Glyphosate alters reproductive function by affecting the gonadotropin expression and spermatic function

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Introduction

Sexual differentiation in the brain takes place from late gestation to the early postnatal days and is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase, and glyphosate was shown to decrease aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure on the reproductive development of male offspring.

Material and Methods

To evaluate the effects of glyphosate male offspring sexual development, eighteen 90-day-old female Wistar rats were mated in a monogamous couple, and the beginning of gestation (GD1) was confirmed by vaginal smear containing spermatozoa. Glyphosate Roundup Transorb was diluted in a watery suspension and administered to the mothers once a day, p.o. (gavage) from GD18 to PND5 [control group (0 mg/kg/day) and treated group (50 mg/kg/day, NOAEL for reproductive toxicity)]. On PND4, the litters were culled to eight pups per female and kept at this proportion until weaning (PND21). The sixty-day-old male offspring were evaluated for sexual behavior and partner preference; serum testosterone, estradiol, FSH and LH concentrations; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles and spermatic functional tests for plasmatic and membrane integrity and mitochondrial activity. The growth, the weight and age at puberty of the animals were also recorded. The analysis of body growth was performed using MANOVA; the sexual behavior and age at puberty were analyzed through Mann-Whitney *U* test; the weights of seminal vesicle (drained and undrained) were compared by paired Student's t-test; and all other parameters were analyzed by Student's t-test (Statistical difference: $P<0.05$; at least 8 animals/procedure/group).

Results and Discussion

The most important findings were increases in sexual partner preference scores (71.6 ± 43.0 *vs* 312.6 ± 160.3 seconds, control and treated respectively, $P<0.01$) and the latency time to the first mount (0.6 ± 1.0 *vs* 5.2 ±7.0 seconds, control and treated respectively, $P<0.05$); testosterone (60 ± 15 *vs* 150 ± 18 ng/dl, control and treated respectively, $P<0.01$) and estradiol (1.3 *vs* 2.9 ± 0.5 pg/ml, control and treated respectively, $P<0.01$) serum concentrations; the mRNA expression (15% increased, $P<0.05$) and protein content (80% increased, $P<0.01$) in the pituitary and the serum concentration (80% increased, $P<0.05$) of LH; total sperm production (52.34 ± 4.2 *vs* 99.22 ± 19.8 $x10^6$/testis, control and treated respectively, $P<0.05$) and reserves (cauda of epididymis ($x10^6$) 53.9 ± 8.2 *vs* 47.7 ± 4.8, control and treated respectively, $P<0.05$); and the height of the germinal epithelium of seminiferous tubules (91.7 ± 2.2 *vs* 97.7 ± 1.1 control and treated respectively, $P<0.05$). We also observed an early onset of puberty but no effect on the body growth in these animals. The plasmatic and membrane integrity and also the mitochondrial activity were decreased possibly causing problems in the sperm function. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.

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Feed efficiency and its relationship to puberty in Nelore heifers

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Introduction

Feed efficiency is measured by the ratio between consumption and weight gain. An alternative measure of efficiency is residual feed intake (RFI), defined as the difference between observed and predicted consumption based on body weight and metabolic average daily weight gain. Another productive feature extremely important is the sexual precocity of females, which has been recommended in zebu cattle breeding programs in recent years. (1) reported that the reproductive traits studied (age at puberty, pregnancy rate, days to calving) did not differ between heifers classified as high, medium and low RFI. This study aimed to evaluate the reproductive tract traits of Nelore (*Bos indicus*) heifers classified according to RFI.

Materials and Methods

Heifers (n=118) assessments started with an initial age average of seven months in the test of individual consumption and 12 months for evaluation of reproductive traits. At the end of the test, described in details by (2), heifers were classified according to RFI: high (n = 37), medium (n = 45), and low RFI (n = 36). Reproductive tract traits that were evaluated by ultrasound (US) were: thickness of the endometrium (ET), average ovarian area (AOV), appearance of the corpus luteum (CL) and follicular population. To evaluate the follicular population it was performed the count of follicles smaller than 4 mm (Small Fol), between 4.1 e 7.9 mm (Medium Fol) and greater than 8 mm (Large Fol). Eight evaluations were performed at intervals of approximately 45 days between 12 and 24 months. The variables were analyzed in the GLM procedure, the class containing the RFI as the only fixed effect. The difference between the adjusted means was compared using the t-test at 5% significance level.

Results and Discussion

The onset of puberty was not influenced by the class of RFI, but the high RFI animals had lower amounts of heifers cycling than animals from medium and low classes RFI (P=0.08) (Table 1). Studies with *Bos taurus* heifers report that the animals evaluated as positive RFI displayed puberty earlier than negative RFI heifers (3), different from the present study, in which no difference was observed between the classes. Our results demonstrate that the reproductive traits evaluated by US were not influenced by the class of RFI.

Table 1. Least square means (± standard error) of reproductive traits in Nelore heifers evaluated as RFI.

	High CAR (n=36)	Medium CAR (n = 45)	Low CAR (n=37)
CL (%)	63.8[a] (23/36)	82.2[a] (36/45)	72.9[a] (27/37)
ET (mm)	11.59 ± 0.2[a]	11.49 ± 0.2[a]	11.73 ± 0.1[a]
AOV (cm²)	3.9 ± 0.1[a]	4.3 ± 0.1[a]	3.8 ± 0.1[a]
Small Fol	31.12 ± 2.3[a]	30.47 ± 2.1[a]	30.35 ± 2.4[a]
Medium Fol	2.71 ± 0.4[a]	2.55 ± 0.3[a]	2.28 ± 0.4[a]
Large Fol	1.2 ± 0.1[a]	1.1 ± 0.1[a]	1.1 ± 0.1[a]

Different letters in the same line ($p<0.05$).

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Distribution of Luteinizing Hormone Receptor (LHR) in sheep cervix during the oestrous cycle

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Introduction

The presence of functional LHR in the bovine cervix was associated with an increase of the cervical prostaglandin E2 (PGE2) synthesis around the oestrus (1). In the other hand, it is known that luteinizing hormone (LH) is involved in the control collagen turnover (2), suggesting that higher estradiol at oestrus increase the cervical LHR, stimulating PGE2 synthesis to regulate the stromal collagen content and produce the cervical relaxation. In the ewe, the cervical LHR mRNA was detected at the time of progestagen-synchronized oestrous (3). The aim of this work was determine the histological distribution of LHR protein in the ovine cervix during the oestrous cycle.

Materials and Methods

Cranial (Cr) and Caudal (Ca) cervical zones were obtained of Corriedale adult ewes at Days 1 (n=6), 6 (n=6) or 13 (n=6) after oestrus detection (Day 0). The distribution of RLH was assayed by immunohistochemistry using a polyclonal antibody (hRLH, H-50, Santa Cruz Biotechnology, Inc.CA-USA, dilution 1:75). In Cr and Ca cervix of each sheep, digitized images of 10 fields (40X) were analyzed in the following histological compartments (HC): apical (ALE) and basal (BLE) luminal epithelium, superficial (SFS) and deep (DFS) fold stroma and superficial (SWS) and deep (DWS) wall stroma, and superficial (SGE) and deep (DGE) glandular epithelium only from the Cr zone, because there are no present in the Ca. Images were cleaned to obtain only the HC to evaluate (Photoshop 6.0) and the percent of positive area (%positive area) respect to the total HC area was measured (Image Pro Express). The results (mean±sem) were analyzed by ANOVA (Mixed Proc, SAS), including the fixed effects of day of oestrous cycle, cervical zone, HC and the interactions between them.

Results and Discussion

The LHR immunostaining was diffusing in the cytoplasm of cells in all the HC studied. There was an effect of day of oestrous cycle ($P<0.0003$) and HC ($P<0.0001$) on %positive area, as well as an interaction between day of oestrous cycle and cervical zone ($P<0.003$). The %positive area was higher on Day 13 (18.3±3.6) than on Days 1 (13.6±3.1) and 6 (15.9±3.9). The %positive area was higher in ALE (39.7±4.0) than BLE (20.1±2.4), and lower in SGE (12.7±2.3), DGE (12.8±2.6), SFS (11.7±1.7) and SDF (12.4±1.6). The lowest %positive area was in SWS (6.8±1.3) and DWS (7. 1±1.2). This data disagree with the lower expression of the LHR mRNA in the subepithelial stroma respect to the luminal epithelium and smooth muscle previously reported (3). The %positive area in the Cr zone on Day 13 was higher than in the Ca zone on Day 13 and was higher than in the Cr zone on Day 1 (Figure). These results shown that cervical responsiveness to LH is higher in epithelia than in stromas, and higher during the late luteal phase of oestrous cycle, in concordance with the cervical COX-2 (a rate limiting enzyme of the PGE2 synthetic pathway) expression assayed in cervix of the same ewes (4). The results suggest that the LHR/PGE2 system could prepare the cervix to the next oestrous cycle.

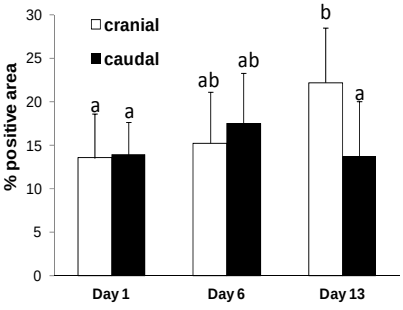

Figure. Percent of positive area (%positive area) of Luteinizing Hormone Receptor (media±sem) in cranial and caudal cervix of sheep on Days 1, 6 or 13 (n=6, each group) after oestrus detection (Day 0) ($p<0.003$).

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Inhibition of prostaglandin F2α biosynthesis by flunixin meglumine during preluteolysis and luteolysis in dairy heifers

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Introduction

Secretion of sequential pulses of prostaglandin F2α (PGF2α) by the endometrium on about 16 days postovulation induces luteolysis in cattle (1,2). Pulses of PGF2α, as represented by a PGF2α metabolite (PGFM), occur during the preluteolytic, luteolytic, and postluteolytic periods in heifers (3). The aim of this study was to determine a minimal dose of Flunixin Meglumine (FM) that effectively blocks PGF2α biosynthesis during the expected period of luteolysis and characterize the effects of short-term FM treatment during the preluteolytic and luteolytic periods on PGFM pulses and luteolysis in dairy cattle.

Material and Methods

In Experiment 1, a single FM dose of 0, 0.5, 1.0 or 1.5g was given to Holstein heifers (n=4/group), 16 days postovulation to inhibit the synthesis of PGF2α, based on concentrations of PGFM. Blood samples were collected hourly for 10 hours after the treatment. In Experiment 2, FM (2.5 mg/kg) was given to heifers at three 8-h intervals, 16 days postovulation (first treatment = Hour 0). Blood samples were collected at 8-h intervals from 15 to 18 days in a vehicle and FM group (n=16/group). Hourly samples were collected from Hours−2 to 28 in 10 heifers in each group. Heifers that were in preluteolysis or luteolysis at Hour 0 based on plasma progesterone (P4) concentrations at 8-h intervals were partitioned into subgroups.

Results and Discussion

The results in Experiment 1 indicated that a total dose of 0.5 g FM was ineffective in preventing PGF2α biosynthesis, whereas 1.0 g was partially effective, and 1.5 g (equivalent to 2.5 mg/kg) was most effective. In Experiment 2, PGFM concentrations during hourly samples and PGFM pulses were reduced ($P < 0.05$) by FM treatment in each subgroup (Fig.1). Concentration at the peak of a PGFM pulse in the FM group was greater ($P < 0.05$) in the luteolytic than in the preluteolytic subgroup. For the preluteolytic subgroup, the first decrease ($P < 0.05$) in P4 concentration after Hour 0 occurred at Hours 24 and 40 in the vehicle and FM groups, respectively. Plasma P4 concentrations 32 and 40 hours after the beginning of luteolysis and length of luteolysis in the luteolytic subgroup were greater ($P < 0.05$) in the FM group. In conclusion, treatment with FM inhibited PGFM production more during preluteolysis than during luteolysis, and a reduction in prominence of PGFM pulses during luteolysis delayed completion of luteolysis in dairy heifers.

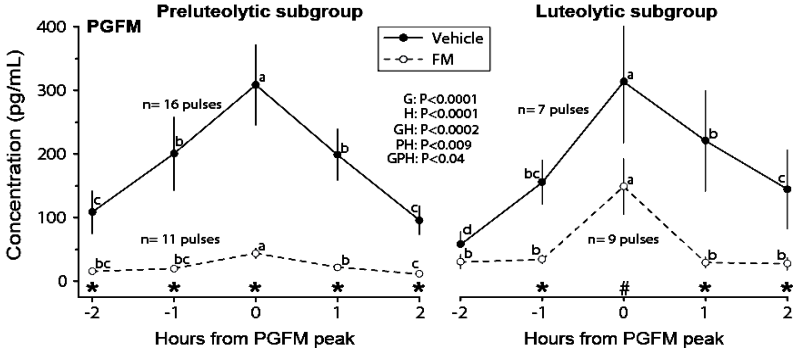

Figure 1. Mean ± SEM concentrations in PGFM pulses. Heifers were in either preluteolytic subgroup or luteolytic subgroup at first treatment. Main effects of group (G), period (P), and hour (H) and the interactions (GH, PH, and GPH) that were significant by factorial analysis are shown. Hours of a difference ($P < 0.05$) and an approaching difference ($P < 0.1$) in PGFM concentrations between groups are indicated, respectively, by an asterisk (*) and hatch mark (#). [a-d]Within a group, means without a common letter differed ($P < 0.05$). Experiment 2.

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Characterization of the effects of the periovulatory endocrine milieu on bovine endometrial gene expression on day 7 post-estrus: a candidate gene approach

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Introduction

In cattle, higher progesterone (P4) concentrations during early pregnancy are associated with higher rates of embryonic development and regulate endometrial gene expression during the first week post-estrus. However, the effects of P4 concentrations on endometrial gene expression have not yet been assessed by an experimental model that closely mimics the physiology of the estrous cycle. Here, we tested a model to pharmacologically manipulate pre-ovulatory follicle growth to ultimately generate contrasting post-ovulatory progesterone concentrations. Objective was to determine the influence of the periovulatory endocrine milieu on the endometrial expression of candidate genes (FABP3, ANPEP, ASGR2 and LPL) as well as genes selected from global gene expression data (NUPR1 and SKALP), in Nelore cows on day 7 (D7) post-estrus.

Material and Methods

Twenty-two cyclic, non-lactating Nelore cows received a P4-releasing device along with estradiol benzoate on day -10 (D-10). Animals were divided to receive a prostaglandin analog (PGF; HP group; N=11) or not (LP group, N=11) on D-10. Progesterone devices were removed and PGF injected on D-2.5 on cows from HP group, and on D-1.5 on cows from LP group. Ovulation was induced with GnRH on D0. Plasma P4 concentrations were measured daily from D0 to D7. On D7 animals were slaughtered and endometrial fragments collected. Complementary cDNA was synthesized from endometrial total RNA extracts. Relative gene expression between experimental groups was determined by qPCR.

Results and Discussion

Expression of the transcripts FABP3, ANPEP, ASGR2, LPL, and NUPR1 was not different between groups HP and LP. Lack of differential expression for candidate genes *ANPEP*, *ASGR2*, *FABP3* and *LPL* is not in accordance with previous reports (1,2). We propose that this discrepancy may be explained, at least partially, by the difference between the experimental model used in our study in comparison to those from other reports (i.e. exogenous P4 supplementation during first week post-estrus). In contrast, expression of the SKALP gene was 2.4 times greater in the LP group in comparison to the HP group ($P<0.05$). SKALP protein stimulates recruitment and function of inflammatory cells but also modulates their activities (e.g. controls tissue damage induced by leukocyte proteases). Our results support a differential pattern of endometrial gene expression in response to the periovulatory endocrine milieu. Additionally, the reduced expression of SKALP could be interpreted to result in an increase in the uterine proteolytic activity associated with a local immune suppression in animals that ovulated larger follicles and reached higher P4 concentrations on D7 post-estrus. These conditions may influence extracellular matrix remodeling of the endometrium and facilitate the establishment of the early interactions between the conceptus and the endometrium and potentially influencing pregnancy success.

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LH plasma concentration in prepubertal Nelore heifers (*B taurus indicus*) supplemented with protected fat or carbohydrate excess

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Introduction

The aim of this study was to evaluate the protected fat supplementation effect and/or carbohydrate excess on plasma leptin level in prepubertal heifers.

Material and Methods

Third contemporary heifers were used with average live weight of 167±13 kg, sorted in 3 groups and receiving or not protected fat (long chain fatty calcium salts acids, Megalac-E®): Control Group (CG, n=10), 4,2 kg of ration associated with sugar cane bagasse, 500 g of grounded corn plus for each animal. Fat Group (FG, n=10) 4.2 kg of ration with sugar cane bagasse, 200g of protected fat per animal. Excess Group (EG, n=10) 4.2 kg of ration with sugar cane bagasse, 500g of ground corn plus 200g of protected fat for each animal, all diet was divided in two periods. The animals had an adaptation period and the treatments started after 12 months of age. Venous blood samples were collected every four days from the 9th to the 18th month of age, every 7 days from 18th to 20th month of age and daily for 17 days during the 10th, 12th, 14th, 16th months and. In three animals (one from each group) and on alternated days (every month 5 animals were collected; all the animals underwent to sequential samples collected every 20min for 12h either before or during feed treatment) during the 11th, 13th, 14th and 16th months. Assays LH quantification were adapted from those described by Bolt et al. (1990) and measured from 8 animals per group. The CV intra LH-test was 5.88% for the high control (3.15 ng /ml) and 5% for the low control (1.07 ng/mL), the inter-assay CV was 8.86% for the high control and 9.08% for the low control and assays sensitivity was 0.058 ng/mL. The results were analyzed by repeated measures ANOVA.

Results and Discussion

There was no difference in mean LH concentration between the three groups either before or during the treatments, either for the samples collected every 4 days or every day for 17 days and also for samples collected every 20min for 12h. Before the treatment period there was no difference between the control groups, and Excess Fat in the number of peaks (1.63±0.7; 2.25±1.2;1.6±1); the total area (112.8±90; 96.7±54.7, 113.8±70 (ng/ml)min). During treatment period the Fat group had a higher ($p= 0.05$) number of peaks (3.12±1.64) than the Excess group (1.86± 0.90) and the control group (2.63± 0.74). But there was no difference in of the mean total LH secretion area ($p>0.05$). It was concluded that nutrition may interfered with puberty age, apparently changing the LH secretion pattern.

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Stimulation of endogenous PRL pulses from luteolysis to ovulation and effects on hormonal and ovarian dinamics in mares

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Introduction

Prolactin (PRL) is a polypeptide hormone that has specialized roles in mammalian female reproduction. In mares, studies were done on stimulation of PRL during transition from anovulatory to ovulatory season, being the increase on PRL related with stimulation of follicle development and advancement of first ovulation (1,2). In addition, during luteolysis and postluteolysis, PRL pulses were greater than on preluteolysis and temporally related with PGFM pulses (3). The aim of the present study during the estrous cycle in mares was to evaluate the effects of a PRL stimulating substance (sulpiride) on: Secretion and pulsatility of PRL and PGF2α, and dynamics of the CL and follicles.

Materials and Methods

A control group (Ct, n = 9) and a sulpiride group (Sp, n = 10) were used. Sulpiride (25 mg/mare) was given every 8 h from Day 13 postovulation until the next ovulation. Blood sampling and ultrasound scanning were done every 12 h until ovulation. In addition, samples were collected hourly for 12 h on Day 14. Hourly sampling encompassed the 8 h period between the fifth and sixth sulpiride treatments.

Results and Discussion

Repeated sulpiride treatment did not appear to maintain PRL concentrations at 12-h intervals beyond Day 14. Therefore, the hypothesis that a long-term increase in PRL altered luteal and follicular end points was not testable. Hourly samples were collected on Day 14 from the hour of a treatment (Hour 0) to Hour 8 for characterization of concentrations and pulses of PRL and a metabolite of PGF2α (PGFM). Concentrations of PRL reached maximum at Hour 4 in the Sp group. The pulses were more prominent ($P < 0.008$) in the Sp group (peak, 19.4 ± 1.9 ng/mL) than in the Ct group (11.5 ± 1.8 ng/mL). Hourly concentrations of PGFM, number and characteristics of PGFM pulses, concentrations of P4, and percentage of CL with color-Doppler signals of blood flow were not affected by the increased PRL. A novel observation was that the peak of a PRL pulse occurred at the same hour or 1 h later than the peak of a PGFM pulse in 8 of 8 PGFM pulses in the Ct group and in 6 of 10 pulses in the Sp group ($P < 0.04$), indicating that sulpiride interfered with the synchrony between PGFM and PRL pulses. The hypothesis that sulpiride treatment during the equine estrous cycle increases the concentrations of PRL and the prominence of PRL pulses was supported.

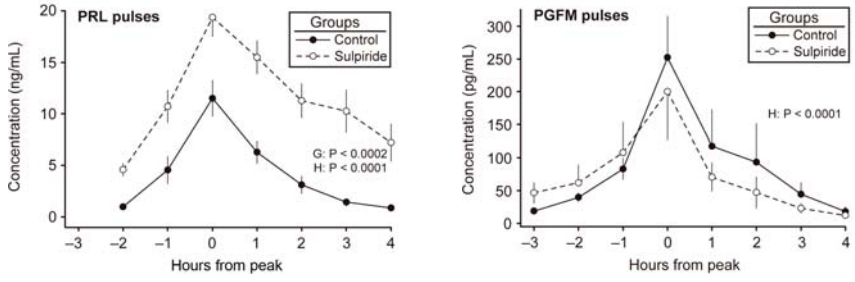

Figure 1. Mean ± SEM concentrations during pulses of PRL and PGFM in controls and sulpiride-treated mares. The hour effect (H) for each pulse was expected, owing to centralization to the pulse peaks. A group effect (G) was found only for the PRL pulses.

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Morphological studies on bovine oocytes matured *in vitro*

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Introduction

In the microscopic evaluation of oocytes, some concepts have appeared to discern the cell quality mainly for the production of embryos *in vitro*. However, after a careful morphological evaluation, some cells do not show satisfactory development for the production of embryos, such as the formation of the follicular *antrum* and the appearance of *cumulus oophorus* that may be related to low cell viability. The aim of this paper is to study a method of morphological evaluation of oocytes, evaluating the dimensions of the *zona pellucida*, *corona radiata* and diameter of the *antrum*. To carry out this work we used oocytes of cattle (Zebu) collected from slaughterhouses.

Materials and Methods

The bovine oocytes used in this work were obtained after maturation *in vitro*, which was performed by the protocol developed by the company Biovitro ®. Subsequently, the oocytes were analyzed by stereomicroscopy and bright field microscopy at 4x and 40x magnification, respectively. Images were obtained by using the camera Samsung SDC - 435, and for the evaluation of the dimensions the program used was EchoImageViewer ® (View) that allowed the morphological evaluation and measurement of the *zona pellucida*, *corona radiata* and *antrum* diameter.

Results and Discussion

One hundred cells were analyzed and classified. Fifteen percent of the functional units were found in follicular development, it was possible to clearly visualize both the *zona pellucida* (average size of 573.1 mm) as the *corona radiata* (average size of 89.8 mm) (1); Ten percent of the cell units evaluated were determined in stage atresia in which many morphological features of apoptosis were observed, in oocytes and granulosa cells, not allowing the determination of the proposed parameters. These results are determinate by the follicular dynamics (2,3). In 75% of the remaining cells was not possible observed clearly and classify the follicular condition, due a progressive degeneration of the follicles. According to the literature, this result may be due to the action of mechanical and physical factors, from the removal of the ovaries, aspiration of oocytes and maintenance means (4). So it was not possible to verify in the present study the determinism of the physiological quality of ovarian follicles, however this work suggested that the quality of the functional units of follicular development are the result of ovulatory cycles earlier (5,6).

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Levels of serum and follicular glucose related with oocyte quality on Girolando cows

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Introduction

The control of ovarian follicle development is associated to the production of factors, acting in systemic or local pathways (1). Metabolic changes related to negative energy balance (NEB) over dairy cows postpartum can modify the microenvironment and the quality of follicular oocytes, leading to reduced fertility (2). The purpose of this study was to establish the correlation of glucose levels in blood and follicular fluid with oocyte quality in dairy cows postpartum.

Material and Methods

Girolando cows (n=162) on third lactating order were submitted to transvaginal follicular aspiration at the 30, 45, 60, 75 and 90 postpartum days. The oocytes were classified according to quality (3) and sent to in vitro maturation for 24 hours. Then the gametes were evaluated concerning to nuclear maturation by the presence of the first polar body. Samples of blood, largest follicle fluid (LF, > 8mm) and small follicles fluid (SF, 2 - 8mm) were collected and glucose concentration (mg/dL) determined by Accu-Check Active® system. The data were analyzed by statistical program BioEstat5.0® and expressed on mean ± s.e.m.

Results and Discussion

The mean glucose levels (mg/dL) of LF (80.18±2.59), SF (47.39±1.94) and blood (60.27±0.95) did not differ ($p>0.05$) between the evaluated moments. Among the parameters, the LF glucose rate remained higher ($p<0.05$) and there were difference between glycemia and SF at the 45 and 60 days postpartum. The average of total oocytes (6.68±0.37) and viable oocytes (3.18±0.30) showed no difference between the aspiration sessions and e the nuclear maturation rates were higher ($p<0.05$) from 45 postpartum day achieving the best result on 75 day (69.9%). It was observed a negative correlation ($p<0.05$) between glycemia and SF glucose criteria ($r = -0.1924$). Furthermore, there was no relation between blood, LF and SF glucose rates with oocytes quality. The results indicate that metabolic changes in serum levels can reflect on follicular environment and that only glucose rates is not able to compromise the morphological quality and competence of in vitro matured oocytes.

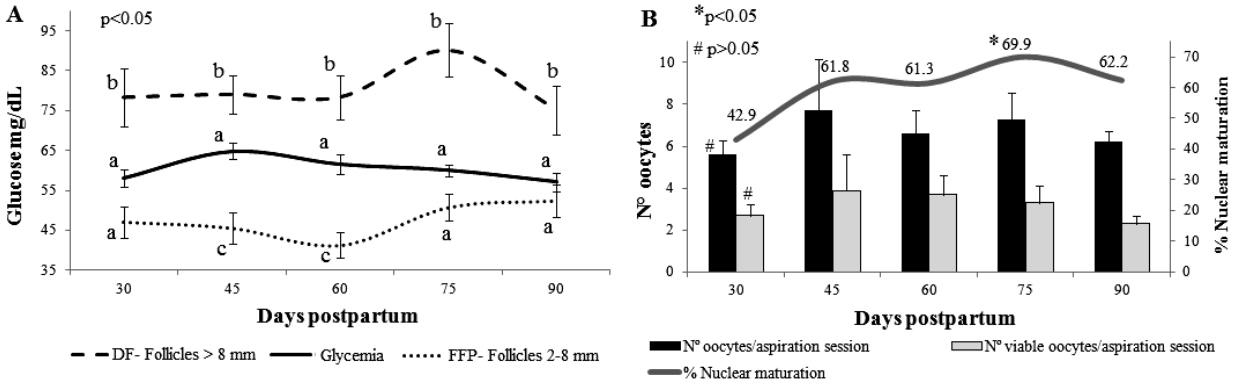

Figure 1. (A) Metabolic oocyte profile and (B) recovered oocyte quality under in vitro nuclear maturation.

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Metabolic profile of follicular fluid and serum from dairy heifers and cows related with oocyte quality

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Introduction

The intrafollicular enviroment features associated to serum profile may influence the oocyte development (1-2). The aim of this work was relate serum and follicular profile with oocyte quality from dairy heifers (n=17) and cows (n=162) between 30 and 90 days postpartum.

Material and Methods

During the female transvaginal follicular aspiration, fluid samples were collected from large follicles (LF; >8mm) and from small follicles (SF; between 2 and 6 mm), beyond intravenous blood. The gama-glutamyl-transaminase (GGT; U/L), total cholesterol (TC; mg/dL) and glucose (mg/dL) concentrations were determined by commercial tests (Labtest®; Accu-Check®). The oocytes derived from the process were evaluated according to their quality (3). Statistical program Bioestat®5.0 analyzed the data, which expressed on mean ± S.E.M.

