Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Chromatin alteration types in bulls with different conception rates at timed-AI

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The aim of this work was to investigate conception rates (CR) at timed-AI of different sires and to assess conventional semen quality parameters, sperm head morphometry, and chromatin alteration types; to compare the pregnancy per AI with in vitro sperm traits. Six Angus bulls were used for timed-AI of 890 lactating Nellore cows at the same farm. The same semen batches used in the field were evaluated in vitro and conventional semen analyses were performed as well as the assessment of sperm head morphometry, chromatin maturity and chromatin alteration types (by Toluidine Blue staining). Logistic regression was used for field data (function "glm") and the laboratory results were submitted to ANOVA and Tukey test by R software, considering P≤0.05. Overall CR was 49.0% and bull was an important factor affecting CR (P=0.007). Bulls 1 (CR=43%; 69/163) and 2 (CR=40%; 48/119) presented reduced (P0.05) CR compared to Bull 6 (61%; 78/128) but no differences (P>0.05) were observed for sperm motility, morphology and concentration between the bulls with different CR. However, Bull 1 presented smaller (P=0.0025) antero-posterior symmetry (APS=0.880±0.002), higher (P=0.0001) shape factor (SF=0.910±0.001) and higher (P=0.0141) Fourier 1 (F1=144.02±6.54) parameters compared to Bull 6 (APS=0.908±0.004; SF=0.889±0.00; F1=110.39±9.21). Higher values of SF are associated with impaired male fertility. Additionally, reduced APS are associated with higher percentages of asymmetric sperm in the sample such as a higher incidence of cells with a narrower posterior portion of sperm head. Moreover, higher values of Fourier 1 are associated with rounder shapes of the anterior portion of sperm head, corroborating with SF and APS findings of this study. On the other hand, Bull 2 presented higher (P=0.0023) percentage of chromatin alteration in the central axis of the sperm head (2.14±0.81) and higher (P=0.0277) percentage of scattered chromatin alteration (3.57±1.65) than Bull 6 (Central=0.25±0.43; Dispersed=0.73±0.75). It is worth mentioning that sperm with DNA damage may fertilize oocytes but embryo development is not likely to occur. Hence, the high incidence of specific chromatin alteration types of Bull 2 may be related to the lower fertility of this sire. In conclusion, bulls with different CR and similar in vitro sperm parameters at conventional semen analyses may present sperm morphometric differences and/or different sperm chromatin alterations. Although further studies are needed to elucidate the real implication of such chromatin alterations on field fertility, sperm morphometric differences and chromatin alterations may be related to, at least in part, the lower pregnancy per AI of the sires with reduced CR at timed-AI.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Sperm evaluation and Doppler velocimetry indices from young Brangus bulls submitted to injectable supplementation with vitamins and minerals

