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Quality of frozen-thawed semen from stallions supplemented with nutraceutical

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Keywords: antioxidants, artificial insemination, spermatogenesis.

The effect of oral supplementation with the main antioxidants and fatty acids involved in spermatogenesis (vitamin E, selenium, L-carnitine, omega-3, and omega-6) was assessed on the seminal quality of frozen semen of stallions (n = 8). The animals, with previously evaluated quality of semen, were divided into Group I (n = 4) and Group II (n = 4) for a 30-week experiment. Group I received the nutraceutical Reproductive® Garanhões JCR (Vetnil, Louveira / SP, Brazil) for 60 consecutive days (treatment) and Group II received the placebo solution in the same period (control), after this period the quality of the cryopreserved semen was evaluated. The animals underwent 60 days of sexual rest, groups were reversed and new assessments of semen were performed. Frozen-thawed semen samples were evaluated regarding spermatozoa kinetics and integrity of plasmatic and acrosomal membrane. Nonparametric analysis was performed using the Mann & Whitney U test through NPAR1WAY procedure of the Statistical Analysis System (SAS).Values are presented as mean ± standard deviation. When the animals received supplementation, higher values ($P < 0.05$) of spermatozoa progressive motility (7.28 ± 4.32 versus 5.25 ± 2.86), integrity of the plasma membrane (43.64 ± 7.53 versus 39.19 ± 5.15) and acrosomal membrane (40.73 ± 7.78 versus 35.73 ± 4.09) were observed. Our results demonstrated a positive and possibly synergistic effect of the antioxidant L-carnitine and selenium on spermatozoa kinetics. Similarly, the increase in plasma and acrosomal membrane integrity may have occurred due to the higher concentrations of polyunsaturated fatty acids, combined with the prevention of excessive lipid peroxidation by antioxidants. Thus, supplementation with a nutraceutical containing fatty acids and antioxidants improved the quality of frozen stallion semen.

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Immunodetection of angiotensin converting enzyme in Nelore sperm

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Keywords: cryopreservation, enzyme, semen.

Testicular form of angiotensin-converting enzyme (tACE) is a ectoenzyme anchored to periacrossomal region of the sperm. This enzyme is able to release the extracellular portion of proteins anchored by glycosylphosphatidylinositol (GPI) that are essential for oocyte fertilization. The study was designed to perform immunodetection in spermatozoa and seminal plasma, and immunolocalization in spermatozoa of tACE before and after freezing semen of Nelore bulls. Semen samples from 10 sexually mature bulls were used. After collection with eletroejaculator, half of the ejaculate was frozen and the other half processed in natura. Immediately after collection or thawing, the semen was centrifuged twice and the pellet resuspended with TALPH. Samples were standardized to a concentration of 100x106 spermatozoa in 100 µl and submitted to SDS-PAGE and immunocytochemistry using anti-ACE antibody (Costa and Thundathil, 2012. Anim Reprod Sci 133: 35- 42). The monoclonal antibody used was able to recognize a single protein band at 100 kDa in sperm suspension of the 10 Nellore as well as seminal plasma of these animals. The protein bands were very well identified, clearly demonstrating the presence of ACE in sperm cells and seminal plasma. The ACE immunodetection in sperm was characterized by intense staining observed on all periacrossomal region showing the location of this enzyme in the sperm. After thawing, it is clearly perceived that the cryopreservation process reduced the intensity of protein bands, suggesting that this enzyme was lost during the protocol used. Corroborating with the western blot data, we also note that the cryopreservation process reduced the intensity of the fluorescent dye in the immunocytochemistry technique, suggesting that this enzyme is lost during the cooling / freezing. However, there was no change in the enzyme's location according to the freezing protocol used. We conclude that the cryopreservation process reduces the amount of TACE in the Nelore bulls sperm.

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Plasmatic and acrossomal membrane integrity and mitochondrial potential evaluation of spermatozoa of the ovine supplemented with selenium

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Keywords: semen, mineral supplementation, small ruminant.

The present study had the objective of evaluating the effects of different concentrations of selenium (Se) supplementation on the integrity of acrosome and spermatic membrane, as well as the mitochondrial potential of ovine spermatozoa. Thirty (30) ovine males, aging from 18 to 24 months old, housed in individual pens, in an intensive system, were divided in 5 experimental groups: GC (n = 5), supplemented with mineral salt without the addition of Se; G1 (n = 5), same mineral salt added with 5mg of Se (sodium selenite)/kg mixed; G2 (n = 5), same mineral salt added with 10mg of Se/kg mixed; G3 (n = 5), same mineral salt added with 15mg of Se/kg mixed; G4 (n = 5), same mineral salt added with 20mg of Se/kg mixed. To each group there was an adaptation time of 14 days, leading to 56 days of treatment. The semen samples were collected using electro-ejaculation. The analyzes were performed with a flux cytometry, BD LSR II (Becton Dickinson, Mountain View, CA, USA) equipped with lasers: blue 488 nm, 100 mW and red 640 nm, 40 mW. The data were evaluated by a program of the same enterprise BD FACSDiva™ software v6.1. In order to evaluate the integrity of acrosome and plasmatic membrane, the propidium iodete (IP; P4170, Sigma), FITC-PSA (L0770, Sigma) and Hoescht 3342 (H342; 14533, Sigma) probes were associated. A 20 0 µL semen was diluted in TALP at the concentration of 10 x 106 spermatozoa/mL, added with 5 µL of H342 (7 µM), 5µL of IP (1,5 µM) and 0,5 µL of FITC-PSA (2ng), homogenized and incubated for 15 minutes at 37ºC protected from light. The experimental design was developed by a Latin square 5 x 5, or five treatments and five experimental periods. The data were analyzed using the GLM procedure of SAS (2009). The mean differences among the groups were verified by Tukey`s test at 5% of significance. The integrity of acrosome and plasmatic membrane, as well as the mitochondrial potential did not show any statistical differences among the treatments, showing no influence of treatment at the parameters evaluated, despite Meseguer et al. (Drug Metab Lett, v.1, p.121-126, 2007), who described an anti-oxidation effect of Se especially at the middle piece`s mitochondrial activity. Due the presented results, the selenium supplementation (with the studied concentrations) did not bring any benefit at the membrane integrity (acrosome and/or plasmatic) and at mitochondrial potential of ovine spermatozoa.