Results and Discussion

Serum levels differed ($p<0.001$) among heifers and cows for GGT (17.7±0.8 vs 29.8±1.1), TC (95.9±2.9 *vs* 133.8±4.3) and glucose (80.7±1.8 *vs* 60.2±0.9), respectively. The LF rates for total cholesterol were higher ($p<0.05$) in cows (68.4±4.2 *vs* 55.6±2.2) and major ($p<0.05$) for glucose on heifers (96.1±7.8 *vs* 80.5±2.7). SF does not differed ($p<0.05$) between heifers and cows for glucose (45.1±4.7 *vs* 47.7±1.6) and TC (52.2±2.7 *vs* 64.8±5.4). Blood, LF and SF glucose concentrations were different ($p<0.01$) in cows (60.2±0.9; 80.5±2.7 e 47.7±1.6) and heifers (80.7±1.8; 96.1±7.8 e 45.1±4.7). Total cholesterol differed ($p<0.01$) among serum, LF and SF in cows (133.8±4.3; 68.4±4.2 e 64.8±4.2) and just between serum and LF (95.9±2.9 e 55.6±2.2) in heifers ($p<0.01$). Aspiration session average of recovered viable oocytes was superior ($p<0.01$) in heifers (5.4±0.7) related to cows (3.0±0.3). Significant correlations ($p<0.01$) were observed in cows among glucose and total cholesterol LF ($r = 0.39$) and SF ($r = 0.34$), furthermore, this associations were observed in heifers, just in LF glucose and total cholesterol ($r = 0.38$). The negative correlations ($p<0.05$) for total oocyte number ($r = -0.31$) and viable oocyte ($r = -0.40$) were associated with heifers serum and SF glucose levels, respectively. Results showed that oocytes develop in different follicular environments which can be modified according to serum profile and these interactions should affect the gamete quality.

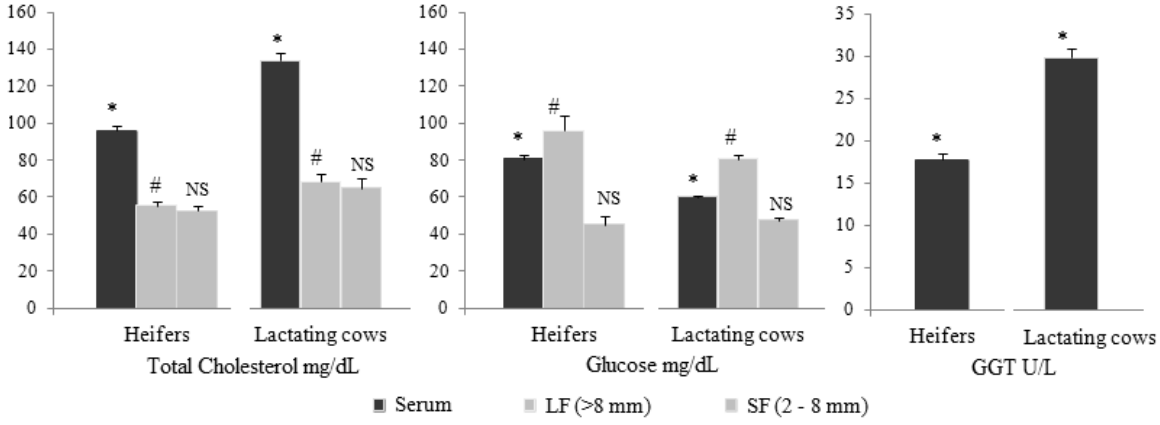

Figure 1. Follicular fluid and serum profile for total cholesterol, glucose and GGT in heifers and lactating cows. *$p<0.001$; #$p<0.05$.

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Induction and synchronization of ovulation in pluriparous goats outside breeding season

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Introduction

Hormonal treatments, like the association of progesterone and equine chorionic gonadotrophin (eCG), are used to synchronize and/or induce estrus in goats, reducing seasonal effects in the reproduction of this specie (1, 2). This study aimed to evaluate the viability of the use of human chorionic gonadotrophin (hCG) as an alternative to the eCG to induce and synchronize estrus in pluriparous goats outside breeding season.

Material and Methods

Eighty three pluriparous goats were used. They received two protocols of induction and synchronization of oestrus: T1 – eCG (n=41) and T2 – hCG (n=42): D0 – insertion of intravaginal dispositive of progesterone in animals of both groups; D5 – application of 75 µg of D-cloprostenol in animals of both groups, animals of T1 received 300 UI of eCG; D6 – withdraw of intravaginal dispositive of progesterone in T1 and T2; D7 – 24 hours after withdraw of intravaginal dispositive of progesterone, animals of T2 received 250 UI of hCG. Twelve hours after removal of intravaginal dispositive of progesterone, observation of estrus was started; it was performed during 72 hours, in intervals of 12 hours with aid of a ruffian. Statistical analyses were performed by chi-square test at 5% of probability.

Results and Discussion

There was no difference between treatments in the estrus rate 12 hours after the removal of intravaginal dispositive ($P>0.05$). However, 24, 36, 48 and 60 hours after withdraw of intravaginal dispositive, T1 showed more animals in estrus than T2 ($P<0.05$). After 72 hours of withdraw of intravaginal dispositive, the number of animals in estrus was similar between treatments ($P>0.05$). Equine chorionic gonadotrophin (eCG) shows function similar to FSH, eCG improves the development of follicles and, consequently, it increases the concentration of oestradiol, inducing estrus (3), this may explain the higher efficiency of eCG to concentrate estrus. Human chorionic gonadotrophin (hCG) shows function similar to LH, therefore, dominant follicle should be responsive to LH, which occurs in the final development of follicle and maturation of oocyte (4), thus, the efficiency of hormonal treatment will depend on phase of follicular wave at the moment of use of hCG. It was concluded that eCG and hCG are efficient to induce estrus in goats; however, eCG was more efficient to synchronize estrus than hCG.

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Fipronil prenatal exposure modifies the strous cycle but not the sexual behavior in female rat offsprings

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Introduction

Fipronil is a broad spectrum insecticide, it acts inhibiting the chloride channels associated to GABA receptors. It is used to control a range of soil and crop pests, as well as fleas and ticks, ants and cockroach (1). Fipronil also interferes with the endocrine system of mammals (2). Pregnant females, when exposed to pesticides, can breed descendants with physical, physiological and neurobehavioral impairment due to critical periods in fetal development (3). Thus, the aim of this study is the assessment of sexual parameters of rat's offspring whose mothers were exposed to fipronil during the pregnancy period.

Material and Methods

40 pregnant female rats received different doses of fipronil *per os* (0.1, 1.0 and 10.0mg/Kg) or water (n=10/group) from the 6th until the 20th pregnancy day. The female offspring were evaluated in adulthood for sexual parameters: estrus cycle and sexual behavior. The estrous cycle was followed by fifteen consecutive days by vaginal smear and the sexual behavior was measured by lordosis coefficient. All results were analyzed using GraphPad Prism version 5.00 for Windows.

Results and Discussion

The female offspring, prenatally treated with 0.1 mg/Kg of fipronil, showed an increase of estrous phase incidence (6,33 ± 0,83 times – fig. 1a) and decreased diestrus phase incidence (2,29 ± 1,70 times – fig.1b), and an irregular estrus cycle was showed on all treated groups. Although the estrous cycle was irregular, there were no differences in the lordosis coefficient, in other words, no alterations in the sexual behavior. These results showed that prenatal exposure to fipronil caused alterations in the estrus cycle of female offspring, but not behavioral alterations, suggest that fipronil has transgenerational endocrine effects on female reproductive parameters.

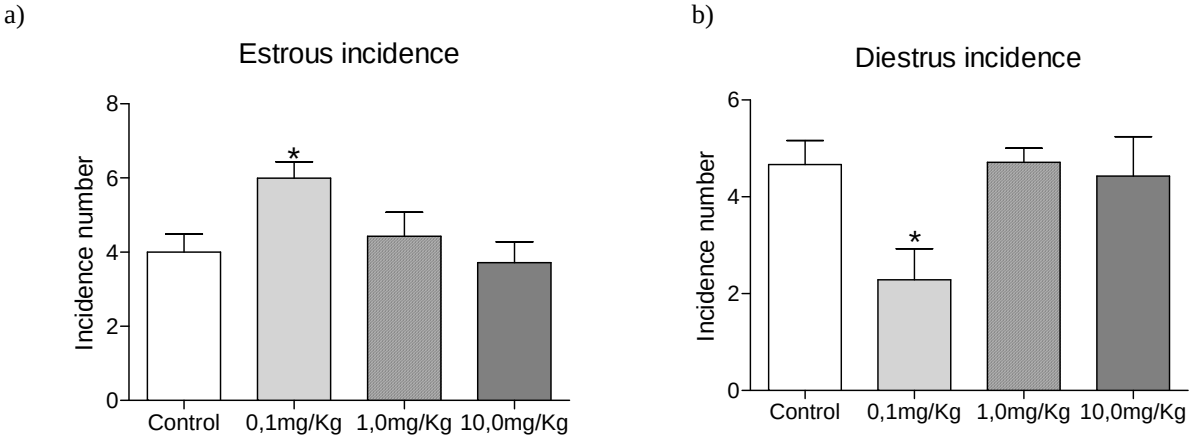

Figure 1. Estrous and diestrus phase incidence in 15 consecutive days of observation: (a) Estrous incidence; (b) diestrus incidence (One-way ANOVA with Dunnett's post. *p<0,05).

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Long form of leptin receptor gene expression in goat follicles

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Introduction

Environmental influence, such as nutrition, has long been known to have a profound effect on ovarian activity (1), but the underlying mechanism remains poorly understood. A growing body of evidence has shown that the metabolic hormone leptin is a potential mediator of the effects of nutrition and metabolism on reproductive process. Actions of leptin are mediated mainly via the long form of leptin receptor (*LEPRb*). Identification of *LEPRb* mRNA in ruminant ovary suggests that leptin may be involved in the ovarian function (2,3). Then, the aim of the present work was to examine the *LEPRb* expression in the goat follicle.

Material and Methods

At a local slaughterhouse, approximately 40 ovaries were collected from adult goats (*Capra hircus*) and transported to the laboratory on ice. Granulosa, theca, and cumulus cells and oocytes were collected from small (1 to 3 mm) and large (3 to 6 mm) antral follicles. Cells that were isolated from the same size of follicles were collected into pool. Total RNA was immediately extracted from each cell pool using TRI® reagent (Sigma, USA) according to the manufacturer's procedure. One microgram of total RNA was reverse transcribed in a 20-µL volume using kit ImProm-II™ (Promega, Madison, WI, USA) as recommended by the manufacturer. PCRs were performed in a 25-µL reaction using Taq DNA polymerase and a specific primer to *LEPRb*. The PCR conditions were 35 cycles of 94°C, 61°C, and 72°C for denaturation, annealing and extension, respectively. A reaction without cDNA was used as negative control. PCR product was visualized in an agarose gel (1.5%) and a standard sequencing procedure (ABI PRISM 3100 Genetic analyzer, Applied Biosystems) was used to verify the specificity of the amplified products.

Results and Discussion

RT-PCR experiments revealed the presence of *LEPRb* mRNA in granulosa, theca, and cumulus cells and oocytes derived from different sizes of follicles. This finding is in agreement with a study in ovine and bovine follicles (2,3). The RT-PCR product was confirmed by sequence analysis, which showed that the DNA sequence generated was 99% homologous to the known sequences of goat *LEPRb*. This finding suggests that leptin may play a role in the ovary in an autocrine/paracrine fashion and takes part in important process concerning goat reproduction.

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Cell-secreted vesicles called exosomes in equine ovarian follicular fluid: characterization during various phases of follicular development

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Introduction

In mammals, ovarian follicles have to be selected, stimulated to growth, and ovulate in order to achieve a successful pregnancy (1). The ovarian follicle is a closed unit containing different somatic cells namely theca cells, granulosa cells, cumulus cells, and oocyte. Communication among these cells is necessary to synchronize the functional movement of this unit until ovulation. It is important to point out that communication between cells occurs in the presence of a fluid-filled cavity called antrum containing follicular fluid (FF) (2). Recently, we identified the presence of cell-secreted vesicles (exosomes) in ovarian FF (3). Exosomes are small membrane nanovesicles (30-70nm) of endosomal origin that contain mRNA, miRNAs (small noncoding RNA), and proteins. Exosomes are released into the extracellular environment and mediate intercellular communication. Our overall goal is to identify the role of exosomal miRNAs during ovarian follicle development. In this study, we postulated that exosomal miRNAs in FF play a role in mediating cell communication during follicle maturation. To test this hypothesis, we characterized the expression profile of exosomal miRNAs in FF at deviation, mid-estrus and prior to ovulation using the mare as a model.

Material and Methods

Granulosa cells and FF were obtained *in vivo* from light-horse mares (aged 3-13 yr). Reproductive tracts were examined daily during the follicular phase to identify and sample the dominant follicle at follicular deviation (~22mm), and mid-estrus (~35mm) follicles. Follicular maturation was induced by administration of deslorelin (1.5mg, iv) and pre-ovulatory follicles were aspirated 32-36 hr later.

Results and Discussion

Exosomes were isolated, and real-time PCR analysis was conducted to examine the relative levels of 384 mature miRNA sequences. Data analysis revealed dynamic changes of different exosomal miRNAs in FF during follicular development. At deviation a total of 288 miRNAs were identified in exosomes isolated from FF, whereas at mid-estrus a total of 125 exosomal miRNAs were identified. Prior to ovulation, exosomes isolated from FF contained a total of 281 miRNAs. Specific examples include significantly ($P \leq 0.05$) higher levels of miR-346 and miR-485-3p at deviation, significantly ($P \leq 0.05$) higher levels of miR-433, miR-323-3p, and miR-140-3p at mid-estrus, and significantly ($P \leq 0.05$) higher levels of miR-143, miR-196, miR-206, miR-323-5p, miR-328, miR-383, miR-448, miR-490-3p, and miR-496 at pre-ovulation. Potential pathways targeted by exosomal miRNAs during follicular maturation include cell proliferation, motility, and prevention of apoptosis. Finally, we also examined the presence of exosomal miRNAs in FF collected from old (n=3) (aged > 20 yrs) mares to examine potential changes related to aging. Eight exosomal miRNAs were present at significantly ($P \leq 0.05$) higher levels at deviation in FF collected from young compared to old mares. Similarly, at mid-estrus 7 exosomal miRNAs were present at significantly ($P < 0.01$) lower levels in FF collected in FF collected from young compared to old mares. At pre-ovulation 26 miRNAs were present at significant ($P \leq 0.05$) higher levels in exosomes of FF collected from young compared to old mares. In conclusion, our data indicate that during normal ovarian follicular development exosomal miRNAs levels change dynamically and potentially are involved to fine-tune cell signaling pathways. Moreover, we postulated that age-related changes in exosomal miRNAs in FF might underlie age-related changes in oocyte maturation.

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The association of prostaglandin with ovulation inducers in mares

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Introduction

The use of ovulation inducers is widely used to enhance the reproductive efficiency of mares. The hCG and deslorelin has its known effect promoting ovulation in up to 48 hours, previous studies have shown that the dose of 1000 IU of hCG was as effective as higher doses (1). The prostaglandin (PGE_2 and $PGF_{2\alpha}$) have a key role in follicular rupture only a few hours preceding ovulation. $PGF_{2\alpha}$ may act by inducing hydrolase liberation by cells of the ovarian epithelium, when $PGF_{2\alpha}$ does not enhance plasmin and collagenase activities the follicle does not undergo its typical changes at the apex. In mammals an increase prostaglandins is noted following the gonadotropin surge (2). The enzyme cyclooxygenase-2 (COX-2) is responsible for synthesizing prostanoids in the ovaries of rats, as well as mares, COX-2 is the enzyme involved in the production of PGF and PGE in the follicle 10 to 12 hours before ovulation. The time sequence in expression of this COX-2 enzyme paralleled the concentration of its products in follicular fluid, PGE and PGF, increased from baseline levels at 30 hours post-hCG treatment to peak concentrations at 36 h, and decreased again by 39 h post-hCG treatment (3). Due to these mechanisms it has been found that mares treated with flunixin meglumine, a prostaglandin synthesis inhibitor, have a higher incidence of ovulatory failure (3). This study aimed to evaluate the time between induction and ovulation in mares using different inducers with or without $PGF_{2\alpha}$.

Materials and Methods

Seventeen estrous cycles were evaluated, divided into four groups according to preovulatory treatment received : **GI**: 5 mg dinoprost tromethamine (Lutalyse®) I.M. (n = 5), **GII**: 1000 IU hCG (Chorulon®) I.V. associated with 5 mg of dinoprost tromethamine I.M. (n = 4); **GIII**: 0.75 mg deslorelin I.V. associated with 5 mg of dinoprost tromethamine I.M. (n = 3); **GIV**: 1000 IU hCG I.V. (n = 5). Follicular dynamics was monitored daily by transrectal ultrasonography and treatments were applied when the largest follicle reached a diameter ≥ 35 mm, in presence of uterine edema ≥ 3.0. After 24 hours of ovulation induction, we proceeded the follicular development monitoring each six hours up to ovulation. For the statistical analysis method was applied to a completely randomized design, taking into account the principles of repetition (with BOX-COX transformation).

Discussion

The time between induction and ovulation was higher in group I (significance between 1% to 5%) when compared to groups II, III and IV, the transformed averages were 78.0, 37.5, 32.0 and 37 , 2 hours, respectively. Prostaglandin is essential for ovulation, when its action is inhibits using anti-inflammatory, ovulation does not occur (3), however from the results obtained for group I, it appears that exogenous administration of prostaglandin, does not anticipated ovulation under these conditions, probably because its effect occur only few hours before the process of follicular rupture (2). The other groups presented similar results, showing that the association of $PGF_{2\alpha}$ with the inducers did not reduce the interval between induction and ovulation. However, these data are preliminary and more studies are being performed in order to evaluate a larger sample size for statistical significance, and to investigate whether the association of prostaglandins with ovulation inducer may increase their effectiveness by reducing ovulatory failure.

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In vitro estradiol production by bovine secondary follicles using two different culture systems

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Introduction

In vitro culture of secondary follicles up to the antral stage is a very important tool for providing information regarding follicular requirements during the late preantral follicle phase. The production of autocrine/paracrine factors by preantral follicles in the culture medium has beneficial effects on the survival and growth of different cell types (1). Therefore, the aim of this study was to compare estradiol production in a system with partial removal and replacement of medium versus the addition of a small amount using α-MEM$^+$ or TCM-199$^+$ during long-term (32 days) in vitro culture.

Materials and Methods

Secondary follicles (n=151) were cultured individually under mineral oil for 32 days at 38.5°C in 5% CO_2 in a humidified incubator. The basic culture media consisted of α-MEM$^+$ or TCM-199$^+$ (pH 7.2 to 7.4) supplemented with ITS (insulin 1000 mg/l, transferrin 550 mg/l and sodium selenite 0.67 mg/l), 2 mM glutamine, 2 mM hypoxanthine, 3 mg/ml bovine serum albumin (BSA), 50 µg/ml ascorbic acid and rFSH (100 ng/ml). The medium change protocols tested were: Conventional (MEM-C and TCM-C) – removal and subsequent addition of the same amount (60 µl) in a 100 µl drop, maintaining the initial volume of 100 µl; and Small Supplementation (MEM-S and TCM-S) – addition of 5 µl of fresh medium to an initial small drop (50 µl), resulting in a final volume of 125 µl on the last day of culture. Medium change was performed every other day. To evaluate follicular steroidogenesis in vitro, concentrations of estradiol were measured by enzyme-linked immunosorbent assay (ELISA) in the culture media before (day 0) and at the end (day 32) of culture in all treatments. Data were analyzed by one-way ANOVA.

Results and Discussion

The mean estradiol concentration in the fresh culture media at day 0 for both media was 0.2 ± 0.0 ng/ml. A group effect ($P < 0.0001$) for estradiol concentration was observed at day 32. Regardless of the type of medium, follicles submitted to the Small Supplementation protocol (MEM-S: 12.6 ± 2.3 and TCM-S: 2.5 ± 0.4 ng/ml) produced more ($P < 0.004$) estradiol than under the Conventional protocol (MEM-C: 3.3 ± 0.5 and TCM-C: 1.5 ± 0.3 ng/ml). However, estradiol production was higher ($P < 0.003$) using α-MEM$^+$ for both change protocols than TCM-199$^+$. Recently, we have observed that bovine secondary follicles are able to grow and acquire an antrum cavity when cultured in vitro (2). In this study, estradiol release by secondary follicles after 32 days of in vitro culture was indicative of follicle differentiation and growth. In vitro differentiation of bovine secondary follicles has been previously obtained (3,4). In conclusion, these results suggest the occurrence of greater cell differentiation of bovine secondary follicles characterized by greater estradiol production in the culture medium when using the Small Supplementation protocol with α-MEM$^+$. The Small Supplementation protocol has demonstrated to be more practical and faster for medium change during preantral follicle culture, avoiding longer exposure of the follicles to outside environmental conditions.

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Growth hormone benefits the in vitro development of isolated canine preantral follicle in the presence of Follicle-Stimulating Hormone

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Introduction

The growth hormone (GH) stimulate the growth and differentiation in almost all mammalian cells and play a essential role on ovarian follicular growth and development (1). However, it is unknown how this hormone acts on *in vitro* culture of canine preantral follicle. To this, the aim of this study was to evaluate the effect of GH addition on *in vitro* culture of canine preantral follicle in the combination or not with Follicle-Stimulating Hormone (FSH).

Material and Methods

Canine secondary preantral follicles were isolated and cultured for 18 days. In order to test the effect of GH in combination or not with FSH, six following treatments were used: The control, which consisted of Minimum Essential Medium (MEM) supplemented with BSA (3 mg/mL), glutamine (2 mM), hypoxanthine (2 mM), transferrin (5.5 µg/mL), selenium (5 ng/mL) and ascorbic acid (50 µg/mL) or plus GH at concentration of 10 or 50 ng/ml, in the absence (GH10 or GH 50) or presence of FSH GH10 +FSH or GH50 + FSH). The parameters evaluated were follicular growth and the rate of antrum formation.

Results and Discussion

The rate of antrum formation after culture was higher in GH-treated groups plus FSH, than in the others treatments, with no significant difference between the two concentrations of GH. Furthermore, at the end of in vitro culture the group GH50+FSH showed a higher follicular diameter when compared to the other treatments. This work is an agreement with Magalhães et al, 2011, which observed that GH associated with FSH acted as a regulator of follicular development. Furthermore, Wang et al (2006), related that the role of GH on follicular growth may be associated with its reported effects on cellular proliferation and steroidogenesis. In conclusion, GH at high concentration (50 ng/ml) associated to FSH, stimulates the *in vitro* development of isolated canine preantral.

Table 1. Antrum formation and diameter after 18 days of culture of canine preantral follicle cultured in differents concentrations of GH in absence or presence of FSH.

	Antrum formation(%)	Diameter D18(µm)
Control	90,00 B	379.01 ± 50.31 B
MEM+ FSH	90,00 B	410.95 ± 64.64 B
GH10	89,47 B	393.31 ± 72.63 B
GH50	87,18 B	387.47 ± 63.33 B
GH10 + FSH	92,50 AB	419.32 ± 59.88 B
GH50+ FSH	100,00 A	481.01 ± 71.85 A

Differents superscripts (A,B) between rows differs significantly.

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Effect of interval to timed artificial insemination after synch treatment on pregnancy rates in Nelore cows

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Introduction

The time of insemination relative to ovulation is an important factor in conception rate due to two physiological factors: 1) time required for sperm transport and capacitation in the female genital tract and 2) survival of both gametes (spermatozoa and oocyte) (Pursley et al., 1998). The present study evaluated the influence of interval from end of synchronization treatment to fixed-time artificial insemination (TAI) on pregnancy rate.

Materials and Methods

The experiment was conducted on the campus of the University of São Paulo, Pirassununga, College of Veterinary Medicine and Animal Science. On Day 0, Nelore cows (n=665) received an intravaginal progesterone-releasing device on a random stage of estrous cycle, and an injection of 2.0 mg of Estradiol Benzoate (EB). On Day 8, the inserts were removed and the cows received an injection of 0.150 mg of PGF2α and 300 IU of equine chorionic gonadotropin (eCG). On Day 9, cows received an injection of 1.0 mg of EB. Cows were randomly assigned to receive TAI at 1 of 3 intervals post-PGF: 30 to 35 h (Group 1, n=257), 36 to 40 h (Group 2, n=252) or 41 to 45 h (Group 3, n=256). Frozen-thawed semen from two Nelore bulls were assinged in a balanced distribution across each TAI group. Ultrasonic diagnosis fo pregnancy status was assessed 30 days after TAI.

Results

Pregnancy rates were influenced ($P < 0.05$) by TAI interval (63.8, 75.4, and 54.7% for Groups 1, 2, and 3, respectively).

Pregnancy rates by interval to AI after PGF

Hours after PGF	Pregnancy Rate (%)	N
30 to 35	63.8ab	164/257
36 to 40	75.4a	190/252
41 to 45	54.7b	140/ 256
MEANS	64.6	494/765

Conclusion

Based on prior studies that have documented the interval to ovulation using this protocol in Nelore cattle, we interpret these data to imply optimum fertility occurs when inseminations are performed between 6 and 4 hours prior to expected time of ovulation.

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Intrafollicular hormones, growth factors and follicle blood flow in mares

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Introduction

Changes in follicular fluid during follicle growth reflect the relationship between follicular cells and gonadotropins, as well as the autocrine and paracrine action of regulators of hormonal function and cellular growth. Follicular fluid contents play an important role in follicular development, oocyte maturation, ovulation and follicle vascularity. Equine follicular fluid from growing, healthy follicles of different diameters has not been evaluated during spontaneous follicular waves, but only partially after induced waves with follicle ablation or hormonal treatments. The objective of this study was to elucidate the changes in follicular fluid concentrations of gonadotropins, steroids, IGF-1, VEGF, PGF2α and nitric oxide during the different phases of spontaneous follicular waves and to correlate those findings with follicle wall blood flow in mares.

Materials and Methods

Non-lactating mares (n=16) were used during the reproductive season in the Northern Hemisphere. Diameters of all follicles ≥6 mm were measured daily by B-mode transrectal ultrasonography after day 10 of the estrous cycle (day 0 = ovulation). The largest growing follicle after at least three consecutive evaluations was distributed in 6 groups (n=8/group) according to diameter (15-19, 20-25, 26-30, 31-35, 36-45 mm) or presence of an impending ovulatory follicle (IOF) in which ultrasound scanning was performed every 6-12 h. Follicle wall blood flow was evaluated using color- and Power-Doppler ultrasonography immediately before ultrasound-guided transvaginal follicle aspiration of the selected follicle. Follicular fluid concentrations of hormones (FSH, LH, estradiol [E2], progesterone [P4] and PGF2α), vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (free IGF-1) were determined by enzyme immunoassay and nitric oxide (NO) was determined by colorimetric assay.

Results and Discussion

Follicle blood flow increased ($P<0.0001$) with follicular development. FSH ($P<0.002$) and LH ($P<0.05$) concentrations increased when the follicles reached 20-25 mm and remained higher in the following groups, with a slight but not significant decrease in FSH concentration for the IOF group. A positive correlation ($r=0.30$, $P<0.05$) between FSH and LH was observed. FSH concentration and blood flow followed the same pattern during follicular development, characterized by an elevated concentration of this gonadotropin in growing follicles after expected follicle diameter deviation (20-25 mm group). E2 concentration increased along with follicle development. The 15-19 mm group had the lowest E2 concentration, while the groups above 31-35 mm had the highest ($P<0.0001$) concentrations. High P4 concentrations were detected in the IOF group, and the E2:P4 ratio increased ($P<0.0001$) until the 31-35 mm group and decreased ($P<0.005$) in the IOF group. E2 and P4 were positively correlated with follicle blood flow ($r=0.56$ and $r=0.48$, respectively, $P<0.001$) and LH concentration ($r=0.36$, $P<0.02$ and $r=0.39$, $P<0.01$, respectively). The correlation between blood flow and steroid hormones may have a role in the systemic/paracrine effects of these hormones during the different phases of an ovulatory follicular wave. IGF-1 and PGF2α follicular fluid concentrations did not differ among groups. The 36-45 mm and IOF groups had lower ($P<0.0007$) VEGF concentrations than the 15-19 mm follicle group. Intrafollicular VEGF had a negative correlation with follicle blood flow ($r= -0.43$, $P<0.005$). Furthermore, VEGF was negatively correlated with E2 ($r= -0.44$, $P<0.004$) and P4 ($r= -0.31$, $P<0.04$) and tended to be positively correlated with NO ($r= 0.29$, $P< 0.06$). NO concentration was lower ($P<0.03$) in the 36-45 mm and IOF groups when compared with follicles at expected diameter deviation; similar results have been observed in cattle (1) and buffalo (2). In conclusion, these results demonstrated for the first time in mares the intrafollicular dynamics of gonadotropins, E2, P4, IGF-1, PGF2α, VEGF and NO according to follicle diameter and indicates relationships with follicle wall blood flow.

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Diestrus progesteronemia affects growth of the pre-ovulatory follicle in Nelore cows

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Introduction

Numerous studies have demonstrated the influence of progesterone (P4) on follicle growth in cattle [1;2]. The aims of this work were (i) to control pre-ovulatory follicle (POF) diameter by manipulating the exposure of the dominant follicle (DF) to P4 and (ii) to evaluate the relationship between the diameter of the POF prior to the induction of ovulation and follicular growth rate from D-1 to D1.

