A001 Male Reproductive Physiology and Semen Technology

Testicular mass and height of the seminiferous epithelium during the postnatal development of guinea pig (Cavia porcellus, Linnaeus, 1758)

A. Gradela[1], A.K.R. Nunes[1], L.F.T. Martins[1], J.M. Santos[1], M.H.T. Matos[1], G.N. Silva[1], V.S. Franzo[2]

[1]UNIVASF, Petrolina, PE, Brasil; [2]UFMT, Cuiabá, MT, Brasil.

Keywords: morphometry, non pubescent, pubescent.

Quantitative parameters relating to the seminiferous tubule have positive relationships with the spermatogenic activity. They are also crucial for studies involving reproductive parameters, once they are constituted into an accurate instrument for evaluating the spermatogenic capacity, both in normal as in pathological or experimental conditions (Castro et al., 1997). Although guinea pigs are excellent experimental models, studies describing quantitative parameters of their seminiferous tubule are nonexistent in this specie. Thus, the objective of this study was to evaluate the seminiferous epithelium height (EH) of guinea pigs during the postnatal development, as contribution to studies focused on their spermatogenic capacity and use as an experimental model. This study was approved by the CEEHA[1] from UNIVASF (protocol nr.22041019). Guinea pigs in the non pubescent (IP), early prepubescent (EP), late prepubescent (LP), pubescent (PU), post pubescent 1 (PP1) and post pubescent 2 (PP2) stages were used (N= 5 animals/group; Gradela et al., 2012, Proceedings...AAAA, 8th). Testicular mass (TM; g) was evaluated and the right testicle (N = 3/group) collected, fixed in buffered formalin at 10% for 18 hours and immersed in alcohol 70% for routine histological processing by HE stain. The EH was measured in 10 cross-sections of seminiferous tubules for every animal with full spermatogenic activity (PU, PP1 and PP2), and 20 cross-sections for those that did not have all cells of the spermatogenic lineage (IP, EP and LP). Measurements were performed with 40x micrometric lenses and 40x magnifications, from the basal membrane to the luminal edge. Images were analyzed with the Motic Image Plus 2.0 ML software. Averages were evaluated by ANOVA followed by Tukey test, and simple correlation coefficients (r) were analyzed among the variables (Assistat 7.6 beta). CM increased from 0.03±0.00 g to 1.07±0.17 g, with evident increase ($P<0.05$) from IP to PP1 stage (0.03±0,00 in IP; 0.16±0,08 in EP; 0.29±0.07 in LP; 0.54±0,14 in PU and 0.87±0,17 in PP1) and discrete increase ($P>0.05$) in PP2 (1.07±0.17). EH varied from 20.94±4.09 μm in the IP stage to 80.9±11.84 μm in PP1, differing significantly in LP (34.12±11.07 μm); PU (63.12±12.98 μm) and PP1 (80.90±11.84 μm). Average EH of PU, PP1 and PP2 guinea pigs is within the range observed in domestic species (60 to 100 μm; França; Russell. Male reproduction: a multidisciplinary overview, 1998). It may be concluded that the increased EH followed the testicular mass increase, reflecting the different stages of testicular development and coinciding with the full establishment of spermatogenesis that occur at puberty. This knowledge supports the use of guinea pigs as experimental models, since it helps to understand its spermatogenic process.

[1]Comitê de Ética em Estudos Humanos e Animais (Ethics Committee for Human and Animal Studies).

A002 Male Reproductive Physiology and Semen Technology

Gonadosomatic index and diameter of the seminiferous tubules during the postnatal testicular development of guinea pigs (Cavia porcellus, Linnaeus, 1758)

A.K.R. Nunes[1], L.F.T. Martins[1], J.M. Santos[1], M.H.T. Matos[1], G.N. Silva[1], V.S. Franzo[2], A. Gradela[1]

[1]UNIVASF, Petrolina, PE, Brasil; [2]UFMT, Cuiabá, MT, Brasil.

Keywords: guinea pig, testicular development. testis morphometry.

Testicular morphometric analyses are important for the description of the spermatogenic process of each species, and all quantitative parameters relating to the seminiferous tubule have positive relationship with the spermatogenic activity (França & Russell. In: Regadera & Martinez-Garcia Male reproduction: a multidisciplinary overview. Madrid: Churchill Livingstone, 1998. 198-219). The use of guinea pigs as an experimental model has great importance in scientific research but analysis of its testis morphometry is scarce. The objective of this study was to evaluate the gonadosomatic index (GSI (%) = TM/CM (g) x 100) and the diameter of the seminiferous tubules (TD) of guinea pigs in the inpubescent (IP), early prepubescent (EP), late prepubescent (LP), pubescent (PU) and postpubescent 1 (PP1) stages (N= 5 animals/group) (Gradela et al., Proceedings... AAAA, 8th, 2012). 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. TD was evaluated in 10 cross-sections of seminiferous tubules for every animal with full spermatogenic activity (PU and PP1) and in 20 sections for those that did not have all cells of the spermatogenic lineage (IP, EP and LP). Measurements were performed with 10x micrometric lenses and 10x magnification and the images were analyzed with the Motic Image Plus 2.0 ML software. Averages were analyzed by ANOVA followed by Tukey test, and simple correlation coefficients (r) were determined among variables (Assistat 7.6 beta). This study was approved by the CEEHA[1] from UNIVASF (protocol nr.22041019). The CM (112.25±1.52 in IP; 225.49±18.53 in EP; 285.04±17.54 in LP; 388.17±29.77 in PU and 471.27±21.32 in PP1) and TM (0.03±0.00; 0.16±0.08; 0.29±0.07; 0.54±0.14 and 0.87±0.17, respectively) increased with age, reflecting the different stages of the testicular development of guinea pigs. Significant correlations ($P<0.01$) were observed between age and CM ($r= 0.95$), age and TM ($r= 0.94$) and CM and TM ($r= 0.94$). GSI was 0.03±0.00% in IP; 0.06±0.04% in EP; 0.10±0.02% in LP; 0.14±0.04% in PU and 0.17±0.04% in PP1. Average TD varied from 70.97±8.44 to 196.60±29.92, differing ($P<0.05$) among LP (124.51±20.96); PU (177.63±30.45) and PP1 (196.60±29.92) and had a positive correlation with age. In conclusion, in guinea pigs, the GSI had significant and positive correlations with the TM at puberty and postpuberty1, reflecting the reproductive activity and testis growth, coincident with the full establishment of spermatogenesis at puberty. This knowledge helps to understand the spermatogenic process of guinea pigs and assist in its use as an experimental model.

[1]Comitê de Ética em Estudos Humanos e Animais (Ethics Committee for Human and Animal Studies).

A003 Male Reproductive Physiology and Semen Technology

The extender osmolarity changes the percentage of hyperactive cells in refrigerated boar semen

A.F.C. Andrade[1], M. Andrade Torres[1], G.M. Ravagnani[1], M. Lima Oliveira[1], M.R. Bianchi-Alves[1], A.S. Moretti[1], F.O. Papa[2], J.A. Dell'Aqua Jr[2], M.A. Alvarenga[2], S.M.M.K. Martins[1]

[1]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Pirassununga, SP, Brasil; [2]Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista, Botucatu, SP, Brasil.

Keywords: osmolarity, hyperactivation, spermatozoa.

Hyperactivation must have its beginning in appropriated time and place for the spermatozoa to be able to perform fertilization. Also, the hyperactive sperm movement characteristics are represented by the increase in ALH (amplitude of lateral head displacement) and VCL (curvilinear velocity). The changes in osmolarity affect in greater proportion the motility, when compared to other parameters related to quality and sperm viability (Yest et al, 2010, Anim Reprod Sci, 119, 265-74). Thus, this study verified that different osmolarity conditions modify the percentage of hyperactive spermatozoa. Were made three semen collections of three boars (n=9). Samples were extended in media with various osmolarities, namely 360 and 404 mOsm (Botupharma®, Botucatu-SP, Brasil), that differ by changing the dilution, 1.1 L and 1 L of ultrapure water, respectively. Raw semen was mixed within extender to obtain a concentration of 30 X 10^6 sptz/ml. Analysis were made at 90 minutes, 24 and 48 hours after dilution. Aliquots of semen were evaluated under cover slide, in computer assisted sperm analysis (SCA-Microptic®, Microptic SL, Barcelona, Spain) under an epifluorescence microscope (Nikon, model Eclipse Ni-U). Adjustments included edit/sort toll for the ALH > 3.5 µm and VCL > 97 µm/s values (Schimidt, 2004, Reproduction, 128, 171-79), to evaluate the percentage of hyperactive swine spermatozoa. The experimental design was in generalized blocks, added to repeated measurements in time. Data were analyzed by SAS program (SAS Institute Inc., 2010), subjected to analysis of variance and interactions by Greenhouse-Geisser test at 5%. There was no interaction between time and treatment (P > 0,05), for all variables. However, there was a significance trend (P=0,0582) to the treatment effect for the sperm hyperactivation, with values as 16,66 ± 2,44 and 24,79 ± 3,46 to the 360 and 404 mOsm treatments, respectively. This results lead us to believe that extenders with greater osmolarity, as represented by the 404 mOsm extender, tends to show induce a higher percentage of hyperactive cells. Such results can be verified in further studies, with a greater number of repetitions.

Acknowledgments: FAPESP process 2011/23484-8 and to Botupharma®.

A004 Male Reproductive Physiology and Semen Technology

Cooling and cryopreservation effect on viability of ejaculated and epididymal spermatozoa of Gir bulls

A.M. Cunha[1], J.O. Carvalho[2], M.N. Diógenes[1], C.F. Martins[3], B.D.M. Silva[4], M.A.N. Dode[4]

[1]Universidade de Brasília, Brasília, DF, Brasil; [2]Universidade de São Paulo, São Paulo, SP, Brasil; [3]EMBRAPA Cerrados/CTZL, Brasília, DF, Brasil; [4]EMBRAPA Cenargen, Brasilia, DF, Brasil.

