Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Sperm morphological changes from the epididymis to the vas deference in rheas (*Rhea americana*)

Luana Grasiele Pereira Bezerra[1]*, Andreia Maria da Silva[1], Artur Pereira Jurema[1], Ana Glória Pereira[1], Maiko Roberto Tavares Dantas[1], Moacir Franco de Oliveira[1], Pierre Comizzoli[2], Alexandre Rodrigues Silva[1]

[1] Laboratory of Animal Germplasm Conservation, Federal Rural University of Semiarid Region – UFERSA, Mossoró, RN, Brazil;
[2] Smithsonian National Zoo and Conservation Biology Institute, Washington, USA.

***E-mail:** luana_grasielly@yahoo.com.br

Financial Support: National Council for the Scientific and Technological Development - CNPq

Abstract

Rheas (*Rhea americana*) are ratite birds from South America, which have great ecological importance in the maintenance of ecosystems. Unfortunately, rheas are classified as an almost threatened species according to the International Union for Conservation of Nature – IUCN. Therefore, research on reproductive biology is critical to protect that species and to develop reproductive biotechnologies that can enhance rhea conservation. However, little is known about rhea reproduction, including sperm production. Knowing that bird spermatozoa leave the testes, go through the epididymis, and are then stored in the vas deferens, the objective of the study was to determine for the first time the morphometric changes that occur in rhea sperm during the transit from the epididymis to the vas deferens. Five adult males from the Center for Multiplication of Wild Animals (CEMAS/UFERSA) were used. Individuals were part of a scheduled culling that were approved by the ethical committee of UFERSA (Opinion no. 09/20200. Individuals were premedicated with intramuscular (IM) administration of 2% xylazine hydrochloride (1mg/kg) plus 10% ketamine hydrochloride (15mg/kg), anesthetized with IM thiopental 1g (150mg/kg), and euthanized with intracardiac administration of potassium chloride 19.1% (2.56 mEq/Kg). After dissection of the reproductive tract, sperm cells were collected from the entire epididymis and vas deferens by a flotation technique using an isosmotic saline solution. Sperm samples then were smeared on a slide and stained with Bengal Rose for morphometric analysis (Chromato®, SP, Brazil; 200 sperm per segment per animal). Images were captured using brightfield microscopy (100x) connected to an image analyzer software LAS EZ Ink. The sperm structures, such as acrosome, head, mid-piece, and tail were measured separately in addition to the total sperm length. Data were expressed as mean and standard error and analyzed (Kruskal-Wallis test) to compare sperm morphometry in the epididymis and the vas deferens. Rhea male gametes were observed as filiform sperm, consisting of head and tail connected by a mid-piece. The spermatic head is relatively straight, but tapered in its anterior portion.. The acrosome was small, conical and pointed. A significant increase in the length of the acrosome ($P<0.05$) from the epididymis (0.932 ± 0.007 µm) to the vas deferens (0.971 ± 0.008 µm) was observed, which may be related to the accumulation of enzymes that will be further involved on acrosome reaction and fertilization processes. Sperm head was wider ($P<0.05$) in vas deferens (0.590 ± 0.003µm) than in the epididymis (0.566 ± 0.003 µm). The sperm midpiece increased in size ($P<0.05$) from the epididymis (2.05 ± 0.01 µm) to the vas deferens (2.12 ± 0.04 µm). An increase in midpiece length is likely related to the development of the mitochondrial sheath giving motility potential to the sperm. In addition, the sperm tail length increased from the epididymis (30.3 ± 0.1 µm) to the vas deferens (31.0 ± 0.1 µm). Finally, it was found that the spermatozoa from the vas deferens (40.8 ± 0.1 µm) were longer ($P<0.05$) than in the epididymis (40.0. ± 0.1 µm). In sum, all parts of the rhea sperm cells increase in size from the epididymis to the vas deferens, which is a fundamental information regarding the sperm maturation process.

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Selection of genetically superior Nellore bulls based on sperm parameters and testicular shape

Luiz Eduardo Natal Bizari[1], Diego Gouvêa de Souza[2], Guilherme Rizzoto[3], Ronério Augusto Bach[3], Ludimila Cardoso Zoccal Janini[3], Alicio Martins Júnior[4], Rafael Silva Cipriano[1]

[1] Salesian Catholic University Center Auxilium- *UniSALESIANO* Araçatuba, SP, Brazil
[2] Masterfertility Animal Reproduction
[3] São Paulo State University (UNESP), Botucatu, SP, Brazil
[4] São Paulo State University (UNESP), Araçatuba, SP, Brazil

Abstract

The aim of this study was to investigate the effects of age, scrotal circumference (SC), testicular volume (TV), and anatomical testis shapes on several sperm parameters in Nellore bulls. SC and testicular biometry were verified using a measuring tape and caliper (length and width), while testicular shape was calculated through the ratio width/length and testicular volume (TV = 2 [(r2) x π x h]). One hundred and one bulls with age ranging from 18 to 35 months were used in the experiment. Data of sperm parameters were obtained through the use of a computerized sperm analysis system and were considered for possible correlations between TV and semen quality parameter as well as total motility, progressive motility, sperm concentration, minor defects, major defects, and total defects. Statistical analysis was applied to verify presence or absence of interaction among SC, TV, testis shapes and sperm parameters evaluated by multivariate linear regression, with positive and negative interactions considered significant when P<0.05. T test was employed to compare SC and TV testis parameters. For the testicular shapes, it was possible to identify the predominance of a width/length ratio between 0.5 – 0.625. Animals with long (<0.5), spherical oval (0.751 – 0.875), and spherical (>0.875) were not observed. There was a positive correlation between SC and TV. Testis with long oval shape (0.626 – 0.750) present higher values (P>0.05) of SC and TV than those classified as moderate long shape (>0.5 – 0.625). There was a statistical difference among testicular volume, concentration and major defects (P>0.05). There were no differences between the other factors and age. However, correlation was found between sperm concentration and testicular shape, with higher number of spermatozoa observed for long oval testis (ratio: 0.626 – 0.750) in comparison with other shapes. No correlation was detected between testicular shape and semen quality, but testis with greater TV and SC showed increased sperm production, evidenced by the highest sperm concentration. In conclusion, it seems that testicular shape has an effective influence on the standard testicular measurements as well as on some sperm parameters and, therefore, it should be considered in the evaluation of bulls in selection programs.

Keywords: Fertility, Genetics, Nelore, Computerized analysis, Bos indicus.

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Butylated hydroxytoluene supplemental increases *in vitro* fertility of cryopreserved boar spermatozoa

André Furugen Cesar de Andrade[1], Kayode Balogun[2], Zoltan Machaty[2], Robert Victor Knox[3]

[1] University of São Paulo, Pirassununga, São Paulo, Brazil
[2] Purdue University, West Lafayette, Indiana, USA
[3] University of Illinois at Urbana-Champaign, USA