Material and Methods

Fifty-one non-lactating, cyclic Nelore cows were pre-synchronized with 2 injections of sodium cloprostenol (PGF) 14 days apart. Ten days after the second PGF (D-10) animals received a new (Experiment 1; Exp1) or a second use (Experiment 2; Exp2) intravaginal P4 device, along with estradiol benzoate. In each experiment, animals were divided to receive (large follicle group; LF) or not (small follicle group; SF) a PGF injection on D-10. Progesterone devices were removed and PGF was injected on D-2 in LF cows, or on D-1.5 in SF cows. Ovulation was induced with GnRH on D0. Analyses were performed including only animals that ovulated between 18 and 48 hours post-GnRH (n=36). Group means were compared by student's t test, frequency data was analyzed by Fisher's exact test and regressions by the Minitab 16 software.

Results and Discussion

Dominant follicle diameter on D-2 and D-1 was larger on LF group than on SF group. On the other hand, DF daily growth rate between D-1 and D1 was greater on SF group than on LF group. Regression analysis, including animals from both experiments, revealed a negative correlation between the diameter of the DF on D-2 and rate of follicle growth from D-1 to D1 (R^2: 0.23; $P < 0.01$; N=36). Furthermore, the diameter of the DF on D-1 and follicle growth rate from D-1 to D1 showed cubic relationship, represented by a greater growth rate of follicles smaller than 9 mm, and smaller growth rate for follicles larger than 12.5 mm, whereas follicles between 9 and 12.5 mm presented similar growth rates (R^2: 0.40; $P < 0.001$; N=36). It was observed that 82% (14/17) of the ovulations on Exp1 occurred between 24 and 48 hours post-GnRH in comparison to 38% on Exp2 (13/34; $P < 0.003$). When considering the first 24 hours post-GnRH, 50% (17/34) of the cows on Exp2 ovulated during this window, whereas no ovulations were observed for animals from Exp1 (0/17; $P<0.0001$). In conclusion, increased exposure to P4 (i.e. P4 device, presence of functional CL and shortened proestrus) during the synchronization protocol leads to a reduction on the diameter of the POF. Moreover, the increased P4 exposure is associated with an enhanced follicle growth rate after P4 device removal. Additionally, it was observed that the frequency of early ovulations was higher in animals treated with P4 devices of second use.

Table 1. Follicle diameter and growth rate on experiments 1 e 2 (mean ± standard error of the mean).

Variables	Experiment 1			Experiment 2		
	LF n = 7	SF n = 7	P	LF n = 11	SF n = 11	P
Follicle on D−2 (mm)	11.2 ± 0.6	8.5 ± 0.8	< 0.01	11.0 ± 0.6	8.7 ± 0.5	< 0.01
Follicle on D−1 (mm)	12.2 ± 0.3	10.0 ± 0.8	< 0.05	12.0 ± 0.6	9.4 ± 0.5	< 0.01
POF (mm)	13.8 ± 0.4	12.1 ± 0.7	< 0.05	13.2 ± 0.5	11.5 ± 0.4	< 0.01
Growth rate between D−1 e D0 (mm/day)	1.4 ± 0.4	1.8 ± 0.3	> 0.05	1.7 ± 0.4	2.2 ± 0.4	> 0.05
Growth rate between D−1 e D1 (mm/day)	0.81 ± 0.1	1.02 ± 0.1	< 0.08	0.70 ± 0.2	1.1 ± 0.2	< 0.07

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Effect of low dose of estradiol cipionate after artificial insemination service on fertitility post- treatment in Holstein cows of a large dairy farm of North México

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Introduction

A 12-month calving interval is generally considered the most economically desirable for dairy cows. To achieve this, the empty cows after Artificial Insemination (AI) service, must be inseminated at soon as possible. Managerial and economic efficiencies of cattle production systems are most likely to benefit from having a compact calving pattern, which can be achieved synchronizing AI to increase breeding efficiency. Macmillan et al. (1997) reported that a small dose of estradiol benzoate (EB) injected 12, 13, or 14 d after a synchronized estrus and AI partially synchronized return to service soon (9 to10 d), and fertility at the estrus associated after return was also increased by treatment, and also the pregnancy rates initial to AI remained unchanged. The current study was done with the objective to analyze the effect of injection of 1 mg estradiol cipionate (ECP) 13 d after AI on pregnancy rate at the estrus associated and ongoing pregnancy in Holstein cows.

Materials and Méthods

Lactating Holstein cows (n=110) were used in this research during two months of a summer season. Body condition scores were similar in all animals. Fifty-five cows out of one hundred ten were injected with 1 mg of estradiol cipionate (ECP) (Lab.Pharmacia & Upjohn, México) 13 days after AI service and the other fifty-five were not injected (control group). All cows had been inseminated after the voluntary waiting period of 50 days postpartum at first service between 60 and 70 days in milk. Heat detection was made by closed visual observation and podometer. Pregnancy diagnosis was performed by transrectal palpation 45±3 days after service.

Results and Discussion

Pregnancy rate was low for both groups possibly due to heat stress during the summer season, but no difference was found between them, what means that injection of 1 mg of ECP d 13 after service didn't affect ongoing pregnancy (23.6% vs 20% injected and non injected cows respectively). Fertility at the first estrus associated with return to service was increased by the injection (50% vs 18% injected and non injected cows respectively, $P< 0.05$). The results are similar to those of Macmillan et al. (1997) and Burke et al. (2000) with estradiol benzoate, but in our case the number of animals used was higher in milk production and during the summer season. In conclusion 1 mg of ECP injected d 13 after AI service increased fertility at the estrus associated with return to service and didn't affect ongoing pregnancy in lactating dairy cows.

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Effect of eCG during Ovsynch protocol and hCG post-insemination on conception rate in high-yielding Holstein cows

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Introduction

Several studies have been done administrating treatments with human chorionic gonadotrophin (hCG) under fertility. There are differences among breeds, applied doses, date of the injection with respect to the Artificial insemination (AI), injections after AI to detected estrus, in comparison with one fixed-time Artificial insemination (FTAI) protocol, type of FTAI protocol, comparison between primiparous and pluriparous cows, season of the year and experimental units by group of treatment (1). It has been shown that the equine chorionic gonadotrophin treatment (eCG) increases the development of the dominant follicle and ovulation of a biggest follicle which result in a rise and functional corpus luteum (2). The objectives were to evaluate the effects of eCG at the time of injection during Ovsynch Cloprostenol and hCG 5 days after AI.

Materials and Methods

Lactating Holstein cows (n=590) from two to four calving and 34 days in milk were pre-synchronized with 500 µg of Cloprostenol (Celosil; MSD, México) 14 days apart (34 and 48 days in milk). One hundred forty cows with a 44lts/day production, which did not were inseminated because no heat was observed, were assigned randomly with 35 replications per treatment. The treatments were : 1) Ovsynch (control); 2) Ovsynch + 400 UI of eCG (Folligon; MSD, México) on day 7 of Ovsynch; 3) Ovsynch + 3500 UI de hCG (Chorulon; MSD, México) by Im injection day 5 after AI and ; 4) Ovsynch + 400 UI of eCG + 3500 UI hCG on day 7 Ovsynch and day 5 after AI, respectively. All treatments began 12 days after the last injection of Closprostenol and ultrasonic equipment was used to evaluate ovaries structures. The pregnancy diagnostic was performed by transrectal palpation 39 days after AI.

Results and Discussion

Treatment 2 was higher ($P<0.05$) in conception rate than the other treatments (Fig 1). At the beginning of the treatments the ovaries status did not influence the results. Likewise, there was no significant effect of treatment 4 related to the conception rate on Holstein cows.

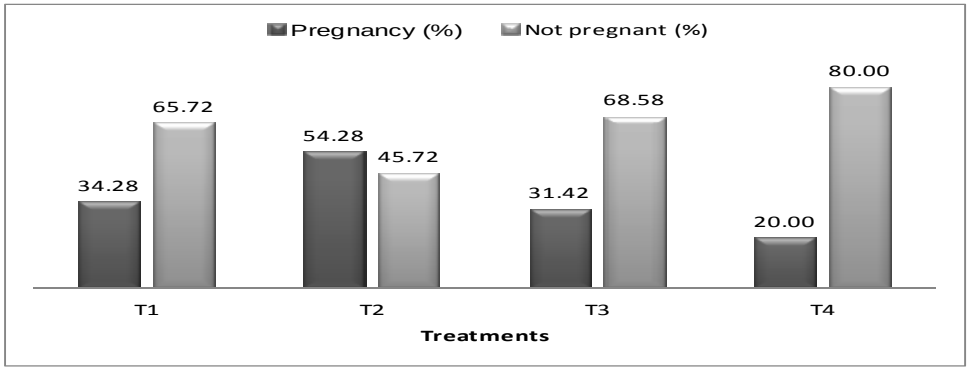

Figure 1. Conception rate in all treatments

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Hormonal treatment to improve embryo survival in beef cows after fixed time artificial insemination

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Introduction

Successful maternal recognition of pregnancy (MRP) is directly involved with early survival of the bovine embryo(1). This study evaluated strategies to enhance ovarian function of cows submitted to fixed time artificial insemination (FTAI) and thereby optimize MRP.

Material and Methods

Twenty-eight cycling non-suckled Nelore (*Bos taurus indicus*) cows received one progesterone (1.9g) intravaginal device and 2mg estradiol benzoate. Seven days later received 250 µg of Dinoprost Thrometramine (PGF2alpha). The device was removed two days later and 0.5 mg of estradiol cipionate was given. FTAI (D 0) occurred 48 hours after device removal (2). Females were assigned to receive either no further treatment (Control, n=14) or 200mcg of gonadorrelin (GnRH, im) on D5 and 2500 IU of hCG (im) on D12 (Treated, n=14). Cows were submitted to ultra-sound examination of ovaries and plasma progesterone determination (P4) on D5, D12, D18 and D28 (3). Categorical data were analyzed by Chi-square test and continuous variables were assessed by ANOVA in a 2x2 factorial design (pregnancy status taken as presence or absence of conceptus vs. treatment group).

Results and Discussion

Conception rate was not different ($P>0.05$) between control (36%) and treated cows (50%). All treated cows developed at least one accessory corpus luteum and had greater P4 on D12, D18 and D28 than control cows ($P<0.05$) regardless of pregnancy status. Treated non-pregnant cows (n=7) had greater ($P<0.05$) dominant follicle diameter on D18 (13.7±3.2mm) than pregnant contemporaries (10.5±2.2mm, n=7). A similar trend was observed among control non-pregnant cows (11.9±3.0mm, n=9) and pregnant cows (9.7±2.2mm, n=5). Hormonal treatment did not influence ($P>0.05$) size of original CL on D12 or D18 as compared with control cows on D12 and D18. Treated cows that were detected as non-pregnant (D28, n=7) had greater ($P<0.05$) P4 on D18 (6.28±3.74 ng/mL) than control cows regardless of their pregnancy status. Among pregnant cows, treated cows (n=7) had greater ($P<0.05$) P4 on D18 (8.63±3.44 ng/mL) and D28 (9.09±4.87 ng/mL) than control cows (n=5; 5.02±2.24 and 5.79±3.30 ng/mL, respectively). In summary, hormonal treatment induced the formation of accessory corpus luteum and increased P4 around the time of MRP regardless the pregnancy status determined through the presence of a conceptus.

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Influence of human GDF9 recombinant protein on the quality of bovine embryos *in vitro* produced

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Introduction

In vitro production (IVP) of embryos is an auxiliary and strategic tool in animal reproduction. During oocyte maturation, complex cascades of activation and inhibition of enzymes, hormones and growth factors occur, resulting in nuclear and cytoplasmatic maturation (1). GDF9 growth factor actively participates in the process of *in vivo* oocyte maturation, which involves the interconnections between oocyte and surrounding cumulus cells, as well as regulation and absorption of amino acids, glycolysis and cholesterol biosynthesis in cumulus cells via oocyte junctions (2,3). The present study aimed to evaluate the effects of human GDF9 recombinant protein (GDF9) added to the maturation medium on cleavage, development and quality rates of bovine embryos *in vitro* produced.

Materials and Methods

Oocytes were *in vitro* matured (IVM) in TCM199 medium containing four different concentrations (0, 50, 100, and 200 ng/mL) of human GDF9 (GDF9 human ®, Sigma-Aldrich), fertilized after 24 h, and subsequently the probable zygotes were *in vitro* cultured. Cleavage and blastocyst rates were evaluated after 48 h and 7 days of culture, and subsequently the quality of embryos produced was verified. For embryo quality evaluation, it was determined the cells number ratio in the inner cell mass (ICM) and trophoblast cells (TF) by modified differential fluorochrome staining (4). The embryos were considered superior when exhibited a proportion ICM:TF greater than 1:2. Cleavage and blastocyst rates were assessed by chi-square (χ^2), while the ratio between ICM and TF were assessed by Tukey test, in SAS v.8.2.

Results and Discussion

GDF9, regardless of the concentration, did not affect the cleavage - 92.06% (603/655); 86.92% (565/650); 91.95% (553/608) and 89.87% (586/652), and blastocyst rates - 49.92% (327/655); 52% (338/650); 46.87% (285/608); and 46.93% (306/652), respectively for each treatment. The results of the present study corroborate with Jee et al. (3) who found no relationship between the addition of recombinant human GDF9 and blastocyst rates. The percentage of superior embryos were 68.33% (82/120); 40.83% (49/120); 50.83% (61/120) and 83.33% (100/120), respectively for each treatment. The higher percentage of superior embryos obtained using the TCM199 supplemented with 200 ng/mL GDF9 confirms the findings by Yeo et al. (5). Therefore, the recombinant human GDF9 increases the proportion of embryos which presents more ICM cells, contributing positively to the ratio between ICM and TF.

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Biological research in primordial canine germ cells

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Introduction

Embryonic germ cells are pluripotent cells derived from primordial germ cells originated during embryonic development. These cells are essential precursors of gamete cells, which fusion will produce a totipotent zygote initiating the embryonic development program that will originate adult cells and a next generation of germ cells (5). Studies of pluripotent embryonic stem cells using animal models have been successfully performed for treatment of several genetic diseases, especially using canine models due to homology with human. Therefore the main of this study is to further understand the cellular biology of canine germ cells via isolation, characterization and establishment of a cellular lineage. Our long term goal is to establish a cell bank to be used as a cell source for therapeutic applications in veterinary and human medicine.

Materials and Methods

Eight canine embryos with approximately 22 days of gestation from females submitted to ovariohysterectomy surgery during neutering campaign were used in this study. After collection, embryos were washed in PBS and transported to a hood with sterile conditions. For isolation and culture of embryonic germ cells, we isolated the paramesonephric region, and after added specific media compost with DMEM, L-Glutamine, noNn essential AMINO acids, 2-mercaptaethanol, penicillin and streptomycin, with and without bFGF. For the microscopic analyses the cells were fixed, submitted to serial dehydration using alcohols after added osmium 2% and analyzed by electromicroscopy and transmission. For immunohistochemistry, embryos were processed as routinely for this technique, and was used Oct4 as pluripotent marker (4).

Results and Discussion

After isolation, cells had active proliferation and multiplication forming compact colonies. With eight days we observed that cell linages were very proliferative, showing early senescence in the tenth passage. The cell replication was done only when the cells are presented with 80% confluence, approximately every 96 hours. Cell culture can be maintained with or without bFGF, however with the addition of bFGF there is an increase in the number of colonies (2). Cell morphology can be described as been round, flat and compact, with large round nucleus, large amount of vesicles, well developed ribosomes and presence of high amount of mitochondria (1-3). By immunohistochemistry, Oct4 signal was present in the paramesonephric region including the genital ridge region (4).

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Effect of meiotic arrest with butyrolactone I on *in vitro* production of bovine embryos

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Introduction

In the cytoplasm of mammalian oocytes, the nuclear and cytoplasmic maturation occur at different times and this fact seriously undertakes the viability of *in vitro* matured oocytes. Thus, one alternative to improve the quality of oocyte and consequently the quality of embryo is the use of drugs that induce inhibition of meiotic maturation (nuclear), causing at the same time a better cytoplasmic maturation. Thus, this study, evaluated the effect of the addition of a meiotic arrest butyrolactone I (BL-I, Enzo Life Sciences International, Inc. 5120 Butler Pike Plymouth Meeting, PA 19462) on *in vitro* production of bovine embryos.

Material and Methods

Nelore oocytes were matured in TCM-199 with Earle's salt + 10% FCS, FSH and LH, in 5% CO_2 atmosphere. To delay meiosis, the oocytes were maintained for 6 hours in medium in presence of Butyrolactone I 100 µM. Then the oocytes were cultured for 18 hours in agent-free medium to meiosis resume, completing 24 hours of maturation. After 24 hours of maturation (day 0), oocytes were fertilized in human tubal fluid (HTF – Irvine, New Zeland) under the same condition above. Semen was selected through Percoll gradient and the concentration adjusted to 2 x 10^6 sperm/mL. The presumably zygotes were culture in 90µL droplets of SOFaa + 0.6% BSA + 2.5% FCS in 5% CO_2, 5% O_2, 90% N_2 atmosphere until day 7, when blastocyst rate was evaluated. There were made 5 routines. Data were analyzed with ANOVA, followed by Tukey test using the general linear model (PROC GLM) of SAS 9.2. The level of significance adopted was 5%.

Results and Discussion

No statistical differences were observed in blastocyst production rate: Control: 42.3 ± 2.7%; Butyrolactone I 100µM: 42.2 ± 2.3% (P=0.97). The butyrolactone I was able to produce embryos without degeneration and with similar qualities to the agent-free group. However, to really prove if its action does not compromise embryonic development, techniques such as TUNEL (Terminal deoxinucleotil transferase Uracil Nick End Labeling) are being performed for the *in situ* detection of apoptotic cells.

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Sorted semen affect expression of genes related to implantation and pregnancy recognition

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Introduction

In vitro produced embryos using sorted semen by flow citometry presents altered mRNA expression patterns of developmentally important genes, showing that sperm sexing by flow cytometry may result in sperm damage, semen fertility reduction (1) and ongoing pregnancy failure (2). The density gradient centrifugation as an alternative method and was able to separate X and Y-bearing sperm without reducing the sperm fertility (3). The purpose of this work was to compare the relative mRNA abundance of gene transcripts related to implantation, pregnancy recognition and placenta formation of bovine embryos produced *in vitro* using unsexed semen (control group), sorted by density gradient centrifugation or by flow cytometry.

Material and Methods

Blastocysts at day 7 of *in vitro* culture were used to examined the relative mRNA expression of four genes: *AKR1B1*, *COX2*, *IGF2R* and *PLAC8*. Poly(A) RNA was extracted and reverse transcription (RT) was realized to produce cDNA. Quantification of mRNA transcripts was performed by real-time quantitative (q) RT-PCR. For qRT-PCR, three groups of cDNA per experimental group, each obtained from 10 embryos, were used with two repetitions for all genes. Experiments were conducted to compare relative levels of each transcript with those of the housekeeping histone *H2AFZ* in each sample. The comparative cycle threshold (CT) method was used to quantify expression levels. Fold changes in the relative gene expression of the target gene were determined using the formula $2^{-\Delta\Delta CT}$, with control group like reference. Data were analysed using one-way ANOVA with 5% of significance.

Results and Discussion

Flow cytometry methodology resulted in a significant reduction in the expression level of *AKR1B1* that could be related to pregnancy failure (4), and reduction of *COX2* levels could be associated to embryos with poor quality (5). Density gradient centrifugation increased the transcripts levels of *PLAC8* that could be related to embryo quality, pregnancy recognition and success (4, 5). Density gradient methodology increased the expression of genes related with pregnancy establishment and embryo quality. These results suggest that density gradient centrifugation produce better quality embryos to pregnancy induction than flow cytometry.

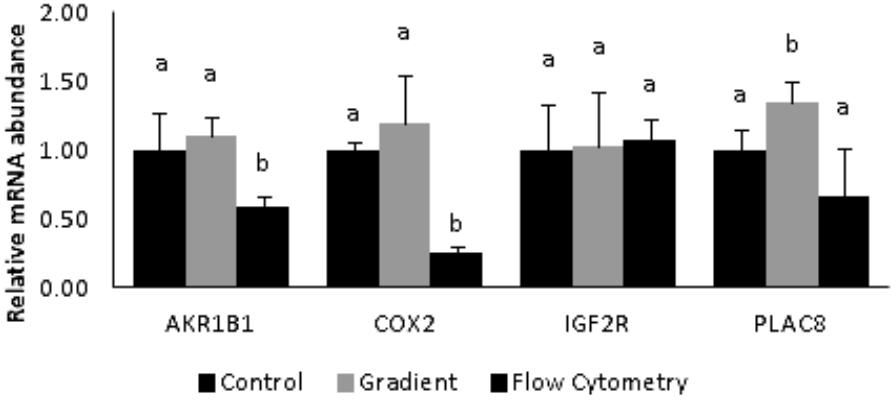

Figure 1. Relative mRNA abundance of genes related to implantation, pregnancy recognition and placenta formation of bovine embryos produced *in vitro* using unsexed semen (control), sorted by density gradient centrifugation and or flow cytometry.

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In vivo evaluation and differentiation of mesenchymal stem cells from the yolk sac of bovine embryos

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Introdution

The embryonic yolk sac (YS) is considered a promising source of stem cells since it contains clusters of both mesenchymal and hematopoietic cells for fetal development. These cells represent a population of multipotent progenitor cells that are capable of differentiating into osteogenic and adipogenic cell linages that have mesenchymal derivation. Our previous results have shown bovine YS-derived mesenchymal cells stem cells (bYS-MSC) is a promising source of mesenchymal stem cells as they meet the criteria of MSC classification, proposed by the International Society for Cell Therapy, in regards of surface markers expression. The objective of this study was to test the capability of the bYS-MSC to differentiate into osteogenic and adipogenic cells and test whether or not these cells are able to promote growth of tumor with three germ layers *in vivo* after injection in immunocompromised nude mice.

Material and Method

Eight bovine embryos at of 38 ± 3 days (range from 33 to 42 days) of development were collected from a local slaughterhouse. The YS were dissected from the bovine embryos using a stereoscope, than washed in saline solution, minced and digested in type IV collagenase for 2 hours at 37°C and 5% of CO_2 for cellular disaggregation and primary cell culture establishment. The dispersed cells were then washed twice in α-MEM (Gibco) supplemented with 10% of fetal bovine serum. The bYS-MSC cells were then cultured at 37°C; 5% of CO_2 for 24 h. The adherent cells were maintained in culture for several passages. The cells were assessed for viability after freezing and thawing cycles. The growth curve was determined plating cells at low density ($2x10^4$ cells) in a 90-mm plastic culture dish, then the cells were ressuspended and the total cell number counted every other day by until the 10^{th} passage. Also, for the ability of colony formation, $2x10^4$ cells were plated in 90-mm plastic culture dishes. Also, bYS-MSC at the 2^{nd} passage were cultured in specific media for induction and differentiating (StemX and VivoTM Differentiation Kits, R & D Systems) to induce bone and fat tissues differentiation while the negative controls were kept in α-MEM medium. The cells then were fixed with 4% paraformaldehyde and stained with Alizarin Red (3,4) to assess the osteogenic differentiation and with Red Oil for adipogenic differentiation (1,2,4). Finally, 1×10^4 bYS-MSC at the 5^{th} passage was intradermally injected into the dorsal lumbar region of the nude mice to evaluate the ability tumor formation and the three germ cell layers of the bYS-MSC.

Results

In culture bYS-MSC were initially fibroblast-like, but after 16 days in culture with osteogenic differentiation medium, the cells acquired polygonal morphology, had sparse cytoplasm and were filled with cytoplasmic vacuoles perhaps as a result to cellular senescence (1,2). The growth curve peaked at 16^{th} passage and the colony formation was observed after 10 days in culture. Osteogenic differentiation was observed after 24 days in culture where slight deposition of bone was observed in the extracellular matrix. The cells cultured with adipogenic differentiation medium contained birefringent granules filled with lipids within the cytoplasm after 16 days in culture and after 20 days showed the accumulation of intracellular lipid vacuoles present in almost all cytoplasm. These adipogenic cells stained positively with Red Oil (1,2,4). The site of bYS-MSC injection in the skin and all organs of the nude mice were examined. There were no signs of tumor formation (5) at the injection site and all the organs analyzed (i.e. liver, spleen, lugs). bYS-MSC showed to possess a multipotent profile to differentiate into cell lineages which arise from the mesenchmal layer during the embryo development *in vitro*. Further studies will be focused on the ability of bYS-MSC differentiation *in vivo* using different site for bYS-MSC injection to evaluate the tumor and three germ cell layer formation.

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Pregnant rate after transfer of bovine embryo produced *in vitro* using sexed and frozen semen from Nelore breed

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Introduction

There is a growing commercial interest in world for the cryopreservation of *in vitro* produced (IVP) bovine embryos, since this technique allows commercializing and transporting of bovine superior genetic inside the country and abroad (1,2 ,3).The present study aimed to investigate pregnancy rate of receptor cows after transfer of embryos produced *in vitro* using sexed and frozen semen.

Material and Methods

The experiment was conducted in three commercial farms (A, B and C) located in the southeast region of Bahia State, northeast of Brazil, among January and April of 2011. Sexed and frozen embryos (130) were acquired from Nelore bred for direct transfer. The blastocysts (D7) were acquire after OPU-PIV, using sexed and frozen semen from Nelore breed (X – chromosome bearing, 2.0 x 10^6 sptz/dose, CRV-Lagoa, Brazil) and cryopreserved for vitrification (Sexing Technologies, Sertãozinho, SP, Brazil). The embryo transfer were performed in receptor cows previously selected and synchronized with two doses IM of prostaglandin F2α (cloprostenol, Ciosin®, Schering-Plough, Brazil) 11 days apart. The evaluation of pregnancy was performed using ultrasound (Aloka SSd 500, linear transducer 5.0 MHz, Tokyo, Japan) between 28 and 32 days after embryo transfer and re-evaluated at 60 days. The data obtained of pregnancy rates were submitted to statistical analyses employing qui-square test.

Results and Discussion

The pregnancy rates obtained were: farm A, 58.5% (38/65); farm B, 60.4% (26/43); farm C, 54.5% (12/22), with no significant differences between farms ($p>0,05$). This results are consistent with other studies that obtained pregnancy rates of 51.7% (2) and 66.0% (3) after embryo transfer in commercial programs, proving technical availability. The pregnancy rate obtained in this study were similar to that observed after the use of sexed and frozen Frisia Holstein embryos imported from Canada, 54.5% (4). Considering that in this preliminary study it was observed a mean pregnancy rates of 63.0 %, these results show that nowadays it is possible to obtain satisfactory results of pregnancy rates from cryopreserved IVP zebu embryos, compared to IVP embryos of fresh transfer.

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Ewes' fertility is negatively affected by vaginitis provoked by the application of intravaginal sponges

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Introduction

Intravaginal sponges impregnated with progestagens (IS) are commonly used for estrous synchronization in small ruminants. However, its application induces bacterial growth, and localized inflammation with accumulation of mucus (1). These effects are similar when sponges with or without progestagen are used (2). Although it is known that the fertility obtained in ewes treated with IS is lower than that of a spontaneous estrus, it is not known if the vaginitis is involved in the fertility decrease. The objective of this study was to determine if the vaginitis provoked by the use of IS affects negatively the fertility, and if this effect is related with the progestagen content in inseminated ewes.

Materials and Methods

The experiment was conducted on a commercial farm, in Tacuarembó, Uruguay, during late April (mid-breeding season; autumn), with 575 mature (4-6 years old) Australian Merino ewes [body condition score = 3.4±0.5 (scale 1 to 5) and weight = 43.5±3.7 kg (mean ± SEM)]. Ewes were presynchronized with 1 dose of a PGF2alpha analogue (75 µg Cloprostenol, Ciclase DL, Syntex, Buenos Aires, Argentina), and estrus was daily recorded with vasectomised rams, during 5 d. Females detected in estrus were randomly allocated in three groups. Two days after estrus, sheep of Group MAP (n= 206) received an intravaginal polyurethane sponge impregnated with 60 mg of medroxyprogesterone acetate (MAP) during 14 days. At the same time, sponges without MAP were inserted in 156 ewes (Group IS). All sponges were previously sterilized with ethylene oxide (Biolene, Buenos Aires, Argentina). Other 213 ewes (Group SE) remained without treatment. Sponges were withdrawn 14 days later, and estrus was recorded with vasectomised rams. Ewes in estrus were cervically inseminated 12 h later with fresh semen (100 million spermatozoa). Conception rates were determined 40 days after insemination by ultrasound, and differences in conception rate were determined with Fisher exacts probability test.