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The aim of this study was to evaluate the effects of parenteral supplementation with vitamins and minerals on sperm parameters and supratesticular artery hemodynamics in young bulls. Healthy Brangus bulls (n=28), aged 15 months, weighing on average 339±38 kg were divided into Control (n=14) and Treatment (n=14) groups. The groups were maintained on *Brachiaria brizantha* cv marandu pasture, supplemented with 1 kg of energy concentrate/animal, and access to water and mineral salt ad libitum during the study. The Treatment group received parenteral supplementation of vitamins (Kit Adaptador Vit®; Biogénesis Bagó, Brazil;1mL/50 kg, SC) and minerals (Kit Adaptador Min®; Biogénesis Bagó; 1mL/75 kg, SC) on the first day of the experiment (D0) and on day 60. The Control group received 0.9% NaCl in the same volume and days as the treatment group. The spermogram (tourbillon 0-5; motility 0-100%; vigor 1-5; sperm/mL concentration and sperm morphology) and ultrasound (Sonoscape S8) of the supratesticular artery [Doppler velocimetric indices mean velocity (MV), resistivity index (RI) and pulsatility index (PI)] were performed on D0, D60 and D120. Data were analyzed by ANOVA using GLM, followed by Tukey's test (5%). At the beginning of the study (D0), the control and treatment groups were similar (P>0.1) for sperm parameters and Doppler velocimetry indices. After 60 days, the control group did not differ from the treatment for tourbillon (2.0±0.6 vs. 1.8±0.5; P=0.62), motility (59.4±12.9 vs. 55.0±9.9; P=0.74), vigor (3.4±0.4 vs. 3.2±0.2; P=0.73), concentration (129.6±58.6 vs. 104.2±44.2; P=0.92), major (24.1±8.3 vs. 12.1±5.3; P=0.78) and minor defects (8.0±2.1 vs. 8.7±2.6; P=0.55). MV (16.4±1.3 vs. 15.9±0.9; P=0.89), RI (0.5±0.1 vs. 0.5±0.1; P=0.72) and PI (0.5±0.1 vs. 0.5±0.1; P=0.44) also did not differ between control and treatment, respectively. At the end of the study (D120) the control group also did not differ from the treatment for tourbillon (2.1±0.4 vs. 2.5±0.3; P=0.42), motility (57.7±9.6 vs. 67.9±8.2; P=0.46), vigor (2.5±0.4 vs. 2.8±0.4; P=0.42), concentration (237.5±80.5 vs. 236.3±89.8; P=0.91), major (18.5±3.9 vs. 20.8±5.7; P=0.71) and minor defects (10.4 ±3.0 vs. 9.9±2.4; P=0.91). MV (13.3±0.6 vs. 14.1±0.7; P=0.20), RI (0.6±0.1 vs. 0.5±0.1; P=0.78) and PI (0.6±0.1 vs. 0.5±0.1; P=0.45) were also similar between control and treatment, respectively. Throughout the study, no variable showed significant variation, except for sperm concentration, which increased (P=0.03) from the beginning (D0: 49.4±12.4) to the end (D120: 236.3±89.8) of the study. There was no significant variation between the parameters, possibly due to the good conditions and absence of nutritional challenges in the system in which the young bulls were managed, therefore, parenteral supplementation with vitamins and minerals did not influence the sperm parameters and the hemodynamics of the supratesticular artery.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Donkey sperm kinetic parameters: preliminary results

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Semen evaluation in donkeys still does not have an established standard. Therefore, studies and field surveys must be carried out in an attempt to establish a standard of andrological examination within Brazilian conditions. This study aimed to evaluate and correlate the kinetic parameters with the results of the pregnancy rate (71,94%) of inseminated donkey females with fresh semen. Six Pêga donkeys of proven fertility, aged between 5 and 15 years were used. Semen samples were obtained from three ejaculates from each of the six donkeys. Semen collection was performed with the aid of an artificial vagina after stimulation of males by the presence of female donkeys in estrus (N=110). Subsequently, the semen was filtered to remove the gel fraction, and only those ejaculates that reached motility equal to or greater than 70% were used. The ejaculates were submitted to evaluations of sperm kinetics by CASA system (Total motility (TM, %), progressive motility (PM, %), average path velocity (VAP, µm/s), straight line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), lateral head amplitude (ALH, µm), straightness (STR, %), linearity (LIN, %) and percentage of cells with fast velocity (RAPID, µm/s; %) and plasma membrane integrity, using the supravital dye eosin/nigrosin. Statistical analysis was performed using Pearson's correlation between kinetic parameters, considering a significance level of 5% (P<0.05). No correlation was identified between kinetic parameters: TM (r= -0.591), PM (r= -0.127), RAPID cells (r= -0.535), VAP (r= 0.400), VSL (r= 0.587), VCL (r= 0.141), ALH (r=-0.337), STR (r= 0.396), LIN (r= 0.313) and plasma membrane integrity (r= - 0.492) with pregnancy rate. However, was identified a high correlation between plasma membrane integrity and the TM (r= 0.960), RAPID cells (r= 0.883), STR (r= -0.714), and LIN (r=-0.737); high correlation positive between RAPID cells with ALH (r= 0.772), and negative with STR (r= -0.741); high correlation between VAP with VSL (r= 0.900), and VCL (r= 0.863); high correlation negative between ALH with STR (r= -0.890), and LIN (r= -0.899). From the preliminary results, it is suggested that males who ejaculate with spermatozoa with a rectilinear pattern, fast velocity, and intact plasma membrane, are more predisposed to present better fertility.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Flow cytometry as an auxiliary tool for evaluating the fertility potential of Nellore bulls