Abstract

The aim of this work to evaluate how supplementing a commercial freezing media with butylated hydroxytoluene (BHT), or reduced glutathione (GSH), or their combination affected in-vitro measures of boar sperm after cryopreservation. Ejaculates were collected from 30 high fertility boars used in a weekly collection rotation. Samples were diluted 1:1 in extender and cooled before overnight shipping at 17 °C to the freezing lab. On arrival, samples were mixed and split into the treatments with the following additions before cryopreservation; 1) semen without additional antioxidants (Control/C), 2) semen with 1 mM BHT(B), 3) semen with 2 mM GSH(G), and 4) semen with 1 mM BHT+2 mM GSH(S). Semen was evaluated for motility kinetics at 30, 120, and 240 min after thawing. Simultaneously integrity of acrosome and plasma membrane (viability), and assessment of intracellular superoxide levels were performed at 60 min after thawing using a Flow cytometry (BD LSR Fortessa; Becton Dickinson, San Jose, CA, USA). There were differences ($P \leq 0.05$) in total motility between Control and GSH at all time points evaluated (T30: C-35.83±2.47%[b]; B-36.23±2.38%[b]; G-39.57±2.37%[a]; S-36.27±1.93%[ab]/T120: C-22.33±2.34%[b]; B-21.73±2.00%[b]; G-26.40±2.17%[a]; S-25.8±1.92%[ab]/T240: C-17.73±1.88%[b]; B-17.53±1.79%[b]; G-24.23±2.11%[a]; S-23.20±1.96%[ab]). No ($P > 0.05$) differences among Control and other treatment groups were observed in viability (C-53.99±2.08%; B-53.29±3.00%; G-54.63±2.82%; S-49.50±1.95%). Differences ($P < 0.05$) in superoxide levels were observed between the control and BHT+GSH (C-2034.63±116.36a.u.[a]; B-1824.85±80.39a.u.[ab]; G-1879.45±142.54a.u.[ab]; S-1679.04±226.75a.u.[b]). There was a higher superoxide anion production in the Control than in the BHT+GSH. For IVF, there were more (+4.25%) blastocysts ($P \leq 0.05$) for BHT compared to Control treatment groups (C-18.64±3.05%[b]; B-22.89±2.07%[a]; G-10.63±2.00%[bc]; S-8.55±1.55%[c]. However, BHT+GSH had a negative ($P < 0.05$) impact on fertilization rate compared to Control (-10.09%) and BHT (-14.34%). These results indicate that commercial media supplemented with GSH increased motility but did not have a positive impact on in vitro fertility rate. On the other hand, media supplemented with BHT improved the in vitro fertilizing ability of the frozen-thawed sperm cells. This research was funded by São Paulo Research Foundation, Brazil (FAPESP 2019/15668-3 - AFCA) and National Council for Scientific and Technological Development (CNPq) grant number 308989/2020-1 (AFCA).

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Number of sperm cells per dose and its correlation with bull fertility

Lucia Helena Rodrigues[1], Marcelo Roncoletta[2]

[1] Médica Veterinária Autônoma, Sertãozinho/SP, Brazil
[2] Inprenha Biotecnologia e Desenvolvimento Avançado S.A., Jaboticabal, São Paulo, Brazil

Abstract

The economic impact of artificial insemination (AI) for livestock development is undeniable, and the maximization of the superior genetical semen donors is crucial and it has been constant pressure on the semen industry. In this scenery, a question that still brings discussions, is the spermatic concentration in the insemination dose, even total and/or motile cell numbers, and its correlation with fertility in the field. It is known that correlations are not direct and there is an inherent individuality in there. The present study tries to demonstrate that the sperm cell number in the insemination dose can be used as a tool for ejaculate maximization in the industry. In 3 different breeding seasons, 0.5mL semen doses of 10 bulls (5 Nellore and 5 Angus, all allocated in a Comercial Center of Semen Industrialization and all semen batches were industrialized according to the company rules), were used in AI procedures at 4 different farms. 2,000 semen doses of each bull were industrialized per each breeding season, divided into 4 groups with different sperm concentrations (10, 15, 20, and 25x10^6 total sperm per dose). The % of sperm motility after thawed (evaluated in contrast microscopy) was correlated with fertility rate too. The distribution of the doses into the herds was aleatory in lots of mature cows and heifers. The fertility rate or pregnancy rate (PR) was obtained by ultrasound diagnostic at 35-40days after AI. Three different Statistical Methods were used. The first one is a Multiple Linear Regression Model (MLR) to estimate the fertility, using variables "bull" and "PR" per "sperm concentration per dose (estimated and the real one)", in different situations (breeding season, farm, and animal category by each bull) and considering the interactions between those variables too. With this analysis, it was observed a big oscillation in the PR considering the variables as "bull", "farm", and "breeding season". The interactions between those variables were high too and because of that, the fertility estimation could be considered just individually per each bull. The 2nd method was the PROCCOR Model (by SAS 6.9), used to estimate the correlation between PR with sperm cell concentrations. To compare the PR obtained with those different sperm concentrations per dose, it was used the *Chi-Square* and *Cochran-Mantel-Haenszel* (CMH) Methods (SAS 6.9). The average PR obtained was 67.51% (in general) and 68.5%, 64.3%, and 66.48%, respectively for each breeding season. Substantial differences in the PR for each bull were observed (range between 60.36 to 86.32%), but no statistical correlation between sperm cell concentration into the dose was observed (r = 0,068 e p = 0,26). The % of PR was not improved with the more sperm cell per dose, the results considering the 4 groups of sperm concentration were 67.34% (10x10^6), 68.87% (15x10^6), 70.30% (20x10^6), and 69.25% (20x10^6). It became quite evident that the % of motile sperm per dose had a high correlation with PR (r^2 =0.9532), and based on the CMH method, we estimate that for each 1 unit more in motility percentage it is possible to improve 0.3842 points in fertility rate (y=0,3842x + 55,295). The 3rd statistical method used was an exponential model to estimate de "optimal dose curve - ODC" (y = a.e(-b/x), where y = pregnancy rate; x = number of sperm cells per dose and a = asymptotic level of the pregnancy, b = growth rate and, e =natural logarithm base), method firstly designed by Den Daas (1997)[1] for Holsteins Bulls. According to the ODC statistical method and its results, it was not possible to determine the "optimal number of sperm cells per dose" per each bull – the PR of each bull demonstrated variation in the different breeding seasons or herds (farms) tested, independently of the sperm number per dose, as demonstrated in the CMH method too. The explanation for the ODC method design by Den Daas (1997) does not demonstrate nice "ODC" in this work, maybe is in the number of sperm cells used in Holland, because there, they designed the experiment using very low sperm concentration at the beginning of the curve (5x10^6/dose) while, here in Brazil, the lower dose tested was with 10x10^6/dose. The method could be an interesting tool for the industry if nicely adapted and thinking in the particularities of each country. The most important conclusion here was - working into a range of 10 to 25x10^6 total sperm cells/dose, independently of the bull tested, there was no impact on the fertility rate of the bulls.

Keywords: Artificial Insemination, Semen Evaluation, Pregnancy rate.

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IMMUNOSTAINING OF OSTEOPONTIN IN STALLION EPIDIDYMIS

Luciana Seeger Bortoluzzi[1], Manuela Tondin[1], Mariana Fries Gerling[1], Verônica La Cruz Bueno[1], Henrique Boll de Araujo Bastos[1], Rodrigo Costa Mattos[1], Maria Inês Mascarenhas Jobim[1]

[1] REPROLAB - Faculdade de Veterinária, UFRGS, Porto Alegre, RS, Brazil

Abstract

Osteopontin (OPN) is a protein related to high fertility, present in the seminal plasma of several species, including in horses. It has several functions, including adhesion, signaling and cell interaction. OPN has been described in the epididymis of rats and sheep, but has never been reported in horses. The objective of this study was determinate immunostaining of OPN in different portions of epididymal tissue in stallions. Biopsies were collected from the caput, corpus and cauda of the epididymis of 6 mixed-breed stallions. The samples were fixed in 4% paraformoldehyde buffered solution for subsequent histological and immunohistochemical evaluation. The tissues were stained with hematoxylin–eosin and the sections were analyzed using optical microscopy. After sectioning the paraffin blocks at 5-um thickness, the samples were fixed on slides that were previously treated with HistoGrip (Life Technologies). An automated system (Benchmark XT, Ventana Medical Systems, Tucson, AZ, USA) was used for immunohistochemistry. Positive immunostaining for OPN was found throughout the entire epididymis, including caput, corpus and cauda. In the caput and corpus sections, the staining occurred along the columnar epithelial cells, stereocilia and basal cells, while in the cauda section, OPN was present only in the apical portion of the columnar cells and in the stereocilia. When passing through the caput and corpus epididymis, the spermatozoa suffers a series of morphofunctional changes in order to acquire fertilizing capacity. The increased presence of OPN in the caput and corpus epididymis may indicate that it helps in the cellular interaction between spermatozoa and luminal epithelium, contributing to the sperm maturation process. In contrast, the cauda of the epididymis has the characteristic of being the main reservoir of matured spermatozoa, not requiring an intense cellular communication as other portions. These findings demonstrate that OPN is present in epididymal caput, corpus and cauda cells in stallions, suggesting participation in complex mechanism of spermatozoa development and maturation.