Results and Discussion

The conception rate of SE group was significantly higher (p = 0.0002) than that of MAP and IS groups (Table 1), without differences between these ones. No significant differences were observed in the conception rates in relation to day after sponge removal (group MAP and IS; p=0.27). The use of intravaginal sponges to synchronize estrus and ovulation in ewes causes vaginitis that reduce the fertility of synchronized estrus despite it includes MAP or not.

Table 1. Pregnancy rate at different moment after sponge removal in ewes treated with or without sponge and with or without MAP (medroxyprogesterone acetate).

	Days after sponge withdrawal				
Treatment	Day 0	Day 1	Day 2	Day 3	Total
Sponge without MAP	2/12 (17%)	28/80 (35%)	22/49 (45%)	2/11 (18%)	54/154 (35%) a
Sponge with MAP	0/0 (0%)	2/4 (50%)	64/163 (39%)	17/33 (52%)	84/205 (41%) a
Without Sponge					118/213 (55%) b

[a]different letters in the same column represent statistical differences between groups.

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Isolation and characterization of mesenchymal stem cells from cat amniotic membrane

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Introduction

The amnion derived mesenchymal stem cells (MSCs) are multipotent cells with high ability to differentiate into multiple lineages. Can be obtained by non-invasive methods and used exempt ethical implications. Moreover, the amnion is discarded after birth and allows the isolation of MSCs with high efficiency. Due to their immunomodulatory and primitive proprieties, low tumorigenicity and high ability to generate specific lineages, the amniotic MSCs are the most widely studied in recent years (1, 2, 3). The aim of this study was to isolate and characterize the progenitor mesenchymal cells from the cat amniotic membrane for future application in cell therapy.

Material and Methods

The cells were isolated from four fetal membranes collected from routine ovariohysterectomy process in cats during the middle and last third gestation. The dorsal portion of amnion was mechanical separated and extensively washed in phosphate buffering solution and subsequently subjected to enzymatic digestion. The isolated cells were extensively propagated and frozen in various passages while the grow kinetics and cell morphology were analyzed.

Results and Discussion

In culture medium DMEN-F12/α-MEM, showed plastic adherence, morphology similar to fibroblast and high ability to propagate indefinitely for at least 10 passages. Immunocytochemical and flow citometry analyses at passages P3-P4, these cells were positive for mesenchymal stem cells specific markers CD73 and CD90 and negative for hematopoietic markers CD34, CD45 and CD79. It suggests that the amniotic mesenchymal cells are non hematopoietic cells and retain the progenitor-like proprieties. In differentiation media showed high plasticity, differentiating into osteogenic and adipogenic lineages. Moreover, these cells can be cryopreserved without functional and morphological depreciation, although the cell viability seems to decrease in high passages than in freshly cells. These findings suggest that the domestic cat amnion can be considered an important and useful source of MSCs with great interest for cell therapy and tissue engineering.

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Dairy cattle embryo production and inovulation in Imperatriz and Porto Franco (MA)

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Introduction

The protein animal demand encourages researchers to deepen studies in pursuit of efficiency in animals exploration. This study was conducted in Imperatriz and Porto Franco (MA), aiming the biotechnology for synchronization and superovulation in embryo transfer procedures and dissemination of technical, contributing to milk production expansion in the region of Vale do Tocantins (MA). The insertion of the projects on biotechnology transfer for milk small producers of Imperatriz and Porto-Franco (MA), may help that producers because it's the base of the income all of them, use low productivity animals and inadequate management practices. The objective was guide these milk producers to adopt the reproductive biotechnology and better general management, such as: rotational grazing, food supplements, and best quality water, to increase the production rates. Initially were established and defined the criteria for benefit of program, "two embryos placed on recipient cows for year".

Material and Methods

Were used six (6) donor cows and one hundred and fifty seven (157) recipient cows which were treated with hormones. Were used 1050 IU of follicle stimulating hormone (FSH) in each procedure in the donor cows by five (5) consecutive days, subdivided this volume in decreasing values, being 300 IU on the 1st and 2nd day and 150 IU in 3th, 4th and 5th days, by intramuscular way divided into two daily doses at each 12 hours to promote superovulation which were started between the 7th and 8th day of the estrus cycle (the estrus was considering as day 0). Were also utilized 500 g of cloprostenol (IM) for synchronization of the donor cow estrous cycle, with the recipient cows is given 24 hours before at the donor. The (30) thirty uterines flushings, correspond to five (5) treatments for each six (6) cows using intervals of about 60 days. Embryos were collected using 1000 ml of Phosphat Buffer Solution (PBS) by washing, for conclude each animal received a dose of 500 mg of cloprostenol (IM).

Results and Discussion

In uterine flushing, were recovered 206 embryos of which 157 were distributed to recipient cows, being transplanted 76.2% of the embryos recovered, the remaining of embryos showed low quality. Were collected on average 7.0 embryos per donor, approximately 6.0 of these were transplanted resulting in 3.8 pregnancies per harvest. Referring to the corpus luteum (CL) of recipient cows was found medium and large size, they had influence on pregnancy rates, signaling that the bigger ones are more desirable for a better pregnancy rate, a greater proportion of ovulation on ovarian corresponding to right side of the bovine cattle without statistical significance ($p < 0.05$), the estrus incidence of in recipient cows stood on the same day or a day after estrus of the donors cows.

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Occurrence of pregnancy, parturition and puerperium disorders in Holstein-Zebu dairy cows

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Introduction

Disorders of pregnancy, parturition and puerperium can reduce cattle reproductive performance and, consequently, decrease milk production. This study aimed to diagnose and quantify occurrence of different reproductive failures in dairy cattle during pregnancy, parturition and puerperium.

Material and Methods

Ten dairy cattle herds were monitored from January to December, in Coronel Xavier Chaves, south Minas Gerais, Brazil. Reproductive failures were studied in 380 adult Holstein-Zebu cows with body condition score over than 3 (1 to 5 scale) and average daily milk production of 15 liters per cow. Animals fed on *Brachiaria decumbens*, 22% protein ration and mineral mixture in semi-confined system. All cows were submitted to clinical and reproductive examinations every fifteen days, when disorders were diagnosed and recorded. Fetal dystocia (fetus presented in abnormal position, which impede that parturition takes place naturally, diagnosed via rectal palpation), maternal dystocia (absence of uterine contraction, which impede the normal parturition, diagnosed via rectal palpation), fetopelvic disproportion (fetus size larger than cow pelvic cavity, not open enough for passage, diagnosed via rectal palpation), retained placenta (retention of fetal membranes after 12 hours of parturition), multiple pregnancy (gestation of more than one fetus, diagnosed via rectal palpation and/or after parturition), prolonged pregnancy (exceeds normal bovine 260 day-pregnancy in 30 days, counted from the record of breed/insemination), uterine infection (strong odor pus in vulva region, or via transrectal palpation with asymmetry between uterine horns, due to purulent content), prolapsed vagina (observation of vagina exteriorization) and prolapsed uterus (observation of vagina, cervix and uterine body exteriorization) occurrences were monitored. For statistical analysis, occurrences were submitted to Chi-square test at 5% probability.

Results and Discussion

Mean of different reproductive failures per herd observed was 3.5, ranging from 2 to 6 disorders. Retained placenta corresponded to major occurrence, being present in 9 of 10 farms monitored. During the study, were observed 1.84% of fetal dystocia, 3.68% of fetopelvic disproportion, 7.10% of retained placenta, 0.53% of prolonged pregnancy, 3.95% of uterine infection, 0.53% of prolapsed vagina and 0.53% of prolapsed uterus. There were no cases of multiple pregnancy and maternal dystocia. Farms did not differ significantly for anyone of the variables studied.

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Application of placental gonadotropins on the *in vitro* production of bovine embryos

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Introduction

The *in vitro* maturation (IVM) is one of the most challenging stages in bovine *in vitro* production (IVP). The IVM medium should provide satisfactory conditions for the oocyte maturation, nuclear and cytoplasmic, to occur as close as possible of the physiology (1). The pituitary gonadotropins are essential components for these changes to occur synchronically in order to produce competent oocytes (2). However, there is controversy whether these hormones (pituitary and/or placental) are really necessary and also about the concentrations to be used (3). Given this disagreement, this study aimed to compare the effect of different combination of gonadotropins (on the IVM medium) on production of bovine blastocysts.

Material and Methods

Cumulus-oocyte complexes (COC, n=399, grades I and II), obtained from bovine ovaries (abattoir Fribom, Assis, SP, Brazil), were divided into three groups: G1) FSH (0.5 µg/mL) and LH (50 µg/mL, n=114 COC in 4 replicates); G2) hCG (2 UI/mL) and FSH (0.5 µg/mL, n=139 COC in 5 replicates); G3) eCG (4 UI/mL) and LH (50 µg/mL, n=146 COC in 5 replicates). The selected COC were matured in TCM 199 supplemented with 10% fetal calf serum. After 24 h matured COC were subjected to *in vitro* fertilization (IVF, D0) in TALP-FIV for 22-24 h. The semen was selected for viability by Percoll gradient method and then evaluated for motility and sperm concentration, to calculate the insemination dose ($1x10^6$ sperm/mL). Presumptive zygotes (PZ) were cultured in SOF (Synthetic Oviduct Fluid) in an incubator (38.3°C, 5% CO_2 and maximum humidity). Embryo development was evaluated by the cleavage, blastocyst and hatching rates (D3, D7 and D10, respectively) compared to the PZ. The average rates of replicates were analyzed by ANOVA and Tukey-Kramer and significance was considered when $P<0.05$.

Results and Discussion

There were no statistical differences between the mean values of cleavage (76, 84 and 73 %), blastocyst (34, 38 and 40 %) and hatching (20, 24 and 22 %, respectively, for G1, G2 and G3; Fig.1). The partial replacement of the pituitary gonadotropins LH (for hCG in G2) and FSH (for eCG in G3) resulted in development rates similar to the G1 (control group with FSH and LH). In this experimental condition, we conclude that the placental hormones are a viable and effective alternative in the replacement of FSH or LH on the IVM of bovine oocytes, to be used in the commercial IVP of bovine embryos.

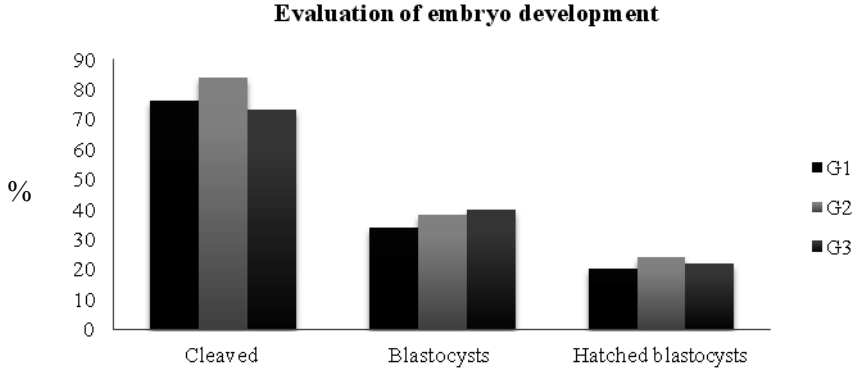

Figure 1. Percentage of cleaved, blastocysts and hatched blastocysts between groups.

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Reconstruction of mouse blastocysts by heterologous aggregation of the inner cell mass with one or two trofectoderm fragments

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Introduction

Somatic cell nuclear transfer (SCNT) is known to cause a high rate of anomalies throughout the conceptus formation which often results in fetal or neonatal losses (1). Placental malformation is one of the most prevalent anomalies and is the major challenge to carry pregnancies to term and to uphold the neonate viability (2). An efficient approach to overcome miscarriage derived from SCNT could be the chimerism by cell aggregation. The aggregation of trophectoderm (TE) vesicles or TE fragments from non-cloned bovine embryos (*in vivo* or *in vitro* produced) could increase the rate of full term pregnancy clones and also neonatal survival. Hence, this work aimed to validate in mice the reconstruction of blastocysts by heterologous aggregation by joining the inner cell mass (ICM) and the TE.

Material and Methods

Swiss Webster (SW) and C57BL/6/EGFP (EGFP) females were used as ICM and TE donors, respectively. Embryos were obtained after superovulation and *in vivo* fertilization. Blastocysts (in expansion, expanded or hatched) were sectioned with a microblade assisted by a micromanipulator (NK2, Eppendorf®) linked on an inverted microscope in order to isolate ICM and TE. The bisection was designed to be tangential to ICM and to produce two fragments, one with just TE and another with the whole ICM and minimum amounts of the TE. In experiment 1, the joining and subsequent aggregation were tested in the 56 heterologous structures (28 pairs of TE/EGFP and ICM/SW). Pairs were laid in microwells. Afterwards, pairs were manually united by an obliterated microcapillary and then were cultured for 24 h (37ºC, 5% CO_2 and saturated humidity) in media KSOMaa. Experiment 2 evaluated the addition of an extra TE fragment, *i.e.*, two fragments of TE and one of ICM to form a triplet (TE-ICM-TE). This experiment used the same methodology of the first experiment, except for the SW strain (ICM and TE). In the triplet (TG; TE-ICM-TE) and duet (DG; ICM-TE) groups we attempted 17 and 20 reconstruction assays for 54 and 20 blastocysts, respectively. The evaluation of reconstructed blastocysts was similar to experiment 1, when aggregation was confirmed in TG whether at least one TE fragment was joined and cohesive with the ICM, forming a typical blastocyst.

Results and Discussion

The aggregation rate of the reconstructed blastocysts (chimerism rate) in experiment 1 was 25% (7/28). The viability rate of sectioned structures (ICM and/or TE) was 84% (47/56) and took into account the separated structures that remained viable after culture. In experiment 2, adding an extra fragment of TE did not increase the aggregation rate in the GT (29%, 5/17) when compared to DG (30%, 6/20; P=1.0, Fisher's Exact Test). Contrary to the inference that the increased contact between ICM and TE fragments would increase the reconstruction rate, the use of only one fragment was as well effective. Despite of the low adhesion of trofectoderm cells in this technique, this model of heterologous reconstruction of blastocyst was considered feasible and effective to embryonic chimera production. Additionally, the presented model established in mice is about to be tested in bovine for further validation.

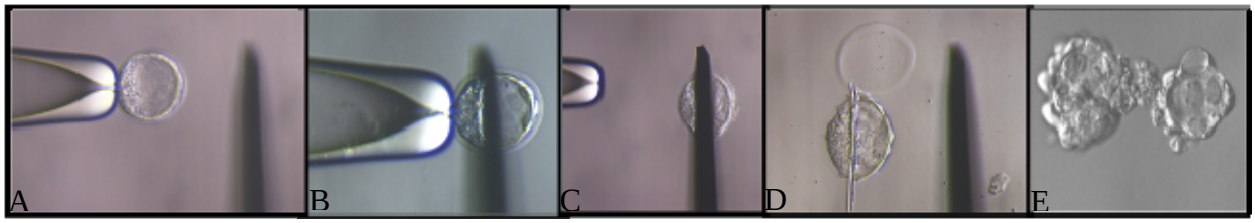

Figura 1. Isolation of MCI and TE in expanded blastocysts with a microblade (Figure A-D). The bisection was designed to be tangential to ICM (Figure C and D). Approximation of structures (ICM- left and TE– right; Figura E).

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Transplacentary infection of Bovine Leukemia Virus in aborted bovine fetuses. Preliminary results

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Introduction

Bovine Leukemia Virus (BLV) is classified into *Retroviridae* family, genus *Deltaretrovirus* (5). Bacteria, virus and parasites are related to causes of fetal losses, and bovine abortion causes serious economic damage in cattle (3, 4). It is known that vertical transmission of BLV, from the mother to the fetus, is possible (2), but it's impacts in livestock due to direct losses from conception and interference in fertility are unknown. The present study investigated the rates of transplacentary infection of BLV in cattle herds in Brazil, by detection of proviral DNA in bovine aborted fetuses.

Material and Methods

A total of 63 bovine aborted fetuses from several Brazilian farms were forwarded to the Centro de Pesquisa e Desenvolvimento de Sanidade Animal of Instituto Biológico for differential diagnosis of abortion causes. Necropsies were done in order to sample lymphatic organs (thymus, spleen and lymph nodes), and the organs were submitted to nested-PCR. The DNA extraction was made from tissue samples using the Wizard® Genomic DNA Purification kit (Promega Corporation, Cat. # A1120). Amplification of the segment that encodes the gp51 env gene of the BVL was conducted by nested PCR, using specific external primers for amplifying a segment of 598 base pairs (bp) (BLV1–5' TCT GTG CCA AGT CTC CCA GAT A 3' and BLV2– 5' AAC AAC AAC CTC TGG GAA GGG T 3'), and specific internal primers for amplifying a segment of 444 base pairs (bp) (BLV3– 5' CCC ACA AGG GCG GCG CCG GTT T 3' and BLV4–5' GCG AGG CCG GGT CCA GAG CTG G 3') [13]. The PCR reaction conditions were: initial denaturation at 94°C/2 min; 40 repeat cycles of denaturation at 95°C/30 sec; annealing at 62°C/30 sec, extension at 72°C/1 min; final extension at 72°C/4 min; the second amplification: initial denaturation at 94°C/2 min; 40 repeat cycles of denaturation at 95°C/30 sec; annealing at 70°C/30 sec, extension at 72°C/1 min; final extension at 72°C/4 min. Analysis of amplified products was by electrophoresis (100 V/60 min) agarose gel at 1.5% in TAE 1x, and stained with ethidium bromide. The image of the gel under UV light was recorded using camera coupled to a computer.

Results and Discussion

It was detected BLV in 14.3% (9/63) fetus with nested-PCR (Fig.1), showing a high rate of transplacentary transmission. These preliminary results confirm the sanitary situation of the Brazilian bovine herds, where BLV is widespread (2). For this reason, sanitary measures should be taken into consideration in order to control the prevalence and economic losses caused by the BLV. The viral DNA of the positive samples will be sequenced, and the phylogenetic analysis will allow for identifying the different genotypes.

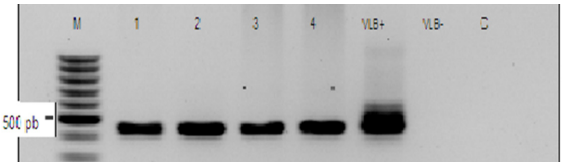

Figure 1. polyacrylamide gel submitted to eletrophoresis. M = ladder 500pb. Numbers 1,2,3,4 = Positive samples to BLV nested-PCR. VLB + = positive control (FLK cells infected with BLV). VLB - = negative control (VERO cells negative to BLV). C = ultrapure water.

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Embryonic fluid as proteic source during *in vitro* culture improves the embryonic criosensitivity

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Introduction

One of the major problems in *in vitro* production (IVP) of bovine embryos is the high embryonic criosensitivity, which is directly related to the use of FBS in IVP medium (1). This study evaluated the effects of FBS, bovine serum albumin (BSA) and embryonic fluid (EF) (Sigma-Aldrich, St. Louis, MO, USA) isolated or in combination during the *in vitro* culture (IVC) of embryos as a proteic supplement on the blastocyst development and viability after vitrification.

Materials and Methods

In vitro maturation (IVM) of oocytes was performed in TCM 199 with 10% FBS, 25mM sodium bicarbonate; 1.0µg/mL FSH; 50UI/mL hCG; 1.0µg/mL estradiol; 0.2mM sodium pyruvate; 83.4µg/mL amikacin and 1µg/mL antioxidant. After 24h of IVM, the oocytes were co-incubated with sperm in TALP-FIV containing 6 mg/ml BSA for 20 h approximately. The *in vitro* culture (IVC) was performed in SOFaa. According to the protein source, the following treatments were delineated: control - BSA (6 mg/mL) + FBS (2.5%); BSA (8mg/mL); BSA (6mg/mL) + EF (2.5%); and BSA (6mg/mL) + FBS (2.5%) + EF (2.5%). All cultures during IVP process were performed in an incubator at 38.5 ° C and in 5% CO_2 in air. After seven days of IVC, blastocyst rates were evaluated, and the embryos produced were vitrified as described by Vajta et al. (2) with some modifications. Embryonic survival after vitrification was assessed by embryonic hatching rate post-thawing; embryos were cultivated for 48h under the same conditions. Three replicates were performed, and results were evaluated by chi-square test (χ^2) in SAS v.8.2 software ($p = 0.05$).

Results and Discussion

Initially, the BSA as unique protein source during the IVC produced the lower rate of embryo development to the blastocyst stage (75/247 – 30.36%[a]). However, when the EF was added to BSA, there was an increase in the embryo production (146/344 - 42.44% [c]). Despite this beneficial effect, when the three protein sources (BSA + FBS + EF) were used together, the blastocyst rates were significantly lower than those displayed by control group (150/328 - 45.73% [c], 165/283 - 58.30% [b], respectively), demonstrating a possible detrimental effect of proteins in excess in the medium or a negative interaction between FBS and EF. When we evaluated the hatched blastocysts rate after vitrification, the best result was obtained from the BSA+EF group (64/110 - 58,4%[b]), that was superior to other groups (BSA: 16/57 – 28.7%[a]; BSA+FBS: 39/124 – 31.45%[a]; BSA+EF+FBS: 35/140 – 25%[a]). Therefore, we conclude that EF is an interesting protein source to replace FBS during embryo culture since it did not impair the blastocyst development and improved the embryonic survival to vitrification, being an alternative when IVP embryos are destined to cryopreservation.

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Influence of different oxygen tensions and fetal bovine serum concentrations on dimethylation of lysine 9 of histone 3 (H3K9me2) in *in vitro* produced bovine embryos

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Introduction

In vitro production of bovine embryos (IVP) is a biotechnology of great economic impact, allowing for a greater reproductive use of genetically superior animals. Despite advances in IVP, the percentage of embryos developing is still below those seen *in vivo* (1). Epigenetics is the regulation of gene expression and function, without alterations to the DNA sequence (2). Epigenetic processes, such as histone remodelling, control gene expression and are vital for proper embryo development (3). Specific histone modifications can stimulate or repress gene transcription (4). The present study evaluated the influence of different oxygen tensions during *in vitro* culture of embryos (5% and 20%), as well as fetal bovine serum (FBS, 0 and 2.5%), in the dimethylation of lysine 9 of histone 3 (H3K9me2), an epigenetic mark associated with repression of gene transcription, in IVP bovine embryos.

Materials and Methods

Bovine embryos were produced using established conditions for IVM, IVF and IVC, and were submitted to four treatments (2x2) during embryo culture. T1: presumptive zygotes were cultured in SOF media, in the presence of FBS (2.5%) in 5% O_2; T2: presumptive zygotes were cultured in SOF media, without FBS in 5% O_2; T3: presumptive zygotes were cultured in SOF media, in the presence of FBS (2.5%) in 20% O_2 and T4: presumptive zygotes were cultured in SOF media, without FBS in 20%. On days 7 and 8 of culture, expanded blastocysts were fixed in 4% paraformaldehyde and maintained in PBS+tritonX 0.1%. Immufluorescence was then performed using a specific antibody to recognize the modification H3K9me2. Embryos were analysed with ImageJ, and results are presented as percent of total DNA.

Results and Discussion

Cleavage and blastocyst development rates did not differ significantly between treatments, with cleavage rates at T1: 83.43% ± 3.75 (604/724); T2: 88.95% ± 3.81 (565/698); T3: 81.75% ± 3.11(569/ 696); T4: 78.56% ± 2.93(546/695), and development rates at: T1: 32.28% ± 5.66 (195/604); T2: 23.72% ± 6.10 (134/565); T3: 35.85% ± 7.25 (204/569) e T4: 22.16% ± 7.75 (121/546). Notwithstanding, treatments that employed FBS (T1 e T3) showed a tendency for higher blastocyst development rates ($P<0.08$). Global staining for the residue H3K9me2 (a repressive mark for transcription) was not affected significantly by the treatments (T1: 17.26 ± 4.64; T2: 25.26 ± 5.62; T3: 23.4 ± 4.39, T4: 25.20 ± 5.55). These results suggest that bovine blastocysts, produced *in vitro*, were not susceptible to changes in this specific represssive histone mark when submitted to varying oxygen tensions, or to the presence or absence of FBS in the media. We are currently evaluating the effects of these culturing conditions in the global marking of residue H3K4me2, a permissive histone mark.

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Effectiveness of parthenote and *in vivo* fertilized embryo aggregation in mouse

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Introduction

Embryonic chimerism - mixing of cells originated from at least two different fertilizations - has been used as a tool for stem cell pluripotency diagnosis, transgenic rodent production and organogenesis studies. Additionally, parthenotes are used for studies related to gene imprinting and the ability of their cells to compose the placenta and/or an adult animal. The aim of this work was to validate the production and characterize developmental kinetic of parthenogenetic embryos (PGE), obtained from C57BL/6 EGFP mice (EGFP), to determine PGE chimerism potential, and to localize PGE cells on the produced blastocyst.

Materials and Methods

For the aggregation were used pre compaction embryos produced either by *in vivo* (Swiss Webster strain; SW) or by *in vitro* (EGFP) and after *in vivo* fertilization (IVvF) or parthenogenetic activation (PGA), respectively. To PGA was used strontium chloride hydrate (5 mM for 6 hours). Two experiments were outlined in order to: i) evaluate the development and kinetic from IVvF (DIV; n=53 embryos) and from PGA (DPG; n=409 embryos) techniques, both from SW oocytes; ii) evaluate the aggregation between pairs of control embryos (C; n=20, IVvF from SW) and IVvF/SW plus PGA/EGFP embryos (PG; n=40). After manipulation (e.g., removal of the zona pellucida and approximation of pairs, for C and PG groups), all the embryos were kept *in vitro* culture (37ºC, 5% CO_2 and saturated humidity) during 48 to 60 hours (C and PG), or up to blastocyst stage (DIV and DPG). Chimerism rate and PGE fluorescence (pre and post culture) were evaluated under an inverted microscope with epifluorescence source and digital images were captured (Eclipse Ti and NIS-Elements, Nikon, Tokyo, Japan, respectively), by merging visible and UV light images.

Results and Discussion

The rate of PGA (EGFP oocytes) assessed by the presence of at least one pronucleus was 54.5% (66/121). The rate of blastocyst production from PGA (oocytes) or 2 cell embryos (IVvF) was significantly different ($P<0.001$; Fisher's exact test) between DIV and DPG (71.4 and 12.9%, respectively). When developmental kinetic was evaluated there was observed a difference in the average time to the majority of embryos reach blastocyst stage for DIV (48 h) and DPG (120 h). Chimerism rate differed ($P=0.006$; Fisher's exact test) between C (55.0%; 11/20) and PG (17.5%; 7/40) groups, after assessment from 48 to 60 h of culture. In PG group, was observed a random incorporation of EGFP cells in the obtained chimeras. In one of the blastocysts obtained, EGFP cells were mostly allocated in inner mass cell. It was concluded that production and *in vitro* development of PGE - until 120 h of culture - is feasible. Although PGE cells have a slower developmental kinetic than embryos derived from IVvF, they were capable to be incorporate into the trophectoderm and inner cell mass of the produced chimeras.

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Shotgun lipidomics of endometrium and uterine flushings in beef cattle

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Introduction

In bovine, failure in conception taking place during the first three weeks of pregnancy make up 20 to 40% of embryonic loss (1). During such period, embryonic development depends upon the uterine secretion called the histotroph (2). The formulated hypothesis was that the biochemical composition of the histotroph is associated with the success of pregnancy (3). The objective of the research was to characterize the lipid profile of the endometrial tissue and uterine washing of Nelore cows.

Material and Methods

Non-lactating Nelore cows were slaughtered on day 7 of a synchronized estrus cycle (day 0 = induction of ovulation with GnRH). Uterine washings (n=10; 500 ul) and endometrial fragments (n=4; 100 mg) were harvested from the uterine horn contra-lateral to the ovary containing the corpus luteum (CL) and were individually investigated through shotgun lipidomics approach. The total lipids in the uterine washing and endometrium were extracted using the Bligh & Dyer method (4). B-sitosterol was used as an internal standard. The analysis was performed in a Synapt G1 Q-TOF (Waters) apparatus, using an electrospray source both in the negative and positive mode (ESI-MS). The MALDI-TOF autoflex III smartbeam (Bruker) equipment was also used in the reflectron mode. All the samples were extracted in triplicate and analyzed in quintuplicate to verify possible variations in the analysis.

Results and Discussion

It was observed from the comparison between the lipid profile of the uterine washings and endometrial tissue that the fatty acid (FA) composition differed qualitatively between the two matrixes. The uterine washing was found to be abundant in free fatty acids such as the palmitic, myristic and arachidonic acids and structural lipids (e.g. ceramids, diacil and triacylglycerols). In contrast, the endometrium was abundant in phospholipids such as lecithins made up by phosphatidylcholine, plasmalogens and FAs of high molecular weight (>1000 Da). These results show the potential of the shotgun lipidomics approach for the analysis of the several lipids classes in bovine biological matrix such as the endometrium and uterine washing. Additionally, the distinct lipid profile between the endometrial tissue and uterine washing suggest a fine regulation in the production, metabolism and/or secretion of such compounds in the uterine environment. The attainment of FA profile in high mass/charge resolution from different matrixes enables the identification of biomarkers associated with uterine environments more or less favorable to pregnancy.