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Influence of mini-Percoll techniques in sperm capacitation and plasma membrane integrity of ram frozen–thawed sperm

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Keywords: mini-Percoll, sperm capacitation, ovine.

The success of IVF and subsequent development of embryos are directly related to sperm selection and quality. This study aimed to evaluate the effect of different forces and time of centrifugation in mini-Percoll (MP) techniques for sperm selection on capacitation status and plasma membrane (PM) integrity of ram frozen-thawed sperm. Commercial semen from 10 rams of Santa Inês breed, aging 2-5 years old, were used. At post-thawing (PT), the PM integrity was evaluated by CASA using the SCA® system (Sperm Class Analyzer – Microptic Automatic Diagnostic Systems, Barcelona, Spain). For traditional Percoll (Sigma Chemical, St. Louis, USA), a 2 mL-gradient (90/45% density) was subjected to a 700 x g centrifugation for 10 min followed by 200 x g for 5 min. The MP techniques consisted in an 800 µL-gradient (90/45% density) subjected to either: I) two centrifugations of 5000 x g for 5 min; II) two centrifugations of 2500 x g for 5 min; III) two centrifugations of 1250 x g for 5 min; IV) 700 x g for 10 min, followed by 200 x g for 5 min. At the end of all treatments, aliquots (post-protocols = 0 h) were taken for evaluation of PM integrity and capacitation status by chlortetracycline stain (Sigma Chemical, St. Louis, USA). Later on, samples of all treatments were submitted to incubation at 37°C, 1 h, 2 h and 3 h and the same parameters were assessed. The variables were subjected to either ANOVA or Kruskal-Wallis tests depending on normality, Tukey and Fisher-LSD analysis ($P < 0.05$). There was no difference ($P > 0.05$) among all treatments for capacitation status and PM integrity. The capacitated rate was higher ($P < 0.05$) at 3 h (28.6 ± 2.5%) when compared with 0 h (23.3 ± 1.7%) and 1 h (23.8 ± 1.4%) of incubation, regardless of the treatment. There was no difference ($P > 0.05$) for PM integrity among the protocols at 0h. Regardless of the treatment, the intact and damaged PM rate, respectively, were similar ($P > 0.05$) for PT (16.4 ± 2.0% vs. 84.0 ± 2.0%) and 0 h (19.0 ± 2.9% vs. 81.1 ± 2.9%; average of all treatments). When analyzing just intact cells, the PT values were greater ($P < 0.05$) than any incubation interval, whereas the latter were lower than 0 h values ($P < 0.05$). In conclusion, MP may be used as an ideal alternative to the traditional Percoll, decreasing costs and time of sperm handling, without cell damages in ram sperm.

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Determination of ovine sperm concentration by spermatocrit

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Keywords: sperm concentration, micro centrifugation, spermatozoon.

The evaluation of sperm concentration by micro centrifugation is described in fish (SHIMODA, Braz J. Vet Res Anim Sci, supplement, p.19-24, 2007; GOO, Reprod Dev v.19, p.253-258, 2015), however, there are no reports in mammals. The ram semen is characterized by low volume and high cell concentration, allowing to correlate the cells sedimentation with the sperm concentration. The objective of this project was to evaluate whether the spermatocrit can be used to predict the ram sperm concentration. This study was conducted at the Animal Reproduction Laboratory of the Federal Institute of Santa Catarina, Campus Concordia. The semen of sixteen 16 fertile rams was collected with artificial vagina, totaling 25 ejaculates. A fraction of the semen was diluted in formaldehyde citrate (1:400) and subjected to direct counting in a Neubauer chamber under optical microscope with magnification of 400 times. The other fraction, in natura, was centrifuged in micro capillary (75 mm length and 1 mm Ø) in a micro hematocrit centrifuge at 153 x g for 2 minutes. The capillaries were measured with a hematocrit reading ruler and the results were correlated with the results of the Neubauer chamber. To verify the relationship between sperm concentrations determined in a Neubauer chamber and the spermatocrit, data were submitted to simple linear regression analysis at 5% probability after the sperm count was converted into logarithm and the diagnosis of residuals to confirm the assumptions to perform the regression analysis with the software R. There was a linear relationship ($P < 0.05$) between the sperm concentration determined in a Neubauer chamber and the spermatocrit described by the equation: $Y = 8.9892 + 4.0077X$, where X is the column height obtained when reading the spermatocrit. The coefficient of determination (R^2) was 64.69%, indicating that a significant fraction of the variation in sperm count can be explained by the regressor variable obtained by reading the spermatocrit (mm of column). In this case we had an equation that can be used to estimate the sperm concentration based on the reading of espermatocrit. This study showed the feasibility of the equation to predict the sperm concentration based on the reading of spermatocrit. New data sets will be used to refine the model, obtaining a reading scale that allows the method to be used quickly and conveniently in practice.