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SOCIAL DOMINANCE AND SEMINAL QUALITY DURING A BREEDING OF SAINT CROIX RAMS

Keyla Mauleón Tolentino[1]*, Fernando Sánchez Dávila[1], Rodolfo Ungerfeld Morón[2]

[1] Posgrado Conjunto Facultad de Medicina Veterinaria y Zootecnia y Facultad de Agronomía, Universidad Autónoma de Nuevo León, México
[2] Facultad de Veterinaria, Universidad de la República, Uruguay

***E-mail:** tolentino_1117@hotmail.com

Abstract

While social hierarchy has direct effects on reproductive performance in rams, the information available about its effects on seminal quality is very limited. Also, as far as we know, there are no studies on how seminal quality varies in dominant or subordinate rams during a breeding period. The present work was developed during four breeding in Nuevo León, Mexico (25°88' N) with Saint Croix sheep, a hair breed, with the very little season. The data were collected in four breedings (two in winter and two in spring), in groups of animals in each one, for 7 weeks. In each group (total=12) 2 rams were used (live weight (LW) of the 24 rams = 73.4 ± 0.3; mean ± SD for which the dominance relationship was determined. Each ram dyad was housed with 15 ewes for the 7 weeks. Weekly LW and scrotal circumference (SC) were recorded, and semen was collected with an electro-ejaculator (21160, Bailey, Colorado, USA), determining the volume, concentration, mass motility, and percentage of sperm. with progressive motility. In addition, total ejaculated spermatozoa and total ejaculated progressively motile spermatozoa were calculated. Data were analyzed with a mixed model, including hierarchy, time (week of breeding), and the interaction between hierarchy and time as fixed effects, and breeding as a random effect. Social rank had no direct effects or interaction over time on any variable analyzed. Live weight, semen volume, sperm concentration, mass motility, percentage of sperm with progressive motility, and total ejaculated sperm varied with time ($P<0.05$), and SC and total ejaculated sperm with progressive motility tended to vary over time. Live weight decreased from the first to the second week [72.3 ± 2.9 kg vs 68.8 ± 2.9 kg (LSmean ± SEM)], and again in the fourth (66.2 ± 2.9; $P<0.0001$), reaching values similar to those of the second week in the seventh (68.8 ± 2.9 kg). Seminal volume decreased on the fourth and fifth weeks, recovering in the sixth (0.8± 0.1). The concentration in the first week was 3273.7± 431.0 spermX10^6/mL, decreased between the second and fourth weeks (2586 ± 431.0 spermX10^6/mL and 2578 ± 431.0 spermX10^6/mL), recovering in the fifth (2766.6± 431.0 spermX10^6/mL). Mass motility decreased in the second week (3.5± 0.1 vs 3.06 ± 0.1) and remained low in the third (3.1 ± 0.1) and decreased again in the fourth (2.6 ± 0.1; $P<0.0001$), recovering in the seventh (3.4 ± 0.1). The percentage of sperm with progressive motility decreased from 3290 ± 0.1% in the first week to 2578.9 ± 0.1% in the fourth. The amount of total ejaculated sperm decreased from 3002.4 ± 474.8 spermX10^6 to 1796.3 ± 474.8 spermX10^6 in the third week, recovering in the fifth (2464.5 ± 474.8 spermX10^6). In general, all the indicators evaluated were affected in the first weeks, when most of the ewes were cycling, recovering as fewer ewes were cycling (more ewes were getting pregnant). However, there were no differences between the dominant and subordinate rams in the profile of the changes. It was concluded that at least under the conditions of this study and the breed used, the position of dominance did not affect the profile of the reproductive variables evaluated throughout breeding.

Keywords: Social hierarchy, sexual behavior, sperm

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STEM CELLS FROM WILD FEATHER FOLLICLE OF BRAZILIAN BIRDS AS A BANK OF GENETIC RESOURCES

Yasmin Godoi dos Reis[1], Luciana Cristina Machado[2], Vanessa Cristina de Oliveira[2], Meline de Paula Coutinho[2], Sarah Ingrid Pinto Santos[2], Daniele dos Santos Martins[1]

[1] Department of Veterinary Medicine, Immunohistochemistry Laboratory and Experimental Physiology, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.
[2] Department of Veterinary Medicine, Innovative Therapies Development Group, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.

Abstract

Brazil is considered the second richest country in the world in terms of bird variety, accounting for 1,919 species. Brazilian birds are animals that target of environmental anthropic action, as well as the trafficking of wild animals. Methods such as conservation of genetic resources presents itself as an alternative for the reduction of the continuous loss of animals and feather follicle stem cells (FFSCs) appear as a great source of germplasm for conservation The samples were obtained from the Zoo das Aves, Poços de Caldas/MG/Brazil and CRAS Pró-Arara, Araras/SP/Brazil. All study procedures were approved by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science FZEA/USP, Brazil (nº 6001150921). The bird's samples were collected in avian immediate after euthanasia or when animals founding death in the facility. There were collected from including 3 Pisitaciformes, 3 Galliformes, 1 Apodiforme, 1 Tinamiforme, and 1 Passeriforme. The histology protocol consisted in maintained the samples in 4% paraformaldehyde solution and the processing following was recommended by the laboratory. The slices were stained with hematoxylin and eosin and analyzed with light microscopy. For the cell culture protocol the samples were placed in Petri dishes containing PBS, sequentially the calamus region was section and a longitudinal section of the calamus for the feather pulp was completely extracted. Mechanical maceration was performed, followed by enzymatic digestion (IV collagen). After the cells were plated in culture with DMEM medium (Gibco®) suplemmented with 15% bovine serum (Hyclone), 1% antibiotic (Gibco®) and 1% fetal bovine (Gibco®) and placed in a 5% CO2 incubator at a temperature of 39ºC. As a result, we could observe in the histological analysis of the calamus that it is composed of feather pulp inside a tubular structure. The cell wall is coated with stratum corneum, and below we can find epidermal cells of keratinized stratified squamous epithelium. The germinal extract is a simple columnar epithelium and it's possible to visualize blood cells inside it, demonstrating that the feather pulp possibly provides nutrients that help the feathers grow. In the epidermal collar found the cells rounded and oval present at the base of the pulp, the FFSCs. When evaluating the bird cells profile in culture, we established that between 24 and 72 hours the fragments continue to dissipate potential progenitor cells. It was possible to visualize cell clusters in species cultures of *Aburria cujubi* species at 48 hours, *Lophura edwards at* 96 hours and up to 240 hours in *Ara arauna*. However in *Amazona Festiva* and *Eupetomena macroura* species it was not possible to visualize the clusters. Morphologically, the cells are rounded and without an apparent nucleus. Although after 336 hours, the morphological cell from *Aburria cujubi* changed and was able to visualize the fibroblastoid cell appearance. The maintenance of these cells in culture, without the loss of morphological quality must be within 96 hours, as our findings show that this cell type enters into quiescence after these period. Thus, we believe that feather follicle cells are a new source of genetic material preservation and in the future provide as a valuable tool for the preservation of endangered bird species. Surveys with FFCs enable the researches about resources for material conservation, genetics analysis and studies of molecular characterizations. In addition, to enabling the formation of a bank of genetic resources aimed at the production of wild birds.