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Homologous recombination and non-homologous end-joining repair pathways in bovine embryos with different developmental competence

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Introduction

Most oocytes that complete the first cleavage in vitro do not develop further than 4- to 8-cells stage possibly due to the inability of early-stage embryos to repair DNA injuries (1). The main genes responsible for controlling the DNA double-strand breaks repair, through the homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways, are expressed in mammalian oocytes and embryos (2). However, little is known about the expression profile of these two repair pathways during early embryo development. The present study was designed to assess the expression profile of important genes of HR and NHEJ DNA-repair pathways in bovine embryos of high, intermediate and low developmental competence.

Material and Methods

Bovine embryos with high, intermediate or low competence of embryonic development were selected based on the cleavage time (28, 32 and 36 hours post insemination, respectively) and removed from in vitro culture before, during or after the expected moment of embryonic genome activation (36, 72 or 96 hours post insemination, respectively). This approach allowed us to evaluate mRNA expression of genes that control homologous recombination (53BP1, ATM, RAD50, RAD51, RAD52, BRCA1, BRCA2, NBS1), and non-homologous end-joining (KU70, KU80, DNAPK) DNA-repair pathways in bovine embryos with high, intermediate or low developmental competence.

Results and Discussion

The timing of cleavage post-insemination was shown to have a major effect on the developmental capacity of the embryos, which was significantly higher in the early-cleaved compared to intermediate and late-cleaved embryos (Fig.1A and B). All evaluated genes were detected before, during and after the expected time for embryonic genome activation in embryos with high, intermediate or low developmental competence. However, only 53BP1 and RAD52 genes were found to be differentially expressed in embryos with higher and lower developmental competence before the expected time for embryonic genome activation (36 hours post insemination; Fig. 1C and D). In conclusion, the main genes responsible for triggering and controlling both HR and NHEJ repair pathways are expressed before, during and after embryonic genome activation in bovine embryos with high, intermediate or low developmental competence. Bovine embryos with low developmental competence were found to have higher mRNA expression of 53BP1 and RAD52 before embryonic genome activation.

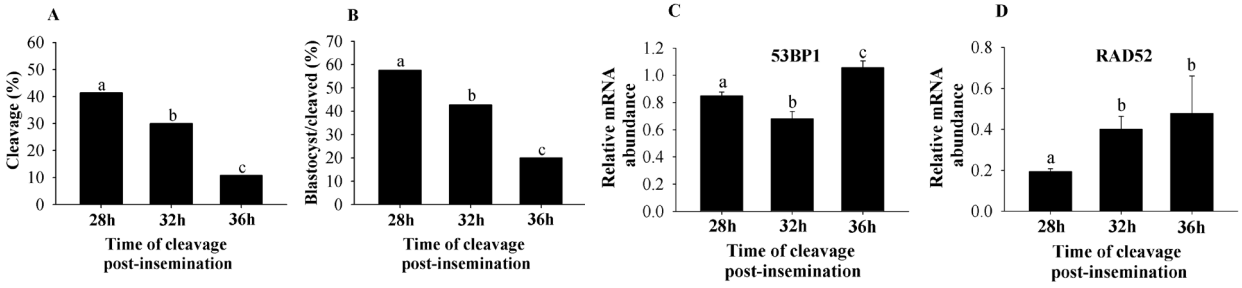

Figure 1. Cleavage kinetics (A) and development of embryos cleaved at 28, 32 and 36 hours post insemination (B). Relative mRNA expression of 53BP1 (C) and RAD52 (D) in bovine embryos with high (28h), intermediate (32h) or low (36h) developmental competence and removed from in vitro culture at 36 h post-insemination. Bars with no common letter are statistically different ($p \leq 0.05$).

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Clinical and subclinical endometritis during the postpartum period of Holstein, Gyr and F1 Holstein x Gyr cows

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Introduction

Clinical endometritis is characterized by the presence of purulent uterine exudate in the vagina 21 days or more postpartum, or mucopurulent after 26 days postpartum (1). Due to the absence of clinical signals, subclinical endometritis can be detected by the relation between neutrophils and epithelial cells from endometrium samples obtained by the cytobrush technique, uterine flushing or swab (2). These diseases are associated with lower conception rates. The objective was to compare the occurrence of clinical and subclinical endometritis in Holstein, Gyr and F1 Gyr x Holstein cows during the postpartum period.

Material and Methods

10 pluriparous Gyr and 14 Gyr x Holstein F1 maintained on pasture, and 17 Holstein maintained in free-stall barns were used in this trial. Holstein cows were separated in retained (n=10) or non retained (n=7) placenta. The cows were monitored weekly until 42 days postpartum. The mean of the body condition score at calving were 3.6 ± 0.4 for Gyr and F1, and 3.7 ± 0.5 for Holsteins. The uterine involution and the return of the ovarian activity were monitored by transrectal palpation and ultrasonography. Endometrial samples were obtained by cytobrush technique (2) at 21, 28, 35 and 42 days postpartum (dpp). The diagnosis of clinical and subclinical endometritis was determined, respectively, through vaginoscopy and cytology: > 18% neutrophils in uterine cytology samples collected at 20-33 days postpartum or > 10% at 34-47 days (1).

Results and Discussion

The incidence of clinical and subclinical endometritis was higher in Holstein than in Gyr and F1 cows during postpartum period. Endometritis was not observed in Gyr and F1 cows at day 42 postpartum. At day 21, the incidence of subclinical endometritis was higher in Holstein cows with retained than in non retained placenta ($P < 0.05$; Table 1).

Table 1. Occurrence of endometritis in Gyr, Holstein and F1 cows between 21 and 42 days postpartum (dpp).

Days postpartum	21 dpp		28 dpp		35 dpp		42 dpp	
Breed	Clinical	Subclin.	Clinical	Subclin.	Clinical	Subclin.	Clinical	Subclin.
Gyr	10%	10%	10%	20%	0%	20%	0%	0%
F1 (Holstein x Gyr)	14%	28%	7%	7%	21%	7%	0%	0%
Holstein without PR	10%	0%*	37%	25%	37%	12%	14%	0%
Holstein with PR	43%	57%*	100%	0%	33%	0%	0%	33%

*Subclinical endometritis are different ($P < 0.05$) by Fisher test.

The uterine involution occurred, on average, at 32.5 ± 5.9, 34.5 ± 6.4, 38.5 ± 3.7 and 37.3 ± 4.0 days postpartum for Gyr, F1, Holstein cows presenting or not retained placenta, respectively. Up to 42 days postpartum, the return to ovarian activity was not observed on Gyr cows. 50% of the F1 cows ovulated until 37 ± 6.7 days, and 100% of the Holstein ovulated until 26 ± 6.4 days postpartum. Retained placenta, milk yield, environment challenges, stress and early postpartum ovulation can explain the differences between genetic bases. Early postpartum ovulation is undesirable because progesterone is immunosuppressive and in presence of contaminated uterus can contribute to the occurrence of uterine infections (3). Cytology and vaginoscopy were effective in identifying infection during uterine involution. Cytobrush is indicated as a complementary test to evaluate the uterine environment at the end of puerperium.

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Gene expression of enzymes from the arachdonic acid metabolic pathway in the endometrium of cows exposed to different periovulatory endocrine milieus

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Introduction

Prostaglandins, thromboxanes, leucotrienes and prostacyclins are eicosanoids generated from enzymatic processing of arachidonic acid (AA) (1). In ruminants, there is extensive participation of eicosanoids in reproductive processes such as ovulation (2), luteolysis (3) and early embryonic development (4). We hypothesize that the concentrations of eicosanoids in uterine tissue and secretions is regulated by periovulatory hormonal fluctuations, leading to uterine oxilipidomic profiles associated with higher or lower embryo receptivity. Objective was to evaluate the effects of the periovulatory endocrine environment on gene expression of the enzymes PTGS1, PTGS2, ALOX12, PTGES, PTGES2, PTGIS and PTGDS, as well as on the concentrations of their metabolites in the endometrial tissue and uterine washings.

Material and Methods

Twenty-two cyclic, non-lactating Nelore cows received a P4-releasing device along with estradiol benzoate on day -10 (D-10). Animals were divided to receive a prostaglandin analog (PGF; HP group; N=11) or not (LP group, N=11) on D-10. Progesterone devices were removed and PGF injected on D-2.5 on cows from HP group, and on D-1.5 on cows from LP group. Ovulation was induced with GnRH on D0. Plasma P4 concentrations were measured daily from D0 to D7. On D7 animals were slaughtered and endometrial fragments as well as uterine washings collected. Gene expression was assessed by qPCR and oxilipids concentrations were determined by mass spectrometry (UPLC-MS/MS).

Results and Discussion

Gene expression of PTGES2, PTGIS and ALOX12 was higher in the HP versus LP group (Table 1; $P<0.05$). In contrast, concentrations of their respective metabolites PGE1 and 8-iso-PGE2 (PTGES), PGE2 (PTGES2), 6-keto-PGF1α (PTGIS), and 12-HETE (ALOX12) were similar between the HP and LP cows in endometrium and washings ($P>0.05$). Abundance of other genes and metabolites were similar between HP and LP. Lack of association between AA metabolizing enzymes gene expression and concentrations of AA metabolites in the endometrium suggests complex regulation of the AA pathway during diestrus in cattle.

Table 1. Gene expression of AA metabolizing enzymes and concentrations of AA metabolites in the endometrial tissue and uterine washings of cows on D7 post-estrus.

Gene	Gene Expression		Metabolite	Endometrial tissue (pmol/g)		Uterine washing (pmol/mL)	
	HP	LP		HP	LP	HP	LP
PTGES2	1.2 ± 0.1*	1.0 ± 0.1	8-iso-PGE2	3.9 ± 0.9	3.7 ± 1.3	-	-
			PGE1	24.8 ± 13.9	20.3 ± 6.7	0.02 ± 0.003	0.02 ± 0.01
			PGE2	38.9 ± 12.9	27.0 ± 4.0	0.3 ± 0.1	0.04 ± 0.2
PTGIS	1.3 ± 0.1*	1.0 ± 0.1	6-keto-PGF1α	215 ± 72.2	225.4 ± 67.9	1.3 ± 0.4	1.4 ± 0.4
ALOX12	1.6 ± 0.1*	1.1 ± 0.1	12-HETE	0.6 ± 0.1	1.1 ± 0.6	2.5 ± 1.1	1.4 ± 0.3

*indicates $P < 0.05$.

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Testis structure, duration of spermatogenesis and spermatogenic efficiency (daily sperm production) in the freshwater turtle *Kinosternon scorpioides*

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Introduction

Kinosternon scorpioides is a freshwater turtle found mainly in the Northern and Northeastern regions of Brazil. Although considered threatened, this species is commercially exploited due to the good quality of its meat. Therefore, accurate studies related to their reproductive biology would provide useful data to help to preserve this species from extinction. Our objectives were to carefully investigate testis structure and function in *K. scorpioides*; particularly the aspects related to the spermatogenic cycle length and Sertoli cell (SC) and spermatogenic efficiencies.

Material and Methods

Nine sexually mature male turtles were utilized in this study. Testis were fixed by immersion in buffered glutaraldehyde and Bouin´s fluid, and routinely processed for histological, morphometric, stereological and immunostaining analyses. In order to determine the duration of spermatogenesis, intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrDU; 150mg/kg) were performed at 1 hour, 7, 15, 22, 23, 24, 30, 31 and 32 days before sacrifice. All data are presented as the mean ± SEM.

Results and Discussion

The body weight and the testis weight were respectively 325 ± 28g and 0.96 ± 0.17g, resulting in a gonadosomatic index (GSI; total testis weight divided by body weight) of 0.59% ± 0.08. Mean tubular diameter and epithelium height were 345 ± 22 and 114 ± 6 µm, respectively. The mean percentage of the seminiferous tubules observed in turtles, 82.3%±2.2, was relatively high in comparison to the values found for the mammalian species already investigated, whereas Leydig cells occupied only 4.5% of the testis parenchyma. Ten stages of the seminiferous epithelium cycle were characterized for this freshwater turtle and, similar to birds, several stages were present per tubular cross-section. The spermiogenic phase was divided in 16 steps and the mature spermatids were released (spermiated) in the tubular lumen at stage IV, which presented the higher frequency (~20.7 ± 1.4%), followed by stages V (~18.4 ± 1.8%), whereas stage X showed the lowest frequency (~3.2 ± 1.2%). The most advanced germ cells labeled in the different time periods after BrDU injection were preleptotene/leptotene at stage VI (1h); pachytene at stage III (7d); secondary spermatocytes at stage X (15d); round spermatids at stage VI (22d); elongating spermatids at stages VI and VII (23 and 24d) and elongated spermatids at stages I, II and III (30, 31 and 32d). Based on the stages frequencies and the most advanced germ cells labeled with BrDU, each spermatogenic cycle and the entire spermatogenic process (4.5 cycles) lasted respectively 11.8 ± 0.2 and 53.1 ± 0.8 days. The SC efficiency (round spermatids per SC) and the daily sperm production per gram of testis (spermatogenic efficiency) were respectively ~27±3 and ~41±6x10^6. These values are situated at the upper level when compared to those described for mammals. To our knowledge, this is the first study to accurately evaluate the testis function in any reptilian species. Besides being useful for studies related to comparative reproductive biology among vertebrates, we expect that our findings will be useful for governmental programs aiming to preserve the *Kinosternon scorpioides*.

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Effect of açaí pulp (*Euterpes edullis*) on the epididymal caput region morphometry of Wistar rats exposed to cadmium chloride

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Introduction

Exposure of humans to toxic metals is a major environmental problem (1). Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life of 10-30 years. Its metabolism and excretion depends on the presence of antioxidants (2). The fruit of *Euterpes edullis*, known as açaí, contains significant amounts of a distinct class of flavonoids, the anthocyanins, which promotes antioxidants properties to this fruit (3). This work aimed to evaluate the effect of açaí pulp on the action of cadmium chloride ($CdCl_2$) in the caput epididymal morphometry when given before or after the açaí.

Material and Methods

Adult Wistar rats (n=25) were exposed to $CdCl_2$ (single dose; 1.2 mg/kg/BW/intraperitoneal – Ip) and açaí pulp (1.5 ml/animal/gavage – Gv), according to five experimental groups (G). The G1 (control group; n=3) received saline (Ip) and oral (Gv) during 56 days. The G2 (n=6) received $CdCl_2$ (Ip) and saline (Gv), and the animals were euthanized after 48h. Group G3 (n=4) was provided with saline (Ip) and açaí pulp (Gv, 56 days). Animals of G4 (preventive action, n=6) were treated with the açaí (Gv, 56 days) and afterward, on the 56^{th} day, they received $CdCl_2$, being euthanized 48h later. Animals of G5 (curative action, n=6) received $CdCl_2$ and they were treated with açaí (Gv, 56 days). Sections of the caput region were fixed in Karnovsky solution and embedded in methacrylate plastic (Historesin®). The following measurements were performed: tubular (TD; µm) and luminal diameters (LD; µm), epithelium height (EH; µm), and volumetric proportion (%), considering tubular (epithelium, basal lamina, lumen with spermatozoa and lumen without spermatozoa) and intertubular compartments (blood vessels, connective tissue, and smooth muscle). The data were obtained from 10 random microscopic fields per segment at 100x magnitude, using a 266 point-grid test system. All the measurements were estimated using the Image Pro-plus software, and the results were analyzed by ANOVA and Newman Keuls tests. Differences were considered significant at $p<0.05$.

Results and Discussion

The TD values obtained in animals exposed to cadmium (G2: 255.5 ± 69.6; G4: 252.3 ± 55.8; G5: 253.7 ± 15.4 µm) were lower than animals of control group (G1: 388.6 ± 21.7 µm; $P < 0.05$). Otherwise, those groups showed increased EH (G2: 35 ± 5.5; G4: 38.1 ± 7.3; G5: 48 ±11 µm) when compared to G1 (32.3 ± 1.9 µm; $P < 0.05$). There were no differences in LD and TD means between G2 and G5 ($P > 0.05$), showing the açaí pulp was not able to restore damaged tissue after cadmium exposition. The percentages of basal lamina and blood vessels at G2 (9.76 ± 1.76; 1.44 ± 0.59%, respectively) were higher than the control group (6.77 ± 0.47; 0.33 ± 0.21%, respectively). The value for lumen with spermatozoa at G4 (12.38 ± 24.76%) was higher than the G2 (4.55 ± 11.16%), and G5 (0.13 ± 0.26%) that showed the lowest percentual. In conclusion, there were morphometric alterations on the epididymal caput region when exposed to cadmium and the açaí pulp had low preventive action and no curative effects on the tissue lesions.

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Efficiency of intratesticular injection of zinc-based solution on male cat sterilization: morphometric testis results

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Introduction

The domestic cat may be the world's most common pet, and chemical sterilization could be an option to interrupt male reproduction and control overpopulation. A zinc-based solution, that is pH neutralized in BioRelease Technologies physiological vehicle (B.E.T. Labs, Lexington, Kentucky, USA), was developed as a chemical sterilization for intratesticular injection for dogs (1) (3). However, there is little information on the testis structure and function on domestic cats (2) and almost no studies regarding chemical sterilization on them.

Material and Methods

Twelve adults' male cats were used. They were divided into two groups: treated and control. Eight treated cats, were given 0.2-0.3mL intratesticular injection of a zinc-based solution and four control cats were given an intratesticular injection 0.2-0.3mL of a saline solution. The testicular width was measured to determine the dosage of the drug and saline solution to be given. In order to compare the action of the drug and its effects on the testis, after 162 days all animals were surgical castrated and the morphometric aspects of the testis were evaluated.

Results and Discussion

The testicular diameter was measured before the intratesticular injection, and the mean of the treated group was 1,45cm and the one of the control group 1,60cm ($P>0.05$). After 162 days of intratesticular injection all cats were surgical castrated, and it was observed, between groups, a significant difference on the diameter and weight for both testis (Tab.1), and the testis were macroscopically evaluated: 50% of the cats of the treated group showed different shape and adherence areas; 25% of treated cats showed severe adherence and fibroses; and 25% of treated cat didn't show macroscopic alteration. To our knowledge, this is the first study of testis morphometric after an intratesticular injection with a zinc-based solution in cats.

Table 1. Cat diameters and weight of right and left testicle (mean ± SEM) 162 days after intratesticular injection, in the treated and control groups.

Parameters	Treated	Control
Diameter right testicle (cm)	0.59 ± 0.35^{a}	1.27 ± 0.47^{b}
Diameter left testicle (cm)	0.52 ± 0.17^{a}	$1,24 \pm 0.50^{b}$
Weight right testicle (g)	0.54 ± 0.33^{a}	$1,62 \pm 0.18^{b}$
Weight left testicle (g)	0.59 ± 0.34^{a}	1.59 ± 0.16^{b}

Different letters in same line indicate difference between treatments ($P < 0.05$).

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Testes weight and comb score of broiler breeder roosters with 71 weeks of age and three body weight categories

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Introduction

Testes morphometric measurements are important markers of the reproductive male fertility in several species. They are difficult to measure in roosters due to testes location into the abdominal cavity (1). Roosters' fertility starts decreasing from 45 weeks of age and it is associated with lower FSH and testosterone circulating concentrations (2). In broiler breeder farms, the replacement of heavy old males or of the ones with poor secondary sexual characteristics seem to improved fertility rates. This study focused on checking if body weight (BW), comb characteristics and testes weight are associated in old broiler breeder roosters.

Material and Methods

In a same farm 30, 60 and 30 broiler breeder roosters were selected randomly according to the BW, respectively: low (≤4.300 kg), medium (between 4.300 and 5.250 kg) and high BW (≥5.250 kg). They were individually identified and comb score (1-poor, 2-fair, 3-good or 4-excellent) was determined for each animal. The roosters were slaughtered according to humane slaughter standards and the testes removed and weighed in a digital scale.

Results and Discussion

Roosters BW varied from 3.3 to 6kg, probably due to the consequence of food restriction related to competition for food among the animals. The roosters of low and high BW categories differed in average testis weight (7.73 ± 3.87 g and 11.06 ± 2.25 g) and the roosters of three BW categories differed in comb score (2.39 ± 0.99, 3.12 ± 0.72 and 3.47 ± 0.68) ($P \leq 0.05$). The BW had moderate correlation with right ($r = 0.376$, $P<0.0001$) and left ($r = 0.371$, $P<0.0001$) testis weight and with comb score ($r = 0.498$, $P<0.0001$). The comb score correlates moderately with right ($r = 0.454$, $P<0.0001$) and left ($r = 0.455$, $P<0.0001$) testis weight. Studies in roosters demonstrate that low energy and protein intakes can reduce testosterone serum concentrations (3,4) and the testicular weight (5, 6, 7). There was up 21.64-fold range in average testis weight of roosters of same BW. Variation greater than 35-fold range was related in average testis weight of 103 great-grandparents roosters not subjected to feed restriction (6) and it is an alert for the little importance given to genetic improvement based on reproductive characteristics (1). It is concluded that BW and comb score are measurable characteristics in farm broiler breeders which are associated directly and proportionally to testicular weight. However, the weight of the testes is a characteristic of high genetic variation.

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Testicular weight and its correlation with body weight and testicular biometry in Japanese quail: implications for selection of breeders

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Introduction

Currently, there is no reliable predictive parameter to estimate sperm production and breeding potential in Japanese quail. Despite intra-abdominal location of the gonads, methods such as ultrasound may be used to assess testicular measures and indirectly estimate testicular volume, which is directly correlated to capacity of sperm production (1). Differences in testicular shape, size and position (2) may also difficult a unique approach between right and left testes. Therefore, the purpose with the present study was to determine which testicular measures better correlate with testicular weight, contributing to create a selection parameter for Japanese quail in breeding colonies.

Materials and Methods

After weighing, 25 male Japanese quails at 60 days old, raised under nutrition and light conditions commonly used, were slaughtered by cervical displacement and testes were dissected, weighted and measured (length, width, thickness and perimeter) with an electronic digital caliper. Width was considered from epididymal to lateral side and thickness was measured in the ventrodorsal axis. Parameters were correlated using Pearson's correlation coefficient (r) at 1% level of significance.

Results and Discussion

Data are presented in Table 1. Even with gonadossomatic index near 4%, body weight was poorly correlated with testicular weight. Perimeter revealed strong positive correlation for both testis, but may be difficult to assess by ultrasonography. Width and thickness showed a higher correlation coefficient, indicating that they could be used to estimate capacity of sperm production in both testes, rather than length. Given the identification of a single parameter for comparison of males, the thickness of the left testis showed linear correlation coefficient of 0.89 with the sum of the weight of both testes. Although different in shape, the right testis was more elongated, and the left one more rounded, and the testicular weight was similar, suggesting equal contribution for sperm production. These findings may be important to develop a practical approach to select genetically superior Japanese quail breeders at the top of the pyramid, based on indirect assessment of sperm production capacity, by means of testicular ultrasonography, although the latter is not yet consolidated in birds

Table 1. Pearson's correlation coefficient (r) between testicular weight and body weight, testicular length, width and perimeter for right and left testes in Japanese quail breeders.

	Body weight	Testicular length	Testicular width	Testicular thickness	Testicular perimeter
Right testis weight	0.12	0.61*	0.75*	0.75*	0.81*
Left testis weight	0.14	0.81*	0.85*	0.90*	0.86*

*Pearson correlation significant at t test ($P < 0,01$)

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Sperm variables along epididymal transit in pigs undergoing puberty

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Introduction

In pigs, there have been several studies describing testicular changes during puberty, recognizing an increase in testicular size and diameter of seminiferous tubules, as well as a dynamic change in the cell populations that make up the stages of the seminiferous epithelium (1 and 2). However, sperm transit variables along the epididimys at this early stage of testicular growth are still largely unknown. This study aims to compare the elongated testicular spermatids along epididymal transit regarding morphology and characterize epididymal sperm chromatin as an indicator of maturation in this period.

Material and Methods

Testes of 8 pigs 6 months of age were obtained from a slaughterhouse, inmediatedly after sacrifice. Testes wear separated from epididymis, which ware sectioned into head, body and tail. Small pieces of each segment were weighed and macerated to evaluate the following variables: Sperm concentration (sperm / g), swelling of chromatin by Sodium thioglycolate (TG) as a reducing agent (3), DNA stability by acridine orange (AO) (4), and sperm morphology (5).

Results and Discussion

Sperm count corrected by the weight of each tissue and expressed per gram of parenchyma shows a progressive increase along the epididymal transit, denoting storage in cauda epididymis, as has been described in other mammals (6). The features of chromatin show a lower number of metachromatic spermatozoa in cauda epididymis (AO), denoting a higher replacement of protamines by histones which is accompanied by greater compaction of chromatin (TG), which involves increased formation of disulfide bridges in it, being highest in the cauda. The teratozoospermia is also lower in cauda epididymis, implying reabsorption (phagocytosis) of abnormal sperm along the epididymal transit. In overall conclusion, these changes are responsible for improving the quality of sperm that will be ejaculated, after completing epididymal maturation (7) (See table below).

	Sperm count (10^5 / gr of tissues).	Metachromasia (%) AO	Swelling (%) TG	Teratozoospermia (%)
Testis	0.15 ± 0.01	13.63 ± 3.42	12.00 ± 2.27	22.63 ± 3.42
Epididymal head	3.53 ± 0.17	6.38 ± 1.60	5.50 ± 2.56	16.13 ± 3.72
Epididymal body	3.66 ± 0.17	2.38 ± 1.60	2.63 ± 1.30	10.00 ± 2.73
Epididymal cauda	3.87 ± 0.20	0.88 ± 0.83	1.25 ± 0.89	7.88 ± 2.53

Mean ± SD.

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Corporal biometrics and testicular volume in Santa Inês ram lambs

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Introduction

Sheep breeding always had great economic importance; the reasons include wool, meat and milk production (1). In this kind of activity is extremely important the correct selection of the male of the herd. Therefore, is essential to know the physiology of testicular, ponderal development and the factors that potentially interfere with these processes (2). For years, the search for indicators of fertility at of the selection ram's programs has been the aim of many studies. Testicular and seminal parameters, sexual behavior, hormonal profiles and their correlations have been evaluated to elucidate the potential of reproductive capacity (3). The biometric of testis is appropriate indicator of the development of other reproductive tract structures and spermatogenic capacity (4). The aim of this study was to correlate the Combined Testicular Volume (CTV), obtained by measuring with caliper or ultrasonography, with the Body Mass Index (BMI) and body weight (BW) in Santa Ines ram lambs at 60 days of life.

Material and Methods

In this study have been used 14 Santa Inês ram lambs, ages 60 to 64 days of life, weighting 18.5 ± 3.4 Kg, born January/2012 at the Laboratory of Biotechnology Applied in Ovine and Caprine Reproduction, located at the São Paulo State University (UNESP, Botucatu, SP, Brazil). Were measured height to withers (HW), and from the sternum (breast-bone) to the ischiatic tuberosity (SIL) data expressed in centimeters, and WB, expressed in Kg. The BMI (5, 6) was calculated using the equation BMI = BW. $[(WH \,.\, 100^{-1})(SIL \,.\, 100^{-1})]^{-1}$. The testicular biometrics was performed using a caliper and ultrasound equipment (Prosound 2 Aloka® with micro convex transducer, UST 9111 – 3.75-7.5 MHz Aloka®). Testicular Anteroposterior axis (AP), lateromedial axis (LM) and height was evaluated not considering the tail of epididymis. For the ultrasonographic measurement skin thickness was not considered. To obtain the Testicular Volume of each testicle, in the two methods (caliper or ultrasonographic measurement), the following equation (7) was used [Testicular Volume = $4/3\pi$ $(AP \,.\, 2^{-1})$ $(LM \,.\, 2^{-1})$ $(height \,.\, 2^{-1})$]. For statistical analysis (Pearson's Correlation), CTV was obtained by adding the values of both testes (right and left).

Results and Discussion

As can be seen in Tab.1, in this experiment, the correlation between both BMI or BW with Combined Testicular Volume measured both with the caliper or ultrasonography was positive and significative ($P < 0.001$). Oliveira and Bicudo (2006) demonstrated in Santa Ines ram lambs aged 70 days that the testicular volume obtained from measurements made with caliper are strongly and significantly correlated with the true testicular volume assessed by the water displacement after slaughter (8).

Table 1. Correlation Coefficients (r) between CTV obtained from the caliper or ultrasonographic measurements with BMI and BW of Santa Ines ram lambs at 60-64 days of age.

Body Biometrics	Combined Testicular Volume (mL)	
	Caliper	Ultrasonography
BMI	0.78	0.76
BW (kg)	0.91	0.87

$P < 0{,}001$ for all the variables.