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Effect of different melatonin concentration in integrity of acrosomal membrane in swine sperm cryopreserved

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Keywords: acrosomal, melatonin, cryopreservation.

Boar semen is extremely vulnerable to temperature variation, and sensitive to lipoperoxidation damage, because its membrane contains high concentration of polyunsaturated fatty acids (BONDAN, C. Am J of Biochm Biotech. in press. 2016). The structural differences in the lipid layers and the process of "cryo-capacitation" can explain the sensitivity of the swine sperm cells to cryopreservation (BAILEY, J.L. J Androl. v. 21, p. 1-7, 2000). Melatonin is an amphiphilic antioxidant capable of penetrating in cellular compartments, protecting the cells from oxidative damage (REITER, R.J. News Physiol. Sci. v.15, p.246-250, 2000). Therefore, the objective of this study was to evaluate whether different concentrations of melatonin have protective effect to the acrosome membrane of cryopreserved swine sperm. For this, the rich fraction of 5 ejaculates from 3 boars was diluted with commercial extender in the ratio 1:1. Samples were kept at 20°C for 120 min and then conditioned at 15°C for 180 min. Then, the ejaculate was centrifuged (1600xg/5min/15°C) and the sediment was resuspended in 1:2 semen:cooling extender (CE - 80% lactose solution 11% and 20% of egg yolk) and kept at 5°C for 90 minutes. At the end of this period, the same volume of freezing extender (FE - 89.5% CE, 1.5% Ex Orvus Paste and 9% glycerol) was added to reach a final sperm concentration of 5x109/ml. Melatonin was added to extenders CE and FE, with a final concentration of 0; 1.25; 2.5 to 5 mM. Semen was then transferred to a 0.5ml straw and kept for 20min in nitrogen vapor and then immersed in liquid nitrogen. Samples were thawed at 37°C for 20 seconds. Further, semen was diluted to 1:4 with commercial extender and centrifuged (800xg/3 min). The pellet was resuspended again in commercial extender. Samples were evaluated for motility (under phase contrast microscopy) and acrosomal membrane integrity using FITC-Pisum sativum (FITC – PSA, 11.7 mg / mL) by flow cytometry (BD-Accuri). Data were analyzed using PROC MIXED (SAS® Institute Inc., Cary, NC, EUA). The Tukey test was used to compare the mean of the minimum squares of the group and the data are presented as mean of percentage ± SD. Regression analysis was performed in Instat® (Graphpad Instat: GraphPad Software Oberlin, San Diego – CA, USA). There was no difference between melatonin concentrations in percentage of motility after thawing [(32.66% ± 2.69 (0); 35.00% ± 2.69 (1.25 mm); 39.50% ± 2.8 (2.5 mM) and 37.3% ± 2.69 (5 mM)]. No effect was identified on acrosome integrity, being the percentage of post-thaw acrosomes integrity of 51.96% ± 3.64 (0); 49.13% ± 3.64 (1.25 mM); 47.48% ± 3.64 (2.5 mM) and 48.80% ± 3.78 (5 mM). There was no correlation between melatonin concentrations and motility ($r = -0.072$, $p = 0.58$) and the percentage of post-thaw intact acrosomes ($r = 0.1419$, $p = 0.283$). We concluded that the addition of melatonin in the concentrations used did not have a protective effect on the acrosomal membrane for cryopreservation of boar semen.

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Effects of serine proteases inhibitors in bovine sperm cryopreservation

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Keywords: Antipain, PAI-1, semen.