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Gross and histological features of male reproductive system of the Jaguarundi (*Herpailurus yagouaroundi*)

Luciana Cristina Machado[1], Sarah Ingrid Pinto Santos[1], Meline de Paula Coutinho[1], Clésio Gomes Mariano Júnior[1], Carlos Eduardo Ambrósio[1]

[1] Faculdade de Zootecnia e Engenharia de Alimentos/Universidade de São Paulo, Pirassununga, SP

Abstract

The *Herpailurus yagouaroundi* is a small wild cat popularly known as Jaguarundi or Moorish cat. This wildcat has an average weight of 5,2kg, the body is elongated, with an average head and body length of 63,7 cm, the tail is very long with an average length of 41,9 cm. The coat pattern varies from dark gray to orange, but the dark gray pattern is most commonly seen. The jaguarondi inhabits a wide variety of habitats and its distribution extends from south to central Argentina to northern Mexico, across Central and South America to east of the Andes. It can be found in all regions of Brazil, however, it is considered a vulnerable species in Rio Grande do Sul (1). Understanding the morphological and microscopic aspects of the reproductive system of wild species is of great value for the advancement of reproductive biotechnologies focused on reproductive management in captivity and, consequently, conservation of the species. Therefore, this work aims to describe macroscopic and microscopical aspects of the male reproductive system of the Jaguarandi that may be useful for the knowledge and reproductive management of the species. The Jaguarundi was received by the Animal Anatomy Laboratory after death by road accident. The animal was about 71 cm in length from head to tail, 30 cm in height and approximately 2.5 kg in weight. Judging by the measurements, a young animal had not yet reached adulthood. The urogenital system was dissected with a stereo microscope, photo documented and the following structures were fixed with 4% PFA during 24 hours for histology: penis, testes, epididymis, prostate and bulbo-urethral gland. After the fixation time, the samples were dehydrated in increasing alcohol series (70% to 100%) and diaphanized in xylol. Then, the samples were embedded in 3x4 cm dimensions in paraffin embedding. Sections at the thickness of 5 µm were made on the paraffinized sample blocks in microtome. The sections were positioned on slides and deparaffinized in an oven at 60°C for 2 hours. The slides were then stained in hematoxylin-eosin. We found the following structures of the male reproductive system of Jaguarundi: scrotum, penis, testes, epididymis, prostate, and bulbo-urethral gland. During the examination of the genital organs of the moorish cat, it was possible to identify in the perineal location the scrotum covered by hair containing the two testicles and the foreskin with an orifice positioned ventrally for the penis exposure. The structures identified and their location were similar to those found in the domestic cat (2). Several authors describe the presence of keratinized papillae, also known as spicules, in the penis of wild and domestic cats (3, 4). However, we did not observe these structures in the Jaguarandi's penis, possibly because it was a young animal (Scanning electron microscopy will be performed to check their presence). From the dissection of the scrotum, it was possible to visualize the septum of the scrotum and the vaginal process covering both testicles. One of the testicles had its fascia dissected until we could visualize the epididymis. Microscopically we observed a thick tunica albuginea, seminiferous tubules and Sertoli cells were observed in a section. Macroscopically, it was possible to identify the head, body and tail of the epididymis. Dissection and microscopic evaluation of the components of the male reproductive system of the Jaguarandi revealed a great similarity with the reproductive system of the domestic and other wild cats. Still, it has its own particularities and even in comparison with other anatomical studies, we found structures and other features that have been poorly described in the literature. More morphological studies on wild species and particularly on the Moorish cat need to be carried out to ensure and provide a solid anatomical knowledge of the species.

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Melatonin supplementation as an antioxidant in goat bucks semen cryopreservation improves seminal quality parameters during the breeding season

Valeria Hernandez-López[1], Fernando Sánchez-Dávila[1]*, Keyla Mauleón Tolentino[1], Viviana A. Martínez-Zuazua[1], Rogelio A. Ledezma-Torres[2], Estela Garza-Brenner[1], Cecilia Zapata-Campos[3]

[1] Universidad Autónoma de Nuevo León, Facultad de Agronomía, General Escobedo N.L., México
[2] Universidad Autónoma de Nuevo León, Facultad de Medicina Veterinaria y Zootecnia, General Escobedo, N.L., México
[3] Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia, carretera Victoria-Mante km 5, ejido santa librada, Cd Victoria, Tamaulipas, México

***E-mail:** fernando_sd3@hotmail.com

Abstract

The use of antioxidants in the cryopreservation of semen in mammalian species has increased in the last decade to expand genetic material via artificial insemination. Despite the good quality of the extenders available on the market, the spermatozoon presents structural and biochemical damage during the freeze-thaw process, damaging motility, viability, and ability to fertilize. One of the main events that the sperm undergoes during this process is oxidative stress, which causes an imbalance between antioxidant systems and reactive oxygen species, which are produced during the cryopreservation process. One of the antioxidants that have been evaluated is melatonin, which has had positive results in other species, however, in goat semen it has begun to be used in combination with other commercial extenders to reduce sperm oxidative stress. This study was conducted to evaluate the effects of melatonin added to a commercial extender using semen from young bucks during the reproductive season. Six goats (four Alpine and 2 Nubian breeds) with one year of age and a live weight of 40 to 48 kg were used. Twice a week and for three weeks, a semen sample was obtained by electroejaculation; subsequently, the semen sample was evaluated in terms of the characteristics of seminal quality. Four repetitions of each treatment/buck were performed for each semen sample, a total of 16 straws, where one repetition was a 0.5 ml straw, with a concentration of 10 million sperm. Four levels of melatonin were evaluated: T1 to T4, where it was added to the diluent: 0.0, 0.5, 1.0, and 1.5 µM, respectively. Once the straws were filled with diluted semen, the refrigeration process was carried out at 5° C for 5 hours to later be subjected to the freezing process with liquid nitrogen for 18 minutes at -192° C. Subsequently, they were thawed at 37° C to evaluate the variables of mass motility (1-5), % of progressive motility and concentration of spermatozoa with progressive motility. As a result of the study, differences were found for mass motility ($P<0.05$) for thawed semen samples that were added melatonin T2= 2.27; T3= 2.53; T4=2.2 compared to T1, Control= 1.82. Likewise, differences ($P<0.05$) were found for progressive motility, where it was higher for T3, which was added 1.0 µM of melatonin = 56.02%, followed by T2 and T4 (47.13% and 47.88%, respectively), being the lowest for T1= 40.4%. No differences were found in terms of the number of sperms with progressive motility. In conclusion, melatonin supplementation in goat bucks semen samples can improve seminal quality parameters such as sperm motility after the freeze-thaw process, with the most optimal dose of 1.0 µM melatonin.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Evaluation of the response of frozen-thawed canine semen from Cimarron breeds dogs using different extenders and freezing processes

Danilo Fila[1,2], Adrian Carzoli[1], Rafael Aragunde[1], Alejandra Berglavaz[2], Jorge Gil[1]

[1] Unidad Académica de Reproducción Animal, Facultad de Veterinaria, Montevideo, Uruguay
[2] Ejercicio Liberal

Abstract

The aim of this work was to determine the variations in sperm motility of frozen-thawed semen with different extenders and freezing processes. 10 clinically healthy Cimarron breed adult dogs, between the ages of 2 and 6, belonging to private breeders, fed a maintenance diet, free access to water, were used. At the beginning of the experiment for their selection, a complete andrological examination will be carried out on each one. A total of four samples will be taken per animal, with a minimum sexual abstinence of one week, since they are reproducers of proven fertility with offspring in the last 4 months. Semen will be collected as described by Linde-Forsberg (1991) (I). The first fraction of the semen, coming from the prostate, will be discarded to preserve and evaluate the second fraction, rich in sperm. A complete spermiogram will be performed as described by Fila (2018) (II). Five groups will be made: two commercial ones, 1) Tryladil plus 20% egg yolk (TH) (III); 2) Andromed (A) (III); two elaborated diluents, 3) tris plus 20% yolk and ethylene glycol (THE) (IV), and 4) Triladil plus 1% soy lecithin (TL) (V), and 5) Deep-frozen (UC) (VI). Those ejaculates that met the following characteristics will be used: volume of the second fraction of the ejaculate greater than 1mL, concentration greater than 200x10^6/ml, more than 75% normal morphology and alive. After the sperm evaluation of each of the second fractions of the ejaculate, aliquots similar in sperm concentration will be taken. Each ejaculate will be divided into 5 similar aliquots and placed in different tubes to be used in each group. The suspension obtained will be diluted (1:3 V/V) and equilibrated at 4°C for 1 hour. After this period, they will be re-diluted to the final concentration (100 x 10^6) according to the different media and forms of freezing. For the first four groups, the sperm suspensions will be placed in 0.5 mL straws and later on liquid Nitrogen vapors for 10 min and later submerged in it. The straws of each group will be thawed in a water bath at 38°C x 1 min. Initial sperm motility will be subjectively estimated in % by visual option in an optical microscope with phase contrast (Olympus, Mod BX41, Japan) and stage tempered at 37ºC at 400x magnification. Post-thawing sperm motility evaluation will be performed by placing the straws in a water bath at 37ºC for 4 hours, where individual motility will be assessed at 0, 1, 2 and 4 hours. Values will be presented as mean ± standard error of the mean. To determine if there were differences between the means of the 5 groups studied, for each group, the ANOVA tests will be performed with the GLM Procedure (SAS) for repeated samples, where the results of individual motility will be compared when thawing the straws during the thermoresistance test. The TH group presented the following results time 0, 43.5%, time 1, 31.2%, time 2, 16.2%, time 4, 1.9%. Group A, time 0, 41.2%, at time 1, 27.3%, at time 2, 16.3%, at time 4, 4.4%. THE group, time 0, 45.8%, at time 1, 34.6%, at time 2, 23.1%, at time 4, 11.2%. TL group, time 0, 44.1%, at time 1, 37.7%, at time 2, 24.2%, at time 4, 13.5%. UC group, time 0, 42.6%, at time 1, 25.4%, at time 2, 12.7%, at time 4, 2.3%. Sperm motility did not present differences at time 0. The motility results of the groups THE and TL were different ($p<0.05$) at times 1, 2 and 4 to the other three groups. In our experimental conditions, the use of media made by the team with the addition of a cryoprotectant different from the commercial ones or the addition of lecithin improved the sperm motility of frozen semen, in contrast to commercial extenders or the UC protocol.