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The aim of this study was to investigate which parameters evaluated by cytometry were related to the fertility index of bulls obtained by natural mating. Thus, the fertility of the bulls was defined based on the quartiles of the data distribution of pregnancy rate after the end of the mating season (90 days duration) and classified as low fertility (LF; n=9) with an index of 66.57±0.62% (129 pregnant cows/192 total cows), medium fertility [MF; n=10) with an index of 76.47±0.51% (160 pregnant cows/210 total cows), or high fertility [HF; n=10) with an index of 84.80±0.60% (193 pregnant cows/277 total cows). Semen from all bulls (n=29) was collected using an electroejaculator (Autojac® (Neovet, Brazil) 10 d before the natural mating season began. The semen was cryopreserved using a portable programmable semen cryopreservation system (TK 4000®, Tetakon, Uberaba, Brazil). After thawing, the samples were analyzed for intact plasma and acrosomal membranes (MPAI), superoxide anion production (SO), lipid peroxidation (LP), and the quality and percentage of cells with high mitochondrial potential (HMP) using a flow cytometer (BD LSR II, Becton Dickinson, Mountain View, CA, USA). The quality of the mitochondrial potential was determined by measuring the fluorescence intensity of cells classified as having HMP using a flow cytometer. Statistical analyses were performed using SAS® version 9.4, SAS Institute, Cary, NC, USA). The UNIVARIATE procedure was used to determine quartiles and classify the groups according to fertility. Analysis of variance was performed using mixed models. No significant differences (P>0.05) were found between the groups in MPAI (LF=36.74±2.59%; MF=35.81±2.15%; and HF=32.81±2.29%), as well as in the quantification of SO (P>0.05; LF=1651±227 AU; MF=2111±189 AU; and HF=2401±201 AU) and LP (P>0.05; LF=125.57±5.64 AU; MF=117.82±4.69 AU; and HF=125.04±4.99 AU). No differences (P>0.05) were observed in the percentage of cells with HMP (LF=40.30±2.91%; MF=41.88±2.42%; and HF=37.85±2.57%), however, differences (P<0.05) were observed in the quality of HMP (LF=13240±1665 AU; MF=18799±1384 AU; and HF=18281±1474 AU), with the MF and HF groups not differing from each other, but having higher quality of mitochondrial potential when compared to the LF group. The results indicate that the evaluation of high mitochondrial potential is an important analysis and can be a differential in the fertility capacity of bull semen. This is because sperm movement is intimately dependent on mitochondrial function, which is indispensable in the energy formation process. In the present study, the high mitochondrial potential evaluated in frozen semen is the one that has the greatest relationship with the fertility of bulls in natural mating.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Royal jelly supplementation in boar semen extender: a preliminary study