Cryopreservation is partially harmful to bovine semen fertility and induces capacitation-like changes in sperm. Seminal plasma contains serine proteases and serine proteases inhibitors, which are involved in mammalian fertilization. Due toits function, serine proteases inhibitors can be applied to prevent cold-induced sperm capacitation. We analysed the effect of differentconcentrations of two serine proteases inhibitors, Plasminogen activator inhibitors 1 - PAI-1 (70 ηg, 140 ηg and 210 ηg) and Antipain (10 µg, 50 µg and 100 µg) in supplementation to the extender of bovine semen cryopreservation. Thirty-six ejaculates from four Curraleiro Pé-Duro bulls were collected by electroejaculation and evaluated for macroscopic characteristics (volume and aspect) and microscopic characteristics (motility, vigor, concentration and sperm pathologies). Posteriorly, semen was diluted in Tris-Egg yolk culture medium according treatments and then placed on 0.5mL straws, frozen in a TK 3000®machine (TK Ltda, Uberaba, Brazil), and stored in a cryopreservation tank (-196ºC). The effect of the inhibitors on the sperm parameters (sperm kinetics - CASA, acrosome integrity, plasma membrane integrity, mitochondrial membrane potential, sperm defects and acrosome reaction rate) were evaluated in the post-thaw semen. Sperm cryopreservation with Antipain (10 µg, 50 µg e 100 µg) decreased post-thaw kinetics parameters of progressive motility (MP - µm/s), straight-line speed (VSL - µm/s), linearity (LIN - %), straightness (SRT - %) and the percentage of hyper-activated spermatozoa in comparison to the control ($P < 0.05$). PAI-1 (210 ηg) decreased VSL and LIN in comparison to the control ($P < 0.05$). Antipain and PAI-1 had no effect on integrity parameters of the plasma membrane, mitochondrial membrane potential and sperm defects. Sperm cryopreserved in the presence of Antipain (10 µg, 50 µg e 100 µg) and PAI-1 (70 and 140 ηg) had acrosome integrity values higher than the control, demonstrating the Antipain inhibitors and PAI -1 ability to preserve the acrosome integrity ($P < 0.05$). There was no statistical difference among the studied treatments for variable rate of acrosome reaction, demonstrating that cryopreserved spermatozoa with Antipain and PAI-1 were able to complete the in vitro acrosome reaction. In conclusion, the serine proteases inhibitors, Antipain and PAI-1 (70 and 140ηg) are able to preserve the acrosome integrity of cryopreserved bovine sperm.

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Time of stabilization and exposure to nitrogen vapor alters the viability of cryopreserved canine semen

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Keywords: dog semen , freezing, progressive motility.

This study evaluated the effect of different freezing curves on the post thaw viability of canine semen, previously transported (up to 2 h). Three male dogs were used to obtain ejaculates by manual masturbation (7 replications). After collected, the semen was diluted 1:1 (v/v) in Tris medium and transported to the laboratory at 10°C, when it was diluted 1:1 (v/v) in Tris with 10% egg yolk, constituting the fraction A. The fraction B was composed by Tris egg yolk, added of 7.0% glycerol (Merck). The addition of fraction B was performed only after semen stabilization at 5°C, obtaining a final glycerol concentration of 3.5%. Subsequently the semen was loaded into 0.25 mL straws and allocated to one of the four experimental groups (according to the stabilization period at 5°C / followed by the period of exposure to the nitrogen vapor), as follows: Group 60m/5m; Group 60m/20m; Group 20m/5m, and Group 20m/20m. The samples were treated with their respective protocol, and then plunged into liquid nitrogen. After storage, the straws were thawed at 37°C / 30 seconds, and submitted to progressive motility (PM) and spermatic vigor (SV) assessment, during the longevity test. Three evaluations were performed at 15 minutes, 1 h and 2 h after thawing. Data were analyzed by ANOVA and when indicated, by Tukey or Student test ($P \leq 0.05$) for comparison between groups and times, respectively. The PM values were similar among different freezing curves. In the longevity comparison, over the time, the PM at 15 min did not differ among groups, being (G-60m/5m 31.2 ± 4.1, G-60m/20m 38.7 ± 8.3; G-20m/5m 38.7 ± 3.6; G-20m/20m 38.1 ± 5.3) ($P > 0.05$). However, the 60m/5m group maintained PM until 1 h after thawing (24.4 ± 3.3), showing a reduction only at 2 h (9.4 ± 3.3). At 1 h evaluation, the PM of G-60m/20m (18.7 ± 5.9), G-20m/5m (21.9 ± 3.4) and G-20m/20m (21.9 ± 3.4) were lower than at 15 min evaluation ($P < 0.05$), however it was similar to that found at 2 h evaluation (G60m/20m (8.7 ± 3.6), G20m/5m (13.1 ± 4.2) and G20m/20m (15.6 ± 3,6). The SV values were also similar among freezing curves ($P > 0.05$). However, all groups showed SV values differences during longevity test evaluations performed at 15 min and 2 h time, being respectively G-60m/5m 2.9 ± 0.2 vs 1.5 ± 0.5; G-60m/20m 2.9 ± 0.2 vs 1.3 ± 0.4; G-20m/5m 3.1 ± 0.1 vs. 1.8 ± 0.4; G-20m/20m 3.1 ± 0.1 vs 2.1 ± 0.4 ($P < 0.05$). However, when comparing the evaluation time points 1 h and 2 h, the Group 60m/20m (2 ± 0.5 vs 1.3 ± 0.4) and Group 20m/20m (2.4 ± 0.2 vs 2.1 ± 0.4) were similar, while groups 20m/5m (2.6 ± 0.2 vs 1.8 ± 0.4), and groups 60m/5m (2.6 ± 0.2 vs 1.5 ± 0.5) showed a significant reduction in SV. Our data show that distinct modulation of freezing curve may change the sperm parameters, and that G-60m/5m treatment provide higher viability of cryopreserved canine semen.

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Buffaloes (*Bubalus bubalis*) sperm cooling face to different extenders and evaluation of sperm in casa motility

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Keywords: extender, cooling, breeding.