Keywords: bovine, epididymis, spermatozoa.

The use of epididymal spermatozoa in various assisted reproductive technologies is an important tool to multiply genetic material from animals that die suddenly and/or have acquired reproductive failure. However, to establish more appropriate procedures to use spermatozoa from epididymis, it is necessary to know about their behavior and viability after stress situations such as cooling and cryopreservation. To evaluate the characteristics and viability of epididymal and ejaculated spermatozoa, seven bulls Gir were used. Ejaculated semen (EJ) was collected by electrostimulation. After collecting the ejaculate, the animals were castrated and the testicles were kept for two hours at 5°C, simulating the transport to the laboratory. Then, spermatozoa from the cauda epididymis were collected by extravasation (PE) method. In both groups, sperm samples were diluted in tris-yolk, packaged and refrigerated for four hours at 5°C and then cryopreserved. After cooling and thawing, samples were taken for evaluation of total and progressive motility in a computerized system (CASA), plasma membrane integrity, assessed with propidium iodide (PI) and 6-carboxyfluorescein diacetate (C-FDA) and acrosomal integrity by the technique of Peanut agglutinin (PNA conjugated to FITC). Data were analyzed by ANOVA and Tukey test ($P<0.05$). After cooling, epididymal sperm had higher total motility, progressive motility and acrosome integrity than the ejaculate, being 58.9 ± 21.2% and 79.5 ± 5.9%, 29.6 ± 13.9% and 46, 1 ± 7.2%, 48.9 ± 20.6% and 69.4 ± 7.3%, respectively. However, the percentage of cells with intact plasma membrane was similar between the two groups (EJ = 56.4 ± 16.6% and PE = 69.7 ± 8.8%). After thawing, no difference was found in any of the characteristics studied: total motility, progressive motility, acrosome integrity and plasma membrane integrity, with 48.4 ± 9.8% and 54.2 ± 10.7%, 26.3 ± 8.8% and 35.4 ± 11.3%, 41.3 ± 12.9% and 46.8 ± 7.6% and 41.6 ± 11.9% and 50.8 ± 9.0% for the EJ and PE, respectively. The results indicate that epididymal spermatozoa are more resistant to cooling than EJ, but behave similarly when submitted to cryopreservation. However, more studies are needed to evaluate other sperm characteristics, such as longevity, capacitation, response to sperm selection for IVF and binding capacity to the zona pellucida, among others.

A005 Male Reproductive Physiology and Semen Technology

The influence of brucellosis on the morphological characteristics of canine epididymal sperm

C.F. Lúcio, D.S. Angrimani, J.D. Agostini Losano, M.M. Brito, M. Nichi, R.A. Silva Junior, L.B. Keid, C.I. Vannucchi

USP, São Paulo, SP, Brasil.

Keywords: brucellosis, dog, epididymal spermatozoa.

Canine Brucellosis is a zoonosis of difficult epidemiological control, can partially compromise fertility and has limited diagnostic definition. The reproductive analysis of a mongrel dog, 2 years old, identified *Brucella canis* infection, through the test named Canine Brucella Ab Rapid Antigen Test (Bioeasy, Minas Gerais, Brazil), which is highly specific for this agent. The physical examination revealed no remarkable change and the dog was submitted to orchiectomy. At macroscopic exam, the testis and epididymides were normal. Due to the frequent location of the bacteria in the epididymis, sperm were harvested from the caput (CP), body (BO) and tail (TA) of the epididymides and evaluated for sperm concentration, motility and morphology (eosin/nigrosin stain), mitochondrial activity (oxidation of 3,3'-diaminobenzidine, DAB), acrosomal membrane integrity (Fast Green-Rose Bengal stain) and flow cytometry with the probes JC1 (mitochondrial potential), FITC (acrosomal membrane integrity) and PI (plasma membrane integrity). The results were analyzed based on the sperm evaluation of 20 serologically negative dogs. The concentration and sperm motility of the tail were lower ($325x10^6$ sperm/mL and 50%, respectively) than the serologically negative dogs ($866 \pm 129x10^6$ sperm/mL and 72 ± 2%). There was a lower mitochondrial activity and potential due to the infectious process in the three epididymal segments (DABIII CP: 20%, BO: 20% and DABIV TA: 20%, control: DABIII CP: 13 ± 1%, BO: 9 ± 1% and DABIV TA: 10 ± 5%; JC1 CP: 20%, BO: 11% and TA 10%, control: CP: 13 ± 1% BO: 40 ± 3% and TA: 65 ± 4%). A higher percentage of acrosome membrane lesions were found in the body and tail samples (BO: 40%, TA: 11%, control BO: 22 ± 4% and TA: 7 ± 1%), teratospermia (major defects: 29%, control: 19 ± 5%), and high frequency of sperm distal droplets on the epididymal tail (21%; Control: 6 ± 1%). Despite the absence of clinical signs and macroscopic alterations of the testis and epididymides, sperm evaluations revealed important data. Alterations of the sperm derived from the epididymal tail suggest that the severity is associated with the increased temporal exposure to the epididymal environment, by means of bacterial toxins or through the local immune response. Due to the inflammatory changes promoted by the bacteria, there was an increased production of free radicals and, consequently, a reduction of sperm mitochondrial activity. The decrease in sperm motility can be attributed to the lower energy production by mitochondria. Lipid peroxidation justifies the changes found in acrosome membrane. The high percentage of distal droplets in epididymal tail spermatozoa may reflect a delayed maturation, probably as a consequence of Brucellosis. *Brucella canis*, though insidious, promotes important sperm changes in dogs, which can serve as a definitive diagnosis, along with the results of canine Brucellosis sorology.

A006 Male Reproductive Physiology and Semen Technology

Sperm profile during epididymal maturation in dogs

D.S. Angrimani, C.F. Lúcio, J.D. Agostini Losano, G.A.L. Veiga, M.M. Brito, M. Nichi, M.E.O.D. Assumpção, C.I. Vannucchi

Universidade de São Paulo, São Paulo, SP, Brasil.

Keywords: epididymis, dogs, sperm maturation.

Sperm maturation through the epididymis is a process that leads to morphologically and functionally changes, enabling fertilization. Therefore, major elucidations regarding the physiology of sperm maturation are necessary for further improvement of biotechnologies uses with epididymal sperm. Thus, the aim of this study was to determine the spermatic profile of canine epididymal samples. We used 21 dogs submitted to bilateral orchiectomy. Until processing, the epididymides were stored at 5°C for up to 24 hours. Sperm were harvested through small incisions (<1mm) in the caput (CAP), corpus (COR) and tail (TAIL) of the epididymides and then deposited in 300µl of PBS. Samples were evaluated for motility, velocity and Computer Assisted Sperm Analysis (CASA). To evaluate the plasma membrane permeability, acrosomal integrity and mitochondrial activity, we performed the eosin/nigrosin, fast green/rose bengal and the oxidation of 3,3 '-diaminobenzidine (DAB) stains, respectively. In order to determine plasma membrane integrity, acrosomal and mitochondrial potential, we used flow cytometry with specific probes (FITC, PI and JC1, respectively). Data were compared by ANOVA and Tukey test ($p \leq 0.05$). Regarding sperm motility and velocity, statistical difference was noticed between the samples of TAIL (mot: 69.7±4%; velocity: 2.6±0.1), compared to COR (mot: 27.7±3%; velocity: 2±0.1), both higher than CAP (mot: 0±0%; velocity: 0±0). The same data pattern was observed for the CASA variables: motile, progressive, rapid, VAP, VSL, straight trajectory and linear. In regards to the percentage of intact plasma membranes, TAIL samples (92.6±1.1%) were higher than COR (74.5±3.2%), which in turn was higher than the CAP (40.1±4.7%). The highest mitochondrial activity (DAB I) was observed for TAIL (75.9±3.4%), with values greater than COR (46.8 ± 2.5%), the latest superior than CAP (26±2.4%). Acrosomal damage was higher in CAP samples (40.2±3.7%), compared to COR (14.4±1.1%) and TAIL (6.5±1.8%). For flow cytometry, we observed lower plasma membrane acrosomal integrity in the COR (20.6±2.6%) and CAP (24.6±2.4%) groups, compared to the TAIL group (38.5±3.4%). Moreover, we found increased mitochondrial potential in TAIL samples (65±4.2%) compared to the COR (38.3±3.6%) and CAP (43.6±4.1%). In conclusion, sperm from the epididymis tail have a higher sperm profile than corpus and caput groups, especially regarding sperm membrane integrity, motility and, consequently, the fertilizing capacity. Our results confirm the occurrence of morphological and functional changes during the transit through the epididymides, enabling the use of epididymal sperm in several reproductive biotechnologies.

A007 Male Reproductive Physiology and Semen Technology

Scrotal thermography in rams after testicular degeneration and low level laser therapy treatment: preliminary results

E.C.C. Celeghini, M.R. Bianchi-Alves, R.P. Arruda, A.F.C. Andrade, L. Batissaco, S.A. Florez-Rodriguez, G.M. Ravagnani, M.A. Torres

Centro de Biotecnologia em Reprodução Animal, VRA-FMVZ-USP, Pirassununga, SP, Brasil.

Keywords: insulation, laser therapy, termography.

