. Semen was diluted in 2.4g of non-fat dried skimmed milk, 4.9g of glucose and 95ml ultrapure water. Each ejaculate was split into six fractions and diluted 1+1, 1+2 and 1+3. Samples were subjected to assessment of motility, mitochondrial activity and pH immediately after collection, 24 and 48 hours after cooling. Mitochondrial activity was evaluated according to Aziz et al., 2005 (Theriogenology, 64, 1350–1356, 2005). The diluted semen was stored in a refrigerator at 5±1.6°C and Botubox® at 15°C for 48 hours. Variance analysis and comparison of means by Duncan's Test were done using SAS® software. Findings among dilution of fresh semen were similar ($P > 0.05$). At 24 and 48 hours of cooling, the 1+1 dilution resulted in lower progressive motility (PM) ($P < 0.001$) at 15°C (22.5±9.11, 11.66±7.66) and 5°C (15.83±11.66, 4.77±7.13). At 24 hours, progressive motility at 5 or 15°C was similar among the dilutions 1+2 (36.66±12.36, 42.77±14.26) and 1+3 (42.5 ± 11.53; 44.72±11.94). At 48 hours, PM was higher ($P <0.001$) on samples diluted in 1+3 at 15°C (39.72±12.65) than at 5°C (31.38 ±14.32). The mitochondrial activity revealed a similarity between dilutions of just diluted semen and cooled semen. The semen pH immediately after dilution did not differ among the three dilutions (7.25±0.17, 7.13±0.21, 7.12±0.15) and increased with cooling. Dilutions 1+2 and 1+3 were similar at 5°C (7.48±0.27 and 7.45±0.14, 7.42±0.11 and 7.35±0.1) and 15°C (7.42±0.2 and 7.32±0.23, 7.36±0.12 and 7.25±0.17) at 24 or 48 hours of cooling. The 1+1 dilution resulted in higher pH values in both 5°C (7.66±0.42 and 7.6±0.25) and at 15°C (7.65±0.24 and 7.5±0.29) in 24 and 48h. At 5°C, the pH remained elevated during the 48 hours of cooling, but at 15°C pH increased in the first 24 hours and decreased again at 48 hours. Those samples never reached the pH of the just diluted semen. For transportation up to 48 hours, the dilution of 1+3 at 15°C was more appropriate. This dilution showed the smallest pH variation and better progressive motility. The dilution of 1+1 should not be used for cooling. Increasing dilution promotes sperm viability and does not interfere with mitochondrial activity. Storage and temperature affect pH of semen diluted in skimmed milk and glucose. The absence of buffer in the diluent may have caused variations in the pH of cooled semen. Changes in pH can also be related to the percentage of live sperm during storage, samples with higher PM showed lower pH values.

A018 Male Reproductive Physiology and Semen Technology

Acute toxicity of cryoprotectants in *Rhamdia quelen* spermatozoa

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Keywords: cryoprotectants, *Rhamdia quelen*, spermatozoa.

Sperm cryopreservation in fish has been developing last years and some protocols were defined. Before any protocol of cryopreservation it should be evaluate the acute toxicity to indicate the leadt harmful cryoprotector. The objectives of the present study were to evaluate the acute toxicity of 5, 10, 20 and 30% methanol, dimethyl sulfoxide (DMSO), and methyl glycol at the times 0, 5, 10 and 25 min. Mature males of Rhamdia quelen were used as gamete donors, they were raised in Aquaculture Station of Faculdade Medicina Veterinaria e Zootecnia - Universidade Estadual Paulista – Botucatu, Brazil. Semen was collected by abdominal massage. After the collection, sperm concentration was checked by using a Neubauer counting chamber. To the acute toxicity test we used spermatozoa samples with 80% motile cels. Three cryoprotectants, methanol, dimethyl sulfoxide, and methyl glycol were used at final concentrations of 5, 10, 20 and 30%. Cryoprotectant solutions were prepared in Hanks (300mOsmol/Kg). Sperm motility was estimated by activating spermatozoa with. The addition of 10 µL of distilled water was used to activate 1 µL of sperm suspension placed on a glass slide without the use of a coverslip. Acute toxicity data were analyzed using ANOVA and Tukey test. No sperm motility was observed when the semen was exposed at concentrations of 20 and 30% of the three cryoprotectants. There was no difference in motility between MeOH (51.11%), MG (46.67%) and DMSO (34.44%) at a concentration of 10% cryoprotectants. The concentration of 5% motility was similar to MeOH (72.22%) and MG (75.56%), and both were higher ($p < 0.05$) than DMSO (46.67%). Within the cryoprotectants, the concentration of 5% was higher ($p < 0.05$) than concentration of 10% MeOH and MG, but it did not differ for DMSO. These results indicate Methanol and methyl glycol as cryoprotectants in *Rhamdia quelen* spermatozoa.