The objective of this experiment was to test the in vitro efficacy of different extenders: TES and TRIS (Brito, 2014) with LDL (low density lipoproteins) concentrations of 10 and 5% on sperm longevity of buffalo in the cooling process at 5ºC. We used 3 ejaculates from 4 male Murrah buffaloes, used to semen collection with artificial vagina. After collection, each sample was subjected to analysis of the physical and morphological characteristics of semen (CBRA, 2013). Immediately after collection, each ejaculate was split into four aliquots, each sample being diluted in extensors to obtain 50x106 SPTZ/mL. The filling was performed in 0.5mL straws, packed in a plastic bag (chup-chup) containing metal balls on the bottom and attached to the edge of a glass container, submerged in a container with water at 27°C. The flask was placed in an environment at 5°C, obtaining a cooling curve of 0.25ºC/minute. The semen straws were kept in active cooling (counter refrigerator) at 5°C and sequentially evaluated, reheating the contents at 37°C 30 seconds before the evaluation of sperm motility (total and progressive), using the computerized system (CASA), in times of 12, 24, 48, 72 and 96 hours, with the evaluations of T0 being made subjectively. Data were analyzed by Anova (split plot) and t test ($P < 0.05$), using the Assistat software 7.7 beta (2016). The average total motility (%) found after cooling regarding the extensor TES 10%, TES 5%, TRIS 10% and TRIS 5% were 68.0a, 66.6b, 63.9c, 63.7c; and for times 12, 24, 48, 72 and 96 hours 91.3ª, 81.8b, 70.5c, 55.9d and 28.4e, respectively. The averages of progressive motility (%) were 44.2a, 43.2a, 41.5b 41.9b regarding the extenders mentioned above; and the times were 68.4ª, 53.5b, 42.4c, 30.9d and 18.1e respectively. Interactions between extender and storage times were not significant for the variables studied. It is concluded that the TES 10% LDL was the best extender in the in vitro preservation of sperm during cooling. The results allow to incorporate the use of semen cooled up to 48 hours, as a management option in the fertilization of buffalo females, to reduce costs and improve reproductive efficiency of TAI programs. However, the research should be continued in order to identify suitable extenders for a greater efficiency of this technique.

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Comparison between automated and conventional sperm freezing systems regarding to *in vitro* semen quality

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Keywords: CASA, Nelore, cooling.

The most commonly used methods for field semen freezing include simple systems using ice in styrofoam boxes or domestic refrigerators for cooling process and styrofoam boxes with liquid nitrogen (N2L) for sperm cryopreservation. However, the problem of this technique is the difficulty of standardizing the cooling curve and its consequences for cryopreservation quality due to the large variation in styrofoam boxes and refrigerators size, which can affect these two processes. Hence, the aim of this study was to simultaneously compare an automated cooling and freezing system with a conventional system using domestic refrigerator for cooling and styrofoam box for freezing (45 liters). Thirty eight batches collected by eletroejaculator from 12 Nellore bulls were used. The bulls were 3.02±0.5 years old, had 631±119 kg of body weight and 33.8±1.89 cm of scrotal circumference. All samples were diluted in BotuBov® (Botupharma®, Botucatu, Brazil) semen extender to a final concentration of 50x106 sptz/mL. After dilution, semen doses were loaded in 0.5 mL straws and simultaneously cryopreserved using two systems. For system 1 (automated), a TK 4000® (Tetakon®, Uberaba, Brazil) freezing machine was programmed to perform a cooling curve of 0.25ºC/min. After 4h at 5°C for stabilization, semen was frozen in the same equipment (curve -15°C/min, 5°C to -80°C, after -20ºC to -140°C). For system 2 (conventional), a domestic refrigerator stabilized at 5°C was used for the cooling procedure. After 4h, straws were placed at 3 cm above N2L during 20 min and then submerged in N2L. Sperm kinetics was assessed by CASA (computer assisted semen analysis - IVOS® version 14) and the following parameters were evaluated: total motility (TM%), progressive motility (PM%), rapid cells (RAP%), path velocity (VAP, µm/s), straight velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), lateral displacement of sperm head (ALH, µm), beat frequency (BCF, Hz), straightness (STR%) and linearity (LIN%). Flow cytometry was used for assessment of plasma membrane and acrosome integrity using the fluorescent probes propidium iodide, FITC-PSA and Hoescht 3342. The data were submitted to analysis of variance by SAS proc MIXED with significance of $P < 0.05$. System 1 (automatic) was superior to system 2 (conventional) regarding to TM (41.1 ± 2.2 vs 26.3 ± 2.2), PM (31.6 ± 1.7 vs 21.3 ± 1.7), RAP (38.7 ± 2.1 vs 23.9 ± 2.1), VAP (79.6 ± 1.1 vs 72.9 ± 2.1), VCL (134.4 ± 2.3 vs 118.1 ± 2.3) and ALH (5.5 ± 0.1 vs 4.9 ± 0.1), and presented reduced STR (81.0 ± 0.8 vs 86.5 ± 0.8) and LIN (51.1 ± 0.8 vs 56.9 ± 0.8). No differences were observed between the two tested systems regarding to plasma and acrosome membrane integrity. We concluded that the slower and controlled cooling/freezing curve presented better sperm kinetics parameters when compared to the conventional freezing system.

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Scrotal temperature patterns and seminal quality of composite bulls during winter and summer

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Keywords: bovine, fertility, testicular thermoregulation.