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Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of melatonin supplementation on goat bucks semen samples refrigerated at 5º C during the breeding season

Viviana A. Martínez-Zuazua[1], Fernando Sánchez-Dávila[1]*, Valeria Hernandez-López[1], Keyla Mauleón Tolentino[1], Rogelio A. Ledezma-Torres[2], Estela Garza-Brenner[1], Cecilia Zapata-Campos[3]

[1] Universidad Autónoma de Nuevo León, Facultad de Agronomía, General Escobedo N.L., México
[2] Universidad Autónoma de Nuevo León, Facultad de Medicina Veterinaria y Zootecnia, General Escobedo, N.L., México
[3] Universidad Autónoma de Tamaulipas, Facultad de Medicina Veterinaria y Zootecnia, carretera Victoria-Mante km 5, ejido Santa librada, Cd Victoria, Tamaulipas, México

***Autor responsable:** Fernando Sánchez-Dávila

Abstract

In recent years, antioxidant supplementation in semen freezing protocols has increased with the aim of using the artificial insemination (AI) technique on a large scale in different species of zootechnical importance. In goats, AI has limited its application due to the dispersion of its herds globally, however, estrus synchronization protocols during the reproductive season can be implemented with the use of semen refrigerated at 5º C. During this process, the sperm is required to remain active for a longer time, and its sperm motility does not decrease. One of the antioxidants proposed to prevent the oxidation process is melatonin, a powerful antioxidant produced naturally by the pineal gland. The objective of this study was to evaluate the supplementation of four levels of melatonin in goat bucks' semen and to determine the sperm motility curve for 36 hours refrigerated at 5º C. Six 1-year-old goat bucks, four Alpine and two Nubian breed, with a live weight of 40 to 48 kg, were used. Semen extraction was performed twice a week/three weeks for each of the males and were obtained by electroejaculation. The treatments to be evaluated were T1= control; T2= 0.5 µM of melatonin; T3= 1.0 µM melatonin and T4= 1.5 µM melatonin added together with the commercial diluent. The semen samples of each treatment (0.5 ml straws, with a sperm concentration of 10 million) were initially kept at 37º C and the cooling curve was immediately started to keep the semen samples at 5º C for 36 hours. The evaluation times of each semen sample were 0, 6, 12, 24 and 36 hours of refrigeration. Melatonin levels (NM) and refrigeration times (RT) for mass motility (MM), progressive motility (MP) and progressively motile sperm concentration (PMSC) were evaluated. Main effects of NM and RT were found, being the best values for T3 to which 1.0 µM of melatonin was added (MM=2.04; MP=43.66%; PMSC= 250.62). For the effect of RT, it was found that from 12 to 24 hours the values of MM, MP and PMSC fell drastically regardless of the level of melatonin supplementation ($P<0.05$). Being that for 12 hours of refrigeration, the values obtained were MM= 2.06; PM=42.70%; and PMSC= 206.79, compared to the 24-hour RT which was MM= 1.07; MP= 19.44; and PMSC= 96.51 respectively. In conclusion, supplementing the diluted semen sample with a 1.0 µM dose of melatonin and keeping it at 5º C for 12 hours will maintain optimal levels of sperm motility in goat bucks semen samples collected during the breeding season.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Morphological variations of the testis in an anuran community in a Brazilian northeastern caatinga fragment during the annual reproductive cycle

Artur da Nóbrega Carreiro[1], Hyldetan Ruan de Araújo Cezar[1], Maycon Rodrigues da Silva[1], Maria Sara Maia de Queiroz[2], Marcela Meira Ramos Abrantes[3], Stephenson Hallison Formiga Abrantes[1], Washington Luiz Silva Vieira[4], Danilo José Ayres de Menezes[1,2]

[1] Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, Brazil
[2] Universidade Federal do Rio Grande do Norte, Departamento de Morfologia, Brazil
[3] UniFIP Centro Universitário, Brazil
[4] Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia, Brazil

Abstract

Little is known about the Pseudopaludicola pocoto species, having been identified in 2014 by Magalhães and collaborators, being a small frog found in the semiarid region of Paraíba, Brazil. Amphibians show greater sensitivity to environmental factors, given their interface between the aquatic and terrestrial environment, which arouses interest in studying them The present study aimed to describe the changes in macroscopic and microscopic morphology, resulting from the interaction of environmental factors in a community of individuals of the species *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014. Thirty-nine mature male individuals were collected in groups monthly samples of three animals in a fragment of Caatinga located in the municipality of Passagem, Pb, Brazil from January 2019 to January 2020. The specimens were identified by external morphology and molecular analysis, with genetic divergence of less than 3% for the species. Histomorphometric and stereological analyzes were performed on the gonads of the individuals collected, where morphological changes were observed in volume and frequency of the germ cell lines as well as in the average amount of Sertoli cells in significant correlations with environmental factors such as ambient temperature and rainfall, with more voluminous cells and a greater frequency in the rainiest months and with milder temperatures. This correlation observed of morphological factors related to the rainy season was observed in several other frogs (1; 2; 3), with higher volumes and cell quantities in periods with higher rainfall (4; 5; 6; 7). The presence of all germlines throughout the year characterized a continuous reproductive cycle adapted to regions where there is a certain unpredictability in the environmental phenomena characteristic of a semi-arid region. The fact that the males collected presented mature sperm throughout the year does not suggest that the species is actually in its reproductive period, since the maturation stage of females is fundamental for the occurrence of the reproductive period (5; 8).

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Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

The feasibility of a swine semen storage system produced with bacteriostatic molecules to control bacterial proliferation

Janine de Camargo[1], Mariana G. Marques[2], Jean Carlo Volpato Faccin[2], Pedro Nacib Jorge Neto[3], Jalusa Deon Kich[2], Claudia Almeida Scariot[1], Ricardo Zanella[1]

[1] Universidade de Passo Fundo, Passo Fundo, RS, Brazil;
[2] Embrapa suínos e aves, Concórdia, SC, Brazil;
[3] Universidade de São Paulo, SP, Brazil.