The body surface emits radiation in the infrared range of the electromagnetic spectrum. The thermographic camera is able to capture the radiation and produce images with different colors that depend of the quantity of radiation emitted. Thus, the thermography is a helpful tool to measure the scrotal surface medium temperature (SSMT). The low level laser therapy (LLLT) induces cellular proliferation and it is possible that LLLT contributes to the restoration of the seminiferous epithelium after testicular degeneration. The biostimulation was observed in the seminiferous epithelium of rats (Taha and Valojerdi, 2004, Lasers Surg. Med., 34, 4, 352-359); however, the temperature of the treated area may increase until 0.5ºC. Thus, the aim of this study was to evaluate the SSMT of rams submitted to scrotal insulation for the induction of testicular degeneration. For this, six rams were divided in three experimental groups: 1) Control, without LLLT treatment (n=2); 2) LLLT treatment of cumulative dose of 28J/cm² (n=2); 3) LLLT treatment of cumulative dose of 56J/cm² (n=2). It was used the output power of 30 mW and the treatment period was of 15 days, every 48h, in groups 2 and 3. Scrotal insulation was done in all rams for 72h before the experimental period. The thermal images were captured in at: T-8, T-5, T-1, T0, T3, T4, T6, T10, T17, T24 and T31 days, as related to insulation, being T0 the time that corresponded to placing the insulation bags. A T640 thermographic camera was used (FLIR Systems, Boston, USA) and thermal images were analyzed by the FLIR Quick Report® Software. The environmental temperature was measured at the moment of image aquisition. SSMT values were corrected by the environmental temperature (Basile et al., 2010, ARS Veterinária, 26, 77-81). The results were analyzed employing the Statistical Analysis System (SAS Institute Inc., 1995). The SSMT data were submitted to ANOVA and the factor repeated measures in time was added. Tukey's test was used to compare the averages when there was time X treatment interaction or time and treatment effect. There is no time X treatment interaction (P=0.48) neither treatment effect (P=0.25). However, it was observed a time effect (P=0.001). The SSMT was significantly (P>0.05) higher in T3 (36.25±0.49ºC) than T-8 (33.72±0.27ºC), T-5 (33.70±0.30ºC), T-1 (33.87±0.41ºC), T0 (33.49±0.24ºC), T6 (32.95±0.26ºC), T10 (33.83±0.24ºC), T17 (33.76±0.34ºC), T24 (33.17±0.36ºC) and T31 (32.78±0.53ºC). T3 was the time immediately after removal the insulation bags. On the other hand, T3 was not different (P>0.05) of T4 (34.75±0.24ºC). In conclusion, thermography is applicable to the evaluation of the SSMT in insulated rams and LLLT does not change the SSMT of treated groups. However, the results are preliminary and more studies are needed.

Acknowledgments: FAPESP process 2012/00040-0 and 2011/16744-3.

A008 Male Reproductive Physiology and Semen Technology

The effect of seminal plasma removal on cryopreservation of semen from Nelore bulls collected by eletroejaculation using different methods of separation (preliminary data)

F.M. Monteiro[1], M.E.Z. Mercadante[1], S.P. Campanholi[1], J.A. Dell'Aqua Jr[2], F.O. Papa[2], C.P. Freitas-Dell'Aqua[2]

[1]Instituto de Zootecnia, Sertãozinho, SP, Brasil; [2]UNESP, Botucatu, SP, Brasil.

Keywords: centrifugation, computerized evaluation of sperm, filtration, seminal plasma.

Seminal plasma, that mixes with sperm in the ejaculate, and serves as a means of transportation to 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 harmful components to sperm viability. An alternative to reduce the concentrations of seminal plasma of the ejaculate is 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 the frozen-thawed semen from 13 Nelore bulls collected by electroejaculation with the removal of seminal plasma through centrifugation and filtration techniques and their consequences on sperm kinetics. The work is being conducted at the Institute of Animal Science Sertãozinho / SP (partial data). Semen was collected using electroejaculation and were performed the physical and morphological analyzes routine before freezing (volume, motility, vigor, concentration and morphology). After collection, ejaculated semen was divided into three treatments before freezing: standard freezing T1, T2 and T3 were used to separate the seminal plasma. T2 semen was centrifuged (10 minutes at 600 xg) and T3 semen was filtered using the Filter Sperm ®. After treatments, semen was diluted with BotuBov® extender to a final concentration of $100x10^6$ sperm/ml and frozen using a freezing machine TK 4000 ®. Semen was thawed at 37 °C for 30 seconds and analyzed by Hamilton Thorne Research - IVOS 12, placing a drop in the Makler chamber heated to 38 °C for the analysis. Sperm variables considered were: total sperm motility (TM), motility (PM), average path velocity (VAP), progressive velocity (VSL), curvelinear velocity (VCL), amplitude of lateral head displacement (ALH), tail beat frequency (BCF), straightness (STR) and linearity (LIN). The statistical analyzes were performed in SAS PROC GLM, using 5% significance. Mean values for MT (T1, 39.5 ± 4.0, T2, 39.3 ± 4.0, T3, 42.6 ± 4.0), MP (T1, 29.8 ± 2.8, T2 , 28.0 ± 2.8, T3, 33.0 ± 2.8), STR (T1, 83.9 ± 1.1, T2, 81.3 ± 1.1, T3, 83.3 ± 1, 1), ALH (T1, 5.3 ± 0.2, T2, 6.1 ± 0.2, T3, 5.9 ± 0.2) and LIN (T1, 55.3 ± 1.5, T2; 52.2 ± 1.5, T3, 53.6 ± 1.5) did not differ between treatments (P>0.05). However, for VAP and VCL, T2 (88.2 ± 2.3 146.3 ± 5.4) and T3 (88.9 ± 2.3 145.0 ± 5.4) differed from T1 (81 10 ± 2.3 130.6 ± 5.4), respectively (P <0.01). For VSL, T3 (74.1 ± 1.5) differed from T1 (67.8 ± 1.5; P <0.01). Based on these preliminary results, it can be concluded that frozen semen after removal of seminal plasma by centrifugation and filtration were better for VAP, VSL and VCL compared to standard freezing.

A009 Male Reproductive Physiology and Semen Technology

Effect of catalase on freezing of sperm cell obtained from the cauda epididymis of Nelore bulls

F.C. Almeida[1], S.V. Silva[2], H.M. Souza[1], J.A.C. Lima Filho[1], A.A. Wicke[3], A.M. Batista[1], M.M.P. Guerra[1]

[1]UFRPE, Recife, PE, Brasil; [2]UFPB, João Pessoa, PB, Brasil; [3]Ciclo, São Gonçalo, BA, Brasil.

Keywords: antioxidant, cryopreservation, germosplasm.

Previous dilution in semen cryopreservation processes promotes reduction in antioxidants concentration, which may cause imbalance between oxidants and antioxidants concentrations, and cell stress (Bilodeau et al., 2000, Mol Reprod Dev, 55, 282-8). Catalase (CAT) is an enzymatic antioxidant that catalyses H2O2 molecules into water and oxygen (Norderberg, 2001, Free Rad Biol Med, 31, 1287-312). This study aimed to evaluate sperm integrity obtained from cauda epididymis of Nellore bulls subjected to freezing in diluents supplemented with CAT. Epididymides were obtained from slaughterhouse, minutes after animals' death. Sperm cells were recovered by flotation technique (Almeida et al., 2012, Anim Reprod, 9, 959), diluted in Tris-yolk egg. Each *pool* of sperm was formed from five epididymis, with a total of 11 *pools*. Samples were divided into experimental groups (control, CAT 50 and 100 U/mL), with $80x10^6$ sperm/mL as the final concentration, packed in straws (0.25 mL) and frozen (TK3000®, TK Tecnologia em congelação LTDA, Uberaba, Brasil). After thawing (37°C/30s), samples were evaluated for motility (CASA/SCA™ software v.5.1; Microptics, S.L., Barcelona, Spain), plasma membrane integrity (PMi), acrosome integrity (ACi) and mitochondrial membrane potential (MMP). We performed analysis of variance (F test) and test of Student-Newman-Keuls ($P < 0.05$). All experimental groups demonstrated post-thaw motility above 60.00%. Integrity assessments revealed no significant difference ($P > 0.05$) between control, CAT 50 and CAT 100 groups, respectively, PMi (49.55±4.46; 48.27±5.53; 50.82±4.89), ACi (32.82±28.34; 51.50±35.82; 53.18±32.66) and MMP (62.86±6.47; 63.73±8.71; 65.05±10.68). Based on results, we conclude that CAT addition at concentrations of 50 and 100 U/mL do not affect the integrity of epididymal spermatozoa.

A010 Male Reproductive Physiology and Semen Technology

Evaluation of canine semen cooled after 120 hours with different means

G.F.O. Menezes[1], R.F. Bittencourt[2], L.G.S. Moura[2], Y.C. Coutrim[2], M. Chalhoulb[3], A.L. Ribeiro Filho[3]

[1]Universidade Federal de Sergipe, Aracaju, SE, Brasil; [2]União Metropolitana para Educação e Cultura, Lauro de Freitas, BA, Brasil; [3]Universidade Federal da Bahia, Salvador, BA, Brasil.

Keywords: botu-semen, dogs, extenders.

The biotech semen in dogs have had great progress in recent years, due to the demand to improve fertility rates in artificial insemination in this species. The cooled semen has proved the best technique for insemination later, being a practical and low cost. After cooling semen adding extenders becomes important factor for enhancing the viability thereof, for extended periods of time. The aim of this study was to evaluate the quality of semen cooled for 120 hours in three different extenders, one milk-based (Botu-semen ® -Botucatu/SP, Brazil) and two based on egg yolk and Tris, Tris egg yolk without glycerol (TRIS) buffer and Tris egg yolk with 6% glycerol (TRIS + GL). Were cooled ejaculates of four dogs that after centrifugation for 10min (600G), were fractionated and diluted in the three extenders (Botu-semen ®, TRIS, TRIS + GL) and assessed every 12 hours to complete the 120 hours. To evaluate sperm parameters were observed: total motility (TM) and progressive (MP), sperm vigor (VIG), morphology and functionality of the membrane hypo osmotic swelling test (HOST). After 120 hours, the evaluations were done and the results demonstrated that TRIS got better indexes in all parameters, in which the MT obtained was 21.3%, 20.0 and 37.5%, for the media Botu-semen ®, TRIS and TRIS + GL, respectively. The MP was 11.3%, 10% and 27.5% means for Botu-semen ®, TRIS and TRIS + GL, respectively. The results of VIG were higher for TRIS (2.5) compared to other means Botu-semen ® (1.3) and TRIS + GL (0.9). The same happened to HOST, in which the group obtained TRIS index with sperm membrane integrity of 39.0%, higher than the results observed means for Botu-semen ® and TRIS + GL, with 20% and 37.5% respectively. After this study it was concluded that the TRIS provided better maintenance rates of sperm parameters after 120 hours, which proves to be the best alternative among the means evaluated for cooling canine semen.