A019 Male Reproductive Physiology and Semen Technology

Inclusion of 10% seminal plasma in swine thawing extender improves motile and progressive spermatozoa

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Keywords: CASA, seminal plasma, spermatozoa.

The presence of seminal plasma (SP) damages spermatozoa during cryopreservation, but it is necessary in thaw media to improve semen quality (Okazaki et al, 2009, Theriogenology, 71, 491-498). Thus, we tested the addition of 10% SP in thawed boar semen. Four sperm-rich fraction collections from six boars (n=24) were made. Samples were divided in three aliquots namely no-centrifuged (NC), centrifuged resuspended (CR) and centrifuged without SP (CS). After centrifugation (500xg/10min) of CR and CS, the supernatant (SP) of CS was removed and the sample recentrifuged (2500xg/30min), after that SP was filtered through 0.22μm membranes and stored at -80°C for future use. CR was resuspended in its own SP. All treatments were extended in freezing extender (Botupharma®, Botucatu-SP, Brazil) to obtain a final concentration of $300x10^6$ sperm/mL. The extended semen was packaged in 0.5mL straws. The straws were freeze in an automatic system (TK Tecnologia em Congelação®, Uberaba-MG, Brazil) using a rate of -0.5°C/min until 5°C, -20°C/min until -120°C and then immersed in liquid nitrogen (-196°C). Two straws were thawed in water-bath at 37°C/30s and extended 1:1 (semen:extender) in freezing extender. From CS added 10% SP (v:v) in freezing media originated treatment centrifuged added SP (CP). The samples remained in water-bath at 37°C until 120 min, and evaluated at 5, 60 and 120 min. The samples were analyzed by the semen computerized analysis system (SCA-Microptic®, Microptic S.L., Barcelona, Spain). The experimental design was randomized blocks added to repeated measures in time factor. The data were analyzed by SAS program (SAS Institute Inc., 2010) and subjected to analysis of mixed models. The results did not show time x treatment interaction, and then the treatment effects were analyzed by orthogonal contrasts (centrifugation effect NCxCR; with SP effect CSxCP; without SP effect CSxNC+CP). The results did not show time x treatment interaction ($P>0.05$) for any of the variables (TM: 9.39 ± 1.11; 8.67 ± 1.05; 9.01 ± 1.3; 11.67 ± 1.24, PM: 7.61 ± 0.99; 6.91 ± 0.91; 7.48 ± 1.18; 9.62 ± 1.24; among NC, CR, CS e CP respectively, $P>0.05$) thus, the principal effect was analyzed. The centrifugation did not affect ($P>0.05$) total and progressive motilities. Therefore, if variables had an effect, it was due to SP presence. Motile and progressive spermatozoa increased ($P<0.05$) with seminal plasma addition in thawed media. Removal of SP did not affect ($P>0.05$) the variables. Thus, addition of 10% SP after thawing improves semen quality.

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A020 Male Reproductive Physiology and Semen Technology

Cellrox deep red® for the detection of oxidative stress in ram sperm by *in vitro* induction

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Keywords: fluorescent probe, reactive oxygen species, semen.