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This study aimed to investigate the effect of royal jelly, rich in bioactive components with antioxidant properties, on swine semen extender to counteract the stress caused by refrigeration. The experiment was carried out at the Animal Reproduction Laboratory at EMBRAPA, in Concórdia, SC. Beltsville semen extender (BTS) was supplemented with lyophilized royal jelly: 0 (control), 0.25 (RJ0.25), 0.5 (RJ0.5), 1 (RJ1), or 2% (w/v) (RJ2). The ejaculate of 5 males, collected using the gloved-hand method, were diluted to a final concentration of $5x10^6$ [sperm cells/mL] and kept at 17ºC for 24 or 120 h. Computer Assisted Sperm Analysis (CASA) was applied to verify royal jelly and the preservation duration's effect on sperm cells. The data were checked for normality and homogeneity of variances and analyzed by two-way ANOVA. The effect of royal jelly concentration, duration of refrigeration, and the interaction between the two factors was observed regarding the percentage of sperm with bent tail (BT; $p<0.0001$, $p<0.0001$, and $p=0.0002$, respectively), total sperm motility (TM; $p<0.0001$, $p<0.0001$, and $p=0.0005$, respectively) and progressive motility (PM; $p<0.0001$, $p=0.0047$ and $p=0.0174$, respectively). On the other hand, the analysis of the percentage of sperm cells with a distal droplet (DD) was affected only by the concentration of royal jelly and the duration of refrigeration ($p<0.0001$ and $p=0.0010$, respectively). In contrast, the percentage of sperm with a proximal droplet (PD) showed only the effect of the preservation time ($p=0.0150$). After 24 h, RJ1 and RJ2 showed a higher percentage of BT than control (9.48±1.37; $p=0.0041$; 11.16±0.92; $p=0.0002$; 3.16±0.53, respectively). RJ1 and RJ2 presented no motility (TM and PM) after 24 h, while lower concentrations of royal jelly did not affect TM at this time. RJ0.5 showed lower PM than control after 24 h (19.16±5.93 vs 40.98±6.89; $p=0.0055$). More prolonged exposure to specific amounts of royal jelly caused a significant increase in BT (RJ0.5, 10.34±1.12; $p=0.0069$; RJ2, 24.78±2.59; $p<0.0001$), a decrease in PM (RJ0.25, 14.58±4.18; $p=0.0017$; RJ0.50, 6.26±2.32 $p<0.0363$) and a reduced TM (RJ0.25, 38.80±7.38; $p=0.0009$; RJ0.50. 24.16±4.48; $p<0.0001$). The combination of higher concentrations of royal jelly and longer storage time increased DD (RJ2-120h, 12.50±0.59; $p<0.05$) but did not affect PD results. Since we observed a toxic effect of royal jelly at tested concentrations on overall sperm viability in this preliminary analysis, the next step will include the evaluation of mitochondrial membrane potential, ROS formation, and membrane integrity through flow cytometry analysis on a different set of lower royal jelly concentrations.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Heat stress experienced in utero induces alterations in the gene expression profile that is transmitted to the next generation, even without changes in sperm and embryonic quality

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The heat stress (HS) caused by hot flashes may have teratogenic potential when experienced in utero, and it might program a new molecular response that is passed on to future generations. This study assessed the HS effect at different pregnancy stages on sperm quality and testicular expression of genes related to i) glucose transport (GLUT-3 and GLUT-8), ii) cellular response to HS (HSPA1A; Hsp60 and HSP90AAI), and iii) elimination of macromolecules and organelles damaged by autophagy (ATG8 and ATG5) in F1 males. The ability to transmit the HS response to the next generation was inferred by analyzing the expression of the same genes in F2 embryos. For this, 40 female mice of the C57BL/6 strain were mated with males (same strain) in a ratio of 1:1. After confirming mating (vaginal plug), females were subjected to HS (41 °C and 65% RH) daily for 2 h during pregnancy in the first half (FP, from D1 to D10; n=10), the second half (SP, from D11 to delivery; n=10), or the total pregnancy (TP, from D1 to delivery; n=10). A control group (CTR; n=10) was kept under normothermic conditions (25°C, 45% RH). At 8 weeks of age, five F1 males from each group were mated with 10 CTR females, previously stimulated by intraperitoneal administrations of 5 IU eCG (Folligon®, Intervet, São Paulo, Brazil), and 5 IU of hCG (Chorulon®, Intervet) after 48 h. After mating (72 h), all animals were euthanized. Sperm retrieved from the cauda epididymis and embryos recovered from uterine washing (PBS plus 0.4% BSA) were evaluated. For gene expression analysis, 20 testes and 100 blastocysts obtained from 20 animals (n = 5/group) were subjected to RNA extraction, reverse transcription, and quantification of selected genes by qPCR. Sperm parameters and gene expression data (normalized with β-actin, GAPDH, and H2AFZ) were evaluated by GLM ANOVA, followed by Tukey test, and superovulatory responses by Kruskal-Wallis test. No difference (P>0.05) was observed in sperm concentration, motility, and normal spermatozoa among male groups, as well as in the average of total recovered structures, viable embryos, unfertilized oocytes, and degenerated embryos among female groups. Nonetheless, a higher (P 0.05) expression of glucose transporters (GLUT-3 and GLUT-8) was observed in the testes and embryos of all HS-groups compared to the CTR group. A similar result (P 0.05) was observed for the genes involved in the cellular response to HS (HSPA1A and Hsp60) and autophagy (ATG8) when HS occurs in the first half of pregnancy (FP and TP groups). The expression of these genes in the FP group was similar (P 0.05) to CTR, and both were lower (P>0.05) than the FP and TP groups. Regardless of the biological sample (testes or embryos) and experimental group, the expression of the HSP90AAI and ATG5 were not affected. In conclusion, HS experienced in utero induces changes in the gene expression profile that is transmitted to the next generation, even without changes in sperm and embryonic quality.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Potential use of hCG to improve buck fertility during non-breeding season: Effects on testosterone profile