A011 Male Reproductive Physiology and Semen Technology

The osmolarity of the extender affects the viability of chilled swine spermatozoa?

G.M. Ravagnani[1], M. Andrade Torres[2], M. Lima Oliveira[1], S.M.M.K. Martins[1], M.R. Bianchi-Alves[1], A.S. Moretti[1], F.O. Papa[3], J.A. Dell'Aqua Jr[3], M.A. Alvarenga[3], F.V. Meirelles[4], A.F.C. Andrade[1]

[1]FMVZ - USP, Pirassununga, SP, Brasil; [2]FMVZ-USP, Pirassununga, SP, Brasil; [3]FMVZ - UNESP, Botucatu, SP, Brasil; [4]FZEA - USP, Pirassununga, SP, Brasil.

Keywords: membrane integrity,osmolarity, spermatozoa.

The present study evaluated the effects of two different conditions of osmolality on plasma membrane and acrosomal integrity, and mitochondrial membrane potential. Therefore, three semen collections from three boars were carried out (n = 9). The samples were diluted in media with different osmotic potentials, namely, 360 (A) and 404mOSM (B) (Botupharma®, Botucatu-SP, Brazil). The osmotic potentials differed by changing the amount of ultrapure water used for dilution, 1.1 L (360 mOsm) and 1 l (404 mOsm). Fresh semen was mixed with extenders to obtain a concentration of 30 x 10^6sptz/mL. Analyses were performed at 90 minutes, 24 and 48 hours after dilution. They were analyzed for the sperm cells that presented simultaneously plasma membrane integrity, acrosomal and mitochondrial membrane potential (PIAIC) (Celeghini et al., 2007, Reprod Domest Anim, 42, 479-88). For this, aliquots of 150µL of semen were stained with Hoescht 33342 (5 mg / ml in DMSO) iodide, propidium (0.5 mg / ml in DPBS), JC-1 (153 mM in DMSO) and 50 l of FITC -PSA (100 g / ml in DPBS). The samples were incubated for 8 minutes at 37°C and then evaluated under epifluorescence microscopy (Nikon Eclipse Model NI-U) in a triple filter (D / F / R, C58420) at 1000X magnification. The experimental design was in generalized randomized blocks added the factor of repeated measures. Data were analyzed using SAS (SAS Institute Inc., 2010), subjected to analysis of variance and interactions by the Greenhouse-Geisser at 5%. Data are presented as mean ± standard error. There was no time x treatment interaction (P> 0.05) for the PIAIC variable. Thus, we assessed the effect of osmolality factor. Apparently, increased osmolality improves the integrity of the plasma and acrosomal membranes as well as mitochondrial membrane potential (p = 0.0571). Extender A showed 54.09 ± 4.14% of cells PIAIC, while extender B had 64.14 ± 3.14%. From these results, it can be concluded that the higher the osmolality, the greater the number of cells with plasma membrane integrity, acrosomal and mitochondrial membrane potential.

Acknowledgments: FAPESP process 2011/23484-8 and Botupharma®.

A012 Male Reproductive Physiology and Semen Technology

Epididymis morphology and morphometry of the trachemys scripta elegans (Wied, 1839) raised in Brazil

I.C. Pires[1], A.C.S. Silva[1], L.C. Souza[1], E.S. Araújo[1], R.T. Silva[1], M.D. Faria[1], V.S. Franzo[2], A. Gradela[1]

[1]UNIVASF, Petrolina, PE, Brasil; [2]UFMT, Cuiabá, MT, Brasil.

Keywords: epididymis, testudines, turtle.

Trachemys scripta elegans (WIED, 1839) (*T. scripta elegans*) is an underwater exotic turtle invasive of the Cerrado[1], being constantly marketed in Brazil. The function of epididymides is the maturation and storage of spermatozoa, providing a favorable environment to physiological and morphological changes. However, information on this species is scarce, which may assist in population control measures or comparative studies. The objective of this study was to describe the epididymides morphology and morphometry of *T. scripta elegans* raised in Brazil and correlate the epididymal morphometry with body biometric data. Eleven males of the species, from the Parque Ecológico do Tietê[2] (IBAMA[3] Record Nr. 2491988), had the reproductive system and the body biometrics (mass (MC), volume (VC), carapace length (CARL), carapace width (CARW), plastron length (PLAL), plastron width (PLAW) and height (HEI)) assessed. The epididymis were dissected for macroscopic and morphometric evaluation (mass (ME), volume (VE); length (EL), width (EW) and thickness (ET)). The Kruskal-Wallis test followed by t test was employed for comparison of means ($\pm$ sd) and the simple correlation coefficient (R) was determined among the variables (Assistat 7.6 beta). This study was approved by the CEDEP[4] from UNIVASF[5] (Protocol nr. 0001/160412). Genitals were a pair of testicles and epididymis, vas deferens, mesorchium present throughout the testicular wall and a penis. Epididymis were convolute structures and were located in the right and left antimeres connected to the medial surface of each testicle and joined to vas deferens, which penetrated into the cloaca, near the opening base of the urinary bladder. MC was587.45$\pm$198.37 g, VC 556.54$\pm$289.75 ml, CARL15.50$\pm$2.35 cm, CARW 12.2$\pm$ 1.39 cm, PLAL13.72$\pm$1.93 cm, PLAW 9.21$\pm$1.48 cm and HEI 5.57$\pm$1.34 cm. ME, VE and ET differed ($P<0.01$) between right and left antimeres (0.28$\pm$0.25 vs 0.29$\pm$0.25 g, 0.24$\pm$0.16 vs 0.26$\pm$0.16 ml and 0.24$\pm$0.23 vs 0.25$\pm$0.20 cm, respectively), while EL and EW did not differ ($P>0.05$) (1.81$\pm$0.42 vs 1.74$\pm$0,43 cm and 0.52$\pm$0.12vs 0.55$\pm$0.27 cm, respectively). It is concluded that in this species, body parameters have significant correlation among themselves except for CARL and HEI. Also, ME correlates with the other morphometric parameters in both antimeres, while VE correlates to EW and ET in the right antimere and only with EW in the left antimere. These data provide information on *T. scripta elegans* epididymis and may be helpful in population control and comparative studies.

References

[1]Vast tropical savanna Eco region in Brazil; [2]Tietê Ecological Park; [3]Instituto Brasileiro de Meio Ambiente e Recursos Naturais Renováveis (Brazilian Institute for Environment and Renewable Natural Resources); [4]Comitê de Ética e Deontologia (Ethics and Deontology Committee); [5]*Universidade* Federal do Vale do São Francisco (Federal University of the São Francisco Valley).

A013 Male Reproductive Physiology and Semen Technology

Flow-cytometer sex sorting affects sperm characteristics related to viability and capacity to bind oviduct cells

J.O. Carvalho[1], R. Sartori[1], L. Rodello[2], S.D. Bicudo[2], G.B. Mourão[1], M.A.N. Dode[3]

[1]Universidade de São Paulo, Piracicaba, SP, Brasil; [2]UNESP, Botucatu, SP, Brasil; [3]EMBRAPA Recursos Genéticos e Biotecnologia, Brasília, DF, Brasil.

Keywords: bovine, fertility, fertilization.

This study assessed the effect of sex process on sperm survival and capacity to bind to oviduct cells, which are essential for IVF. Each ejaculate of Nelore bulls (n=4) was collected and separated into three fractions: non-sexed (NS), sexed for X-sperm (X), and sexed for Y-sperm (Y). A fourth group was formed by pooling X and Y samples (XY). Semen from each group was assessed for sperm viability after thawing (0-), after washing (0) and 2, 4, 8 and 12 h after incubation in synthetic oviduct fluid. In each moment, the samples were analyzed for sperm motility by computer-assisted semen analysis (CASA), plasma membrane stability (PM, merocyanine 540), PM integrity (Syber green), acrosomal integrity (PNA) and mitochondrial membrane potential (Mitotracker green) in flow cytometer. For sperm binding test, sperm from each group/bull was incubated for 30 min and 24 h with oviduct explants. Data were analyzed using generalized linear models (SAS$^{(R)}$; $P<0.05$). The percentages of sperm motility (58.1±4.3 and 35.2±4.4), progressive motility (46.1±6.1 and 25.7±4.8), PM integrity (91.0±3.9 and 79.5±6.0), mitochondrial membrane potential (79.2±9.3 and 69.0±10.6), PM stability (77.4±4.6 and 19.4±4.2), and live sperm with intact acrosome (57.2±8.5 and 31.3±7.9) were higher in non sexed than in sexed sperm. Moreover, percentages of sperm with destabilized PM and live sperm with reacted acrosome were higher in sexed sperm (65.7±5.5 and 35.6±8.9) than in non sexed (2.5±0.9 and 15.7±3.8). Those differences were kept up to 8 h of incubation. However, after 12 h sperm quality was similar between groups. By comparing the X and Y groups it was observed that after wash the X sperm (56.6±13.9) had higher percentage of sperm with mitochondrial potential than Y sperm (41.7±13.8), and higher percentage of live sperm with reacted acrosome at 0, 2 and 4 h of incubation. The sexing process did not affect sperm binding to the oviduct cells after 30 min. However, after 24 h, the XY group (6.7±2.0) had less sperm bound in oviduct explants than NS group (23.6±7.2). After 30 min, the Y group (84.9±17.0), showed higher number of sperm bound than the X group (72.1±20.3), bud there was no difference between them at 24 h. In conclusion, sexing process affected structural characteristics of the sperm cell, inducing a precapacitaion status and acrosome reaction as well as its capacity to remain bound to the oviduct explants. Regarding to X and Y sperm, it was identified that X sperm was more sensitive to the sexing process than Y sperm. Those pieces of information can be used to develop new alternatives to improve the results when sexed sperm is used for AI. The alternatives can be changes in protocols for AI, such as different moments for insemination, or in cryopreservation processes, such as adding protein extract of seminal plasma after sexing to increase the capacity of the sperm to remain bound to oviduct cells.