The objective was to compare the behavior of scrotal surface temperatures during extreme weather conditions recorded during winter or summer and evaluate their impact on raw semen quality, in order to expand the knowledge about reproductive physiology of composite bulls raised on tropical climate. The experiment was conducted in Embrapa, São Carlos-SP (tropical climate, subtype Cwa according Köppen). The average of minimum air temperature is 14.1°C and the maximum reaches 26.9°C during the winter. On the other hand, during the summer, minimum is 19.4°C and maximum average is 29.4°C. Seventeen Canchim bulls (⅝Charolais x ⅜Zebu) were utilized as semen donors. Bulls were 3 years old and weighted 504kg on average. Animals were kept in a single group in a pasture-based production system. Evaluations were performed monthly during winter (July to September) and summer (December to March). The scrotal surface temperature was measured by infrared thermometry (ST-600, Incoterm®). The three anatomical references chosen to describe the thermoregulatory efficiency were the spermatic cord (SpC, °C), dorsal testicular pole (DTP, °C) and tail of the epididymis (TEp, °C). Semen was monthly collected by electroejaculation and it was submitted to quantitative and qualitative laboratory evaluation (CBRA, 2013). Laboratory analysis included the assessment of sperm concentration (SC x109sptz/mL), progressive motility (PM%), total sperm defects (TDef %), fragmentation of chromatin (FC, % using toluidine blue technique) and sperm plasma membrane integrity (SMI, % using hiposmotic test). Data were analyzed using BioEstat 5.0 Version. Variables presenting abnormal distribution were transformed using linear transformation method and data were submitted to ANOVA. Means were compared by Tukey test ($P < 0.05$). Temperatures of SpC, DTP and TEp were lower in summer (32.57 ± 0.76 vs 33.79 ± 0.69°C; 31.88 ± 0.75 vs 32.31 ± 0.58°C; 28.15 ± 1.65 vs 30.01 ± 1.14°C, respectively; $P < 0.05$). This indicates that animals activated very efficiently their testicular thermoregulation system during the hottest season. The SC was higher in the summer (2.09 ± 1.51 vs 1.23 ± 0.87 x109sptz/mL, $P < 0.05$), while the PM was higher during the winter (69.72 ± 8.78 vs 56.18 ± 18.43%, $P < 0.05$). There were no significant differences in winter or summer for TDef (21.3 ± 11.1 vs 17.9 ± 11.4%), FC (3.2 ± 3.4 vs 2.4 ± 3.0%) and SMI values (66.2 ± 23.2 vs 67.0 ± 17.5%). Although the progressive motility was slightly higher in the winter, quantitative sperm production was higher during the summer. The similar incidence of morphologic defects, chromatin fragmentation and integrity of sperm membrane during the seasons demonstrates the potential fertilizing capacity of semen, regardless the climatic seasons. Thus, it was concluded that composite bulls showed functional scrotal thermoregulation system, which was able to efficiently compensate the bioclimatic adversities intrinsic to the summer season, and they kept the semen quality during the hottest part of the year.

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Correlations between testicular surface temperatures measured by infrared thermography and seminal parameters in rams of distinct genotypic groups

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Keywords: Ovis aries, testicular temperature, semen.

The aim of this study was to correlate the testicular surface temperature with rectal temperature and semen quality in rams of different genotypic groups. The experiment was conducted at Embrapa Pecuária Sudeste, São Carlos-SP, where the climate is characterized by Köppen as Cwa type. The experiment was conducted from October/2015 to February/2016. Twenty rams (20.1 months; 67.6 kg) of four genotypes were used as semen donors: Morada Nova (n = 5), Santa Inês (n = 5), Dorper (n = 5) and Texel (n = 5). The animals were kept in confinement in a 570 m2 barn partly covered by a roof of 196 m2. Every thirty days in the morning, it was held thermography infrared of scrotal region (T300, FLIR® Systems, Wilsonville, USA). Simultaneously, the rectal temperature was being measured (RT, °C). The thermograms were analyzed using FLIR® Tools Plus 3.1 software and average testicular temperature (ATT,°C) and temperatures of the dorsal pole (DPT, °C) and ventral pole (VPT, °C) of the testicles were determined. Based on the difference between DPT and VPT, it was calculated testicular temperature gradient (TTG, °C). Semen samples were collected monthly by artificial vagina. We evaluated the progressive motility (%), gross motility (0-5), sperm count (x109 sperm/mL), major sperm defects (%), minor sperm defects (%), total defects (%) and DNA fragmentation index (%). Data were analyzed for normality by Lilliefors test. Correlations were calculated by Pearson correlation test, first based on the overall data set and then grounding the data by genotype. The data were evaluated in BioEstat 5.0 program with significance level of 5%. The ATT showed a positive and significant correlation with minor sperm defects (0.68; $P < 0.05$) and total defects (0.57; $P < 0.05$) in Dorper animals. The TTG was positively correlated with rectal temperature (0.36; $P < 0.05$), being higher in Santa Ines animals (0.69; $P < 0.05$). In Morada Nova breed, TTG was positively correlated with mass motion (0.76; $P < 0.05$). In rams of Texel breed, TTG was correlated with sperm count (0.65; $P < 0.05$). These results demonstrate individuality in testicular thermoregulation of each genotypic group and its response as for the seminal quality, which has lower degree of effect in animals considered naturalized. It strengthens the hypothesis that abnormal testicular temperature can negatively affect semen quality, especially in exotic breeds animals. The gradient increase, which stands for larger temperature difference between testicular poles, presented with positive results for semen, highlighting the importance of their use within the complementary breeding soundness evaluation. In addition, this study reinforces the contribution of the infrared thermography use in reproductive evaluation of males.