The sperm cell is highly susceptible to damage caused by oxidative stress (OS). It is one of the factors that are associated with male infertility. Therefore, it is very important to find new analyses methods that are easier to perform and read. The CellROX Deep Red Reagent® fluorescent probe (CAT 10422, Life Technologies, New York, USA) presents a number of advantages, but there are no published studies of its use on sperm. Thus, the aim of the present study is to evaluate the effectiveness of this method in samples of ram semen induced to *in vitro* OS. The analyses were conducted in four ejaculates of three White Dorper rams (n=12) that were between 14 and 24 months of age. Semen was collected by artificial vagina. The ejaculates were treated in T0, T50 and T100. T0 corresponded to the control semen sample that was not submitted to OS induction, T50 corresponded to the semen sample content 50% without OS induction and 50% inducted to OS, and T100 corresponded to the semen sample that was entire submitted to OS induction. The OS induction was performed by adding iron sulfate (4 mM) and ascorbic acid (20 mM). For the preparation of the technique, 200 µL (25x106 spermatozoa/mL) of each semen sample (T0, T50 and T100) was used and 0.5 µL of CellROX® (1 mM) and 2 µL of Hoechst 33342 (2.5 mg/mL, Life Technologies) were added. The semen sample was incubated at 37° C/30 minutes. After incubation, the sample was centrifuged at 2,000g/5 minutes, the supernatant was removed and the pellet was resuspended in 200 µL of TALP sperm. The cells were classified as sperm under mild or no OS (unstained midpiece), sperm under moderate OS (midpiece stained pale red), and sperm under intense OS (midpiece stained strong red). Data obtained from T0, T50 and T100 treatments were evaluated by analysis of variance (ANOVA) and the means were compared using Fisher's LSD test. OS data in T0, T50 and T100 treatments were subjected to linear regression analysis by Statview software (Stat View 1998, SAS Institute Inc., Cary, NC, USA). The experimental model used was: $Y = a + bX$, wherein Y is the estimative of OS due to treatment, a is the linear regression coefficient corresponding to the value of Y when X is 0, b is the regression coefficient for the percent of X on the response Y and X is the treatment. A significant difference ($p<0.0001$) in OS was observed between T0 (0.25±0.11%a), T50 (16.37±1.75%b) and T100 (30.83±3.57%c) treatments. As there were differences between the treatments, the results were submitted to linear regression analysis, resulting in the equation $Y=12.33+2.381x$ and determination coefficient was of 0.728. Thus, it is possible to conclude that the CellROX® Deep Red probe is able to detect ROS production in spermatozoa, using the ovine species as the experimental model and the OS induction model with iron sulfate and ascorbic acid.

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A021 Male Reproductive Physiology and Semen Technology

IVF vs. ICSI: efficiency and "bull fertility effect" using sex sorted or non sorted semen in bovine

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Keywords: bovine, ICSI, sex sorted sperm.

The generation of bovine embryos by IVF is a biotechnology in expansion with a huge potential for animal production. However, in some cases, high value semen is not efficient for IVF. In those cases, ICSI is recommended. The application of sex sorted semen to both IVF and ICSI is one of the current main goals of applied reproductive biotechnology. The aim of this work was 1) to compare IVF and ICSI blastocyst rates, using sex sorted (X) and non sorted (NS) sperm; and 2) to evaluate the prevalence of bull factor over ICSI procedures, using both types of semen. To this aim, COCs were collected from slaughtered cow ovaries and IVM for 21 h. For IVF, Brackett and Oliphant protocol (1975) was used. Sperm concentration was $16x10^6$/mL for NS groups (IVF-NS) and $5x10^5$/mL for X groups (IVF-X). For ICSI, NS or X straw were thawed (ICSI-NS and ICSI-X groups) and co-incubated with 50 ng/µL of pCX-EGFP. The expression of EGFP was considered an indicator of fertilization. Sperms were chemically or physically broken and used for ICSI. Oocytes were activated with 5 µM ionomycin for 4 min, placed 3 h on TCM-199 and 3 h on 1.9 mM DMAP. For each repetition, 1-2 frozen straws were used for IVF and 1/4 of straw for ICSI. In assay 1, the medians and variances from all the embryos were compared between groups (IVF-NS, IVF-X, ICSI-NS and ICSI-X) by Kruskal-Wallis and Dunn's multiple comparisons test. For assay 2, only data from procedures made with bulls whose straws were available in X and NS format were used. Blastocyst rates from each bull (3 bulls for IVF-NS vs. ICSI-NS and 5 for IVF-X vs. ICSI-X) were analyzed by Two-way ANOVA, not RM and Tukey's multiple comparisons test. Results: In assay 1, means±SD of blastocyst/total oocytes were 24.8±20.7 (n=1972) for IVF-NS, 17.3±21.8 (n=1324) for IVF-X, 15.2±12.6 (n=350) for ICSI-NS and 9.3±5.5 (n=511) for ICSI-X, with no differences between them. In Experiment 2, bull effect ($P<0.01$) and the interaction bull-treatment effect ($P<0.05$) was significant for non sorted groups. For sex sorted groups, the interaction bull-treatment effect was considered very significant ($P<0.01$). Bull effect ($P<0.05$) was significant for IVF-X, while ICSI-X was the only group which did not show differences between bulls. Conclusion: The ARTs assayed are equally efficient for in vitro blastocyst production, using X and NS semen. If in addition to the efficiency of the techniques, we consider the volume of X semen used and its cost, ICSI shows an advantage over IVF. We can also say that bull fertility affects IVF results, with X and NS sperm, as was previously described. For the first time, we report a bull effect over ICSI procedures, which is not evident when X sperm is used. These results suggest that ICSI could be a good strategy for embryo production, in particular cases (poor sperm quality, quantity, or availability) of sex sorted sperm from bulls of high genetic value.