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This study evaluated the effectiveness of hCG administered in bucks during non-breeding season on testosterone profile. A total of 20 mature bucks (1.8 ± 0.1 years, 54.3 ± 3.0 kg, 3.0 ± 0.0 BCS) were equally assigned to receive four hCG administrations (G-hCG; n = 10, 300 IU, 1 mL, im, Fertcor, Ceva, Brasil) or Saline (G-Control; n = 10, saline, 1mL, im) 14 days apart. Blood was sampled by jugular vein puncture in vacuum tubes without anticoagulant on Days -7, 0(1º dose), 1, 7, 14 (2º dose), 15, 21, 28 (3º dose), 29, 35, 42 (4º dose), 43, 49 and 56. The measurements of serum testosterone concentrations were performed using the solid phase radioimmunoassay (RIA) technique using a commercial kit (Beckman Coulter, Immunotech, Prague, Czech Republic), following the manufacturer's recommendations. The sensitivity and intra-assay coefficient of variation were 0.05 ng/mL and 8%, respectively. After the normality test, parametric data were analyzed by one-way ANOVA followed by a post hoc Tukey test at 5% significance. No difference (P > 0.05) was observed in the first two moments (Day -7 and 0) between groups (G-Control 2.5 ± 0.5, 4.4 ± 0.6; G-hCG 2.2 ± 0.4, 3.4 ± 0.9 respectively). The day after (Day 1, 15, 29, 43) and 7 days later each administration (Day 7, 21, 35, 49) were observed greater (P < 0.01) concentrations of testosterone in G-hCG (12.1 ± 1.6, 18.0 ± 2.6, 12.6 ± 3.0, 10.9 ± 2.2) and (4.3 ± 1.3, 3.4 ± 0.7, 4.8 ± 0.6, 23.1 ± 5.1 ng/mL), than G-Control (5.3 ± 1.0, 5.4 ± 1.2, 5.1 ± 1.0, 8.1 ± 2.7) and (3.6 ± 0.7, 3.7 ± 1.2, 5.1 ± 1.3, 16.0 ± 3.4 ng/mL), respectively. The strategic hCG administrations two weeks apart proved to be efficient for increasing testosterone concentrations in bucks during the non-breeding season.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

The impact of semen extenders on the segregation of somatic cells and spermatozoa: optimizing biologic samples for cloning and *in vitro* fertilization