Financial support: CNPq, EMBRAPA and FAPESP (2010/00585-0 and 2010/15054-0).

A014 Male Reproductive Physiology and Semen Technology

Standardization of sperm binding assay in cryopreserved bull semen evaluated by computer assisted sperm analysis and conventional microscopy

J.D. Agostini Losano[1], D.S. Angrimani[1], T. Santos Criscuolo[2], M.M. Brito[1], R.J.G. Pereira[1], A.M. Rocha[1], V.H. Barnabe[1], R.C. Barnabe[1], M. Nichi[1]

[1]Universidade de São Paulo, São Paulo, SP, Brasil; [2]Huntington Medicina Reprodutiva, São Paulo, SP, Brasil.

Keywords: bull semen, functional tests, sperm interaction.

The interaction between spermatozoa and zona pellucida occurs through binding of sperm acrossomal receptors to zona pellucida ZP3 and ZP2 proteins (Yanagimachi, 1994, Zygote, 2, 371-72). The perivitelline membrane of chicken eggs shows homologies with these proteins, allowing the binding of sperm from several species (Barbato et al., 1998, Biology of Reproduction, 58, 686-99). However, adequate standardization is required in order to verify the possible application of this technique for semen evaluation in determined species. The present study aimed to validate the sperm binding assay in cryopreserved bull semen by evaluating the results of the test using different proportions of live / dead sperm (0%, 25%, 50%, 75% and 100%). Cryopreserved samples (n=6) were thawed and divided in two aliquots; one kept in water bath at 37°C (live) and the other immersed in liquid nitrogen and thawed 3x (dead). Both samples were then mixed in different proportions which were then submitted to computerized motility analysis (CASA; Hamilton-Thorne Ceros 12.3), membrane integrity (eosin / nigrosin), acrosomal integrity (Fast-green/Rose-bengal) and mitochondrial activity (3'3 Diaminobenzidine) evaluations. Shortly thereafter, membrane squares measuring 0,5 cm^2 were incubated with the live/dead proportions in a concentration of 100.000 spermatozoa per ml, at 37°C for one hour. Sperm binding was assessed by both conventional microscopy and CASA. Results were expressed as the number of sperm bound per mm^2 of membrane. Statistical analysis used SAS System for Windows. With the increase in the proportion of live sperm, there was an increase in the number of spermatozoa bound to the membrane, in evaluations performed using both CASA and microscopy. Linear regression showed a high relationship between the proportion of live sperm and the number of sperm bound to the membrane (CASA, $R^2 = 0.92$, $p < 0.0001$, Microscope, $R^2 = 0.91$, $p < 0.0001$). There was a positive correlation between the number of spermatozoa bound to the membrane and acrosomal integrity (%, CASA 0.846, $p < 0.0001$; Microscope 0.788, $p < 0.0001$) membrane integrity (%CASA 0.845, $p < 0,0001$; Microscope 0.846, $p < 0.0001$), high mitochondrial activity (%; CASA 0.759, $p < 0.0001$; Microscope 0.768, $p < 0.0001$), motility (%; CASA 0.890, $p < 0.0001$, Microscope 0.806 $p < 0.0001$) and progressive motility (%; CASA 0.861, $p < 0.0001$, Microscope 0.919, $p < 0.0001$). We can conclude that the sperm binding assay may be used in for cryopreserved bull semen as an alternative tool to indirectly assess several sperm functions, with affordable costs and straightforwardness methodology.

A015 Male Reproductive Physiology and Semen Technology

Effect of busulfan on spermatogonial markers expression in different mouse strains

K. Gutierrez, W.G. Glanzner, R.O. Chemeris, A. Bridi, P.A. Anezi Jr, A.M. Pereira Dau, M.L. Rigo, J.F.C. Oliveira

Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.

Keywords: gene expression, spermatogonia stem cells, testicular degeneration.

Spermatogenesis is a complex process where many spermatozoa are produced from a small number of spermatogonial stem cells (SSC), which are responsible for the maintenance and constant sperm production throughout the male life (Brinster and Zimmermann, 1994, PNAS, 91, 11298-302). Although the gene expression pattern of spermatogonial stem cells is not completely elucidated, some fundamental genes related to the maintenance and self-renew of these cells and some specific molecular markers are well established (Tsuda et al., 2003, Science, 301, 1239-41; Costoya et al., 2004, Nat Genet, 36, 653-59; Meng et al., 2000, Science, 287, 1489-93). Among the various models used in testicular degeneration studies, one of the most utilized is the administration of busulfan (Zohni et al., 2012, Hum Reprod, 27, 44-53). However, the effect of bulsufan treatment on the expression of spermatogonial molecular markers in mice has not been established yet. The aim of this study was evaluate the mRNA expression of spermatogonial markers (*Nanos2*, *Nanos3*, *GDNF* and *PLZF*) after busulfan treatment in two different mice strains. Balb/C and Swiss male mice were used in this study. Animals of each strain were randomly divided in control groups C30 and C90 (30 and 90 days after vehicle administration) and into busulfan treated groups (B30 and B90, 30 and 90 days after busulfan administration). Animals from busulfan treated groups receive a unique intraperitoneal injection (i.p.) of 40 mg kg^{-1} of busulfan, while animals from control group receive just vehicle. After 30 and 90 days of the busulfan treatment, the animals were, anesthetized and had the testes surgically removed. Total RNA was extracted from the testes using Trizol and the mRNA expression analyzed by qRT-PCR. The mRNA expression was corrected by amplification of *Gapdh* housekeeping gene. Gene expression were analyzed by ANOVA and means compared by a Student's *t* test. A significant reduction in *Nanos2* mRNA expression was observed at 30 ($P<0.01$) and 90 days ($P<0.05$), whereas the expression of *Nanos3* mRNA was only observed at 30 days after busulfan administration ($P<0.001$) in treated Balb/C mice. The *Gdnf* and *Plzf* mRNA levels increased by more than 2.0-fold, independently of the time lapse between busulfan treatment and control group in Balb/C ($P<0.05$). On the other hand, the expression of *Nanos2*, *Nanos3* and *Plzf* mRNA revealed no statistical differences between the experimental groups in Swiss mice ($P>0.05$). However, the *Gdnf* mRNA levels increased after 30 days of busulfan treatment in Swiss animals ($P<0.05$). This study demonstrated that busulfan changes the expression of spermatogonial molecular markers probably depending on cell type present in the seminiferous tubules and these alterations are dependent on mice strain.

A016 Male Reproductive Physiology and Semen Technology

Osmolarity and motility characteristics of swine sperm: changes in characteristics of sperm movement

M. Andrade Torres[1], G.M. Ravagnani[1], M. Lima Oliveira[1], S.M.M.K. Martins[1], M.R. Bianchi-Alves[1], A.S. Moretti[1], F.O. Papa[2], J.A. Dell'Aqua Jr[2], M.A. Alvarenga[2], A.F.C. Andrade[1]

[1]FMVZ/USP, Pirassununga, SP, Brasil; [2]FMVZ/UNESP, Botucatu, SP, Brasil.

Keywords: motility, osmolarity, spermatozoa.

Motility is the most affected parameter by variations in osmolarity (Yest et al., 2010, Anim Reprod Sci, 119, 265-74), and osmotic tolerance is a potential indicator of sperm function (Lechniak et al., 2002, Redrod Domest Anim, 37, 379-80). Thus, this study evaluated the effects of different osmolarities of boar extender in sperm characteristics: total motility (MT,%), progressive motility (MP,%), average path velocity (VAP, μM / s), straight velocity (VSL μM/s), curvilinear velocity (VCL, μM/s), amplitude of lateral head displacement (ALH, μM), beat cross frequency (BCF, Hz), straightness (STR,%) and linearity (LIN,%). Were made three semen collections of three boars (n=9). Samples were extended in media with various osmolarities, namely 360 (T1) and 404 (T2) mOsm (Botupharma®, Botucatu-SP, Brasil), that differ by changing the dilution, 1.1 L and 1 L of ultrapure water, respectively. Raw semen was mixed within extender to obtain a concentration of 30 X 10^6 sptz/mL. Analyses were made at 90 minutes, 24 and 48 hours after dilution. Aliquots of semen were evaluated under cover slide, in computer assisted *sperm* analysis (SCA-Microptic®, Microptic SL, Barcelona, Spain). The experimental design was in generalized blocks added to measures repeated in time. The data were analyzed by SAS program (SAS Institute Inc., 2010), subjected to analysis of variance and interactions by Greenhouse-Geisser test at 5%. There was no interaction between time and treatment ($P>0.05$; T1 and T2) for any of the variables (MT, MP, VAP, VSL, VCL, ALH, BCF, STR, LIN). However, a treatment effect ($P<0.05$) for the characteristics VAP (33.27 ± 1.67, 38.77 ± 1.97), VCL (58.12 ± 2.8, 68.81 ± 3.04), STR (58.55 ± 1.65, 49.04 ± 1.41) and LIN (33.03 ± 1.89, 27.78 ± 1.18) were found. A significance trend ($p = 0.0548$) for the ALH variable with values of T1: 2.53 ± 0.1 and T2: 2.83 ± 0.11. Nevertheless variables MT (89.92 ± 2.64, 91.31 ± 1.21), MP (73.33 ± 4.07, 76.35 ± 3.27), VSL (19.61 ± 1.09 and 18.4 ± 0.78) and BCF (8.75 ± 0.43, 9.3 ± 0.25) not differ ($P> 0.05$) between T1 and T2 respectively. Thus, changes in osmolarity did not alter ($P>0,05$) motility characteristics, but extender with 404 mOsm increase VAP, VCL and ALH, while LIN and STR decrease. The 404 mOsm extender interferes in different characteristics (VAP, VCL, ALH, LIN, STR) suggest that it increases the number of hyperactive cells. Hypermotility as well sperm capacitation should occur at a favorable time and local, since that the beginning of these processes by sperm cells reduce their survival in the female reproductive tract, thus leading to a possible decrease in fertility.