Biometric analysis of testicular development has great importance because it is significantly correlated with future reproductive capacity (9). In conclusion, our data strengthen the hypothesis that even before puberty testicular measures have a positive correlation with body development (4, 10) and must be monitored by methods here employed.

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Prostate adenocarcinoma in dogs naturally infected by *Leishmania infantum*

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Introduction

Canine visceral leishmaniasis (CVL) is an important parasitic zoonosis endemic in Brazil, caused by the protozoa known as *Leishmania infantum.* In the urban area, the dogs are considered the main reservoir of this parasite (1). Although after the infection the parasite multiply and migrate from lymphoid tissue to other organs, particularly male genital system can also be affected by this parasite (2). In humans, chronic inflammatory diseases could be related with prostatitis and prostate cancer (3) and the development of hepatocellular carninoma was associated with *L. infantum* infection (4). Thus, the purpose of this study was to evaluate the structural changes and amastigotes (*L. infantum*) immunohistochemical detection in prostate of dogs with CVL.

Material and methods

Six adult male dogs (excluding senile and pre-pubertal animals), seropositive for visceral leishmaniasis were selected at the Center for Zoonosis Control (CZC) in Bezerros (State of Pernambuco, Brazil). These animals were submitted to euthanasia as part of the official program for zoonosis control. Fragments of prostate were collected for histopathology and immunohistochemistry diagnostic. All prostate samples were fixed in 10% neutral buffered formalin for 24-48 hours and embedded in paraffin. Tissue sections (5µm) were mounted on slides and stained routinely with hematoxylin and eosin (HE) or further processed for immunohistochemistry.

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Results and discussion

All animals showed some type of histopathological lesion in the prostate. The most frequent injury was prostate adenocarcinoma observed in 100% (6/6) of these organs. Chronic and multifocal inflammatory infiltrates were found in 50% (3/6) of the dogs, while diffuse inflammatory infiltrates composed by macrophages, lymphocytes and plasma cells were observed in 33.33% (2/6) of these. Another predominant lesion was prostatic hyperplasia observed in 66.66% (4/6) of these organs. Furthermore, immunohistochemical detection of amastigotes of *L. infantum* inside of macrophages was observed in the prostate in 16.66% (1/6) of the dogs. Chronic inflammation of prostate may induce or increase susceptibility of the carcinogenic factors (4) and CVL like a chronic infectious disease may be related to this process. In conclusion, visceral leishmaniasis should be included in the differential diagnosis of any animal that presents prostate disorders from endemic areas.

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Low incidence of *Leishmania infantum* in the testis of infected dogs

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Introduction

Dogs are the most important reservoir of *Leishmania infantum* and phlebotomine vectors are the primary way of transmission (1). Although after the infection the parasite multiply and migrate from lymphoid tissue to other organs, particularly male genital system can also be affected by this parasite (2). Hence, the aim of current study was determine by immunohistochemistry the *L. infantum* amastigotes presence in testis of dogs naturally infected.

Material and Methods

Five adult male dogs (excluding senile and pre-pubertal animals), seropositive for visceral leishmaniasis were selected at the Center for Zoonosis Control in Bezerros (State of Pernambuco, Brazil). These animals were submitted to euthanasia as part of the official program for zoonosis control. Fragments of both testes were collected for histopathology and immunohistochemistry diagnostic. All testes samples were fixed in 10% neutral buffered formalin for 24-48 hours and embedded in paraffin. Tissue sections (5µm) were mounted on slides and stained routinely with hematoxylin and eosin (HE) or further processed for immunohistochemistry**.**

Results and Discussion

Histopathological lesions in both testes were observed in 80% (4/5) of animals. The major structural change observed was Sertoli cells vacuolization in 60% (3/5) of the dogs, followed by seminiferous epithelium desquamation (20%) (1/5), presence of proteinaceous acidophilus fluid in the tubular lumen (20%) (1/5) and diffuse inflammatory infiltrated composed by macrophages, lymphocytes and plasma cells in 20% (1/5) of these animals. Only 20% (1/5) of the dogs showed macrophages containing amastigotes of *L. infantum* in the intertubular space of testis. This found could be associated or not with a chronic inflammatory infiltrated composed by macrophages, lymphocytes and plasma cells. Our findings are in agreement with AMARA et al, 2009, which demonstrated that there is no relationship between the presence of amastigotes of *L. infantum* and the intensity of testicular damage. In conclusion, amastigotes of *L. infantum* were only observed in macrophages of the intertubular space as well as the testicular alteration could not be related with *L. infantum* in the testicular tissue.

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Ultrastruture of epididymal principal cells of aged Wistar rats

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Introduction

Although many studies have described the histology of the young and adult epididymis, in both humans and experimental animals (rats; humans; monkeys), very few have focused on how the epididymis is affected during aging (1). The most frequently employed animal models for studying basic mechanisms of aging are rodents (2), specially the Brown Norway rat. The epididymis epithelium contains several cell types: principal, basal, clear, narrow, halo and apical cells. Principal cells are the most abundant cell type and play a major role in secretion and absorption (1). In this study, we examined the ultrastructural changes taking place in principal cells of the epididymis caput and cauda in male Wistar rats of 18 months of age.

Material and Methods

Eight animals (18 months old) were studied. The animals submitted to Ketamine (80 mg⁄BW) and Xylazine (5 mg⁄BW) anesthesia were fixed by whole body perfusion. Briefly, after a saline wash to clear the vascular bed of the testis, they were perfused with glutaraldehyde 5% in sodium caccodylate buffer 0.105 M (pH 7.2) for 20 min. Epididymis were removed and post fixed in the same solution overnight. The tissues were postfixed with 1% osmium tetroxide in the same buffer at 4°C, dehydrated in acetone, and embedded in epoxy resin. Ultrathin sections of caput and cauda were cut with a diamond knife and stained with 2% uranyl acetate and 2% lead citrate prior to observation with a transmission electron microscope (Zeiss, Leo 906).

Results and Discussion

The tall columnar principal cells extend the full thickness of the epithelium from basement lamina to lumen. They have a single irregular nucleus, numerous cisternae of rough endoplasmic reticulum, a very prominent Golgi apparatus and a free surface bearing long stereocilia. Numerous coated vesicles and multivesicular bodies were seen in the apical cytoplasm. The free surface of the cell between the bases of the stereocilia is highly irregular in contour and has numerous depressions and deeper invaginations. According to Hoffer et al. (3) these invaginations are evident stages in the formation of coated vesicles, since they found particles of the tracer in these invaginations, as also observed in the coated vesicles and multivesicular bodies. Principal cells also had lipid droplets in the perinuclear region, lysosome-like bodies, large electron-lucent vacuoles resembling giant endosomes and lysosomes. Many lysosomes have round membranous electron-dense profiles. The same ultrastruture was observed in aged hamsters (4) and Brown Norway rats (1). There were a great number of lipofuscin deposits. Some authors reported (4) the most reliable and widespread cytological change correlating with aging is the accumulation of lipofuscin, a pigment derived by oxidation of lipid or lipoprotein sources. An accumulation of lipopigment has been found in aged neurons, bone cells, muscle and in the epididymis. In conclusion, aging causes morphological alteration of the epididymal epithelium, which probably affects their contribution to sperm maturation.

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Medium-term treatment with Cyclosporin A and *Heteropterys tomentosa*: Lack of effects on the epithelium of testis and epididymis of Wistar rats.

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Introduction

Cyclosporin A (CsA) is a widely used immunosuppressive drug, however its side effects are reported on reproductive organs. Treatment with CsA is associated with structural changes of the testis (1) and epididymis (2). *Heteropterys tomentosa* (A. Juss.) is a medicinal plant efficient against the effects of CsA on the testis of Wistar rats treated during 56 days (1). The present work aimed to evaluate the effects of CsA, *H. tomentosa* and of both treatments on the testis and epididymis of Wistar rats.

Material and Methods

Twenty Wistar rats (eight week old) were separated in: **Control group** receiving 0.5mL of water; **CsA group** receiving 15mg/kg-bw/day of CsA diluted in 0.5mL of water; **Ht group** receiving 0.5mL of *H. tomentosa* infusion; and **CsA+Ht group** receiving the CsA dose diluted in 0.5mL of infusion (n=5 in each). After 21 days of treatment the animals were weighed and euthanized. Testis and epididymis were dissected and weighed. Testis, epididymis caput and cauda were fixed, paraffin embedded, sectioned and stained with hematoxilin and eosin. The diameter of the seminiferous tubules were measured; as well as the thickness of the germ epithelium, epididymis caput and cauda epithelium. The means were compared using ANOVA *post hoc* with Duncan's test.

Results and Discussion

Cyclosporin A did not cause variations in body weight gain and final body weight. Previous studies showed reduction of body weight gain of rats treated with CsA in the same dose but maintained for 56 days (1). Testis weight also did not vary among the experimental groups. CsA did not cause any alteration of epididymis weight, but it was higher in Ht group when compared to the others. In a previous study using the same treatments administered during 56 days there was no variation in the testis and epididymis weights caused by CsA, *H. tomentosa* or both (1). The epithelium thickness of the epididymis caput and cauda did not vary among the experimental groups (Table 1). No significant variation was found for the seminiferous tubule diameters and germ epithelium thickness (Table 1). These results showed that the treatment with CsA during 21 days did not damage the structure of testis and epididymis epithelium of Wistar rats.

Table 1. Biometrical parameters and morfometry of testis and epididymis of Wistar rats (means ± SD).

Parameters	CG	CsA	Ht	CsA+Ht
Body weight gain (g)	106.4 ± 22.38	107.4 ± 16.77	104.0 ± 8.03	91.4 ± 12.38
Final body weight (g)	357.2 ± 44.94	364.4 ± 30.73	358.0 ± 15.98	348.4 ± 16.56
Testis weight (g)	1.62 ± 0.05	1.53 ± 0.12	1.70 ± 0.79	1.55 ± 0.15
Epididymis weight (g)	0.42 ± 0.03[a]	0.43 ± 0.03[a]	0.49 ± 0.04[b]	0.43 ± 0.03[a]
Epithelium thickness (µm)				
Epididymis caput	19.89 ± 1.33	19.69 ± 2.15	19.74 ± 1.64	19.36 ± 2.32
Epididymis cauda	18.45 ± 3.90	17.75 ± 0.96	15.65 ± 1.09	18.50 ± 2.39
Seminiferous tubules	82.46 ± 8.50	82.15 ± 2.63	78.73 ± 5.85	76.33 ± 4.49
Diameter (µm)				
Seminiferous tubules	239.46 ± 13.63	246.29 ± 12.60	243.15 ± 19.84	230.57 ± 23.20

CG= Control group; CsA= Cyclosporin A treated group; Ht= *H. tomentosa* treated group; CsA+Ht= group treated with CsA and *H. tomentosa* infusion.
Different superscriptions indicate significant difference according to Duncan's test ($p<0.05$)

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Intratesticular injection of zinc gluconate as a permanent contraceptive for cats

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Introduction

Recently, we reported that a commercial zinc gluconate (ZG) preparation (Testoblock®) disrupted spermatogenesis and caused apparently permanent sterilization in male dogs (1). However, there is a paucity of reports regarding similar approaches in the male cat. Therefore, the objective was to evaluate the efficacy of intratesticular injection of ZG (Testoblock®) as a permanent contraceptive for domestic male cats.

Material and Methods

Sixteen sexually mature mongrel cats were assigned to two groups, Control (n=5) and Treated (n=11), received a single injection of saline or Testoblock®, respectively, into each testis on Day 0. The volume injected was based on 1mL per 27mm testis width, approximately twice the dose (per gram of testis) injected in dogs. Physical examination, testis width, semen characteristics, hematology, clinical chemistry (hepatic and renal function), and libido were assessed on Days 0 (before injection) 60, and 120.

Results and Discussion

Testis width did not change significantly in Control cats. However, in Treated cats, there was evidence of testicular atrophy, based on reductions in testis width on Days 60 and 120 relative to Day 0 (30% and 37%, respectively, $P<0.05$), with reductions (relative to Control cats) on those 2 moments of evaluation of approximately 17% ($P=0.06$) and 23% ($P=0.118$). Regarding sperm parameters, on Day 60, the ejaculates of 10 Treated cats were azoospermic, whereas the remaining cat had lower sperm counts and reduced motility, whereas on Day 120, the ejaculates of 8 (72%) cats were azoospermic, 1 had necrospermia and 2 still had viable sperm. In contrast, Control cats had excellent semen quality throughout the study. Values for hematology and clinical chemistry consistently remained within reference ranges for all cats, with no significant difference between groups. In Treated cats, there was substantially reduced libido, less mounting, aggression and urine marking (spraying) from Day 60 to the end of the study. Furthermore, penile spines (which are testosterone-dependent) were either decreased (6 of 11) or absent (4 of 11) in Treated cats, except for 1 cat which still had well-developed penile spines on Days 60 and 120. In contrast, penile spines remained well-developed in Control cats. To our knowledge, the present study was the first report of a careful clinical/spermatological investigation of the effects of sterilization of cats by one-time bilateral intratesticular injection of ZG. We concluded that intratesticular injection of the zinc gluconate-based chemical sterilant Testoblock® has great potential as a permanent contraceptive for male cats.

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Testicular and hepatic pigmentation of the Anuran *Eupemphix nattereri*: morphological and stereological effects of testosterone cypionate

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Introduction

In amphibians, pigmented cells appear in tegument and in lung as typical melanocytes and in spleen and liver as melanomacrophages or also known as Kupffer cells. The present study aimed at characterizing morphological and stereological patterns of pigmented cells in the liver and testis of the anuran *Eupemphix nattereri*, under effect of sexual hormone.

Material and Methods

Ten adult males, collected in Sao Paulo State, Brazil, received a 5 mg/kg dose of testosterone cypionate solving in vegetal oil during 7 days, with subcutaneous injections. Group of 5 animals was euthanatized after 24h and other group, after 15 days of recovery. Testes and liver are submitted to morphological studies with light microscopy and TEM. The control group received only vegetal oil at the same concentration. Were analyzed 25 histological fields for each animal using an image analyzing system software (Image ProPlus).

Results and Discussion

In the testes, melanocytes-like cells are present in the interstitium, with large and irregular aspect and a great amount of intensely pigmented cytoplasm. In the hepatic tissue were found kupffer cells, characterized by multivesicle bodies in the cytoplasm, including large amount of melanosomes. Was observed an increase of approximately 2x in the occupied area by the pigmented cells in the liver and 4x in the testes, comparing the treated group and the control. Between the treatments, an increase of approximately 1,8x was observed in the liver of animals euthanatized after 15 days.

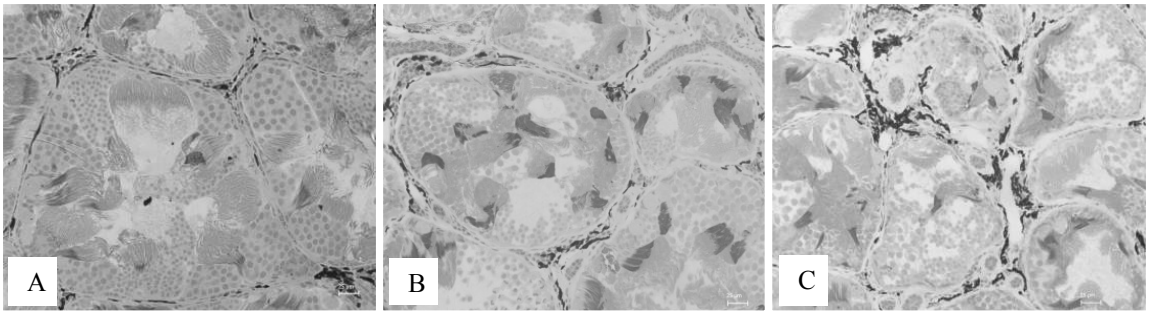
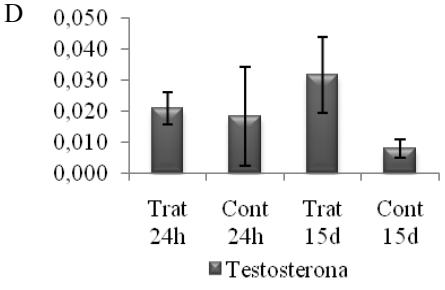


Figure**:** Testicular sections of *E. nattereri* showing seminiferous locule (L) and melanocytes (pigmented area) (arrow). **(A)** Control group. **(B)** Experimental group after 1 day. **(C)** Treatment after 15 days. **(D)** Differences in the pigmented area of the testes between the treated groups and control group.



Keywords: melanocytes, Kupffer cells, liver, testes, testosterone.

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Cryopreservation of collared peccary (*Tayassu tajacu*) epididymal sperm using coconut powdered water (ACP®) and Tris based extenders

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Introduction

The potential use of epididymal sperm is attractive, especially following the death of a valuable male. In that regard, epididymal sperm could be recovered postmortem, cryopreserved with appropriate extender, and used for in vitro fertilization when appropriate oocytes became available (1). The recovery and conservation of the epididymal sperm have been reported for several domestic species, and also demonstrated as an alternative for wild animals (2). The aim of this study was to compare the use of coconut powdered water (ACP®) and Tris extenders for recovery and cryopreservation of epididymal sperm from collared peccaries (*Tayassu tajacu*).

Material and Methods

Six sexually mature male collared peccaries were submitted to an annual planned slaughtering for population control, respecting all ethical and animal welfare standards. The animals belonged to the Centre of Multiplication of Wild Animals from UFERSA. Epididymal sperm were obtained by retrograde flushing. Each cauda epididymis was subjected to a retrograde flush with 5 mL of solution (at 37ºC), being the first washed with ACP, and the other with a buffered media based in Tris. Sperm evaluation was immediately conducted for the following parameters: number of sperm recovered (sperm concentration × volume), motility, vigor, viability, morphology and functional membrane integrity. Then, the samples were centrifuged at 3 G during 10 minutes. The supernatant was removed and the pellet was ressupended in ACP or Tris with 20% of egg-yolk. The procedures for cryopreservation and thawing were conducted as recommended by Castelo et al. (3) for the ejaculated sperm from same species. The statistical analysis was performed using the StatView software. The results were expressed as mean ± SEM. Data were submitted to analysis of variance followed by Student's t-test ($P < 0.05$).

Results and Discussion

A total of 1023 (± 251.5) × 10^6 sperm were recovered by flushing with ACP, and 1566.3 (± 725.1) × 10^6 sperm by flushing using Tris. The sperm parameters are shown in Table 1; no statistical difference was verified between the extenders. These results are similar to those previously described for the agouti epididymal sperm (4). The present study generated new knowledge regarding collared peccary epididymal sperm. In conclusion, both extenders can be used efficiently for the cryopreservation of collared peccary epididymal sperm.

Table 1. Values for sperm characteristics of collared peccaries epididymal sperm, both after initial recovery by retrograde flushing, and after frozen-thawed samples.

	Fresh samples		Frozen-thawed samples	
Parameters	ACP	Tris	ACP	Tris
Motility (%)	87.5 ± 5.3[a]	70 ± 17.5[a]	35.0 ± 5.6[b]	38.3 ± 6.5[b]
Vigor (1-5)	4.0 ± 0.4[a]	3.6 ± 0.6[a]	2.0 ± 0.2[b]	2.0 ± 0.3[b]
Viability (%)	79.1 ± 2.3[a]	79.5 ± 2.6[a]	36.8 ± 3.1[b]	38.1 ± 6.4[b]
Functional membrane integrity (%)	74. 3 ± 4.8[a]	78.1 ± 2.7[a]	52.2 ± 5.3[b]	49.8 ± 8.3[b]
Normal sperm (%)	79.8 ± 2.3[a]	62.7 ± 14.5[ab]	55.2 ± 6.6[b]	40.8 ± 8.9[b]

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The relationships between testicular volume, sperm quality and scrotal surface temperature in adult stallion

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Introduction

Testicular thermoregulation in domestic animals is dependent on the contraction and relaxation of the dartos and cremaster muscles, the activity of the sweat glands, heat irradiation of the scrotal surface, arteriovenous heat exchange via the pampiniform plexus counter–current mechanism and distance between testes and abdomen (1,2,3). In horses, normal spermatogenesis occurs at an average intratesticular temperature of 35°C and this mechanisms for testicular thermoregulation are essential (4).The objective of this study was to determine whether there is a correlation between the testicular volume (TV), sperm quality and scrotal surface temperature (SST) in adults horses.

Materials and Methods

Fifteen healthy Quarter Horse stallions, ranging in age from five to 15 years, were used. Every animal were previously conditioned in a shady environment with temperature of 25 °C and SST was measured using an infrared thermographer (Infra CamTM, FLIR Systems Inc.). In all of the animals were measured too the anterior-posterior axis (AP, cm), medial-lateral axis (ML, cm) and height (HGT, cm) of the testes. The testicular biometry data of the both testes were used in the formula $V = (3/4) \times \pi \times (AP/2) \times (ML/2) \times (HGT/2)$ in cm^3 (4) to calculate the testicular volume (TV). In addition, the sperm motility parameters were analyzed using Computer-Assisted Semen – CASA (Hamilton-Thorne, Beverly, MA, USA). Correlations between the parameters were performed using Pearson's test.

Results and Discussion

There was a negative moderate correlation (r=-31) between TV (cm3, 183.06±65.33) and SST (oC, 34.27±0.68). There was positive moderate correlation (r=0.55) between TV and total sperm motility (%, 68.42±22.27). There was also a positive moderate correlation (r=0.47) between TV and rapid sperm (%,56.82±22.56). The correlation between SST and total sperm motily was r=-0.26 and between SST and rapid sperm was r=-0.29. The results of this study indicate that animals with a larger testicular volume also have better thermoregulation testicular capacity and smaller testicular temperature and therefore have better sperm quality. These results together with the measurement of scrotal superficial temperature have potential for use as a complementary examination technique in andrological evaluations.

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17β-estradiol increased testicular pigmentation in *Eupemphix nattereri* (Anura: Leiuperidae)

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Introduction

Ectothermic vertebrates have a well-developed system of melanin-containing cells, which are distributed throughout several organs and tissues (1). The functional paper of these pigment cells in the organs is not well defined, but there are hypotheses that may have cytoprotective functions against free radicals, detoxification of pollutants and pathogens. Melanin has a protective role against the effects of bacteria, and testicular pigmentation increased after infection (2). This protection is very important in these animals because they are commonly in contact with some pathogens in the habitats where they live. Therefore, we believe that sex steroid hormone 17β-estradiol increased testicular pigmentation due protective actions of melanin.

Material and Methods

We used 10 adult males of *Eupemphix nattereri* that receiving subcutaneous doses containing 5mg/kg de 17β-estradiol, dissolved in vegetable oil, during 7 days. We analyzed the testes of five animals 24h after the last injection, and another five animals after 15 days of recovery. The control group received only vegetable oil.

Results and Discussion

We did not observe differences in the group treated with 17β-estradiol and analysed after 24h. However, in the animals examined after 15 days of recovery, was observed an increase of the pigmented area in the testes ($F=45.01$; $P<0.0001$). These results indicate that the sex hormone caused changes in the testicular pigmented area of *E. nattereri* after 15 days of recovery. The increase of melanin can indicate a defensive reaction against to endocrine disruption caused by sex hormonal treatment.

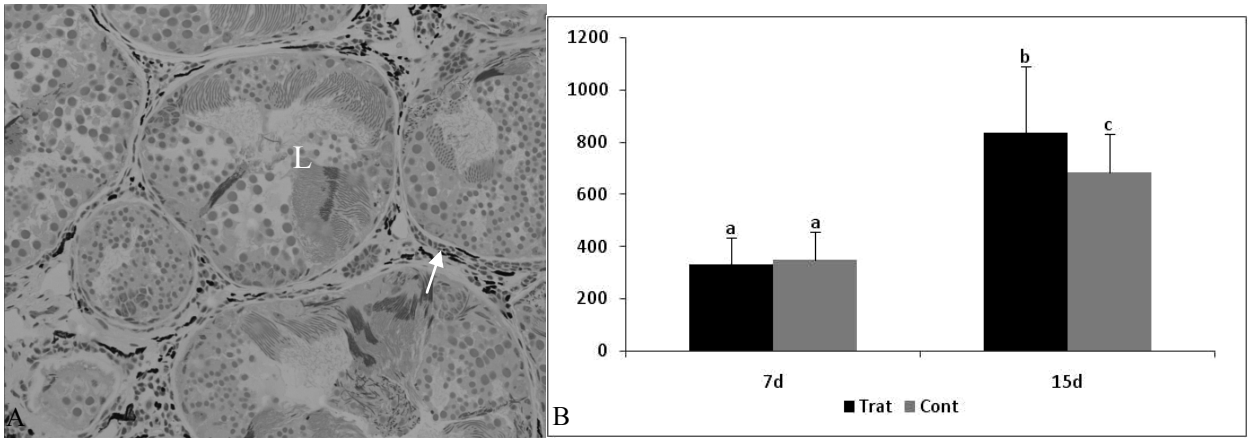

Figure 1. **A:** Pigmentation in the interstitial testicular area of *Euphemphix nattereri* (arrow), around seminiferous locule (L). **B:** Quantity of testicular pigmentation in treated animals (24h) and after fifteen days (15 d) of exposure to 17β-estradiol. Different letters indicates statistical differences ($P<0.05$).

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Measurements of reproductive organs and the ventral prostate epithelia of Wistar rats treated with Cyclosporin A and *Heteropterys tomentosa*

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Introduction

Cyclosporin A (CsA) is a widely used immunosuppressive drug. However, according to previous study, treatment with CsA caused reduction of ventral prostate epithelium thickness and epithelial atrophy (1), probably due to both the reduction of plasma testosterone levels and increase of plasma glucose levels (1). *Heteropterys tomentosa* (A. Juss.) is a Brazilian medicinal plant popularly known for its hypoglycemic and aphrodisiac properties (2, 3). *H. tomentosa* was efficient against the effects of CsA on the testes of Wistar rats treated during 56 days (1). The present study was undertaken to evaluate the effects of CsA, *H. tomentosa* and of both treatment on the ventral prostate of Wistar rats.

Material and Methods

Forty rats (8 weeks) were separated in 2 groups, one treated during 21 days and another during 56. Each group was subdivided into 4 subgroups (n=5). A control group received water(0.5mL). The CsA group received 15mg/kg/day of CsA, while the Ht group received 0.5mL *H. tomentosa* infusion. The CsA+Ht group received both treatments in 0.5mL. The animals were euthanized, the ventral prostate, coagulating gland and seminal vesicle dissected and weighed. The ventral prostate was fixed, Historesin embedded, sectioned and stained with hematoxilin and eosin. The epithelium thickness of the ventral prostate was measured. The means were compared using Duncan's test.

Results and Discussion

Treatment during 21 days: The coagulating gland weight was significantly higher in the Ht group (Table 1). The vesicular gland weight was significantly higher in CsA and CsA+Ht groups when compared to the control (Table 1). The thickness of the ventral prostate epithelium did not vary (Table 1). *Treatment during 56 days:* The ventral prostate weight was significantly reduced in the CsA-treated group when compared to the control; however, this treatment did not have the same effect on the CsA+Ht group (Table 1). The coagulating and vesicular gland weights were reduced in all treated groups, when compared to the control (Table 1). The ventral prostate epithelium thickness did not vary (Table 1). Previous study (1) showed that the treatment with CsA during 56 days caused atrophy of the ventral prostate epithelium. The absence of alteration in the treatment during 21 days could be due to the short treatment period. However, the absence of variation in 56 days treatment groups was unexpected and should be investigated.

Table 1. Biometrical parameters and morphometry of Wistar rats ventral prostate (means ± SD).

Parameters	21 Days				56 Days			
	CG	*CsA*	*Ht*	*CsA+Ht*	*CG*	*CsA*	*Ht*	*CsA+Ht*
Ventral prostate*	0.32 ± 0.06	0.28 ± 0.02	0.43 ± 0.23	0.32 ± 0.08	0.33 ± 0.03^{a}	0.22 ± 0.05^{b}	0.27 ± 0.03^{ab}	0.31 ± 0.1^{a}
Coagulating gland*	0.13 ± 0.01^{ab}	0.12 ± 0.01^{a}	0.14 ± 0.02^{b}	0.11 ± 0.02^{a}	0.17 ± 0.02^{a}	0.11 ± 0.01^{b}	0.13 ± 0.03^{b}	0.12 ± 0.02^{b}
Vesicular gland*	0.66 ± 0.13^{a}	1.49 ± 0.16^{b}	1.42 ± 0.11^{ab}	1.44 ± 0.13^{b}	0.81 ± 0.04^{a}	0.53 ± 0.07^{b}	0.60 ± 0.10^{b}	0.63 ± 0.10^{b}
Epithelium thickness (μm)$^{\#}$	21.19 ± 1.16	20.97 ± 0.89	19.80 ± 2.76	19.32 ± 3.66	17.04 ± 2.61	16.96 ± 2.02	17.65 ± 2.74	17.00 ± 3.10

CG= Control group; CsA = Cyclosporin A treated group; Ht = *H. tomentosa* treated group; CsA + Ht = group treated with CsA and *H. tomentosa*. *Organs' weights (g); $^{\#}$Ventral prostate epithelium thickness.
Different superscriptions indicate significant difference according to Duncan's test ($p<0.05$)

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Isolation and partial characterization of Binder of Sperm Protein 1 (BSP1) from three bovine subspecies raised in Brazil

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Introduction

Binder of Sperm 1 (BSP1) is the major protein in bull seminal plasma and mediates sperm capacitation and binding to the oviduct. However, little information about the structure and function of BSP1 from other bovine subspecies is available. *B. indicus* evolved in the Indian subcontinent and is well-adapted to hot climates, while *B. ibericus*, on the other hand, developed in the Iberian Peninsula, from European *B. taurus*, and were brought to Brazil in the early 1600s. Brazilian *B. ibericus* breeds are small-sized and tropically-adapted, developed mostly in semi-arid regions. Therefore, the aim of this work was to purify and characterize the major BSP1 from the seminal plasma from *B. taurus*, *B indicus* and *B. ibericus* bulls.