A022 Male Reproductive Physiology and Semen Technology

Epididymal and testicular temperatures of Morada Nova and Santa Inês sheep breeds during Summer in a tropical environment

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Keywords: homeothermy, testicular thermoregulation, tropical climate.

High relative humidity and ambient temperature are characteristics of a tropical climate and influence negatively animals mechanisms of heat exchange. Testicular thermoregulation is dependent on the individual ability thermolysis and failures in this process lead to functional impairment of testes. Sheep kept under high temperatures and humidity can present temporary or permanent infertility. For this reason, search and selection of heat tolerant breeds favor reproductive function, despite challenges posed by tropical climate. Native breed Morada Nova is considered adapted and the interest for their productive attributes and rusticity has grown each day, constituting an alternative for meat production and quality leather. However, for greater integration in production systems, this germplasm needs to be better investigated and understood. The aim of this study was to evaluate rectal temperature, testicular and epididymal surface temperatures of individuals Breeds Morada Nova (MN) and Santa Inês (SI) in order to compare efficiency racial regarding maintenance of body and testicular homeothermy. The experiment was conducted at Embrapa Southeast Livestock, in Sao Carlos - SP (21 ° 57'42 " S , 47 ° 50'28 " W , elevation 860m), local of altitude tropical climate type (Cwa). 16 adult sheep (*Ovies aries*) were used, 7 MN and 9 SI, kept in paddocks of star grass (*Cynodon nlemfuensis*), supplemented with mineral salt, with access to drinking fountain for water consumption *ad libitum*. Between the months of December 2013 and February 2014, monthly campaigns of three consecutive days were conducted to measure rectal temperature (RT), with clinical thermometer, surface temperatures of dorsal poles (TESTD) and ventral poles of the testes (TESTV) and surface temperature tail of the epididymis (EPID), obtained with infrared thermometer. The measurements were performed during the morning (07h30 to 10h00) and afternoon (13:30 to 16:30). Experimental data were analyzed using the program BioEstat 5.3. Descriptive analysis was followed by an evaluation of the normal distribution of the same, using the Kolmogorov - Smirnov test (KS test). Analysis of variance was applied followed by comparison of means using Tukey test at 5 % significance level. MN sheep showed lower rectal temperature than SI sheep (38.7 °C vs 38.9 °C, $P < 0.01$). There was no difference in TESTD (31.3 °C vs 31.3 °C), TESTV (30.3 °C vs. 30.5 °C) and EPID (29.2 °C vs 29.4 ° C) for MN and SI. Thus, Morada Nova breed resemble Santa Inês on the ability for testicular and epididymal thermoregulation.

A023 Male Reproductive Physiology and Semen Technology

Equine lyophilized seminal plasma improves the fertilizing capacity of frozen ovine semen

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Keywords: CASA, flow cytometry, mitotraker.