Acknowledgments: FAPESP process 2011/23484-8 and Botupharma®.

A017 Male Reproductive Physiology and Semen Technology

New insights on the use of soybean lecithin for cryopreservation of bull semen

M.P. Rodrigues[1], O.F. Bao Tarrago[1], R.P. Arruda[1], C.M. Mendes[1], A.F.C. Andrade[1], J.N.S. Sales[2], R.C. Barnabe[1]

[1]FMVZ-USP, São Paulo, SP, Brasil; [2]UFPB, Areia, PB, Brasil.

Keywords: antioxidant, bull, sperm cryopreservation.

Nowadays, significant discussion exists about the use of seminal egg yolk-based extender because of bacteriological contamination risks (Gil et al., 2003, Theriogenology, 59, 1157–70). However, products that could replace the egg yolk on sperm extenders do not show satisfactory results (Celeghini et al., 2008, Anim Reprod Sci, 104, 119-31). With this in mind, the aim of the present experiment was to compare the effects of egg yolk-based extender and soybean lecithin-based extender, supplemented with two types of antioxidants, on maintenance of bovine cryopreserved sperm samples. Seminal samples from 20 Brangus bulls were collected by electroejaculation and samples were divided in 3 extenders groups: LA- soybean lecithin supplemented with ascorbic acid (AA, 4.5mM); LS- soybean lecithin supplemented with superoxide dismutase (SOD, 60UI/mL) and GO-egg yolk-based extender, without antioxidant. Semen was cryopreserved and samples were analyzed by laboratorial tests such as computer assisted sperm analysis (CASA); plasma (eosin/nigrosin) and acrosome (fast green/ bengal rose) membrane integrity; mitochondrial cytochemical activity (DAB); DNA integrity (SCSA), and induced oxidative stress index (TBARS). Data were analyzed using the software Statistical Analysis System-SAS® (SAS, 2001), UNIVARIATE procedure was used and variables were tested by Barlett test, moreover, GLIMMIX procedure were used for variance analysis and for determining treatment meanings differences. The level of significance considered was 5%. It could be verified that the presence of antioxidant on semen extender did not protect sperm cells from oxidative stress (LA:640.36; LS:564.13; GO:255.23ng/10^6sptz), and GO extender showed lower levels of TBARS. On the other hand, SOD was as efficient as egg yolk in preserving sperm cells with high mitochondrial potential (66.05%, 76.90%), respectively, in view of superoxide anion produced by mitochondria during the respiration process is catalyzed by SOD, protecting mitochondrial integrity from oxidative damages. In addition, AA exerted a greater protection on DNA induced denaturation, when compared to GO group (0.51%; 2.61%), respectively. Despite the protection of AA on DNA has been correlated, by several authors, to the capacity of reducing oxidative stress, it was verified that this protection involves mechanisms different from its antioxidant role. As it can be seen, soybean lecithin-based extender supplemented with SOD or AA would be an option for definitive replacement of egg yolk-based extender, avoiding sanitary barriers that prevent the international commercialization of bovine cryopreserved semen samples.

A018 Male Reproductive Physiology and Semen Technology

Comparison of the kinetics of sperm movement of boar semen subjected to room temperature or 37°C

M.A.O. Muçouçah, F. Morotti, F.Z. Machado, C.A.A. Melanda, F.C. Souza, C.A. Silva, M.M. Seneda, M.I.M. Martins

UEL, Londrina, PR, Brasil.

Keywords: room temperature, sperm parameters, swine.

The aim of this study was to compare the influence of temperature on the quality of boar semen before dilution, by means of the computerized analysis of sperm kinetics. Were used ejaculates from four Large White boars, with an average age of 3 years, kept in a system of weekly semen collection. The samples were collected by the gloved hand technique with the aid of a dummy. Semen was collected in plastic tubes, protected by Styrofoam, prepared with sterile gauze on the top to separate and discard the gelatinous fraction of the ejaculate. After collection, the container was sealed and taken to the laboratory for evaluation. The ejaculate was aliquoted into two samples, one of which was kept in a Styrofoam container at room temperature (G1) and another was maintained at water bath at 37°C for 10 minutes (G2). The semen samples were evaluated for motility by CASA (Computer Assisted Sperm Analyzer, Hamilton-Thorne IVOS, Beverly, MA, USA) and further diluted 1:1 in 0.9% NaCl solution. The values of sperm velocity index and sperm movement index were calculated according to Núñez-Martínez, Moran and Peña (Reproduction in Domestic Animals, Volume 41, PP. 408-415, 2006). Sperm parameters for the two groups were tested for normality by the Shapiro-Wilk test followed by Mann-Whitney statistical method ($P < 0.05$). The averages found for MOT and PROG MOT were respectively 84.3 ± 8.7 and 41.5 ± 16.3 for G1 and 86 ± 8.7 and 35.5 ± 14.4 for G2. The index sperm velocity and index movement sperm showed an average of 210.9 ± 38.2 and 165.5 ± 17.4 for G1 and averaged 186.3 ± 23.1 and 159.9 ± 17 for G2. There were no statistically significant differences between groups. It can be concluded that there are no differences in semen quality and sperm movement during storage in isothermal container or at 37ºC. Thus, there is greater economy with the equipment to keep the semen at 37°C and more convenience in seminal evaluations at pig breeding farms.

A019 Male Reproductive Physiology and Semen Technology

Canine antioxidant profile of ejaculated and epididymal fluid

M.M. Brito, D.S. Angrimani, C.F. Lúcio, G.A.L. Veiga, F.M. Regazzi, L.C.G. Silva, M. Nichi, C.I. Vannucchi

FMVZ USP, São Paulo, SP, Brasil.

Keywords: antioxidants, dogs, epididymal fluid.

Epididymal maturation is a process that modifies sperm cells allowing them to fertilize properly, but these alterations increase sperm susceptibility to the reactive oxygen species attack (lipid peroxidation). As a mechanism of protection, the epididymis and the prostate must provide antioxidants in order to avoid the damage caused by the oxidative stress. So, this research aimed to compare the antioxidant enzymatic profile of the ejaculate and the distinct parts of the canine epididymis. We used five dogs of different breeds aged 1 to 6 years. From the same animal, the semen sample was obtained by digital manipulation and, after 1 week, from the epididymal segments (caput, corpus and tail), after orchiectomy. The epididymis were stored at 5°C for up to 19 hours and samples were subsequently collected through small incisions (<1mm) in each segment, aspirated separately by automatic pipette and maintained in 100µl TALP extender. We evaluated the activity of catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the three fractions of the ejaculate (fraction I, II and III) and in each epididymal segment. Quantification of catalase activity was measured by the consumption of H2O2, GPx activity was evaluated by the consumption of NADPH and SOD activity, by c-citocrome reduction by superoxide anion, using spectrophotometry. Data were compared by ANOVA and Tukey test ($P \leq 0.05$) and submitted to Pearson and Spearman correlation test. There was no statistical difference regarding the concentration of SOD among samples from fraction I (80±35.6 UI/mL), fraction II (116.1±17 UI/mL) and fraction III (91.2±10.3 UI/mL) of the ejaculate, as well as among samples from the epididymal caput (80.9±16.4 UI/mL), body (102.2±19 UI/mL) and tail (94.5±19.3UI/mL). For catalase, we obtained reduced enzymatic activity on fractions I (0.8±0.8 UI/mL), II (1.7±0 UI/mL) and III (2.9±1.8 UI/mL), but we didn't find activity it in the epididymal segments. For the analysis of GPx, we found a higher activity in fraction II (45.5±15.7 UI/mL), when compared to fractions I (15.5±2.2 UI/mL) and III (8.7±1.7 UI/mL) of the ejaculate. For the epididymis, the tail (80.6±9.2 UI/mL) had superior concentration of GPx when compared to the body (49.4±7.2 UI/mL) and caput (24±3.9 UI/mL). The epididymal tail presented higher concentration of the GPx enzyme by having a higher protein secretion along sperm maturation. Moreover, we observed a positive correlation between SOD and GPx concentrations in the tail ($r=0.86$, $P=0.0012$) and body ($r=0.79$, $P=0.0064$) of the epididymis, which confirms the oxidative homeostasis against the lipidic peroxidation during sperm maturation through the epididymis. We can highlight the importance of GPx and SOD, which protects sperm during maturation. These enzymes must be considered in the composition of the extender for epididymal fluid used for reproductive biotechnologies in the canine species.

A020 Male Reproductive Physiology and Semen Technology

Assessment of sperm characteristics of rams treated with low-level laser therapy: preliminary results

M. R. Bianchi-Alves[1], R. P. Arruda[1], A.F.C. Andrade[1], L. Batissaco[1], S. Florez-Rodriguez[1], G.M. Ravagnani[1], M.A. Torres[1], E.C.C. Celeghini[1]

[1]Center of Biotechnology in Animal Reproduction, Department of Animal Reproduction, Faculty of Veterinary and Animal Science, USP, Pirassununga, SP, Brazil.

Keywords: insulation, laser therapy, rams, spermatozoa.