Material and Methods

Seminal plasma (SP) samples from 4 Brown Swiss (*B. taurus*), 13 Guzerat (*B. indicus*) and 10 Curraleiro (*B. ibericus*) bulls were obtained by semen centrifugation and pooled for each breed. For purification of BSP1, 25 mg of SP proteins from each pool were subjected to heparin affinity chromatography. Heparin-binding proteins were further separated by gelatin affinity chromatography. Proteins with affinity for both heparin and gelatin (HBPGBPs) were separated by 2D-PAGE and identified by ESI-Q-ToF mass spectrometry. MS/MS spectra were searched against NCBInr database using the MASCOT tool. The N-terminal sequence of BSP1 isolated from each of the three subspecies was determined by Edman's degradation. Sequences were blasted (blastp) against *B. taurus* sequence available at the NCBI database.

Results and Discussion

BSP1 was efficiently isolated from all three genotypes after two chromatographic steps followed by molecular exclusion separation. 2D gels of B. Swiss bulls HBPGBP had 6 low molecular weight spots, while gels from Guzerat had only 4. MS/MS analyses from all spots were identified as *B. taurus* PDC-109 (BSP1). N-terminal sequences of all three genotypes were aligned with that of PDC-109 and displayed extensive identity (Figure 1). *B. ibericus* BSP1 is 100% identical to the sequence in the NCBI, while those from Brown Swiss and Guzerat were 81% and 83% identical, respectively. Substitutions on amino acid sequence between all three subspecies are for amino acids of the same non-polar group, suggesting small structural differences, if any. The next step will consist in completing sequencing of the protein and functional characterization of those proteins, to get more insights on how they affect sperm from these tropically-adapted bulls.

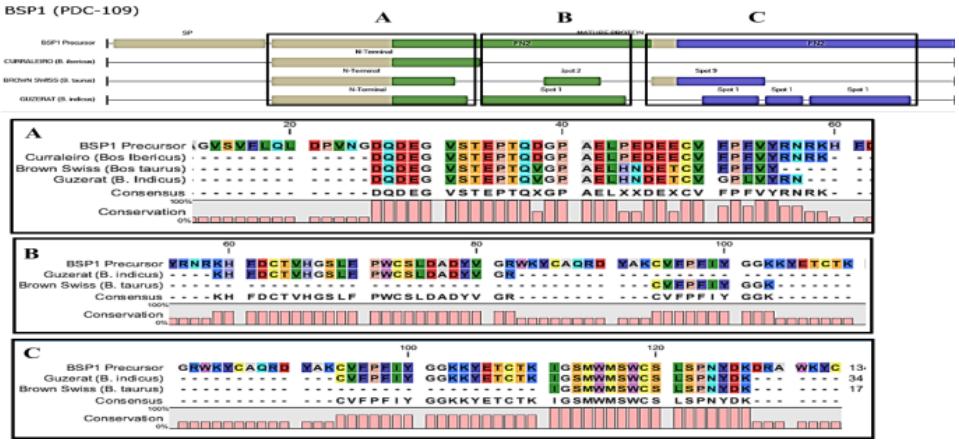

Figure 1. N-terminal sequences and peptides identified by MS/MS of BSP1 from the three bovine subspecies.

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Influence of melatonin on sperm parameters and antioxidant system in diabetic rat epididymis

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Introduction

Diabetes mellitus unbalances male sexual functions due androgen withdraw and hyperglycemia (1). Indeed, the contribution of increased oxidative stress cannot be neglected, particularly in chronic diabetes. The specific action of the neurohormone melatonin, a powerful antioxidant, against the reproductive damage caused by diabetes is poorly understood. This study evaluated the impact of treatment with low doses of melatonin from weaning to adulthood on oxidative stress biomarkers and sperm parameters in the rat epididymis and the interferences on damages induced by experimental diabetes.

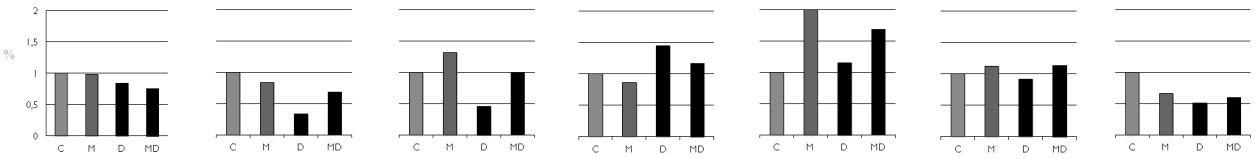
Material and Methods

Male Wistar rats (13 weeks old) were assigned into four groups (n=8/group): control (C), treated with melatonin (M), diabetic (D) and diabetic treated with melatonin (MD). Melatonin was provided in drinking water from 5th to 13th weeks (2.5µg/Kg b.w. in 0.001% ethanol per day) and diabetes was induced at 12th week of age with a single dose of streptozotocin (4.5mg/100g b.w., i.p.). In addition to epididymal weight (**W**), plasma testosterone levels (**T**) were assayed by ELISA. The activities of oxidative stress biomarkers - catalase (**CAT**), glutathione-S-transferase (**GST**) and glutathione peroxidase (**GPx**) - were quantified in plasma and epididymal extracts using biochemical tests. Sperm counts (**S**) and transit time (**TT**) were estimated in epididymis *caput/corpus* (**Cp**) and *cauda* (**Cd**).

Results and Discussion

Results of the study are shown below. Numerical data (Table) are expressed as mean ± SEM and different letters indicate statistically significant values. The graphs show the variations in comparison to control (C).

Groups	W (g)	SCd (x10^6/organ)	TTCd (days)	CAT(U/mg)	GPx (U/mg)	GST(U/mg)	T (ng/dl)
C	0.58 ± 0.1^a	196.09 ± 34^a	6.38 ± 1.67^a	2.86 ± 0.2 ^{a}	0.13 ± 0.05^a	0.58 ± 0.1^a	249.56 ± 43^a
M	0.57 ± 0.1^a	163.83 ± 34^a	8.35 ± 2.0 ^{a}	2.47 ± 0.4 ^{a}	0.26 ± 0.03^b	0.65 ± 0.1^a	165.91 ± 25^a
D	0.49 ± 0.1^b	65.43 ± 10^b	2.91 ± 0.63^b	4.15 ± 0.6^{b,c}	0.15 ± 0.04^{a,c}	0.53 ± 0. ^{a,b}	126.33 ± 47^{b,c}
MD	0.44 ± 0.1^b	134.62 ± 58^c	6.31 ± 2.24^a	3.35 ± 0.6^{a,c}	0.22 ± 0.02^{b,c}	0.66 ± 0.1^{a,c}	150.13 ± 92^{a,c}



Exposure of weaned rats to low doses of MT did not affect epididymis weight, sperm counts or transit in epididymal segments, but improved GPx and GST activity in extracts of whole organ. One-week-diabetes led to a reduction ~ 50% in testosterone levels and epydidymal atrophy and also reduced the sperm counts ~ 67 and TT ~54% in Cd. Neither experimental diabetes nor MT affected the oxidative stress biomarkers in plasma, probably due to the short period of disease. Melatonin treatment preserved the TT in Cd and ameliorated the sperm reserve and circulating testosterone. Such treatment also improved GST and GPx. In conclusion, administration of low melatonin doses to prepubertal rats could not avoid epididymis atrophy after one week of diabetes, but this neurohormone seems to be effective in ameliorating fertility, by normalizing transit time and sperm number in epididymal *cauda* and influencing antioxidant activities, specially CAT. Besides, our previous studies showed that the epydidymal antioxidant system was modified ealier by experimental diabetes than ventral prostate and testis.

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Contributions of the epididymis and accessory sex glands to the proteome of seminal plasma and acessory sex glands in Morada Nova rams

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Introduction

Seminal plasma is mainly composed by secretions of the epididymis and accessory sex glands and contains proteins associated with various characteristics such as sire fertility (1) and semen freezability (2), among others. Given their importance on numerous functions in sperm, it is essential the development of strategies for the identification of components of seminal plasma and the study of the interaction of these with spermatozoa.

Material and Methods

Six Morada Nova rams had their semen collected, and the spermatozoa and seminal plasma were separated by centrifugation. The rams were then vasectomized, and fluids from the accessory sex glands (AGF) were collected. After all collections, the animals were slaughtered and fluids from the vesicular gland, bulbourethral gland were obtained. Cauda epididymal fluid and sperm were also collected. Membrane and fluid proteins were extracted and subjected to 2-D (12,5%) electrophoresis. Western blots using anti-bodhesin-2 were performed for fluids and sperm membrane proteins.

Results and Discussion

Major proteins of seminal plasma, such as RSVPs and spermadhesins (Fig. 1, box b), previously identified by our team (3) come from the vesicular glands. Others, such as clusterin (Fig. 1, box a), appear to come mainly from the epididymis, although less abundant in the AGF. Albumin (Fig. 1, box c), in turn, is shown in most fluid, including cauda epididymal, vesicular gland and bulbourethral gland fluid. Ejaculated sperm membrane-rich proteins appear markedly different from those of cauda epididymal sperm (Fig. 2A and 2B, respectively). Major seminal plasma proteins, such as bodhesin 2, are only seen in ejaculated sperm. This finding is confirmed by immunoblots, showing that bodhesin 2 is present as a series of 4 low molecular weight spots (Fig. 2C), as has been shown in the seminal plasma and vesicular gland fluid (Fig 2D), as shown for Santa Inês rams (3). Other spots, previously identified as actin and alpha-2-HS glycoprotein originate from multiple fluids. We also show that fluids from different accessory sex glands differ markedly from each other, but protein maps from AGF (all fluids mixed, collected from vasectomized males) resemble that of vesicular gland fluid, suggesting that this gland contributes most intensely with AGF. Taken together, our results suggest that different regions of the reproductive tract contribute with different proteins for seminal plasma, and that these proteins change significantly sperm membrane protein pattern after ejaculation, potentially playing important roles in the modulation of sperm function.

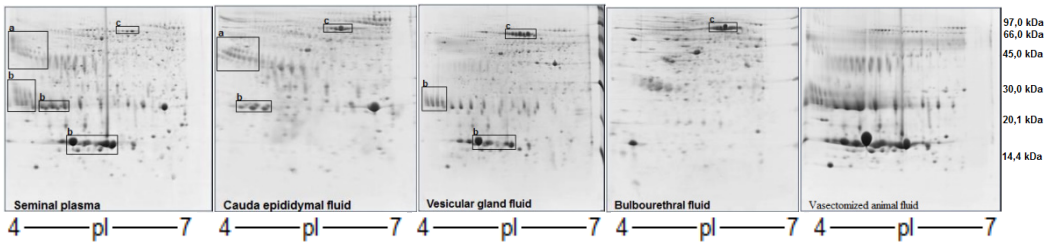

Figure 1. 2-D maps of seminal plasma, AGF, vesicular gland, bulbourethral and cauda epididymal fluid.

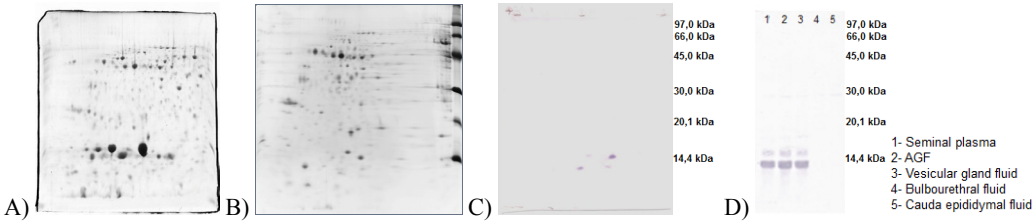

Figure 2. 2-D map of sperm membrane-rich proteins from ejaculated (A) and cauda epididymal (B). Immunoblots against bodhesin-2 of sperm membrane from ejaculated (C) and reproductive tract fluids (D).

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Spermadhesins from ram seminal plasma: gene expression in the reproductive tract, biochemical attributes and interactions with the sperm membrane

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Introduction

Spermadhesins are among the major ram seminal plasma proteins. They were identified by mass spectrometry as bodhesins 1 and 2, and are predominantly secreted by the vesicular glands (1). In ungulates, spermadhesins mediate sperm capacitation and formation of the oviduct reservoir (2). However, in hairy rams, there is no information available about their ability to bind sperm or their chemical attributes. Such information is crucial for understanding how they affect sperm function and, ultimately, reproductive outcomes. Therefore, the purpose of this research was to study if ram seminal plasma spermadhesins have the ability to interact with the sperm membrane and possible ligand candidates, such as heparin and gelatin.

Material and Methods

Semen samples were collected from five adult and reproductively sound Morada Nova rams and centrifuged to separate sperm from seminal plasma. Sperm membrane proteins were extracted and separated using 2-D electrophoresis. One gel was transferred to a PVDF membrane and blotted with anti-Bodhesin 2 (BDH2) polyclonal antibodies. Seminal plasma proteins were separated by SDS-PAGE with or without DTT, transferred and blotted as described. Spots that reacted with anti-BDH2 were trypsin-digested and identified by tandem mass spectrometry (ESI-Q-ToF MS/MS). Seminal plasma proteins were also separated using heparin- and gelatin-affinity liquid chromatography. Both fractions were separated by SDS-PAGE and blotted against anti-BDH2. Tissue samples from testis, epididymis and vesicular glands were collected from 5 other rams at a local abattoir, total RNA was extracted and real-time PCR analysis were run in duplicates to determine the sites of bodhesin 2 expression in the reproductive tract.

Results

Our results showed that bodhesin 2 was not detected in membrane extracts from epididymal sperm but present as major spots in extracts from ejaculated sperm. There was a single band around 14kDa under non-reducing and denaturing conditions, against two bands with slightly different molecular weight in the reduced form. Two spots reacted with anti-BDH2 antibodies (13.4 and 14.1 kDa, pIs 5.5 and 5.6) and their identities were confirmed as bodhesin 2 by mass spectrometry. Peptides identified from such spots matched to the C-terminal region of the multifunctional CUB conserved domain. Based on immunocytochemistry associated with confocal microscopy, using the same antibodies, we show that BDH2 binds to the acrosome, equatorial region and, with less intensity, to the midpiece of ejaculated sperm. This binding pattern changed with capacitation, with BDH2 relocating from the acrosomal to the equatorial and post-equatorial regions. Despite a high homology in amino acid sequence, biochemical differences do exist between goat and ovine spermadhesins. Hairy ram spermadhesin identified in this work binds to heparin, but not to gelatin, while others have shown that bodhesin 2 from goat seminal plasma has no affinity for heparin (3). As evaluated by qPCR, BDH2 gene is mainly expressed in the vesicular glands, and this protein is the second most abundant in the vesicular gland fluid (10.8%). In conclusion, our findings show that spermadhesins are expressed and secreted predominantly by the vesicular glands, and bind to ovine sperm at ejaculation, suggesting its participation in the modulation of sperm functionality. In pigs, where most studies have been conducted, spermadhesins mediate sperm capacitation, formation of the oviduct reservoir and sperm-oocyte binding. The finding that sheep spermadhesins bind to heparin, contrarily to those from goats, suggests that the protein found in the ram sperm membrane might play different roles towards regulation of sperm function.

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Tissue alterations in dorsolateral prostate of rats exposed to obesogenic environment from gestation to adulthood

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Introduction

Obesity is considered a serious public health problem worldwide and its associated metabolic disturbances can affect negatively male reproductive function (1,2). Most experimental studies concerning the effects of obesity on prostate gland are based on ventral lobes and the response of dorsolateral lobes, the rodent complex components which are homologues to the peripheral region of human prostate, is neglected (3,4). The aim of this study was to evaluate if obesogenic environment due high-fat diet power supply from gestation to adulthood interferes on dorsolateral prostate histology of adult rats.

Materials and Methods

Obesity was initially induced on female Wistar rats (4w old) by using a high-fat diet (20%fat, 4.9kcal/g) during 15 weeks whereas control animals received balanced chow (3%fat, 3.5Kcal/g). The progeny obtained by crossing these females with normal rats received the same feed of their mothers for 15 weeks. Following the animals' death, the dorsolateral prostate lobes were removed, weighed and processed for paraffin embedding. The lateral e dorsal counterparts were stained with Tuchmann's Blue and analyzed using stereological methods. The cell proliferation activity was estimated using immunohistochemistry for PCNA.

Results and Discussion

The exposure to obesogenic environment from pregnancy to adulthood was effective in inducing obesity, marked by significant increased body weight, adiposity index, and hyperglycemia accompanied by insulin resistance, as indicated by ipITT. This condition promoted the growth of dorsolateral prostate lobes like previously observed to ventral lobes (5,6). The prostate growth was accompanied by disturbances in the relative proportion of tissue gland components, i.e. decrease in acinar epithelium and stroma and increase in acinar lumen (Table). Obesogenic environment led to discrete reduction in relative frequency of fibromuscular layer and increase in the collagen amount. The remaining stroma constituents such as blood vessels and other compounds were unaffected by obesogenic environment. Cell proliferation activity increased 2.5-fold in lateral lobes of obese rats and were unaltered in dorsal lobes. The histological alterations observed in lateral and dorsal prostate lobes under obesogenic conditions are similar to those previously described to ventral lobe. However the proliferative response is more pronounced for lateral lobes in comparison to ventral ones.

Table 1. Relative frequency (%) in main tissue components of dorsal and lateral rat prostate lobes. Values represent the mean and ± standard deviation. a: different from its respective control; *: percentage of the total stroma.

				Stroma		
Groups	Lobe	Epithelium	Lumen	Total	Fibromuscular*	Collagen*
Control	Dorsal	15.69 ± 0.7	45.26 ± 1.5	39.05 ± 1.4	23.53 ± 1.1	33.76 ± 1
	Lateral	29.83 ± 1.3	42.33 ± 1.7	27.85 ± 1.5	30.98 ± 1.8	35.42 ± 1.8
Obese	Dorsal	14.42 ± 0.7	50.5 ± 2[a]	33.08 ± 1.9[a]	19.57 ± 1.6[a]	41.39 ± 2.4[a]
	Lateral	18.96 ± 0.9[a]	54.56 ± 1.7[a]	26.49 ± 1.4	27.85 ± 1.6	41.36 ± 2.2[a]

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Effects of continued exposure to high-fat diet on ventral prostate structure and cell proliferation

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Introduction

Clinical studies have shown an increased incidence of prostatic lesions and worse prognosis for prostate cancer in obese and overweight patients (1). In addition, experimental data evidenced that the high-saturated fat (HF) intake during prostatic maturation significantly enhances androgen receptor expression and rat ventral prostate growth (2). The effects of continued exposure to HF from intrauterine life to adulthood in prostate gland have not been investigated. The present study evaluated the consequences of obesogenic environment induced by HF diet during gestation to adulthood on the ventral prostate structure and cell proliferation levels.

Materials and Methods

Male Wistar rats (4w old), born from obese and control mothers, were fed for 15 weeks with balanced (3% satured fat, 3.5Kcal/g; Control group - C) or high-fat (20% satured fat, 4.9kcal/g; Obese group - O) diet. After experimental period, rats were killed, blood samples were collected and the ventral prostate was removed, weighed and processed for light microscopy. Immunohistochemistry for proliferating (PCNA) cells and stereological analysis were performed in prostate sections.

Results and Discussion

Obese rats presented an increase of 65% in body weight, 170% in adiposity index and hyperleptinemia. Also exhibited hyperinsulinemia (C: 1.9±0.4; O: 14.6±1.6 mg/ml) and insulin resistance that was detected by ipITT. It was observed in obese rats a diminution in circulating testosterone accompanied by rise in estrogen plasma levels. It is well established that androgens are decreased in obese individuals (3), and this reduction can be correlated with increased insulin (4) and leptin levels (5) as well as with the peripheral conversion of androgens to estrogens (6), which would explain the increased estrogen levels. In addition, obesity led to ventral prostate growth (2) and periprostatic fat gain. The continued exposure to obesogenic environment promoted histological changes in rat ventral prostate, as increase of acinar epithelium height, folding and nuclei density resulting in foci of stratification and epithelial dysplasia. Significant differences in epithelial morphology were observed within the acinus such that areas exhibiting high stratified epithelium areas were continuous with epithelial atrophic areas. The stereological analysis indicated a discrete increase in acinar epithelium and stroma and decrease in acinar lumen. The density of proliferating cells (PCNA-positive) increased in the acinar epithelium of the prostate obese rats, but did not change in the stromal compartment, which indicates an imbalance between epithelial and stromal compartments. These analyzes showed that continued exposure to obesogenic environment led to significant changes in several metabolic parameters and subtle morphological alterations on ventral prostate.

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Epididymal morphology of *Oligoryzomis* sp. from Atlantic Forest

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Introduction

The Brazilian ecosystems are undergoing an accelerated process of fragmentation, in which most species are represented by sets of small isolated populations (1). The destruction and great biological richness have put the tropic forests in the spotlight (2, 3), specially the Atlantic Forest. Small rodents have an important influence on the dynamics of Neotropical forests, and are good indicators of local habitat changes. The study of the reproductive physiology of wild rodents in order to understand the functioning of your epididymis is essential to the understanding of reproductive events. Therefore, the aim of this study was to evaluate the histological characteristics of the epididymis in *Oligoryzomys* sp.

Material and Methods

Four adult male rodents (*Oligoryzomys* sp.) were captured at a Private Reserve of the Atlantic Forest called Rubens Rezende Fontes, in Viçosa – MG, where is located the BIOMA (*Centro de Biodiversidade da Mata Atlântica*). This experiment was authorized by IBAMA (number 22289-1) and approved by Ethic Comite (CEUA; number 09/2012). The animals were euthanized and their epididymis weighed, fixed in Karnovsky solution for 24 h, and embedded in methacrylate plastic (Historesin®). The slides were stained with Toluidine Blue/ Sodium Borate 1%. Four epididymal regions, initial segment, caput, corpus, and cauda, were analyzed under light microscopy, considering the cellular distribution and histology characteristics of the tubular and intertubular compartments.

Results and Discussion

The weight (mean ± SD) of the right and left epididymis was respectively 23.87 ± 1.6 mg and 19.38 ± 3.0 mg. In the descriptive histological analysis was observed difference among epididymal regions for presence and cellular distribution, and size of tubular diameter and epithelium height. This pattern is according to Dimeniconi et al. (4) that characterized the epididymal histology in gerbil. The epithelium height was thicker in the initial segment when compared to the other regions, and the cauda presented the lowest height of this tissue. At the apical area of the epithelium in the initial segment was possible to observe the presence of narrow cells, characteristic of this region. The epithelium of the caput region had abundant principal cells. Moreover, we observed the presence of clear cells intercaled between principal cells in the caput region. Although the nucleous of the principal cell of caput has presented oval shape, its shape was more elongated in the corpus, and located in the basal area of the cell. Clear cells showed to be more numerous in the corpus and specially in the cauda region, when compared to caput. All animals presented mitosis events of their principal cells, suggesting an epithelial renewal process. Finally, the cauda region showed the highest tubular and luminal diameters when compared to the other regions, specially the initial segment. Clear cells showed various shapes, being distributed in sequence or intercaled with principal cells at the tubular epithelium.

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Spatial distribution of prostaglandin D Synthase mRNA in the reproductive tract of *Somalis brasileira* rams

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Introduction

Knowledge of genetic profile of the male reproductive tract from *Somalis brasileira* ram is crucial for studies on the basic reproductive physiology of these animals, information which make possible the use of genes as molecular markers for fertility, to assist the selection of sires with proven fertility in the race in question. The prostaglandin D synthase (PGDS) is a protein associated with fertility (1), in ram it is expressed in the testis and epididymis during fetal development; however the protein is only detected in the epididymis during puberty (2). Its relationship with animal's fertility seems to be related to its ability to transport hydrophobic substances among the different tract reproductive compartments (3). The aim of the present study was to analyze the expression of PGDS in *Somalis brasileira* rams along the male reproductive tract and its relation with the sperm maturation.

Material and Methods

Seminal vesicles, epididymides, bulbourethral glands and testicles from four pre-pubertal *Somalis brasileira* rams were collected, transported to the laboratory on ice and immediately dissected. The epididymides were divided into three parts: caput, corpus and cauda. Total RNA from all tissues was isolated using 1 ml of Trizol® reagent (Life Technologies - Invitrogen). The extraction protocol used was the recommended by the manufacturer. In order to get the RNA pure without reducing it quantity, it was added to the Trizol methodology the extraction and purification by columns (PureLink™ RNA Mini Kit Anbion®). Total RNA quantification was performed by spectrophotometry (NanoDrop 2000 - Thermo Scientific). The amount of 1 µg RNA was used for reverse transcription and the cDNA generated was tested for the candidate genes. Amplification detection was performed by qPCR using Power SYBR® Green PCR Master Mix (Applied Biosystems) in the equipment iQ5 Real-Time PCR Detection System (Bio-Rad) and the GAPDH was used as endogenous gene. Relative expression values ($2^{-\Delta\Delta Ct}$) were analyzed with the SAS 9.0 software package (SAS Inc, Institute, Cary, NC), subjected to the Shapiro-Wilk normality test and the Mann-Whitney U.

Results and Discussion

In the present study, PGDS mRNA expression was detected in the caput and cauda of the epididymis and testis. The corpus of the epididymis, seminal vesicles and bulbourethral glands showed no measurable levels of amplification for PGDS. In the epididymis the expression of PGDS was significantly higher ($p < 0.05$) at the initial part and caput of the epididymis, even when compared with the testis. Although not statistically different, the expression of PGDS in testis was greater than in the cauda epididymis, corroborating the data from (2). The biochemical changes occurring in the male gametes from the epididymal transit to ejaculation gives the sperm mature so that it becomes suitable for fertilization. PGDS protein, found in cattle seminal plasma, was related to sexual capacitation (4, 5) and the function of PGDS at fertilization has been suggested in studies of in vitro maturation of oocyte-sperm binding and fertilization (6), in which more sperm bind to the zona pellucida when oocytes and/or sperm were treated alpha L-PGDS (a recombinant L-PGDS). These results show the PGDS expression in specifics epididymis segments, suggesting a possible interaction of this protein with events which modulate the sperm maturation during epididymal transit.

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Relation of photoperiod with spermatogenic cycle in Viscachas (*Lagostomus maximus*)

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Introduction

Most rodents from South America have seasonal reproductive cycle that is characterized by changes in reproductive morphology and activity according to the environment factors. Therefore the length of photoperiod, temperature, and food and water availability have an influence their reproductive cycle and spermatogenesis process (1). The viscacha is seasonal rodent that belongs to the suborder Hystricognathi, presents very distinguished quantitative and morphological variations in its spermatogenesis in consequence to the photoperiod. These variations are more evident in three distinct periods: in summer, which there is high rate of reproductive activity due to high testosterone levels (2); winter when testicles undergo to regression and spermatogenesis is ceased; and spring when there is gradual recovery of the reproductive activity (3). The aim of present study was to quantify spermatogenesis rate through assessment of the germ cell population during the spring and summer periods by the expression STRA8 and Dazl proteins that are essential proteins to spermatogenesis process by immunohistochemistry.

Material and Methods

Six adult male viscachas were captured in May/2011, during the period of reproductive activity and in November/2011 during the period of testicular regression. Quantitative analysis of germ cells and Sertoli cells present in the seminiferous tubules was performed in 30 transversal cross sections were randomly selected and counted for each animal as previously described (4). Therefore, the diameters of 10 nuclei or nucleoli of each analyzed cell type were measured to obtain the least square means. The calculation of intrinsic efficiency of spermatogenesis was determined using the following variables: *efficiency coefficient* of spermatogonia mitosis (PL/L: A), meiotic yield (AR:PQ), overall spermatogenesis yield (AR:A) and Sertoli cell index (Ar:CS).