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Seminal somatic cells (SSC) can be utilized for IVP through somatic cell nuclear transfer (SCNT). This study evaluated the effects of different extenders for cooling and freezing of bovine and ovine semen on the segregation of SSC and sperm cells (SPTZ) using a 20%-50%-90% Percoll® gradient (PG®) to improve the utilization of biological material and enable in vitro culture of isolated SSC (NEL-THEMAAT et al., Cloning and Stem Cells 10:143-160, 2008). Semen from adult and fertile Texel crossbred rams (n=3) and Brangus bulls (n=4) was divided into eight groups: fresh semen, Tris-Yolk (TY), Citrate-Yolk (CY), Tris-Yolk-Glycerol (TYG), Citrate-Yolk-Glycerol (CYG), BotuBOV®, Skimmed Milk (SM), Skimmed Milk-Glycerol (SMG) for rams; fresh semen, TY, CY, TYG, CYG, BotuBOV®, Lactose-Yolk (LY) and Lactose-Yolk-Glycerol (LYG) for bulls. The SSC segregation profile was assessed at different times: post-collection (-3h), at equilibrium (5°C) after dilution (0h), and either 24h after 5°C equilibrium (cooled semen) or post-thawing (frozen semen). Samples were centrifuged in the PG®, and four fractions were collected: 20% layer (FT1), between 20% and 50% (FT2), 50% layer (FT3), and 90% layer (FT4, Sptz pellet). Cell quantity and viability were evaluated using a Neubauer chamber and Trypan blue staining at 0.4%. Data were analyzed by ANOVA/Tukey, Kruskal-Wallis, X^2, and Pearson's correlation tests, for $P<0.05$. No significant differences were detected between animals and species in the number or proportion of recovered SSC. However, distinct migration patterns were observed in the Percoll® fractions based on each extender, indicating species-specific variations while maintaining consistency within each extender over time. In rams, SM yielded a high number and proportion of SSC in FT1 (78.1%), while SMG concentrated SSC in FT2 (70.5%). Fresh semen, CY, and TY had a higher number and proportion of SSC in FT3 (61.9%, 68.1%, and 68.3%). CYG concentrated SSC in FT2 (62.5%), while TYG and BotuBOV® segregated SSC in FT4 at 0h (64.6% and 66.6%) and in FT3 at 24h (70.2% and 50.7%). In bulls, fresh semen and CY concentrated SSC in FT1 (49.1% and 57.5%), while TY and LY concentrated SSC in FT3 (60.7%) and FT4 (68.0%). The addition of glycerol altered the migration profile of SSC in TYG for FT4 (52.1%) and in LYG for FT3 (63.9%). BotuBOV® segregated SSC in greater proportion in both FT3 (31.1%) and FT4 (44.6%). CYG segregated SSC in FT3 at 0h (46.7%) and in FT4 at post-thawing (42.0%). The presence of glycerol in the extenders dehydrates cells, which reduces damage and affects cell volume, likely interfering with cell migration. Also, the egg yolk and milk constituents may influence extender density, affecting the migration process. In summary, migration of SSC remained similar between individuals of each species. However, the proportion of segregated SSC was influenced by extender composition (egg yolk vs. skimmed milk) and presence or not of glycerol.

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Prepubertal male mice exposed to heat stress experience short-, medium- and long-term reproductive dysfunction

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Extensive studies have demonstrated that exposure of pubertal males to heat stress (HS) compromises spermatogenesis, reduces sperm motility, fertilizing capacity and ultimately fertility. However, little is known about the effects of HS in prepubertal animals. Therefore, the aim of this study was to determine whether prepubertal male mice exposed to HS experience short- (pre-pubertal period/ P22-25), medium- (pubertal period/ P91-122) and long-term (middle-age period/13-15 months) effects on reproductive traits. Lactating Swiss mice and postnatal day 10 (P10) offspring were exposed to HS (35°C 12h/light and 21°C 12h/dark) or control conditions (21°C for 24 h) for 11 days. At P21, offspring were weaned, sexed and prepubertal males maintained under control conditions until sample collection. Parametric data were submitted to ANOVA (main effects: temperature and time) and non- parametric data were analyzed by Wilcoxon. Each analysis was replicated 3-4 times using 3-4 animals/treatment/replicate. In the first experiment, testicular histology was examined in prepubertal P22-25 (short-term) and pubertal P91-122 (medium-term) animals. Exposure of prepubertal male to HS reduced the number of spermatocytes ($p=0.0076$), round ($p<0.0001$) and elongated ($p=0.0016$) spermatids, and Leydig cells ($p=0.0065$) in prepubertal mice. HS also increased the percentage of seminiferous tubules with atrophy ($p=0.0123$), vacuoles ($p=0.0300$), abnormal residual bodies ($p=0.0017$), germ cells loss ($p=0.0017$) and reduced intact germinal epithelium ($p<0.0001$). When HS animals reached puberty, there was an increase in the number of spermatogonia ($p=0.0044$), rounded spermatids ($p<0.0001$) and Sertoli cells ($p<0.0001$) as compared to controls. In addition, HS reduced the number of spermatocytes ($p<0.0001$) and tended to reduce the number of elongated spermatids ($p=0.0832$). HS increased the percentage of seminiferous tubules with abnormal residual bodies ($p=0.0002$), germ cell loss ($p<0.0001$) and disorganization of the germinal epithelium ($p<0.0001$). In the second experiment, HS and control males were subjected to computer-assisted sperm analysis (CASA) when they reached puberty or middle-age. A contemporary group of young control animals was euthanized simultaneously to middle- aged animals as a positive control for sperm parameters. HS reduced sperm hyperactivation ($p=0.0239$) at puberty and reduced epididymal sperm count ($P< 0.05$) in middle-aged animals. However, there was no long-term effect of HS on sperm parameters. In a third experiment, HS and control males were mated to determine their reproductive potential at puberty. Mating naïve females with HS males led to decreased blastocyst yields ($p=0.0130$), viable embryos ($p=0.0130$) and increased incidence of unfertilized oocytes ($p=0.0459$). In conclusion, exposure of prepubertal animals to HS caused severe immediate and medium-term testicular alterations, compromising sperm hyperactivation, in vivo fertilization and embryo production.