Seminal plasma (PS) is pointed as responsible for the protection and reversion of cell damage due to freezing. The limitations of using ovine seminal plasma, such as its reduced volume and the decrease in sperm concentration after its addition, can be circumvented by the use of lyophilized equine seminal plasma (PSLE). This study evaluated the addition of PSLE to the freezing medium of ram semen. Equine PS obtained by artificial vagina (AV) was centrifuged, lyophilized and subjected to protein dosage. The semen was obtained by AV from Lacaune breed rams, and diluted 1 + 3 with yolk Tris glycerol medium without lyophilized PS (control group - CG) or with the addition of the PSLE equivalent to 600µg/mL protein (PSLE Group). The diluted semen was loaded, cooled and frozen in TK 3000 machine, performed in eight repetitions, being evaluated two straws of each repetition. After thawing, the sperm kinetic was evaluated by CASA system and by flow cytometry on acrosome integrity (FITC - PSA), membrane fluidity and stability (YOPRO/M540 respectively), chromatin integrity (acridine orange), apoptosis (Annexin) and potential of mitochondria (Mitotracker). Finally, semen was subjected to heterologous IVF with bovine oocytes, and cleavage rates assessed. Data were subjected to analysis of variance and T test, with significance level of 5 %. No differences were observed on VAP (CG- 87.5 ± 3.9, 95.8 ± 3.8 - PSLE), VSL (CG- 69.3 ± 3.4, PSLE - 76 ± 4.5) BCF (CG- 31.5 ± 1.3, PSLE- 30.3 ± 1.4), MT (CG- 54.4 ± 4.2, PSLE - 56.5 ± 3) , MP (CG- 22,3 ± 3.1, PSLE - 25.3 ± 3), STR (CG- 75.9 ± 1.3, PSLE- 75.9 ± 1.7) and LIN (CG- 42.6 ± 1; PSLE- 40.4 ± 1.4) in the CASA system. Significant differences were observed in the parameters VCL (CG -163.5 µm/s, PSLE -186.2 µm/s) and ALH (CG- 9 µm, PSLE- 8.2 µm) of CASA. In flow cytometry the PSLE (38.9 %) showed higher amount of viable cells (non-apoptotic or necrotic) against CG (32.1%). In the other evaluations, no differences were observed, FITC - PSA (CG- 22.1 ±1.6 % and PSLE- 24.1% ±1.3), YOPRO/M540 (26.1 ±2.7 vs 28.3 ±2.3, viable with membrane stability) for PSLE and CG groups, respectively. In the Mitotracker evaluation, the values for high potential (27.9% ±2.2; 27.9% ±2.2) were observed in both groups. With Acridine Orange, similar levels of intact (95.11% vs 95.12%) and DFI (DNA Fragmentation Index) (4.89% vs 4.88%) were observed in PSLE and CG groups. Cleavage in the PSLE group was higher (71.37 ±2.9%) than CG (43.23 ± 3.2%). The addition of PSLE to the diluent of ram semen improves post thaw viability parameters, increasing the ability of in vitro fertilization, which may represent an alternative to the use of frozen semen in sheep cervical insemination.

A024 Male Reproductive Physiology and Semen Technology

Effect of broiler chicken lines on sperm quality

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Keywords: chicken, genetic selection, semen.

In the last decade an increase in the intensity of selection in broiler lines for faster growth rate and meat yield was observed. Recent studies have shown that the intensification of genetic selection has not just influenced production traits but also embryo development and their metabolic characteristics (DRUYAN, Poult Sci. v. 89, p.1457-67, 2010). Therefore it was hypothesized that this intense selection has also affected reproductive traits as well as semen quality. The objective of this study was to compare the semen quality of two broiler chicken lines developed by the Embrapa Swine and Poultry National Research Center (TT and KK) that are under multi-trait selection. The TT is a broiler male line selected mainly for performance and carcass traits, and the KK is a broiler female line selected also for egg production. For this study, semen samples from 36 males with similar age from each line, housed in the same environmental conditions were collected using dorsum-abdominal massage. The semen was evaluated for: motility, plasma membrane integrity (Eosin/Nigrosin staining), acrosomal integrity (Pope staining) and mitochondrial activity, which was evaluated according to Hrudka (1987, Int J Androl 10, 809-28), modified for 3 degrees (high, medium or absent, DAB1-3). Data was presented as percentage and it was evaluated using One-way Anova and Tukey test. Significance was considered if a $p<0.05$. Plasma membrane integrity was the only evaluated trait with significant difference between both lines (TT 89.30±1.02 and KK 93.25±0.76; p=0.0029). Even knowing that this two lines share similar genetic background and are imposed to different selection strategies, both lines presented high values for sperm membrane integrity, suggesting no influence on fertility. The average sperm motility was 48.88±3.21 and 55.13±3.8 (respectively for TT and KK; p=0.2139); the average acrosomal integrity was 90.86±1.1 and 92.94±0.66 (respectively for TT and KK; p=0.1099). Mitochondrial activity index was observed with high prevalence of active sperms in class 1 and 2, DAB1 52.7±3.53 and 49.03±3.81 (respectively for TT and KK; p=0.4823) and for DAB2 41.32±3.05 and 39.74±2.72 (respectively for TT and KK; p=0.702). These results suggest no significant differences in semen quality between female and male broiler lines, indicating that the selection imposed for different production traits in those lines will probably not interfere with loss of sperm fertility.