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Oxidative stress as fertility indicator in bulls

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Keywords: ROS, antioxidant, semen.

The bull fertility can be obtained by pregnancy rate obtained on females with appropriate reproductive capacity. In vitro, many analyzes are made in attempt to predict fertility of bulls, however, the results are variable and controversial. Biochemical assessments have been suggested as an effective tool of analysis, but diluents, cryoprotectants and seminal plasma may interfere with the results. This study aimed to perform biochemical analyzes in previously selected sperm cells, and correlate with the fertility data field. Three repetitions were performed using frozen semen of bulls of different categories: High Fertility and Low Fertility (pregnancy rate of 57.98 and 38.42%, respectively), according to Fertility Index in IATF - IFERTTM. Each category being composed by 3 bulls. For evaluations, 3 samples of each bull were thawed and homogenized, resulting in a pool, which was analyzed post-thawing and after spermatic selection by the discontinuous Percoll® gradient method (Guimarães, A. G., Anim. Reprod. Sci., v. 146, p. 103-10, 2014). The biochemical tests used to evaluate the production of reactive oxygen species and antioxidant activity were: Superoxide Dismutase (SOD; Misra, H., J. Biol. Chem., v. 247, p. 3170-3175, 1972), Ferric Reducing Antioxidant Power (FRAP; Benzie, I. F., Anal Biochem., v. 239, n. 1, p. 70-6, 1996), Thiobarbituric acid reactive substances (TBARS; Ohkawa, H., Anal. Biochem., v. 95, p. 351–358, 1879) and Reactive oxygen species (ROS; Loetchutinat, C., Radiat. Phys. Chem. v. 72, p. 323–331, 2005). The results were analyzed by T-test, with 5% of significance level. TBARS levels were higher in the High Fertility group (403.9 ± 43.2 nmol/ml) when compared to the Low Fertility group (205.2 ± 184.4 nmol / ml) right after thawing. After Percoll®, levels of TBARS and ROS, presented higher concentrations in the Low Fertility groups (TBARS = 12.09 ± 2 mmol / ml; ROS UF = 10.3 ± 3.3) compared to the High Fertility group (TBARS 17.2 ± 4.9 nmol / ml and 15.4 ± 4.9 ROS UF). This difference of values was probably due to the diluent used to freeze the semen, which was distinct between the bulls, and when removed after the selection, the dilutive effect was eliminated. In the other evaluations, SOD and FRAP, no significant difference was observed between treatments. Low fertility bulls have higher levels of ROS and lipid peroxidation, indicating oxidative stress. This values suggest that the evaluation of ROS and TBARS after sperm selection can be used as bull fertility indicators.

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Software efficiency for counting bovine spermatic cells: preliminary results

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Keywords: Java, open source, semen.

The evaluation of motility and sperm concentration are essential in the analysis of semen quality. In order to increase the reliability of results, automatic systems of semen analysis (Computer-assisted Sperm Analysis - CASA) have been developed. The CASA can be found as hardware or software enabling displaying, scanning and analyzing successive images, providing accurate, precise and meaningful information about the individual movement of each cell as well as sperm cell subpopulations (Amann, Journal of Andrology, 25, 317, 2004; Matos, Revista Brasileira de Reprodução Animal, 32, 225, 2008). This study aimed to develop a software for bovine sperm cell count, from images taken under a microscopy. This program should be compatible with notebooks and PCs for home use, easy to use, in the Portuguese language and free. It is intended, with this software, to standardize the sperm concentration analysis carried out by veterinarians during andrological examinations at field, lowering the cost of acquisition of specific equipment. Ten images of bovine semen were made by microscopy from thawed semen doses. The 0.5 mL reeds were thawed for 30 seconds in a water bath at 37°C. Semen drops were deposited on slides and covered with cover slip. The images were obtained from a domestic digital camera of 14 MP (Samsung, Manaus, Brazil) supported by an improvised backing of paper card that prevented the interference of external light. The software was developed from resources already available in an open source Java solution called ImageJ. The approximate count of the sperm contained in the image was possible through particle analysis capabilities. Initially, the video images were converted into frames and subjected to some treatments, using only 8-bit color and segmenting grayscale so that the software could do the analysis of the image particles. The 10 images were analyzed by the software and by the technician for spermatozoa count. For statistical analysis, the results of the counts were subjected to analysis of variance and the mean of each group was submitted to Tukey test (SAS, 2012) at a significance level of 5%. The average values of the sperm cell counts were 184.2 ± 8.42 and 179.6 ± 8.04, obtained by the software and by the technician, respectively ($P > 0.05$). The software was highly efficient for sperm cell count, being a convenient and easy solution. The CASA instruments have shown high levels of accuracy and reliability using different methodologies of classification that provide a great tool to improve our knowledge and ability to analyze sperm (Verstegenet, Theriogenology, 57, 149, 2002), making it essential to research, personnel training and standardization between laboratories (Amann, Biology of Reproduction, 23, 6478, 1980). Our differential is the gratuity and ease of use, since it is a specific software for sperm analysis and available in Portuguese. In conclusion, the developed software showed the same efficiency as the count carried out by the technician. Acknowledgments to CNPq by financial support and ITI and PIBIC scholarships.