Animal Reproduction

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PHYSIOLOGY OF REPRODUCTION IN MALE AND SEMEN TECHNOLOGY

Vitamin C and L-arginine supplementation prevent increased apoptosis in testicles of rats submitted to a single dose of 5-fluorouracil chemotherapy

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The 5-Fluorouracil (5-Fu) has been the most indicated in systemic combined chemotherapy for the treatment of several types of cancer, however, it is associated with severe toxic effects that sometimes lead to discontinuation of chemotherapy. Treatment with 5-fu has demonstrated toxicity to multiple organs, including sexual organs (Al-asmari et al., Hum Exp Toxicol, 35:10-20, 2016). The aim of this study was to verify the effects of L-arginine and vitamin C supplementation on presence de apoptosis mediators in rats submitted to 5-FU chemotherapy. For this purpose, Wistar rats were divided into 5 groups (n=3): Control group (Gc); 5-FU Group (G5-FU): who received intraperitoneal application of a dose of 5-FU of 50 mg/Kg of body weight; 5-FU + L-arginine group (G5-FU+Arg): who received 213 mg of/ L-arginine/ day in water and application of 5-FU; 5-FU + Vitamin C Group (G5-FU+VitC): who received 5.71 mg of ascorbic acid/day in water and application of 5-FU; Group 5-FU + L-arginine + Ascorbic Acid (vitamin C) (G5-FU+Arg+VitC): who received L-arginine, ascorbic acid and application of 5-FU. L-arginine and vitamin C were administered in the water for 7 days and on the eighth day a single dose of 5-Fu was applied intraperitoneally. After 72 hours of application of 5-FU, the rats were anesthetized and killed by exsanguination, the testes were collected and stored in the freezer at -80 ° C. Western Blotting was realized in testicles for BAX (proapoptotic protein), BCL-2 (antiapoptotic protein) and caspase 3 (protease active in cell apoptosis). The values of each protein then were normalized by the values of the respective βeta-actin. The expression of the bands (animals/group) was quantified by the ImageJ. Statistical analysis used was ANOVA with Tukey ($P < 0.05$). No differences were found among the groups for BCL-2 ($P > 0.05$). The BAX was higher in G5-Fu than in G5-FU+Arg+VitC ($P < 0.05$). The means and error deviation for BAX were: Gc = 0.71 ± 0.02; G5-Fu = 1.23 ± 0.12; G5-FU+Arg = 1.68 ± 0.17; G5-FU+VitC = 1.16 ± 0.09; and G5-FU+Arg+VitC = 0.66 ± 0.06. Caspase 3 was higher in G5-FU+Arg than in all groups, except in G5-Fu ($P=0.022$). The means and error deviation for caspase 3 were: Gc = 0.68 ± 0.06; G5-Fu = 1.08 ± 0.17; G5-FU+Arg = 1.67 ± 0.25; G5- FU+VitC = 0.97 ± 0.07; and G5-FU+Arg+VitC = 0.59 ± 0.02. The BAX/BCL2 ratio defines which cells will undergo programmed cell death. Caspase 3 is activated by BAX in the process of apoptosis and is related to the fragmentation of genetic material (Liu et al., Cell, 89:175–84, 1997). Vitamin C and L-arginine supplementation reduce apoptosis mediators in testicles of rats that submitted to a single dose of 5-FU.

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