The low-level laser therapy (LLLT) is an important tool to induce cellular proliferation and to stimulate mitosis. This biostimulatory effect was observed in the seminiferous epithelium of rats treated with laser therapy (Taha and Valojerdi, 2004, Lasers Surg. Med., 34, 4, 352-359). In this way, the aim of this study was to evaluate different LLLT protocols in testes of rams submitted to scrotal insulation for the induction of the testicular degeneration. For this, six rams were divided in three groups: 1) Control, without LLLT treatment (n=2); 2) LLLT treatment of cumulative dose of 28J/cm² (n=2); 3) LLLT treatment of cumulative dose of 56J/cm² (n=2 The treatment was performed once a day and repeated 48h later during 15 days in the treated groups (2 and 3). The output power used was the same for the treated groups (30mW). The scrotal insulation was done in all rams for 72h before the beginning of the treatment period. After 10 days of scrotal insulation, four semen collections were performed during the experimental period in intervals of 7 days each. The Sperm-Class Analyser (SCA; Microptic S.L., Barcelona, Spain) was used to the computer-assisted assessment of sperm motility. The differential interference-contrast microscopy (model 80i; Nikon, Tokyo, Japan) was used to assess sperm morphology. The epifluorescence microscopy (model 80i; Nikon, Tokyo, Japan; filter D/F/R, C58420, Nikon, Tokyo, Japan) was used to evaluate acrosomal membrane integrity (FITC-PSA), plasma membrane integrity (PI+Hoescht 33342) and mitochondrial function (JC-1). The data was analyzed employing the Statistical Analysis System (SAS Institute Inc., 1995). The treatment effect was tested for ANOVA, the repeated measures factor was added. The Tukey test was used to compare the averages when there was time X treatment interaction or time and treatment effect. There was no time X treatment interaction (P>0.05) for the variables analyzed. There was no difference in the total motility (TM, P=0.56), the progressive motility (PM, P=0**.**54) and the minor defects (MD; P=0.2) among the groups 1 (TM: 41.36±9.07, PM: 29.51±7.75%, MD:12.56±3.3%), 2 (TM: 60.11±9.60%, PM: 45.55±10.28%, MD: 8.56±2.22%) and 3 (TM: 58.03±7.24%, PM: 43.58±7.12%, MD: 10.43±1.74%). Treatment effect was observed only in acrosomal membrane integrity. The group 1 (85.53±2.08%) presented higher (P<0.05) percentage of acrosomal membrane integrity than group 2 (74.50±2.72%) and 3 (6**9**.43±3.67%). There was time effect (P<0.05) to the plasma membrane integrity and mitochondrial function. Thus, it is concluded that LLLT no change the total and progressive motility, minor defects, plasma membrane integrity and mitochondrial function, but decreases the rate of sperm acrosome integrity. However, the results are preliminary and more studies are being done by our group.

Acknowledgments: FAPESP process 2012/00040-0 and 2011/16744-3.

A021 Male Reproductive Physiology and Semen Technology

Post-cryopreservation semen viability in bulls supplemented with protected fat and/or antioxidants

M.M. Guardieiro[1], L. Rodello[2], C.D.M. Toma[2], M. Nichi[3], J.D. Agostini Losano[3], F.L.M. Silva[1], P.L.J. Monteiro Jr[1], A.B. Nascimento[1], F.A.P. Santos[1], S.D. Bicudo[2], R. Sartori[1]

[1]ESALQ/USP, Piracicaba, SP, Brasil; [2]FMVZ/UNESP, Botucatu, SP, Brasil; [3]FMVZ/USP, São Paulo, SP, Brasil.

Keywords: fatty acids, selenium, spermatozoa.

Cryopreservation of semen is important for the expansion of reproductive techniques such as AI and *in vitro* fertilization; however, it induces oxidative stress in sperm cells. To enhance cell survival after freezing, the plasma membrane needs sufficient fluidity that is guaranteed by polyunsaturated fatty acids and antioxidant defense to protect against lipid peroxidation. The aim of this study was to evaluate post-thaw sperm characteristics from bulls fed a diet with or without rumen protected fat and / or antioxidants. A total of 48 Nelore bulls was confined (three animals per pen) and assigned to four treatments according to the addition of rumen protected fat and/or antioxidants to the diet. During 30 days (d), bulls were fed the same adaptation diet (sugar cane bagasse, citrus pulp, corn gluten meal, urea and mineral salt). Thereafter, for 75 d, the same diet was offered, differing in the addition of: F) fat protected from rumen degradation (rich in linoleic acid; Megalac-E®, 1.5% DM, n=12); A) antioxidant (EconomasE®, 3g/head/d, n=12); FA) Megalac-E and EconomasE (n=12); or C) control group (n=12). Semen collection and freezing were performed seven times: 0, 15, 30, 45, 55, 65 and 75 d after start of the experimental diet period. After thawing at 37°C for 30 s, semen analyses were performed by means of computerized analysis of sperm kinetics, flow cytometry for plasma membrane (PM) and acrosome membrane (AM) integrity, fluidity of PM and mitochondrial potential of spermatozoa. Evaluation of lipid peroxidation of sperm (Nichi et al., Theriogenology, 66, 822-28) was also done. Data were analyzed by repeated measures of GLIMMIX of SAS. Lipid peroxidation and the kinetics of thawed sperm were similar among diets ($P>0.10$). Moreover, diets without fat (C and A) increased the percentage of intact PM and unreacted AM (62.2±2.87 vs 53.3±2.87%, $P<0.05$). In addition, diets without antioxidants (C and F) caused an increase in the number of sperm cells with damaged PM and with induction of sperm *acrosome reaction*. After 65 d of supplementation, sperm of bulls fed diets with fat (F and FA) had higher PM stability, compared to the other treatments (60.4±2.62 vs. 52.7±2.62%, $P=0.05$), however there was a trend to increase the number of dead sperm. The mitochondrial potential of spermatozoa was not affected by treatments ($P>0.10$). A beneficial effect to the sperm cells after cryopreservation was found when bulls were supplemented with diets containing antioxidants, compared to diets containing fat.

Acknowledgments: Financial support from CNPq, FAPESP (Proc. 2010/00586-7 and 2010/15054-0), EMBRAPA (Innovation Network on Animal Reproduction - 01.07.01.002) of Brazil, ARM & HAMMER, Alltech and TK Tecnologia em Congelação Ltda.

A022 Male Reproductive Physiology and Semen Technology

Effects of shadow availability on reproductive traits of Nelore bulls

O.F. Bao Tarrago, M.P. Rodrigues, R.P. Arruda, A.F.C. Andrade, F.G. Zaffalon

Faculdade de Medicina Veterinária e Zootecnia, Pirassununga, SP, Brasil.

Keywords: artificial shading, Nelore, semen.

Bull is responsible for more than 90% of all pregnancies in herd, and its reproductive failure exerts much more impact on economy than reproductive failure coming from the bovine female. One of the main causes of bull reduction in reproductive performance is heat stress, which causes testicular degeneration (Hansen, 2004, Animal Reproduction Science, 82, 349-60). Also, Zebu bulls represent more than 80% of bovine males raised in Brazil. This study aimed to evaluate the effect of thermal stress reduction, through shade availability, on reproductive characteristics of Nelore bulls (*Bos indicus*). For this, ten bulls were divided in two experimental groups: available artificial shade (CS, n=5) and unavailable shade (SS, n=5). Each group was kept in two-hectare paddocks, in which shade availability for group CS was artificially created, using an area with poliethylen Sombrite (Polysack®), with 90% of solar light retention and a total of 10 m^2 of shade per animal. In SS group, animals were maintained in paddock without shadow access. Animals were submitted to semen collection every 15 days, for four months. Samples were evaluated according to laboratory standards, with a computer assisted sperm analysis (CASA), morphology (DIC) and sperm viability (fluorescent probes: IP, FITC-PSA and JC1). Data were analyzed using ASA statistical software SAS (2004). Statistical analysis included the factor of repeating measures in time, referring to several times of the sampling. The probability of interaction with the time was determined by the Greenhouse-Geisse test, using the REPEATED command generated by the GLM proceeding (PROC GLM of the SAS). Level of significance considered was 5%. No interaction was observed between treatments (CS and SS) and time (8 collections) for all analyzed variables (P>0.05). No significant effects of treatment were observed (CS *vs* SS) for studied variables such as: progressive motility (77.7 ± 1.3 vs 79.7 ± 1.2, P=0.35), morphology (23.7 ± 2.3 *vs* 16.7 ± 1.2, P=0.28) and sperm viability (66.8 ± 1.7 *vs* 70.9 ± 1.2, P=0.64). So, it can be concluded that the absence of shaded areas, during summer, does not negatively affect reproductive characteristics in Nelore bulls raised in the Southeast of Brazil. These results also suggest that, even in an adverse condition of ambient temperature, Zebu bulls were able to maintain all thermoregulation mechanisms.

A023 Male Reproductive Physiology and Semen Technology

Effect of seminal plasma and egg yolk concentration on freezability of buck semen

R.R.C. Mello, J.M. Cardoso, V.S. Ferreira, Á.C.F. Dias, R.B. Silva, W.P. Martins, M.R.B. Mello

UFRRJ, Seropédica, RJ, Brasil.

Keywords: centrifugation, extender, male goat.