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Evaluation of reproductive potential of new race Blonel bulls: sperm analysis

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Keywords: andrological examination, sperm evaluation, bovine.

There are many registered breeds of cattle that are being largely used for meat production. The newest cutting race, the Blonel, was created and recognized in Brazil by the Ministry of Agriculture, Livestock and Supply (MAPA) from the cross of animal breeds Blonde and Nellore. The Blonde breed, from France, is one of the most important selective genetic programs in the world. His genetic selection had special attention to sexual precocity, fertility, for maternal ability and quality meat production. On the other hand, Nellore is a traditional race in the production of Brazilian beef, its brings hardiness and adaptation to geographical and climatic conditions. This report aimed to evaluate the reproductive performance in andrological examination of bulls of Blonel race. A collection of reproductive data of the year 2012 was made in commercial cattle herd of Blonel race of Três Furnas Farm in Buriti Alegre - GO. For this survey, we studied nine animals that were subjected to andrological exam between 21 and 26 months of age in that year. The andrological examination (according to the Andrology Manual of Brazilian College of Animal Reproduction – CBRA, third edition, 2013) was conducted by the same veterinarian, during the months of August and September. Immediately after collection, the immediate analyzes of semen were conducted by: gross motility, motility, vigor and sperm concentration (according to the Andrology Manual of Brazilian College of Animal Reproduction - CBRA, 3th edition, 2013). From a drop of semen, were made a smear microscopy slide.Subsequently, in the laboratory, this slide was stained (Gram stain) for conducting the mediate analysis of sperm cells (sperm morphology) under microscopy, when were evaluated all types of defects and their frequencies. The results of these evaluations mentioned above are part of the andrological exam report. The data of the reports were collected and typed in a spreadsheet, subjected to analysis and mean and standard deviation calculation. Since there is no treatment or another race for comparison, the statistical analysis is not required. Remember that this is a new race and there are no records of this data in the literature. Results were: 2.67 ± 0.50 of gross motility, 54.44 ± 5.83% of motility, 3.33 ± 0.87 of vigor and 666.67x106 ± 100.00x106/ml of sperm concentration. CBRA recommends values of motility ≥ 60%, vigor ≥ 3 and sperm concentration ~ 350 x 106/ml. Motility and vigor values of the sperm of this report were higher than those reported by Dias (Vet Notícia, 13, 31, 2007) of 45.9 to 51.0% motility and 2.6 to 2.9 of vigor in a study with 22 months of age Nellore bulls raised on pasture. Major (7.78 ± 1.20%) and minor (5.00 ± 2.65%) defect rates were observed, within the recommended by CBRA. Among the major defects were observed: acrosome defects, proximal cytoplasmic droplet, sperm underdeveloped, curled tail to the head, abnormal small, abnormal color, teratological forms and pathological middle piece. The major defects observed were: 2.00 ± 1.50% of pathological isolated head, 0.67± 0.50 % narrow at the base, 0.33 ± 0.71% of piriformis, 0.89 ± 1.05% of abnormal contour, 0.44 ± 0.88% pouch formation and 3.44 ± 2.24% tail tightly folded/rolled. The following minor defects were observed: 2.67 ± 2.00% of normal isolated, 0.33 ± 0.50% of abaxial, retroaxial, oblique, 1.67 ± 2.12% of tail folded or rolled and 0.33 ± 0.50% of distal cytoplasmic droplets. The results of this study were fine, but the Blonel bulls reproductive potential has to be more studied.

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Seminal characteristics in Nelore bulls supplemented with polyunsaturated fatty acids in pasture conditions

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Keywords: PUFA, bulls, semen.

Puberty of bulls is an important indicator for the reproductive improvement of bovines. Semen production and quality are influenced by several factors, especially nutrition. Thus, diets with polyunsaturated fatty acids (PUFA) have effect on reproductive performance of males. Thus, the aim of this work is to evaluate of effects the supplementation with PUFA in the seminal characteristics of Nelore bulls in pasture. Young bulls of 14 months (n = 24) of traditional herd Animal Science Institute of Sertãozinho, SP, were used. The animals were divided into 2 groups with (n = 12) and without supplementation with PUFA (n = 12). The experimental diets were isoproteic and isoenergetic. The animals were kept in Brachiaria brizantha cv. Marandu pasture in continuous stocking during the experimental period. The bulls were submitted every 28 days until they had completed 24 months of age (a total of 12 repetitions) to measurement of scrotal circumference and semen collection in order to investigate the seminal characteristics: subjective motility and CASA (HAMILTON THORNE RESEARCH, Ivos-14, USA), vigor, concentration and morphology. Statistical analyzes were done in the MIXED procedure (SAS Inst., Inc., Cary, NC) using REPEATED command to model the residual covariance structure in animal. There was no detectable effect of treatment on scrotal circumference, motility, vigor, concentration and larger defects, smaller and total. The total motility (MT%), progressive motility (MP%) and rapid (RAP%) analyzed by computer did not suffer effect between treatments. However, an effect of age was noted, the animals showed an improvement in sperm quality from 16 months ($P < 0.0001$). We need more studies with larger numbers of animals and different fat sources to test the real influence of PUFA in bulls.