A025 Male Reproductive Physiology and Semen Technology

Correlation between spermatic concentration and vascular characteristics of testis using doppler ultrasonography in young Holstein bulls

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Keywords: bull, doppler, testis.

The goal of this study was evaluate the correlation between testicular parenquima vascular perfusion, fluxometric parameters and spermatic concentration in young Holstein bulls. Twenty three bulls between 10 and 23 months age, maintained in intensive production system, in Pernambuco, northeast of Brazil were evaluated. The semen was collected by electroejaculation, submitted to semen quality assessment according to CBRA (2013) patterns, and the sperm concentration was performed using Neubauer's chamber. The Doppler Triplex ultrasound exam was performed at the moment of clinical evaluation, to determine the testis parenquima perfusion (% of vascularized parenquima) and fluxometric parameters (Pulsatility and Resistence Indexes) of testicular artery in the spermatic cord. The data were analized by ANOVA, Student's t test and Pearson's correlation. The sperm concentration range from 10 x 10^6 to 690 x 10^6 spermatozoa/mL. No significant correlation was observed between parenquima perfusion and spermatic concentration ($r=-0.046$, $P > 0.05$). However, spermatic concentration was negatively correlated with IP ($r = -0.446$, $P= 0.04$) and IR ($r= -0.449$, $P = 0.04$). These results demonstrated that even though the spermatic concentration was not related to vascular perfusion, the fluxometric parameters of testicular artery could influence the sperm production. Thus, more studies are necessary to better understand the role of IP and IR in the testicular function.

A026 Male Reproductive Physiology and Semen Technology

Addition of seminal plasma to thawed sperm boars and effect on plasma and acrosomal membrane integrity

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Keywords: acrosome reaction, plasma membrane, seminal plasma.

Sperm plasma membrane is the first structure to suffer the detrimental effects induced by cryopreservation (Bailey et al, 2008, Theriogenology, 70, 1251-1259). However, addition of seminal plasma (SP) to thawed sperm increases the percentages of cell with intact plasma and acrosomal membranes in equine (Andrade et al, 2011, Reprod Dom Anim, 46, 682-686). Thus, this trial was conducted to evaluate the effect of addition of 10% of SP to thawed sperm boars on plasma and acrosomal membranes integrity. Sperm-rich fraction from four ejaculates each from six boars were collected (n=24) and divided into three treatments (no centrifuged -NC, centrifuged resuspended-CRES and centrifuged without SP-CWSP). CRES and CWSP were obtained by centrifugation at 500xg/10min. CRES was resuspended in its own SP and CWSP supernatant was removed and then centrifuged at 2500xg/30min again and filtered through membranes with 0.22µm pores and stored at -80°C. The pellet of CWSP, CRES and NC treatments were extended in freezing extender (Botupharma®, Botucatu, Brazil), to obtain a final concentration of $300x10^6$sptz/mL, and stored in 0.5mL straws, after this, straws were freezed in a automatic system (TK Tecnologia em Congelação, Uberaba, Brazil) using a cooling/freezing rate: -0.5°C/min until 5°C; -20°C/min until -120°C and immersed in liquid nitrogen (-196ºC). Two straws per treatment were thawed using a water bath at 37°C/30sec and diluted in a 1:1 ratio in freezing extender. The centrifuged added SP-CASP treatment was originated from the CWSP after thawing. In CWSP was added 10% of SP (obtained after centrifugation) to extended thawing medium (v:v). The samples after remaining in water bath at 37°C were analyzed at three times 5, 60 and 120min. An aliquot was extended in TALP to concentration $5x10^6$sptz/mL and stained with 2µL Hoechst 33342, 3µL propidium iodide and 10µL FITC-PSA. After 10min of incubation at 37°C, the samples were analyzed in flow cytometer (BD FACSAria-Becton Dickinson, San Jose, USA) to evaluate the population of spermatozoa with plasma and acrosomal membranes integrity (MIAI). Data were analyzed using the MIXED procedure (SAS, 2010). Treatments were evaluated using orthogonal contrast: centrifugation effect (NCxCRES); with SP effect (CWSPxCASP) and without SP effect (CWSPxNC+CASP). Differences were considered significant when $p<0.05$ and all results were expressed as means±SEM. There was no time x treatment interaction ($p>0.05$) and thus was only assessed the contrast effects. No difference were found ($p>0.05$) for any contrasts (4.5±0.6 %; 4.52±0.62 %; 5.65±0.77 %; 5.49±0.32 %, NC, CRES, CWSP and CASP, respectively). Thus, it can be stated that the removal of the SP before cryopreservation or the addition of SP after thawing did not affect ($p> 0.05$) the plasma and acrosomal membranes integrity of boar semen.