Abstract

The development of a sustainable swine industry is driven by current social and environmental concerns. Utilizing antibiotics in semen extenders is one of the issues associated with pig breeding. It causes the emergence of multidrug-resistant bacteria due to its widespread application. In addition, the antibiotic may have an adverse effect on the viability of sperm and the vaginal and cervical microbiota of sows. Consequently, animal reproduction biotechnologies have sought out products with technologies capable of minimizing antibiotic use. In this study, we assessed the efficacy of the Bactibag swine semen storage bag, which features a bacteriostatic mechanism to restrict bacterial growth. Six boars' ejaculates were collected using the "gloved-hand" technique. To produce insemination doses, the ejaculate was assessed for volume, sperm motility, and concentration. The semen was diluted with and without antibiotics (0.25 g/L of gentamicin sulfate) at 37°C at in-house made Beltsville Thawing Solution (BTS). Semen doses were produced with a total motility sperm concentration of $2,5 \times 10^9$ sperm in a volume of 90 mL, filled into two different plastic semen storage: Bactibag blisters (Bactibag, IMV Technologies, France) and conventional blisters (GTB Bag, IMV Technologies, France), resulting in four experimental groups: semen diluted in BTS with antibiotics and added to the Bactibag blisters (BBA group) and conventional blisters (GTA group) and semen diluted in BTS without antibiotics and added to the Bactibag blisters (BBN group) and conventional blisters (GTN group). The doses of semen were then kept at 16°C for up to 120 hours (5 days). On a plate, bacterial growth was assessed after 72 hours and 120 hours of storage. We found a relationship between the storage blisters, the use of gentamicin, and time. The use of gentamicin in both type of blisters effectively inhibited bacterial growth from storage day 3 to day 5 ($P < 0.05$). After 72 hours of storage, the results of the BBN group were comparable to the GTA group. There were no differences in bacterial growth between 72 and 120 hours of storage in BBA group. At 120 hours of storage, the BBN group was as effective as the GTA group at inhibiting bacterial growth. Upon examining the bacterial growth over the assessed period (from 72h to 120h), we found that Bactibag blisters alone, without the addition of gentamicin, was able to restrict the bacterial growth. Our results demonstrate the feasibility of the Bactibag blisters as an essential tool for preventing bacterial growth at porcine insemination doses stored at 16°C for up to 5 days.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Endocrine disruptor mixture increases expression of tumor progression-related genes and promotes pre-neoplasic lesions development in prostate microenvironment

Odin Serodio Mettifogo[1], Edson Assunção Mareco[2], Victor Hugo Garcia de Oliveira[2], Ana Beatriz Ratto Gorzoni[3], Thaina Cavalleri Sousa[3], Maria Luiza Silva Ricardo[3], Gisele Alborghetti Nai[3,4], Leonardo de Oliveira Mendes[3,4]

[1] Faculty of Medicine of Presidente Prudente – UNOESTE
[2] Graduate Program in Environment and Regional Development - UNOESTE
[3] Graduate Program in Animal Science – UNOESTE
[4] Graduate Program in Health Science – UNOESTE

Abstract

Endocrine disruptors (ED) have been introduced into the daily lives, disrupting hormonal biosynthesis and inducing genetic alterations capable of establishing cancer. However, most studies refer to drugs alone or in small groups and in short periods of time, not mimicking the total exposure to which humans are subjected. Thus, studies using experimental protocols that mimic human exposure throughout life are important to characterize the gene-environment interaction and its effects on health/homeostasis. Therefore, the aim was to evaluate the effects of exposure to an ED mixture based on human exposure, from intrauterine life to adulthood, on the prostate global gene expression and the tissue repercussions. Sprague-Dawley pregnant rats were divided into 2 groups: Ctrl (vehicle: corn oil, by gavage); ED Mix: 32.11 mg/kg/day of a mixture consisting of 12 DE (phytalates, pesticides, u.v. filters, bisphenol A and butylparaben) diluted in corn oil (2ml/kg), by gavage. Pregnant or lactating rats received treatment from gestational day 7 to postnatal day 21 (DPN21). After weaning, at DPN22, the male offspring continued to receive the mixture until 220 days of age, when they were euthanized. The ventral prostate was dissected and processed for next-generation sequencing (HigSeq-2500 Illumina) and histological analysis (CEUA: 6034). After filtering readings (Fastqc) with low quality and removing adaptors (Trimmomatic), mapping (Hisat2) and transcriptome annotation (HTSeq-count) were performed using reference genome data (rn6). Histological sections were stained with hematoxylin and eosin. Differential expression analysis (DESeq2) of the transcriptome revealed the presence of 52 differentially expressed genes. Of these genes, 49 genes are upregulated and 03 were downregulated in the ED Mix. Among these, 32 genes presented a change (fold-change) greater than 2 times in relation to the control group. Such genes are related to increased cell survival, such as Cacybp (4.54-fold) and Bclaf1 (4.14-fold) or tumor progression and metastasis, such as Need4 (3.56-fold) and Prpf4b (3.55-fold). The genetic alterations were reflected in the tissue microenvironment, with 80% of the animals in the ED Mix group presenting pre-neoplastic lesions. Thus, we can conclude that exposure to a mixture of ED alters the expression level of genes related to processes of cell proliferation/death and epithelial-mesenchymal transition, showing a relationship between gene, environment and prostatic carcinogenesis (Funding: FAPESP – 2018/24044-0).

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

PHENYLALANINE MAY BE PROPOSED AS A BIOMARKER OF FERTILITY IN STALLIONS

Verônica La Cruz Bueno[1,2], Henrique Boll de Araujo Bastos[2], Rodrigo Costa Mattos[2], Sandra Fiala Rechsteiner[1,2]

[1] HISTOREP - Federal University of Pelotas, UFPEL, Pelotas-RS, Brazil
[2] REPROLAB - Faculty of Veterinary Medicine, UFRGS, Porto Alegre-RS, Brazil

Abstract

Stallions' selection as sires is based on three qualities: pedigree, performance record, and conformation. Fertility or fertility potential is usually, at best, a secondary consideration. Fertility can be determined by parameters that reflect breeding success rates or by using sperm characteristics, which have reported inconsistent results. Metabolomics is one of the most recent -OMICS techniques developed after genomics, transcriptomics, and proteomics. Analyzing the entire metabolome makes it possible to access the final products of the metabolism within a biological system. The metabolites are small molecules, less than 1kDa, derived from metabolic pathways and include hormones, amino acids, lipids, carbohydrates, and nucleotides. The composition of the metabolome determines the current phenotypic state of a cell. It actively changes in response to cellular and extracellular stimuli, making metabolomics studies more informative than the other -OMICS approaches, particularly in the investigation of cellular response to exogenous stimuli or in the case of pathology. The metabolomic profile of the seminal plasma was already analyzed in different species like humans, rhesus monkeys, mice, turkeys, bull, carp, rainbow trout, boar, donkeys, goats, and giant panda. In this study, 24 Criollo stallions were used, with ages ranging from 4 to 20 years old and weighing 450 to 500 Kg. The animals were housed in breeding centers in southern Brazil (30ºS, 51ºW). The Committee of Ethical Use in Animal Experimentation at Universidade Federal do Rio Grande do Sul, Brazil approved the study (protocol number 38666). One ejaculate was collected from each stallion during the breeding season. The selected stallion's fertility was evaluated based on the pregnancy rate per cycle on the 16th day after insemination. At least 30 inseminated mares per stallion and two breeding seasons were considered. Pregnancy rates ranged from 20% to 95%. The animals were divided into two groups: group High Pregnancy (HP), with a pregnancy rate per cycle over 50%, and group Low Pregnancy (LP), with a pregnancy rate per cycle below 50%. The identification of the target metabolite was performed by liquid chromatography. Chromatography was performed using Kinetex® Phenyl-Hexyl analytical column (100 x 2.1 mm, 1.7 µm) with the binary mobile phase at a flow of 350µL min^{-1} and injection volume of 10 µL. The LC-QTOF-MS system was a Nexera x2 liquid chromatograph (Shimadzu) coupled to an Impact II mass spectrometer (Bruker Daltonics). The software utilized for system operation and data acquisition were Compass Hystar 3.2 and TASQ. TOF-MS and bbCID (MSMS/MS) data were acquired in the m/z range of 50 to 1000 Da. Metabolite phenylalanine - an amino acid- presented the largest area in the LP group (4,402,336 + SD 1,225,568) compared to the HP group (2,763,743 + SD 1,237,550) P= 0.008. This amino acid contributes to several cellular and biological processes, such as antioxidant detoxification, metabolic processes of reactive oxygen species, and oxidoreductase activity. In bulls, adding small concentrations of phenylalanine resulted in adverse effects on the viability of sperm. In conclusion, Phenylalanine metabolite may be proposed as a marker of low fertility for stallions, given its positive correlation with the conception rate.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

