Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Male reproductive physiology and sperm technology

Sperm head morphometry may be related to reduced semen fertility at timed-AI

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Resumo

The aim of this study was to evaluate conventional in vitro sperm characteristics, morphometry and DNA integrity and to compare them with in vivo fertility. Six semen batches (from six different Angus bulls; a single batch of each bull) were utilized for Timed-AI of 890 lactating Nelore cows (30-40 DPP) at the same farm. All batches were equally divided into seven breeding groups (approximately 125 cows per breeding group) and three AI technicians. In the lab, the same semen batches used in the field were evaluated, being performed three replicates per batch (i.e., 3 repetitions from the same batch). Semen (500µL) was thawed (37°C/30s) and the following parameters were assessed: motility, vigor, concentration, morphology, computer assisted semen analysis and analysis of chromatin integrity and sperm morphometry by toluidine blue. Field data were analyzed by stepwise logistic regression (mixed generalized linear model with binomial distribution) and laboratory results by Tukey test or Kruskal-Wallis test (according to statistical premises). For all analyses, it was considered 5% of significance (R software). Overall conception rate (CR) was 49%. The semen batch was an important factor affecting CR (P=0.007). No effect of BCS (P=0.459), AI technician (P=0.562) or breeding group (P=0.398) were detected, nor their interactions (P>0.05). For each batch (B), the following CR were observed (B1: 42.6%a, n=162; B2: 40.3%a, n=119; B3: 52.3%ab, n=174; B4: 44.6%ab, n=139; B5: 53.0%ab, n=168; B6: 60.9%b, n=128). Considering the semen batches that presented different in vivo fertility (single batches from bulls 1, 2 and 6), no differences were observed in the in vitro sperm analyzes performed, except for some sperm head morphometric traits. B1 presented higher (P=0.0001) shape factor (SF; T1: 0.910±0.001a; T2: 0.896±0.005b; T6: 0.889±0.003bc), smaller (P=0.0025) anteroposterior symmetry (APS; T1:0.880±0.002b; T2:0.908±0.010a; T6:0.908±0.004a) and higher (P=0.0141) Fourier 1 (T1:144.02±6.539a; T2:112.78±17.813ab; T6:110.39±9.206b) than B6. Thus, B1 presented sperm morphometric traits that can be considered inferior to B6. Higher values of SF are associated with sperm immaturity and reduced APS with higher percentages of asymmetric sperm in the sample (cells with a narrower posterior portion of sperm head). Higher values of Fourier 1 are associated with rounder shapes of the anterior portion of sperm head, corroborating with SF and APS findings. Although further studies are needed to elucidate the real implication of these sperm traits on field fertility, one cannot fail to consider that the sperm morphometric differences observed may be related to the reduced fertilizing capacity of B1, thus justifying, at least in part, the lower field fertility of this semen. Still, among the in vitro sperm traits assessed in the present experiment, only SF, APS and Fourier 1 seemed to be related to the in vivo fertility results of the batches evaluated.

Acknowledgements

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Male reproductive physiology and sperm technology

Effects of thawing and storage temperature on sperm viability of Nelore and Holstein bull's