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A027 Male Reproductive Physiology and Semen Technology

Effect of water intake with different salinity levels on the apoptosis of germinal epithelium cells from Morada Nova lambs

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Keywords: NaCl, ovine, seminiferous tubules.

The aim of this study was to evaluate the influence of different concentrations of water salinity on the apoptosis of germinal cells of the seminiferous tubules from Morada Nova lambs. Thirty two Morada Nova lambs with seven months were used. The animals were distributed into four treatments according to the levels of sodium chloride (NaCl) added to the water for them to drink, and consequently, the levels of total dissolved solids (TDS): 665 (control - treatment 1), 3.322 (treatment 2), 5.958 (treatment 3) or 8.614 (treatment 4) mg/L TDS. After 75 days, animals were killed and the testes were destined to histology and therefore to analysis of apoptosis in the germinal cells through TUNEL technique, using a kit for cell death detection (Roche Diagnostics Ltd., Indianapolis, USA). Sections of 5 µm were mounted on glass slides. Antigen retrieval was performed in sodium citrate buffer (Dinâmica, São Paulo, Brazil) for 10 min. Endogenous peroxidase activity was blocked by 3% H2O2 (Dinâmica) in methanol (Qeel, São Paulo, Brasil) for 10 min. The sections were incubated with TUNEL reaction mixture for 30 min. Then, the specimens were incubated with Converter-POD for 30 min. The DNA fragmentation was revealed by incubation of the tissues with diaminobenzidine (DAB; 0.05% DAB; Sigma, St. Louis, EUA) during 1 min. Finally, sections were counterstained with haematoxylin (Vetec, São Paulo, Brazil). For negative controls, slides were incubated with label solution (without terminal deoxynucleotidyl transferase enzyme) instead of TUNEL reaction mixture. The number of TUNEL positive cells was counted in randomly fields per treatment using Image-Pro Plus® software. The cells were considered TUNEL positive when they showed a brown staining. All data were submitted to Qui-square test ($P<0.05$). The results showed that there was a significant increase in the percentage of TUNEL positive cells in treatment 4 (9.33%), i.e. the treatment with higher proportion of TDS, when compared with the control (0.4%) and other treatments (1.23% e 0.8% for treatments 2 and 3, respectively). There was no staining in the negative control. In conclusion, 8.614 mg/mL NaCl added to the water increases apoptosis in early spermatogonia and spermatide of the seminiferous tubules from Morada Nova lambs.

A028 Male Reproductive Physiology and Semen Technology

Characterization of the L-ARG/PRKG1/CGMP signaling pathway in the in vitro capacitation of bovine spermatozoa

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Keywords: bovine, nitric oxide, sperm capacitation.