ASSESSMENT OF HISTOLOGIC AND ULTRASOUND IMAGE OF TESTICULAR´S RAM AFTER SERIAL FREQUENT BIOPSIES

Paula Zanin Rattes[1], Renan Denadai[1], Antônio Guilherme Roncada Pupulim[1], Giovana Wingeter Di Santis[2], Felipe Martins Negreiros Navolar[2], Jaqueline Candido de Carvalho[1], John Patric Kastelic[3], João Carlos Pinheiro Ferreira[1]

[1] Department of Veterinary Surgery and Animal Reproduction, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, SP, Brazil
[2] Department of Preventive Veterinary Medicine. Londrina State University, Londrina, PR, Brazil
[3] Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada=

Financial support: CAPES (fomento nº 0001), FAPESP (processo nº 2018/02007-6) e CNPq (processo nº 2021/3845-4).

Abstract

Testicular biopsy is an important technique that complements the andrological examination. However, the consequence of serial biopsies at short intervals is not known. The objective of this study was to evaluate the ultrasound image and histologic of testicular parenchyma of rams after serial frequent biopsies. Six 18-month-old rams (Dorper x Santa Inês) were used. The rams were fed twice a day with a total mixed ration and water *ad libitum*. Testicular biopsies were performed with a TRU-CUT 16 G needle (Unit® São Paulo, Brazil) at times 0 (M0 = first biopsy), 3, 6, 12, 24, and 48 hours, alternating the testes and site of the biopsies at each time. Previously, the rams received antibiotics (benzathine penicillin G; 20,000 IU/kg - single dose) and anti-inflammatory drugs (flunixin meglumine; 1.1 mg/kg every 24h for 3 days). Before each biopsy, asepsis and local anesthesia (1 mL of 2% lidocaine) of the scrotal skin was performed. The testes were assessed by B-mode ultrasound (MyLabFive® equipped with a 7.5 MHz linear-array transducer, Esaote - Italy) before (day 0 = D0) and on days 1, 3, 5, 9, 13, 17, and 21 after the first biopsy. The rams were slaughtered 28 days after the first biopsy, and fragments of testicular parenchyma were collected near and far from the biopsy sites and fixed in 10% buffered formalin solution for 48 h, and then immersed in 70% alcohol. The samples were submitted to dehydration, diaphanization (bleaching), and paraffinization. Then, 5 µm sections were stained by hematoxylin and eosin, mounted on histological slides, and evaluated under a light microscope. No changes were observed in the overall ultrasonographic echogenicity and heterogenicity of the testicular parenchyma. However, on D1, near the biopsies sites, it was observed hypoechoic areas (~0,6mm) surrounded by a hyperechoic line. Gradually, these areas became thinner and more hyperechoic, and remained until the last assessment. The histological analysis revealed moderate to severe, focal or multifocal tubular degeneration, peritubular necrosis and calcification, and mononuclear inflammatory infiltrate at the biopsies site. There were no histological alterations on the testicular parenchyma regions far from the biopsy site. Albeit, the focal degeneration occurs at the biopsy site, serial frequent testicular biopsies in rams did not promote ultrasound and histological changes in the testicular parenchyma far from biopsies site. In conclusion, the short-time-interval biopsies did not cause diffuse alterations in testicular parenchyma.

Keyword: TRU-CUT, breeding soundness evaluation, morphology

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Muscarinic Receptor Agonist Transactivates EGF Receptor in Rat Spermatozoa

Lucília Silva Gontijo[1], Clara de Oliveira Guimarães[1], Thainá Lima Corrêa Ramos[1], José Antônio Silva Ribas[1], Catarina Segreti Porto[2], Elisabeth Maróstica[1]

[1] Laboratory of Experimental Pharmacology, Universidade Federal Fluminense - Niterói, RJ
[2] Experimental Endocrinology Sector, Universidade Federal de São Paulo - SP, Brazil

Abstract

The expression of M_1, M_2 and M_3 muscarinic receptor (mAChR) subtypes was shown in efferent ducts and epididymis (1), as well as acetylcholinesterase positive nerve fibers with free endings to the lumen were detected in the epididymis cauda (2), suggesting the release of ACh into the epididymal fluid and its interaction with mAChRs on the spermatozoa (sptz). EGF receptors (EGFR) are also express in mature sperm of mammals and they can be transactivated by G protein coupled receptors (3). Thus, the aim of this study was characterize mAChRs in rat sptz and their possible correlation with EGFR transactivation in sperm function. Male Wistar rats (120-days old) were anesthetized with ketamine/xylasine (80/10mg.Kg^{-1}, i.p.) and the epididymis were removed and dissected (CEUA/UFF: 1026/18). The sptz were obtained from caput and/or cauda of epididymis for sperm evaluation and western blotting (WB) assays or separated in Percoll gradient for immunofluorescence (IF) assays. IF studies were performed with polyclonal goat anti-mAChR primary antibody (M_1-M_5) and rabbit anti-goat IgG, conjugated with Alexa Fluor 594. Blocking peptide was used for the negative control. For *in vivo* sperm evaluation, the rats were treated (i.v.) and sptz were isolated from epididymis cauda. For *in vitro* evaluation, sptz were incubated with 10^{-5} M carbachol (CA) or 10^{-5} M bethanechol (BE) in the absence and presence of 10^{-5} M atropine (AT) and progressive motility, vigor, membrane integrity and functionality were evaluated. WB assays were performed with primary mouse anti-EGFR and anti-pEGFR antibodies and secondary anti-mouse IgG conjugated with peroxidase. Values were expressed as mean±SEM; ANOVA, Newman-Keuls, $P<0.05$. Immature sptz showed the presence of M_1 subtype in the head and flagellum, M_2 in a punctate pattern that outlines the head region, and M_3 in the acrosome, centriole and flagellum. Mature sptz showed M_1 subtype only in the flagellum, M_2 mainly in the head and M_3 concentrated in a punctiform pattern only in the centriole region. In the sperm evaluation, no statistical differences were found among different treatments, *in vivo* or *in vitro*, when compared to the control group, in any parameter analyzed. WB assays for EGFR showed the expression of these receptors in both immature and mature gametes, with greater expression in the latter. The preliminary results also showed that in immature sptz, treatment with BE, increased the p-EGFR Tyr845 expression (intracellular via), effect abolished in the presence of AT. On the other hand, mAChR activation by BE seems to decrease the expression of p-EGFR Tyr1068 (extracellular via) in these sptz. In mature sptz, BE inhibited EGFR activation by the intracellular pathway and activated this receptor by the extracellular pathway. However, the AT was not able to block any of these effects. Our studies showed the presence of mAChR subtypes M_1, M_2 and M_3 in rat sptz, but not M_4 and M_5. These mAChR subtypes are redistributed in the gamete during its transit through the epididymis, suggesting their involvement in the maturation process. They are not directly involved with motility, but appear to be able to transactivate EGFR, both through intracellular and extracellular pathways mediated by MMPs, modulating cell transduction related to gamete capacitation and acrosome reaction. These data may contribute to the knowledge of the physiological role of these receptors in the gamete and their involvement in male (in)fertility processes, expanding the possibilities for new pharmacological targets. (Financial support: CNPq, CAPES, PROPPI/UFF).