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Resumo

Artificial insemination is the one of the most used biotechnologies in animal production. Although well established and used worldwide, some critical steps such as thawing still need improvement. Therefore, we aimed to investigate the effect of thawing temperature on cryopreserved sperm and to evaluate sperm viability after storage for 8 hours. Frozen semen from Holstein (Hol, n=5) and Nelore (Nel, n=5) bulls were thawed at 37°C or at 4°C. After thawing, the samples were kept in the same temperature up to 8 hours. Samples were evaluated at 0, 2, 4, 6 and 8h for total (TM) and progressive motility (PM) by CASA (Hamilton Thorne Biosciences, Beverly, Massachussetts – USA) and membrane (MI) and Acrosome Integrity (AI) by flow cytometry (AMNIS Flow Sight, Amnis Corp., Setattle, WA). Data were analyzed by ANOVA using a 2X2 factorial experimental design, based on breed (Hol or Nel) and thawing temperature (4°C or 37°C), and their interactions. Data were compared among groups at the same time point and within group as repeated measure. TM was affected by breed and time ($P<0.05$). It decreased over time ($P<0.05$) for Nel (0h= 78.4% vs 8h =50.6%) and Hol (0h= 63.4% vs. 8h = 30.2%) semen, when thawed at 37°C. However, time did not affect TM ($P>0.05$) when Nel (0h= 62.2% vs 8h =65.5%) and Hol (0h= 43.8% vs. 39.6%) semen was thawed at 4°C. Regarding PM, except for Hol semen thawed at 37°C that had a decrease ($P<0.05$). After 4h of incubation, in all other treatments, PM was not affected by time and at 8h it was similar among all three groups (Nel 4=24.8%; Nel 37=26.6%; Hol 4= 26.6%). The AI and MI were affected by treatment ($P<0.05$) and time ($P<0.05$). The percentage of sperm with AI and MI was similar among all groups at 0h ($P>0.05$) and were preserved up to 2h. After 4 h there was a decrease in AI and MI on Hol and Nel semen thawed at 37oC ($P<0.05$) but had no impact on those at 4oC ($P>0.05$). No interactions were found between breed and treatment ($P>0.005$) for any of the assessments. In conclusion, Hol and Nel semen are susceptible to be thawed and stored at 4oC or 37oC until 8h post thawing. However, 4°C thawing/storage, is able to maintain the sperm quality for longer storage time than 37oC. Further studies in vitro and in vivo are needed to confirm such fertilization potential.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Male reproductive physiology and sperm technology

A preliminary analysis of commercial trans-anethole effects during ram semen cryopreservation

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Resumo

Cryopreserved semen is used in artificial insemination, positively impacting the genetic improvement of many species. Oxidative stress, a major event caused by cryopreservation, can cause cellular damage and reduce sperm viability. Trans-anethole, a natural antioxidant found in plants (e.g. fennel), appears to improve sperm cell survival during semen cryopreservation. This study assessed the effects of different concentrations of trans-anethole (117870, Sigma) added to sheep semen extender on post cryopreservation survival, kinetic parameters, and sperm binding to egg perivitelline membrane. Semen was collected from six rams using an artificial vagina, on five different days. Ejaculates with sperm motility >70% were used (n=22) and diluted individually with Tris-Egg Yolk-Glycerol extender, with trans-anethole, according to the treatment: CONT (control group, 0 µM), AN10 (10 µM), AN50 (50 µM) and AN100 (100 µM), with a final concentration of 400 x 106 spermatozoa/mL. Cryopreservation was carried out using a freezing machine. A cooling rate of 0.25 °C/min was applied until 5 °C, and this temperature was maintained for 4 h. The freezing rate used was −20 °C/min from 5 to −120 °C, when the straws were immersed in liquid nitrogen (−196 °C) and stored. Thawing was performed at 37 °C for 30 s. Samples were evaluated regarding sperm kinetics (by objective Computer Assisted Semen Analysis, CASA), plasma membrane integrity (PMI, staining with acridine orange and propidium iodide), and functionality (PMF, hypoosmotic swelling test) as well as the sperm binding to egg perivitelline membrane test. Data were analyzed using a generalized linear mixed model, and the results are presented as mean ± SEM. After thawing, the progressive motility of AN100 was higher ($P<0.05$) than AN50 (13.8 ± 1.8 vs 10.2 ± 1.8%), and both were similar ($P>0.05$) to CONT (11.5 ± 1.8%). The medium velocity of AN100 (11.3 ± 1.4%) was higher ($P<0.05$) than AN50 (8.5 ± 1.4%) and CONT (8.1 ± 1.4%). Differences ($P<0.05$) were also observed in average path velocity between AN100 (33.6 ± 2.6 µm/s) and AN50 (29.5 ± 2.6 µm/s), both being similar to CONT (32.1 ± 2.7 µm/s); and straight-line velocity between AN100 (24.0 ± 2.0 µm/s) and AN50 (20.7 ± 2.0 µm/s), with CONT presenting an intermediary value (23.3 ± 2.1 µm/s). The number of sperm bound to the egg perivitelline membrane was higher ($P=0.05$) between AN100 and CONT groups (4293.0 ± 483.9 vs 3076.2 ± 483.9 sperm/mm2, respectively). There was no difference ($P>0.05$) among groups regarding other kinetics parameters, PMI and PMF. In conclusion, the addition of 100 µM trans-anethole to cryopreservation media leads to an improvement in sperm kinetics as well as its fertilizing capacity assessed by the binding test, without disrupting their membrane integrity and functionality.