The sperm capacitation and acrosome reaction (AR) are essential events for successful fertilization of the oocyte, both in vitro and in vivo and, in cattle, are modulated by the action of nitric oxide (NO) (LEAL et al., 2009, Anim Reprod Sci, 116, 38-49). In humans, it was demonstrated that the NO-induced capacitation is mediated by NO/cGMP/PRKG1 pathway (ZHANG et al., 2007, Mol Reprod Dev, 74, 497-501). The addition of 10 mM of L-arginine (L-arg, a precursor of NO synthesis) to the culture medium in vitro improved the parameters of motility, vigor, sperm capacitation and acrossome reaction of in natura bovine spermatozoa and contributed to a higher percentage of penetration of homologous oocytes (LEAL et al., 2009, Anim Reprod Sci, 116, 38-49). The Rp-8-Br-PET-cGMPS (RP) is an antagonist of cGMP-dependent protein kinases G1 (PRKG1) and acts as a competitive inhibitor of cGMP by blocking ion channels in cGMP binding sites. In humans, RP inhibits the motility of spermatozoa when associated with a NO donor (MIRAGLIA et al., 2011, Reproduction, 141, 47-54). Therefore, the aim of this study was to determine whether the addition of L-arg is involved in capacitation of bovine spermatozoa via cGMP and/or PRKG1. Cryopreserved sperm from three Nellore bulls (*Bos indicus*) were used with six replicates for each bull (n=18). In vitro capacitation was induced in 200 µL of capacitating medium supplemented with 20 µg/mL heparin (control) plus treatments, as follows: G1) 1mM L-arg; G2) 50 µM RP; G3) 50 µM RP and 1 mM L-arg. The progressive motility and sperm vigor were subjectively assessed with the support of optical microscopy (100x amplification). Sperm capacitation was evaluated by fluorescent labeling of chlortetracycline (CTC), and the measurement of the concentration of cGMP was performed by ELISA. The results were assessed by analysis of variance (SAS, Statistical Analysis System, 1996). Spermatozoa from G1 showed a higher motility percentage ($P<0.05$) compared to control, G2 and G3 (60.56 ± 7.87 vs 50.56 ± 7.87, 51.67 ± 8.41, 51.11 ± 10.03, respectively). The vigor was higher ($P<0.05$) in G1 (2.67 ± 0.48) and lower in G2 (1.94 ± 0.53). G1 had a higher percentage ($P<0.05$) of capacitated sperm (69.82 ± 3.42) compared to control, G2 and G3 (59.89 ± 2.40, 51.06 ± 2.09 and 51.16 ± 3.00, respectively). The concentration of cGMP was higher ($P<0.05$) in G2 (3.65 ± 0.42) and G3 (3.76 ± 0.77) compared to control and G1 (2.45 ± 0.25 and 2.25 ± 0.72, respectively). There was no difference ($P>0.05$) in the concentration of intrasperm cGMP between control and G1. These results suggest that during in vitro sperm capacitation: 1) L-arg is not related to the increase of cGMP; 2) the activity of PRKG1 decreases the concentration of cGMP; and 3) the action of PRKG1 is independent of the action of L-arg.

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A029 Male Reproductive Physiology and Semen Technology

Biotechnological applications of equine cooled semen after local treatment of seminal vesiculitis

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Keywords: cooled equine semen, local treatment, seminal vesiculitis.

The stallion affected by seminal vesiculitis presents a history of reduced fertility, ejaculatory disorders, transmission of pathogens sexually transmitted and changes in semen with reduction of it quality and longevity. The deleterious effects on semen are due to contamination with bacteria and inflammatory cells that promote agglutination, precipitation, decreased sperm motility and increased reactive oxygen species (ROS) and proinflammatory cytokines, which lead to deleterious effect more pronounced during the storage. Therefore, the aim of this study was to evaluate the quality of semen cooled to 5°C for 24 hours from stallions with seminal vesiculitis before and after local treatment. Five stallions with seminal vesiculitis were used and local treatment was performed by endoscopy for 10 consecutive days. This consisted of flushing with Ringer Lactate solution followed by infusion of the antibiotic of choice selected after antibiogram of bacterial culture of seminal vesicles flush. Two semen collections with 48 hours of interval were performed before (M0), after a week (M1) and after one month (M2) therapy. After collecting the semen was mixed to the extender BotuSemen (Botupharma ®, Botucatu / SP - Brazil) at a concentration of $50x10^6$ sperm/mL. The sample was stored at 5°C in the isothermal container Botuflex (Botupharma ® Botucatu / SP - Brazil) for 24 hours. After the storage period semen was placed in a dry water bath at 37°C for 5 min and subsequently evaluated for sperm kinetics, by CASA method, and the plasma membrane integrity was performed by the epi-fluorescence microscopy, using the combination of fluorescent probes carboxyfluorescein diacetate and propidium iodide. The data were analyzed by (two-way ANOVA) followed by Tukey's test ($p<0.05$). The values (mean±SE) of seminal parameters on M0, M1 and M2 were the following, respectively: sperm kinetics (% total motility: 25.5±3.74a; 38.2±6.59b; 23.4±4.01a; % progressive motility: 9.0±2.12a; 15.2±2.95b; 9.5±2.06a; % rapid sperm: 13.4±2.03a; 20.5±3.71b; 11.9±2.83a) e plasma membrane integrity (% sperm with intact plasma membrane: 31.7±3.56a; 41.1±4.47b; 31.7±2.90a). In conclusion, the local treatment used in this study to seminal vesiculitis enabled better biotechnological utilization of equine cooled semen at 5°C for 24 hours after one week of therapy, since it promoted increased sperm quality regarding the sperm kinetic parameters and plasma membrane integrity. However, this effect was not maintained after one month of therapy, due to the similarity of sperm parameters at that time with the pre-treatment moment which could be explained by the recurrence of the disease.