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Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Use of "*pera*" orange peel essential oil in the freezing of bovine epididymal spermatozoa

Christianne Emmanuelle Andrade Pires Brilhante[1*], Camilla Flávia Avelino de Farias[2], Elisângela Afonso de Moura Kretzschmar[3], Sildivane Valcácia Silva[3]

[1] Pós-graduanda do Mestrado em Biotecnologia, Centro de Biotecnologia, UFPB, João Pessoa, PB, Brasil
[2] Pós-graduanda do RENORBIO, UFPB, João Pessoa, PB, Brasil
[3] Docentes do Bacharelado em Biotecnologia, UFPB, João Pessoa, PB, Brasil

***E-mail:** chrisbrilhante@gmail.com

Abstract

Seminal extenders are used in sperm cryopreservation in order to minimize the damage caused by temperature reduction. This protection is the result of the addition of several compounds that provide a favorable environment for the sperm cell. Orange is a fruit rich in secondary metabolites, such as carbohydrates and phenolic compounds, showing high antioxidant capacity. Orange essential oil is a co-product obtained from the fruit's peel, which is considered to be an agricultural residue from the juice industry. The aim of this study was to test the cryoprotective potential of "*pera*" orange essential oil (OEO) in bovine epididymal spermatozoa subjected to freezing. Two oils were used, the first one was extracted from the "*pera*" orange (*Citrus sinensis*) peel by the hydro-distillation method and the second one was obtained from the commercial brand Laszlo®. As a control group, TRIS-egg yolk (TG; 3.605 g of TRIS; 2.024 g of citric acid; 1.488 g of fructose in 100 mL of double-distilled water; 20% of egg yolk) was used and the other groups were used to to form two extenders: TY + 2% extracted OEO and TY + 2% commercial OEO. All groups were evaluated immediately after adding the extender to the sperm cells (described as 0 h) and after thawing. Subjective sperm motility (0-100%, mean of two evaluators), plasma membrane integrity (PM; double eosin-nigrosin stain) and PM functionality (hypo-osmotic test) were evaluated. After dilution, samples were added in 0.25 mL straws and submitted to the refrigeration curve (5 °C; 4 h) and, subsequently, submitted to the freezing curve in liquid nitrogen vapor (15 minutes). The straws were thawed (37 °C/30 s) and subjected to evaluation. As for motility, at 0 h, the two groups containing the OEO (17.5 ± 18.4; extracted) and (15.0 ± 10.8; commercial) had lower values than TRIS-egg yolk (75.0 ± 15.8; $p<0.05$). The same result was obtained after thawing, reaching zero motility in this condition for the groups with OEO. In the plasma membrane integrity test, the results at 0 h showed a decrease in the number of cells with intact PM within the two groups containing OEO (28.1 ± 24.8; extracted) and (10.7 ± 6.5; commercial) compared to the TRIS-egg yolk group (67.1 ± 10.1; $p<0.05$). Post-thawing results for this parameter showed no difference ($p<0.05$) to the 0 h data. As for the plasma membrane functionality test, it was observed that at 0 h there was no difference ($p>0.05$) between the number of cells with functional PM of both groups with OEO and the TRIS-egg yolk group. After thawing, there was no difference ($p>0.05$) between the TY+ 2% extracted OEO group (18.5 ± 11.7) and the TY group (38.7 ± 18.2), however, there was a decrease in the number of cells with functional PM in the TY + 2% commercial OEO group (6.25 ± 4.0). This result may be related to the high concentration of limonene present in the essential oils used (97.58%; extracted and 97.77%; commercial). Although this compound is reported to have a good tolerance in animal models, its effects in sperm cells is still poorly investigated. Based on the results found, it is concluded that the "*pera*" orange essential oil (both extracted and commercial), at a 2% concentration, does not present cryoprotective activity. Besides that, it reduced the effectiveness of the standard extender (TRIS-egg yolk) in terms of motility, integrity and functionality of the bovine epididymal spermatozoa plasma membrane.

Keywords: bull spermatozoa, cryopreservation, limonene.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

SCREENING IN SILICO OF POTENTIAL PORCINE CATHEPSIN B INHIBITORS AND THEIR BENEFITS IN SPERMATIC PRESERVATION IN PIGS

Manoela Pasini[1], Ivan Cunha Bustamante Filho[1]

[1] Laboratório de Biotecnologia da Reprodução Animal, Programa de Pós-graduação em Biotecnologia, Universidade do Vale do Taquari, Lajeado, Brazil

Abstract

Cathepsin B is an enzyme that, under normal physiological conditions, is mainly involved to intra and extracellular proteins turnover, maintaining the regulation of physiological processes. Recently, it was identified a greater amount of Cathepsin B in the seminal plasma of pigs with lower seminal quality and resistance to preservation at 5°C (1). Considering the need to inhibition of Cathepsin B, this study aims to find new approaches to enzyme inhibition of this enzyme, identifying new potential inhibitors of porcine cathepsin B through bioinformatics. Therefore, using the porcine Cathepsin B protein sequence available in UniProtKB, its tertiary structure was predicted using the Phyre2, I-TASSER and Robetta servers, and refined using the 3Drefine server. The quality of the predicted models was evaluated using the Swiss Prot Structure Assessment, Verify3D and ProSA-web servers. The best evaluated model was submitted to ligand prediction analysis using the Coach server, and the best evaluated small molecule was investigated for its pharmacophoric properties with the Pharmmit server. In the structural modeling of cathepsin B, the best results were obtained with the Phyre 2 and I-TASSER servers, the first being used for the analysis. Using the Coach server, it was possible to identify a binding site with a small molecule in the residues of the PDB protein. These binding sites are identical or very similar to the residues that compose the active site that confers the enzyme activity of cathepsin B. Based on these results, it was searched in the Pharmmit server other small molecules in the ZINC database that had similar pharmacophoric characteristics. Nine compounds were suggested, of which one was submitted to molecular docking studies using the DockThor server. In addition to this molecule, other molecules will also be evaluated by molecular docking. The molecule that best presents binding affinity for the catalytic site of cathepsin B will be tested in vitro and in vivo to validate its enzyme inhibition potential and the potential benefits in sperm preservation in porcine.

Reference

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Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Viability of cheese whey as an additive to liquid-stored swine semen extender

Anna Flávia Tischer da Silva[1]*, Manoela Pasini[1], Claucia Fernanda Volken de Souza[2], Ivan Cunha Bustamante Filho[1]

[1] Laboratório de Biotecnologia, Universidade do Vale do Taquari – Univates
[2] Laboratório de Biotecnologia de Alimentos, Universidade do Vale do Taquari – Univates

***E-mail:** aftdsilva@universo.univates.br

Abstract

The use of artificial insemination (AI) in has been widely used because it is a quick, safe and guaranteed form of fertilization, bringing economic gains to animal production. AI relies on the use of semen extenders for the maintenance of sperm traits such motility, metabolism, capacitation and fertility potential. Currently, several countries in the European Community and the Americas are banning the use of antimicrobials in semen doses for AI. Such a scenario demands the development of new technologies to inhibit the contamination of semen doses and the transmission of diseases through AI. Several new molecules and compounds have been suggested as a substitute for antimicrobials, but an ideal solution has not yet been identified. The microencapsulation of molecules with antimicrobial activity can optimize the use of these bioactive agents, being able to optimize the concentration of molecules, enabling the release of the active compound at a site of action (drug delivery) (1). The objective of this work was to determine the sperm cytotoxicity of cheese whey as a matrix for the elaboration of microcapsules. Samples (n = 5) of swine semen were collected from a commercial boar stud in Estrela, RS, Brazil. The experimental design followed the split sample model, and the addition of different concentrations of powdered and particulate cheese whey to the semen (different whey manipulations) were tested. The treatments were: 0% (negative control), 0.1%; 0.5%; 1%; 2% 5%; 10% and 20% (w/v). The following parameters of sperm motility were evaluated: total motility, progressive motility and localized motility. Motility analysis was performed using a computerized system (AndroVision, Minitube). Data were analyzed by one-way ANOVA followed by Tukey's test, assuming an alpha of 5%. It was observed that both powdered and particulate cheese whey did not change the motility parameters when added up to 10% (w/v). When 20% was added, there was a reduction from 90% (other treatments) to 42% of total motility ($P < 0.01$). It is concluded that the addition of powdered and particulate cheese whey to diluted swine semen has no deleterious effect on sperm motility when used up to 10%. New experiments with flow cytometry are under way to confirm the safety of using this additive in swine semen.

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