Keywords: sperm, antioxidant, freezing, fennel

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Male reproductive physiology and sperm technology

Early acrosome reaction in heat-shocked bovine sperm is associated with changes in Ca2+/calmodulin-dependent protein kinase II (CaMKII) signaling

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Resumo

Heat shock (HS) during bovine sperm capacitation affects its quality, resulting in low fertility. Capacitation is a biochemical process that renders the sperm competent to fertilize, resulting in a cell prone to undergo acrosome reaction. It is known that CaMKII is an important regulator of this event, in which CaMKII activity during sperm capacitation inhibits early acrosome reaction. Thus, the present work aimed to evaluate the effect of HS on acrosome reaction and phosphorylated CaMKII (pCaMKII) localization during bovine sperm capacitation. For that, bovine sperm were processed immediately after thawing (0h) or incubated in capacitating medium (1x106 cells/mL) at 38.5 °C (control) or 41 °C (HS) for 1h, 2h, 3h, and 4h. Were performed 3 replicates, in which 100 spermatozoa were evaluated per group/replicate. pCaMKII was analyzed by immunofluorescence using antibody anti-CaMKII phosphorylated at T286 (1:100). The DNA was stained with Hoescht 33342 (5 µg/mL) and the acrosome was labeled with Pisum sativum agglutinin (FITC-PSA; 100 µg/mL). The sperm were evaluated by fluorescence microscopy and the data were analyzed by two-way ANOVA followed by the post-hoc Tukey's test with a significant difference when $p \leq 0.05$. No interaction between variables (temperature x time) was observed. The acrosome integrity was negatively affected by 4h of in vitro capacitation. This negative effect on acrosome integrity was more pronounced in spermatozoa incubated at 41 °C (28.5%) compared to 38.5 °C (49.75%), in which HS increased the early acrosome reaction. Similar results were observed on pCaMKII localization. In post-thaw semen, pCaMKII was observed in the apical region of the acrosome (90%). In vitro capacitation gradually decreased the percentage of spermatozoa with pCaMKII localized at the acrosomal region. However, this effect on pCaMKII localization was higher during 4h of in vitro capacitation at 41 °C (27.75%) compared to 38.5 °C (45.75%), evidencing the negative effect of HS on pCaMKII acrosomal localization. The HS effect on pCaMKII localization was associated with early acrosome reaction during in vitro capacitation. In addition, HS affected the pCaMKII localization in sperm with intact acrosome (35.75%) compared to the control group at 38.5 °C (78.25%), showing that the lack of pCaMKII at the acrosomal region precedes the early acrosome reaction in HS sperm. In conclusion, the data presented here suggest that early acrosome reaction induced by HS may be at least in part through the HS effect on CaMKII signaling.