Goat semen has a feature to be considered for cryopreservation because the seminal plasma interaction with egg yolk may be detrimental to sperm (Roy, 1957, Nature, 179, 318-319; Gibbons, 2002, Revista Taurus, 16, 24-32). Therefore, the aim of this work was to evaluate, *in vitro,* the effects of seminal plasma and egg yolk on the viability of cryopreserved goat semen. The extender used was the yolk citrate, where it was split into two equal aliquots: one of the samples was added 5% egg yolk (2.5 mL egg yolk: 47.5 mL citrate solution) and another was added 10% egg yolk (5.0 mL egg yolk: 45 mL citrate solution). Semen was cryopreserved using traditional refrigerator and liquid nitrogen vapor (N_2L). After a minimum of 24 hours in N_2L, the straws were thawed. Sperm motility and vigor after thawing and thermo resistance test (TRT) were evaluated. Data were submitted to analysis of variance and means compared by the F test, at 5.0% significance level. The observed values for motility and vigor after thawing and fast and slow thermo resistance test (TRT), according to the presence of seminal plasma and egg yolk percentage, were: 5% egg yolk with plasma (25.0% and 3.3; 1.60% and 0.7; 12.36% and 1.6, respectively); 5% egg yolk without plasma (23.61% and 3.1; 1.25% and 0.2; 9.93% and 1.3, respectively); 10% egg yolk with plasma (30.8% and 3.3; 4.4% and 1.9; 19.5% and 2.7, respectively); and 10% egg yolk without plasma (13.4% and 2.5; 4.1% and 0.5; 17.0% and 1.0, respectively). There were significant differences between the analyzed data ($P < 0.05$) as related to semen with or without plasma, with different percentages of egg yolk. The group presenting the best results was 10% egg yolk citrate in extender with plasma. These results may have occurred because of the seminal plasma with higher concentration of egg yolk promotes greater protection to sperm during cryopreservation. This combination stabilizes the plasma membrane and reduces its disruption in the freezing step, by decreaseing the activity of oxygen reactive species, which can cause damage to sperm DNA (Azerêdo, 2001, Small Ruminant Research, 41, 257-63). The positive results for the presence of plasma can be explained by the fact that the work was conducted in the breeding season, where levels of phospholipase A are lower as compared to the non breeding season. Also, we used young animals (10-month to 1-year old), which are still in the process of development of the reproductive system. These animals have lower phospholipase A compared to adults, explaning the results obtained in this study. Thus, we conclude that the presence of seminal plasma and higher concentration of yolk extender provided greater viability of cryopreserved goat semen.

A024 Male Reproductive Physiology and Semen Technology

Glycerol and dimethylacetamide association for the ovine sperm cryopreservation

R.F. Bittencourt[1], A.L. Ribeiro Filho[2], P.A. Ferraz[2], W.M. Barreto[2], G.F.O. Menezes[1], A.S. Rodrigues[2], M.V.G. Loiola[2], D.B.V. Ribeiro[2], B.M. Pinto[2], M. Chalhoulb[2]

[1]Faculdade de Ciências Agrárias e da Saúde - UNIME, Lauro de Freitas, BA, Brasil; [2]Escola de Medicina Veterinária e Zootecnica - UFBA, Salvador, BA, Brasil.

Keywords: cryprotectants, ovine, sperm cryopreservation.

Ten semen samples of five Santa Ines rams were cryopreserved with the objective of analyzing the use of cryoprotectants glycerol (GL) at 6% (GL6%) and dimethylacetamide (DMA) at 3% (GL3%) or in different levels of association of both (GL5%+DMA1%, GL4%+DMA2%, GL3%+DMA3%, GL2%+DMA4% e GL1%+DMA5%). The base extender was Tris-egg yolk. Right after evaluation, semen was diluted in the different extenders, cooled to 5°C and samples were frozen in liquid nitrogen vapor. After thawing, the kinetic sperm parameters were analyzed (total motility-TM, progressive motility-PM and sperm vigor-VIG). Aliquots were collected for the supravital test with dye eosin (EOS); the sperm morphology was analyzed and the percentage of bent tails (BT) calculated. The functional integrity of the plasmatic membrane was studied by using osmotic shock (OS) to which part of the semen was added to 10 parts of the final solution with deionized water. After the diluted semen incubation for five minutes at 37°C, it was fixed with 10 µL of formaldehyde buffered saline. The percentage of reactive spermatozoa to the OS was determined by subtracting the percentage of spermatozoa with OS-induced BT, from the BT obtained after thawing. These evaluations were carried out in phase contrast microscopy (1000x) and one hundred cells were analyzed per semen sample. All the statistical analysis were performed by using the SAS software, version 5.0 (1996) (MEANS and GLM Proceedure with $p<0.05$). The averages (%) of post-thaw total motility, viability by the supravital and osmotic shock were as follow: GL6%: 66.0; 36.2 and 53,2; DMA3%: 40.0; 46.2 and 33.2; GL5%+DMA1%: 69.0; 40.4 and 42.2; GL4%+DMA2%: 69.0; 33.6 and 29.4; GL3%+DMA3%: 60.0; 40.2 and 28.6; GL2%+DMA4%: 49.0; 39.6 and 29.8 and GL1%+DMA5%: 43.0; 33.0 and 32.0. We observed the greatest ($P<0.05$) rates of total motility for GL6%, GL5%+DMA1% and GL4%+DMA2% extenders in relation to the group with higher level of dimethylacetamide (GL1%+DMA5%) or when the dimethylacetamide was used alone (DMA3%). Despite the large numerical variations, the rates of EOS, OS and BT did not differ ($P>0.05$) among the groups. It can be concluded that the dimethylacetamide and glycerol association was effective for maintenance of sperm viability. Therefore, dimethylacetam.

Financial support: FAPESP- Brazil.

A025 Male Reproductive Physiology and Semen Technology

Are DNA fragmentation, sperm viability and oxidative stress correlated in rams?

T.R.S. Hamilton, A.F. Siqueira, C.M. Mendes, L.S. Castro, P.M. Assis, J.C. Delgado, J.A. Visintin, M.E.O.D. Assumpção

FMVZ-USP, São Paulo, SP, Brasil.

Keywords: DNA fragmentation, ovine, sperm.

Sperm DNA integrity is essential for embryonic development (Fatehi et al., 2006, Journal of Andrology 27, 176-88). Protamination damage, apoptosis and oxidative stress can cause changes in sperm DNA (Tsakmakidis, 2010, Small Ruminant Research, 92, 126-30), acting alone or in combination. Changes in chromatin lead to a more vulnerable sperm, which could turn on the apoptotic process and generation of reactive oxygen species. Little information is known about sperm DNA fragmentation in rams. The aim of this study was to investigate correlations between changes in DNA sperm, oxidative stress and sperm viability in rams. We used 12 rams to collect semen once a week during 9 weeks by artificial vagina. To study seminal viability, we conducted the following analysis: sperm motility, vigor, mass motility, concentration, and morphology; integrity of plasma and acrosomal membranes (propidium iodide / FITC-PSA) and mitochondrial membrane potential (JC1) by flow cytometry. To evaluate sperm DNA damage, the sperm chromatin structure susceptibility assay and alkaline Comet were used. Oxidative stress was measured by lipid peroxidation, using spontaneous and induced TBARS, and intracellular hydrogen peroxide was stained by dichlorofluorescein. Spearman correlation was determined between the dependent variables and significant correlations was considered when $p<0.05$. In the ram semen, sperm motility, vigor and mass motility are highly correlated ($0.75 < r < 0.86$). These variable are inversely correlated with morphological changes ($-0.34 < r < -0.41$), mitochondrial activity ($-0.19 < r < -0.25$) and lipid peroxidation ($-0.17 < r < -0.31$). In addition, the presence of intracellular free radicals were correlated with low mass motility ($r = -0.18$). The greater the percentage of sperm with fragmented chromatin, the greater the morphological defects ($r = 0.18$) and spontaneous lipid peroxidation ($r = 0.19$); and lower values of sperm motility ($r = -0.22$), vigor ($r = -0.18$), mass motility ($r = -0.22$) and integrity of plasma and acrosomal membranes ($r = -0.26$). Finally, higher DNA fragmentation was associated with higher susceptibility to oxidative stress, as measured by the increase in induced lipid peroxidation ($r = 0.19$). In conclusion, there are significant correlations among sperm viability, oxidative stress and DNA fragmentation, given that the magnitude and significance of such correlations depend on the studied parameter and analytical method. Thus, we suggest that a cause and effect relation exists among those criteria or actions of common factors are involved in the physiological events during spermatogenesis.

A026 Male Reproductive Physiology and Semen Technology

Pregnancy rate in Santa Inês ewes inseminated with different sperm concentrations

V.F.C. Garcia, A.L. Gusmão, M.V. Resende, G.A. Campos, L.F.M.C. Silva, C.K. Kiya, C.E.A. Biscarde, L.E.P. Martins, J.R.A. Costa, C.V.D. Carvalho

Universidade Federal da Bahia, Salvador, BA, Brasil.

Keywords: insemination, ovine, sperm concentration.

The objective of the present study was to evaluate pregnancy rates of Santa Inês ewes inseminated with low concentrations of sperm cells. This work was conducted in the city of Esplanada, in the state of Bahia. We used 32 Santa Inês sheep divided into groups according to the concentrations of insemination doses. The animals were divided into four groups containing 8 animals each, named G90, G60, G30, and G15, with 90, 60, 30, and 15 million viable sperm cells, respectively. The sheep were subjected to a synchronization protocol, as follows: on D0, the progesterone implant was inserted (Progespon ®, Schering-Plough Intervet), on D6, applied 300UI eCG (Novormon ®, Syntex) and 0.5 mL of prostaglandin (Ciosin®, Shering-Plough) were injected; on D7, the implant was removed, on D8, 300UI HCG was given to the animals (Vetecor ®, Hertape Calier). Artificial insemination occurred on D9, laparoscopically, between 52 and 56 hours after removal of progesterone implant. Pregnancy diagnosis was performed 30 days after AI via transrectal ultrasound, with a frequency of 5 MHz A statistical analysis was performed using the Tukey test for ANOVA, with PROC GLM, in SAS 8, with significance levels of $p < 0,05$. Pregnancy rates for G90, G60, G30 and G15 were 50%, 37.5%, 25% and 25%, respectively, showing no statistical differences ($P > 0.05$) between groups. These results are consistent with studies previously presented, demonstrating that the use of low sperm concentration can be applied. However, further studies are needed to seek its improvement, since it generates savings because of increased doses per ejaculate.