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Male reproductive physiology and sperm technology

Effect of quercetin on the expression of genes related to oxidative stress in testes of rats fed a high-fat diet

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Resumo

The aim of this study was to verify the effect of quercetin supplementation on the expression of genes related to oxidative stress in testes of Wistar rats fed a high-fat diet. For that, the experiment used 48 Wistar rats in 6 experimental groups: CT (rats fed commercial diet and supplemented with 0 mg / day of quercetin gummy); Q10 (rats fed commercial diet and supplemented with 10 mg / day of quercetin gummy); Q20 (rats fed commercial diet and supplemented with 20 mg / day of quercetin gummy); HF (rats fed with high-fat diet and supplemented with 0 mg / day of quercetin gummy); HFQ10 (rats fed with high-fat diet and supplemented with 10 mg / day of quercetin gummy); and HFQ20 (rats fed with high-fat diet and supplemented with 20 mg / day of quercetin gummy). At the end of the experiment, the rats were anesthetized and killed by exsanguination, the testes were collected and stored in the freezer at -80 ° C. The stored testes were analyzed by RT-qPCR for quantitative expression of genes related to oxidative stress: glutathione synthetase (Gss) and superoxide dismutase 2 (Sod2). The results passed the Shapiro-Wilk normality test and the statistical analysis used was ANOVA. No differences were found among the groups for gene expression of Gss or Sod2 ($P > 0.05$). The means and the standard error of gene expression of Gss were: CT = 1.02 ± 0.09; Q10 = 1.15 ±0.07; Q20 = 1.18 ± 0.13; HF = 1.18 ± 0.12; HFQ10 = 1.16 ± 0.17; and HFQ20 = 1.07 ± 0.10. Quantitative gene expression of Sod2 were: (CT = 1.01 ± 0.06; Q10 = 1.24 ±0.12; Q20 = 1.09 ± 0.13; HF = 1.37 ± 0.12; HFQ10 = 1.04 ± 0.09; and HFQ20 = 0.98 ± 0.07). In another study, it was observed that high-fat diets increased a malindialdehyde (marker of oxidative stress), enzymatic activity of glutathione peroxidase and caspase 3 immunostaining in testes (Migliaccio et al., Cells, 8:443, 2019). Based on that, we expect that the high-fat diet would increase the expression of the studied oxidative stress genes, but this did not happen, which is probably why quercetin had no effect on the expression of these genes. It is concluded that the high-fat diet or quercetin supplementation do not alter the gene expression of Gss or Sod2 in testes of Wistar rats. The effects of a high-fat diet on oxidative stress and testicular damage should be better elucidated.

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Male reproductive physiology and sperm technology

Bulls Exposure to High Temperature-Humidity Index Correlates with Impaired Kinetics of Bovine Spermatozoa

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Resumo

This study aimed to assess the effect of bull exposure to high temperature-humidity index (THI) on spermatozoa kinetics variables. In partnership with a commercial bull semen processing stud, we used data of 2,758 semen batches from 57 bulls. Semen samples were collected by artificial vagina between July/2017 and July/2020. Bulls were housed at Uruguaiana, RS, Brazil (29° 45′ 42.884″ S 57° 5′ 9.034″ W), in individual stalls, fed twice daily according to the nutritional demand for bulls in a twice a week semen collection program. Animals had access to ad libitum water and shade, but no additional temperature or humidity control. Weather data were obtained from a public weather station located approximately 30 km away from the bull stud. We tested 25 statistical models for each dependent variable considering five THI equations and five THI variables. Bull was included as covariate in all models. The statistical model using the number of days when THI reached 74 or above, calculated by the Thom equation (Cooling degree-days, Weather Bureau, 1958) best explained (higher determination coefficient) the changes in all sperm kinetics (data not shown). Then semen batches were separated by quartiles according to the number of days when the THI was greater than 74 (THI>74) during the 60-day window prior to the semen collection day. Quartiles (Q) 1, 2, 3 and 4 represent 0 to 9, 10 to 21, 22 to 41, and more than 42 days of exposure to THI reaching 74 or higher values, respectively. The effect of the quartiles on the dependent variables pre-freezing motility (%), mobile cells (%), motility loss (percentage points), progressive cells (%), fast cells (%), concentration, VSL (mm/s), VCL (mm/s), VAP (mm/s), LIN (%), STR (%), and ALH (mm) was assessed by mixed models using bull as random effect. A significant Q effect, where all Q were different from each other, was observed on motility loss (greater Q = greater increase), mobile cells and fast cells (greater Q = greater reduction). There were also Q group differences for progressive cells and semen concentration, with Q4 reaching lower values than Q1 and Q2. Quartile 4 had lower values of speed variables than Q1 and Q2 (VAP, VCL, VSL), whereas rectilinearity variables (LIN, STR) demonstrated that Q4 samples had greater % of cells with a more rectilinear path. Quartile 4 cells had lower amplitude of movement from their projected real path (ALH) than Q1 and Q2. Quartiles had no effect on pre-freezing motility. In conclusion, a greater the number of days of bull exposure to THI>74 during the 60 days prior to semen collection seems to lead to a greater negative impact on post-freezing sperm cells kinetics. Overall, the data suggest functional impairment of the sperm cells as bulls are exposed to a THI associated with heat stress.

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Male reproductive physiology and sperm technology

Delivery of exogenous sperm microRNAs increases cleavage rates and change gene expression in embryos from low IVP fertility bulls.

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Resumo

Different bulls present high or low in vitro fertility. This lower efficiency on in vitro embryo production (IVP) may represent financial losses and delays in breeding programs. Sperm RNAs play roles in early embryo development by the action of non-coding RNAs, such as microRNAs, which could influence bull fertility. Recently, four microRNAs were identified exclusively in sperm from high IVP fertility bulls (Hamilton et al. Reproduction, Fertility and Development, 33:157, 2021). We hypothesized that these microRNAs could improve in vitro embryo development of low IVP fertility bulls. We performed two functional experiments called Rescue and Proof of Principle experiments with 6 Nellore bulls, 3 with high and 3 with low IVP fertility, retrospectively selected from commercial IVP manipulations (n=7000) from 2016 to 2018. In the rescue experiment, we performed IVP using the 3 bulls with low IVP fertility and the zygotes, 18 h after IVF, were microinjected with 5 to 7 pl mimics of the 4 miRNAs (100 nM, miRCuryLNA®, miScript®, Qiagen - mimic group). In the proof of principle experiment, we used the other 3 bulls with high IVP fertility, and then the zygotes were microinjected as above with inhibitors of the same 4 miRNAs (inhibitor group). Zygotes in control groups were microinjected with negative control mimic or inhibitor molecules (scramble groups) in both experiments. We performed 6 IVP manipulations per bull with 30 oocytes microinjected by experimental group in each replicate. In vitro embryo development rates and gene expression (q-RT-PCR) of miRNAs target transcripts in 2-4 cell embryos were evaluated. After microinjection, on the second day of in vitro culture, 5 embryos at 2-4 cell stage from each group were collected for gene expression of target transcripts: TGB1, CDKN1, HDAC1, PTEN, BCL6 and IRF1. SAS System for Windows 9.3 was used to evaluate IVP data by GLM procedure and qPCR data by mixed procedure (Steibel et al. Genomics, 94:146-153, 2009). In the rescue experiment, cleavage rate was increased in mimic group compared to the scramble group (cleaved structures/total oocytes; 68.30 ± 2.65 vs. 54.23 ± 3.69 respectively; $p < 0.0001$). A lower expression of HDAC1 was found in 2-4 cell embryos in the mimic compared to scramble group. No differences were observed in the proof of principle experiment. As it was hypothesized, the results showed a positive action of exogenous sperm miRNAs in embryo cleavage when using bulls with known low IVP fertility, probably as a result of the increase in global gene expression, as a consequence of lower HDAC1 expression, enzyme responsible for histone deacetylation.