Results and Discussion

The spermatogenesis in viscacha is a discontinuous process. In the winter, there is a decrease in testosterone serum levels and there was a hypertrophy of Leydig cells. Through test T student (P<0,05) the animals captured in the spring showed inferior values of type A spermatogonia when compared to the animal captured in the summer. The Sertoli cells of viscacha change their morphology by the influence of photoperiod, the pineal gland and the melatonin hormone. The Sertoli cell provides structure to a limited number of stem cells (6). In the summer, the viscacha Sertoli cells showed reduced capacity to support stem cells. In the spring, the rate of meiotic pachytene spermatocytes exhibited a yield of 48.21% round spermatids. These values were shown to be similar, when compared to other histricomorphas species. This study suggests that viscacha is phylogenetically close to the capybara and paca, by their reproductive characteristics, even though the viscacha inhabiting different sites (7). In regards of spermatogenic performance, the viscacha showed a lower sperm production in spring compared to the summer, suggesting a seasonal impact on spermatogenesis. According to (8) the female reproductive cycle of viscacha expresses estrus during summer and lactation during spring period. Synchronically, males showed a high rate of sperm production and minimal cell loss. Overall yield of spermatogenesis was about 4 sperm cells in the spring and 38 in summer, respectively, agreeing to (9), showing that during summer, the number of viable sperm is higher than other periods for this species. The expression of STRA8 and Dazl proteins was detected in both periods, showing that during spring there is an increase of cellular apoptosis due to low gonadosomatic activity; however the spermatogenesis was not complete arrested (5). These data suggest that in the viscacha is seasonal rodent, and photoperiod promotes significant changes in germ cells population and spermatogenesis yield.

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Testicular histometry and gonadosomatic index in Japanese quails between 15 and 60 days old

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Introduction

Quail husbandry has attracted many supporters, mainly due to the rapid return of the investment, and also the Japanese quail stands out for a high egg production. The male has intra-abdominal testes, proportionately large, representing 2,26% of body weight, and adapted for rapid production and sperm transport, necessary due to the high daily frequency of copulations (1). Some authors suggest that birds subject to sperm competition have high spermatogenic activity, because of polygamy (2). Current breeding management practices submit the male to consecutive mating for several months, without any rest. Little is known about gonadosomatic index and testicular histometry of quails in tropical conditions. Therefore, the purpose with the present study was to determine gonadosomatic index and testicular histometric features of Japanese quails between 15 and 60 days old raised in a semiarid region of the Minas Gerais State.

Materials and Methods

Twenty Japanese quail males from the same incubation batch, raised under nutrition and light conditions commonly used, were equally distributed among groups 1, 2, 3 and 4, respectively at 15, 30, 45, and 60 days of age. After weighing, the birds were slaughtered by cervical displacement and testes were dissected, weighted and subjected to routine histological processing. Volumetric proportion of testicular components, seminiferous tubules diameter, width of the seminiferous epithelium and width of the testicular capsule were determined. Gonadosomatic index was calculated by summing the weight of both testes divided by body weight and multiplied by one hundred. Data were compared with Student's t test at 5% significance level.

Results and Discussion

Histometric data are presented in Table 1. There were significant differences for all parameters evaluated in birds with 15 and 30 days old, period in which testis weight increased 74 times. Spermatozoa in the lumen of the seminiferous tubules were first identified at 30 days old. Significant differences were also observed for all parameters between males with 30 and 45 days old, when there was substantial increase in the gonadosomatic index, reaching the mean value of 3,82%, largely superior to the found in the literature (1). The testicular development stabilized at 45 days old, evidenced by the lack of statistical difference in testicular weight, gonadosomatic index and all histometric parameters compared to 60 days old males. In conclusion, the Japanese quail in tropical conditions of a semiarid region of the Minas Gerais State showed high proportion of seminiferous tubules, seminiferous tubules diameter and width, and high gonadosomatic index at 45 and 60 days old.

Table 1. Volumetric proportion of testicular components, seminiferous tubules diameter, width of the seminiferous epithelium and width of the testicular capsule in Japanese quails at 15, 30, 45 and 60 days old (mean values ± standard deviation).

Group (days-old)	Seminiferous tubules (%)	Intertubular compartment (%)	Seminiferous tubules diameter (μm)	Width of the seminiferous epithelium (μm)	Width of the testicular capsule (μm)
1 (15)	63.7 ± 6.4^{a}	36.3 ± 5.2^{a}	52.5 ± 7.1^{a}	26.9 ± 4.3^{a}	13.2 ± 4.3^{a}
2 (30)	78.4 ± 5.6^{b}	21.6 ± 3.8^{b}	94.0 ± 15.3^{b}	38.9 ± 6.7^{b}	27.4 ± 5.2^{b}
3 (45)	87.9 ± 4.4^{c}	12.1 ± 4.6^{c}	287.5 ± 29.6^{c}	75.6 ± 12.2	39.3 ± 8.6^{c}
4 (60)	91.2 ± 4.9^{c}	8.8 ± 5.7^{c}	312.4 ± 34.1^{c}	83.2 ± 15.0^{c}	44.7 ± 10.1^{c}

Different letters in the same column indicate difference in Student's t test ($P < 0,05$).

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Proliferation and cell death process in rat testes induced by Brazilian green propolis

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Introduction

Propolis is one of the few natural remedies that have maintained its popularity over a long period of time. However, it has been used indiscriminately, without medical prescription, forgetting its pharmacological potential as well as the possible effects when ingested over prolonged periods and in doses that are often not listed (1,2). Descriptions from the literature showed that effective dilutions of six propolis samples on periodontopathogen microorganisms were found to be cytotoxic to gingival fibroblasts (3), which demonstrates the necessity to determine secure doses that guarantee beneficial propolis effects. Thus, this study aimed to evaluate the cytotoxic potential of Brazilian green propolis, investigating its potential to induce proliferation or cell death in rat testes after propolis intake over a prolonged period and in various doses.

Material and Methods

Twenty-four adult male Wistar rats were treated during 56 days by gavage with 3, 6 and 10mg/kg/day (4) (T1, T2 and T3 groups) of aqueous extract of Brazilian green propolis type 12 (5), collected from *Baccharis dracunculifolia* D. C. by *Apis mellifera* L.. The control group (Co) received only water. After anesthesia, they were perfused with glutaraldehyde 4% and paraformaldehyde 4% in sodium caccodylate buffer 0.1 M (pH 7.2) for 25–30 min. Testes were removed, post fixed overnight in the same solution. The study was approved by the Ethics Committee of UNIARARAS (protocol 860/2009). Six paraffin-embedded testes sections of each animal were submitted to immunodetection of DNA fragmentation using the ApopTag® Plus Peroxidase *In Situ* Apoptosis Detection Kit (Chemicon International), according to manufacturer's instructions. Proliferating cell nuclear antigen (PCNA) immunohistochemistry staining was developed by indirect method (6), using rabbit polyclonal antibody for PCNA (Abcam, diluted 1:1000 in PBS+1%BSA) and goat polyclonal secondary antibody for rabbit IgG (Abcam, diluted 1:1000 in PBS+1%BSA) conjugated with horse radish peroxidase (HRP), both at 37°C for 1h. Positive TUNEL nuclei and PCNA-labeled cells were identified by counting brown staining nuclei. The values obtained were submitted to statistical variance analysis (one-way Anova), followed by Tukey's test; and $p < 0.05$ was considered statistically significant.

Results and Discussion

Although no significant difference has been observed in treated groups when compared with control, our results demonstrated that Brazilian green propolis induced a tendency to higher number of PCNA-labeled cells at T2 group (fig. 1), while this number decreased at T3 group. On the other hand, we can observe a higher number of nuclei positive for cellular death at T3 group (fig.2). These data suggest that propolis interferes with the cell cycle and it can cause an imbalance of the cell physiology, altering reproductive functions. So, caution should be used when taking propolis, to identify a curing dose.

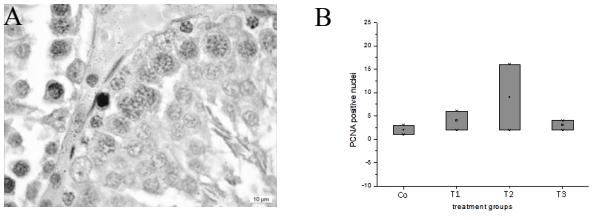

Figure 1.PCNA-labeled germ cell in seminiferous tubules (A) and PCNA-positive cells for group (B).

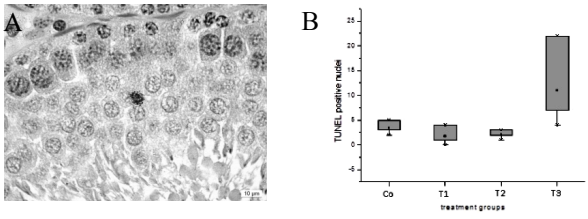

Figure 2.TUNEL-positive nucleus in seminiferous tubules (A) and TUNEL-positive nuclei for group (B).

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Stereological study of testes of curimbatá (*Prochilodus lineatus*)

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Introduction

Curimbatá (*Prochilodus lineatus*) is a South American species of teleost with great economic potential. The objectives of this study are: to determine the volumetric percentage of each one of the testicular components (germ and interstitial) by stereological study; to statistically compare this volumetric percentage among the anterior (A), medial (M) and posterior (P) regions of the testis.

Materials and Methods

The animals used (n=5) were adult male fish, obtained in Aquaculture Center of UNESP in Jaboticabal (São Paulo), in May 2009. The testes were in middle maturation stage (1). The structures considered for counting the points in the intertubular compartment were Leydig cells, blood vessels and connective tissue, and in the tubular compartment, the different types of germ cells (primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa), Sertoli cells, lumen and tunica propria. For counting the testicular components, we used the ImageJ program containing a grid with 432 intersections (points) inserted on each image. 12.960 points were counted for each animal in each region (A, M and P) at 1000 X magnification (modified from 2 and 3). The analysis of variance (ANOVA) was used to compare the volumetric percentage among the three regions of the testis. For each test, a significance level of 5% was considered.

Results and Discussion

Considering a significance level of 5%, there was no difference in the volumetric percentage of each testicular component (germ and interstitial) among the regions A, M and P. The average volumetric percentage (%) (± average standard error) of each one of the testicular components of the A, M and P regions can be seen in Tab. 1. Data obtained by stereological analysis suggest that the components (germ and interstitial) of the curimbatá testes have uniform distribution among the regions A, M and P in the studied phase of the cycle. The testes of *P. lineatus* showed a high percentage of cysts of secondary spermatogonia, suggesting high proliferation rate, and a predominance of cysts of primary spermatocytes and spermatids, indicating that spermatogenesis began and meiosis and spermiogenesis phase predominate in the tissue. The results of this analysis will contribute for a better understanding of functional morphology of the testes of this species, and may serve as parameter for related studies.

Table 1. Average volumetric percentage (± average standard error) of the testicular components of the anterior, middle and posterior regions of the testis of *P. lineatus*.

Testicular component	Anterior (%)	Middle (%)	Posterior (%)
Primary spermatogonia	0.56 (±0.13)	0.59 (±0.1)	0.68 (±0.09)
Secondary spermatogonia	16.0 (±1.93)	16.82 (±1.46)	12.89 (±1.86)
Primary spermatocytes	25.51 (±2.65)	29.18 (±0.98)	28.84 (±2.92)
Secondary spermatocytes	1.15 (±0.33)	0.87 (±0.11)	0.92 (±0.18)
Spermatids	22.69 (±2.65)	21.58 (±1.55)	22.49 (±1.87)
Sperm	4.42 (±0.67)	2.51 (±0.44)	4.07 (±0.65)
Sertoli cells	10.77 (±0.89)	12 (±0.76)	9.48 (±0.96)
Lumen	3.98 (±1.11)	2.05 (±0.44)	4.13 (±0.89)
Tunica propria	4.86 (±0.56)	4.77 (±0.5)	3.64 (±0.36)
Leydig cells	0.87 (±0.24)	0.9 (±0.11)	0.98 (±0.11)
Blood vessels	1.2 (±0.28)	0.81 (±0.04)	1.54 (±0.3)
Connective tissue	7.97 (±2.55)	7.92 (±1.3)	10.34 (±1.87)

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Morphometric changes of Sertoli cells caused by incisive duct obstruction in adult New Zealand white rabbits

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Introduction

The vomeronasal organ (VNO) opens into the incisive duct which communicates the oral and nasal cavities in rabbits (Oryctolagus cuniculus) (1), allowing the pheromones to reach and stimulate it. The VNO modulates neuroendocrine functions and behavior, through excitatory connections with the limbic system and hypothalamus (2). Additionally, the incisive duct obstruction decreases the Leydig cells stimulation, decreasing testosterone release, inducing testicle and animal behavior alterations. The present study was undertaken to evaluate the morphometric changes of rabbits Sertoli cells after incisive duct obstruction.

Material and Methods

The incisive duct of rabbits (n = 8, 2 months old) was blocked by thermocautery after local anesthesia (treated group). The control group (n = 8) was submitted to the same procedure except for the cauterization. Four months after the procedure (180 days of age), rabbits were weighed and anesthetized with thiopental and venous blood was collected through cardiac puncture. Subsequently, the animals were perfused with glutaraldehyde through the left ventricle, and the testicles were removed, weighed, sectioned, and preserved in glutaraldehyde. The fragments were dehydrated, embedded in historesin and sectioned (4μm thick). The sections were stained with toluidine blue and mounted on slides. Sertoli cells were quantified using a grid with 441 intersections attached to a light microscope (3). The gonadosomatic index (GSI) was calculated (gonad weight/body weight x100). The blood was used for plasma testosterone measurement. The results were analyzed using Student's T test with a significance level of $p < 0.05$.

Results and Discussion

The percentage of Sertoli cells in the testicular parenchyma in treated animals was significantly lower (9.50 ± 0.80%) than in control animals (13.84 ± 0.81%). The treatment did not alter the nuclear volume of these cells. However, the total cell volume decreased in treated animals (2708.8 ± 71.9 μm^3) compared to control animals (3064.0 ± 46.8 μm^3). The number of Sertoli cells per testis (67.04 ± 0.12 $x10^6$ treaties and 77.23 ± 1.88 in control $x10^6$) and per gram of testis (22.68 ± 0.62 and 35.00 $x10^6$ treaties ± 0.77 x 10^6 in the control) was significantly reduced in treated animals. There was no significant difference in serum testosterone levels (2.52 ± 0.55 ng/ml in treated and 2.24 ± 0.36 ng/ml in control animals) nor in GSI (0.16 ± 0.01 in treated and 0.14 ± 0.01 in control animals). This study shows that the incisive duct obstruction causes a decrease in the number of Sertoli cells caused by different forms of cell death. The proliferation of Sertoli cells occurs after birth and decreases in the neonatal period (3). Then the incisive duct obstruction performed after the period of proliferation could explain our results. Sertoli cells death and volume cannot be attributed to low levels of testosterone once it was not changed. Nevertheless, we can be hypothesized that other hormones involved in homeostasis of these cells, such as FSH, IGF-1, leptins, and even growth hormone, may play a role in these changes. In conclusion incisive duct obstruction may affect spermatogenesis in rabbits, although these changes did not affect the size of the testicles as shown by GSI among the groups.

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Testicular damage in acute and chronic intermittent simulated hypoxia in mice. Protective role of Ketoprofen

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Introduction

It has now been established that Intermittent Hypobaric Hypoxia (IHH) affects male fertility (1,2). This is an important issue for mining workers in northern Chile since major mining settlements are all located over 3000 meters above sea level, (MASL) (defined by UNESCO as high altitude ecosystem). A mice model mimicking the conditions using simulated IHH is analyzed, with exposure of the animals during 8.3 and 33.2 days, corresponding respectively to one and four cycles of the seminiferous epithelium (3,4).

Material and Methods

Six groups of 6 three months old mice were used and identified as follows: Group 1: Normoxia (Nx) at 8.3 days. Group 2: IHH at 8.3 days. Group 3: IHH + ketoprofen (IHHK) at 8.3 days. Group 4: Nx at 33.2 days. Group 5: IHH at 33.2 days and Group 6: IHHK at 33.2 days. Animals were exposed in a 4/4 days system (4 days hypoxia/4 days normoxia). IHH was simulated at 4200 masl in a hypobaric chamber and normoxia (Nx) condition was established at 500 MASL, in Santiago Chile. Testes were dissected out and fixed in Bouin`s fluid. Parafin sections stained with hematoxylin-eosin were prepared for semi quantitative histopathological analysis, histometric mensuration of tubular and luminal diameter, epithelial height and percentage of tubular vs interstitial area and immunohistochemical assessment of COX-2 and Hif-1α antigens was also performed. All results were expressed as mean $\pm$ standars deviations. Statistical analysis employed a non-parametric Kruskal-Wallis ANOVA test and the Dunn multiple comparison test to determine if there were significant differences between the two experimental groups ($p<0,05$).

Results and Discussion

See table below. Histopathologic data showed that vacuolization is grater at 33.2 days (*) and ketoprofen did not protect. Concerning plugging in lower values are found in IHHK at 33.2 days (*). Tubular diameter decreased at one cycle and recuperate in IHHK at 4 cyles. Histometrical data showed both in acute and chronic IHH that tubular diameter decreases. Tubular lumen and epithelial height does not show great differences. % of tubular v/s interstitial area does not vary significantly in any case. COX-2 and Hif-1α antigens are overexpressed in IHH but decrease in the percentage of positive tubules in IIHK group in acute conditions and also in IHHK at 33.2 days.(#)

Table 1. Testicular parameters in mice exposed to hypobaric hypoxia at one and four cycles of the seminiferous epitelioum and the effect of ketoprofen.

	IHH 8.3 days	IHHK 8.3 days	IHH 33.2 days	IHHK 33.2 days
Vacuolization (%)	14 ± 3.8*	20 ± 3.8	27 ± 2.2*	22 ± 3.0
Tubular plugging (%)	12 ± 2.6*	8 ± 1.5	9 ± 1.6	4 ± 2.8*
Tubular diameter (μm)	170 ± 4.8*	180 ± 5.3	210 ± 5.1*	195 ± 3.0
Luminal diameter (μm)	78 ± 1.2	82 ± 2.1	82 ± 0.97	81 ± 1.62
Epithelial Height (μm)	55 ± 2.3	62 ± 1.2	58 ± 0.6	60 ± 0.9
% Tubular versus interstitial area	82 ± 1.8	78 ± 1.2	80 ± 3.4	82 ± 1.7
COX-2 expression (%)	71 ± 4.3*	24 ± 2.1#	24 ± 1.8*	16 ± 1.3#
Hif-1 α expression (%)	75 ± 2.3*	30 ± 2.0#	28 ± 1.7*	5 ± 0.9#

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Leydig cell stereology of 18 months-old Wistar rats Treated with *Heteropterys tomentosa* A. Juss. infusion

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Introduction

The effects of aging on testicular endocrine function are better known in rats and humans than in other mammals. The plasma testosterone concentration is significantly lower in old age compared to young adulthood (1), and this reduction is mainly caused by decrease of gonadotropin stimulation (2). The Brazilian flora is diverse and most of these plants could be potentially used as natural tonics and remedies, however scarce plant species have been used in laboratory studies. Among them, *Heteropterys tomentosa* (previously known as *Heteropterys aphrodisiaca*) was proved to be useful as a sexual stimulant (3) and against reactive oxygen species (4). This study was undertaken to evaluate the effects of *H. tomentosa* infusion on the testes of 18 months-old rats.

Material and Methods

One group (HA, n=5) was treated with *H. tomentosa* infusion (212 mg/animal/day), and the control group (n=5) received distilled water. Both treatments were administered daily by gavage, for 70 days. Each animal received 0.5mL of either water (sham) or infusion (HA). Rats were anesthetized with Xylazine and Ketamine (5:80mg/kg) and perfusion-fixed with glutaraldehyde 2.5% in cacodylate buffer (0.05M). The testes were removed, weighed and routinely processed for light microscopy (glycol methacrylate). The slides were stained with Toluidine Blue/Sodium Borate 1%, and evaluated using stereological tools under light microscopic examination.

Results and Discussion

The light-microscopic measurements of seminiferous tubules, interstitium and capsule in aging rats agree favorably with those of Ichihara et. al (1). Tubule and interstitial volume were not altered after treatment. The administration of *H. tomentosa* infusion did not alter significantly the Leydig stereological parameters (nucleus and cytoplasm volumes and cell's number per gram of testes). Histological analyses of both control and HA treated testes showed vacuolization of Sertoli cells cytoplasm, germ cell degeneration and exfoliation and swelling of intercellular space. Therefore, even after long-term treatment with *H. tomentosa* infusion, there was no substantial improvement of data regarding to testicular dynamics and integrity, although no hazardous correlations could be drawn between the plant infusion and aging.

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Leydig cell stereology of 15 and 18 months-old Wistar rats

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Introduction

The main effect of aging on male reproduction is the progressively diminishing sexual activity from adolescence into old age due to reduced circulating testosterone levels, which occur in all mammalian species studied to date (1-5). The major producers of androgen in the testis are the Leydig cells (6). Leydig cell studies have demonstrated that aging is accompanied by functional deficits of individual cells, leading to lower androgen content in circulating plasma (3, 7). The present study aimed to evaluate the Leydig cell stereological parameters of Wistar rats with 3, 15 and 18 months of age.

Material and Methods

Fifteen animals were divided into three groups (n=5): 3, 15 and 18 months-old. The animals were housed in a controlled environment with free access to water and rat chow. Rats were anesthetized with a mixture of xylazine and ketamine (8:50mg/kg) then perfused and fixed using saline solution (0.9%) followed by 2.5% glutaraldehyde in 0.05M cacodylate buffer, for 20 minutes each. The testes were removed, cleaned, weighed and sliced into small fragments, which were then routinely processed for embedding in glycol methacrylate. Stereological studies were performed using 4 μm thick sections, toluidin blue stained, in order to quantify the individual volume of LC, relative and absolute volume of nucleus and cytoplasm, as well as the number of LC per gram of testis. The data was analyzed via ANOVA (Tukey test) and the significance level was $p<0.05$.

Results and Discussion

The stereological parameters obtained for old animals in relation to testicular parenchyma agreed favorably with those found by Ichihara et al. (3). The relative volume of Leydig cells per testis, as well as the nucleus, cytoplasm and the individual volume of the Leydig cell did not alter with aging. The number of Leydig cells per gram of testis increased significantly in 15 and 18 months-old rats, compared with young ones. Histological analysis of the old animals showed a wide variety of seminiferous epithelium injuries, such as Sertoli cell vacuolization, cell degeneration, swelling of intercellular space and loss of germ cells by exfoliation. Although only the number of Leydig cell showed significant alterations, future studies using transmission electron microscopy could clarify whether the organelles involved with steroidogenesis have suffered alterations with aging.

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Morphometrical and stereological parameters of Wistar rat testes after Brazilian green propolis intake

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Introduction

Propolis is a resinous substance collected by honeybees from a variety of plant sources that presents polyphenols in its composition and has nutritional as well as therapeutical value. Some authors have demonstrated that their pharmacological effects can be associated with an antioxidant activity (1). Because propolis is not listed as a phytotherapic product, it is often used indiscriminately, without a prescription. There is very little information about the effect of propolis on the reproductive system but there is evidence that long-time exposure to moderate or high doses of reproductive toxicants will cause primary testicular damage (2). This study was undertaken to investigate whether a chronic dose of aqueous Brazilian green propolis extract could disrupt the morphology of Wistar rat testes.

Material and Methods

Twenty-four adult male Wistar rats (90 days old) received, by gavage, an aqueous extract of green propolis, classified as type 12 (3), that was collected from *Baccharis dracunculifolia* D. C. by *Appis mellifera* L. in the city of Araras (SP - southeast Brazil). The animals were divided into four groups with 6 animals in each: a control group (Co) that receive only filtered water and three experimental groups (T1, T2 and T3) treated with 3, 6 and 10mg/kg/day of the extract (4), respectively, for 56 days (5) (protocol 860/2009 approved by the Ethics Committee of FHO-UNIARARAS). After anesthesia, they were perfused with 4% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium caccodylate buffer (pH 7.2) for 25–30 min. Testes were removed, post fixed overnight in the same solution then weighed. Historesin (Leica) embedded testis fragments were sectioned at 3μm thickness and stained with toluidine blue in 1% sodium borate. Stereological analysis was performed with a 120 point grid on 12 random testicular cross sections per animal, to determine the proportion of the testis components (epithelium, lumen and interstitium). Morphometric analysis was performed measuring the larger and smaller diameter of 30 Leydig cell nuclei per animal. Mean values for each experimental group were submitted to statistical variance analysis (one-way Anova), followed by Tukey's test and a value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Stereological data revealed significantly higher tubular epithelium proportions in T1 compared with Co and a significant decrease in T3 when compared to T1. The tubular lumen proportion was significantly smaller in T3 when compared with both groups Co and T2. Interstitial proportion was significantly higher in T3 when compared to the other groups, a fact that can be noted in microscopic images. Leydig cell morphometry showed a significant increase of Leydig cell nuclei diameter in the T3 group in comparison with other experimental groups. Our results clearly demonstrate the potential of propolis to induce an imbalance of the testis as shown by their morphology that is usually a sensitive sign of physiological alterations. They support the increased testosterone levels described in the literature for rats and rabbits treated with propolis (6,7). This fact raises a concern regarding propolis effects on the male reproductive function and, in spite of the well known beneficial effects of propolis, this effect suggests caution in relation to indiscriminate ingestion.

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Histopathological analysis of testes of domestic cats after intratesticular injection of zinc gluconate solution

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Introduction

Intratesticular injection of zinc gluconate (Testoblock®) has been used for sterilization of dogs [1], but the use in cats is still limited. The aim of this study was to evaluate, by light microscopy analysis, if a single intratesticular injection of zinc gluconate will have a sterilizing effect in male cats.

Material and Methods

Sixteen sexually male cats were assigned to two groups, Control (n=5), and Treated (n=11) received a single injection of saline or Testoblock®, respectively, into each testis on day 0. The volume injected was based on 1mL per 27mm testis width, approximately twice the dose (per gram of testis) injected in dogs. At 120 days after injection 11 cats, four belongs to the control group and seven of the treated group, were submitted to orchiectomy. Testicular samples were removed, fixed in glutaraldehyde, embedded in glycol methacrylate resin and fragments with 4μm thickness were stained with toluidine blue and submitted to histological examination.

Results and Discussion

The volume of Testoblock® injected into testis of cats in the present study varied from 0.44 to 0.51ml. In the testis of the control group was not observed morphological changes related with testicular degeneration and all stages of seminiferous epithelial cycle could be noted as well as no changes were noted in Leydig cells and vessels. On the other hand, testis of treated group had germ cells degeneration, multinucleated giant cells and a lack of elongated spermatids in atrophic seminiferous tubules. The majority of seminiferous tubules were lined only by Sertoli cells, which were vacuolated. Leydig cells had varying degrees of lipidic degeneration. According to the histological changes observed in testis of the treated group, we can suggest that the zinc-based solution Testoblock®, can be used as an effective and permanent contraceptive method for cats.

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Modification of bovine spermatogonial stem cells to produce transgenic animals

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Introduction

Mammalian spermatogenesis is sustained by self renewal and differentiation of spermatogonial stem cells (SSCs). The study of these cells provides a model to better understand adult stem cell biology and the mechanisms that control SSC functions. Besides the biomedical potential to perform studies of infertility in many species, SSCs hold a promising biotechnological application at animal transgenesis. In this manner, the goal of this study was to answer the question: "Can LacZ+ bovine SSCs be integrated into seminiferous tubule of prepubertal Nelore bulls subjected to autologous transplantation?"

Materials and Methods

5 months old bulls (n=16) were hemi-castrated and spermatogonial cells were isolated by a two step enzymatic digestion procedure. After differential plating, cells were transduced with a lentivirus vector carrying the LacZ reporter gene sequence. Animals were randomly allocated in four experimental groups: LacZ+/PKH26+, LacZ+/PKH26-, LacZ-/PKH26+, LacZ-/PKH26-. After 60 h of the onset of in vitro culture, spermatogonial cells were autologously transplanted to the remaining testes by an ultrasound guided needle injection at the testis mediastinum. The transplanted testes were surgically removed after 45 days and testicular tissue samples were subjected to x-gal staining to assess the integration of transgenic spermatogonial cells to seminiferous tubule.

Results and Discussion

Spermatogonial cells were successfully isolated and *in vitro* cultured. However, no SSC-enriched population of cells was obtained by differential plating. Although spermatogonial cells were transplanted instead of pure SSCs only, the expression of SSC marker genes ITGA6, PGP9.5, GFRα-1 and the affinity to DBA were detected in all samples collected before the transplant. Cryosections of x-gal stained testicular tissue samples allowed the observation of transgenic cells in all animals that received LacZ+ cells. However, all transgenic cells observed were located at the interstitial space. In conclusion, it was not possible to observe the integration of the transplanted transgenic cells into seminiferous tubule of prepubertal Nelore bulls subjected to autologous transplantation using an ultrasound guided needle injection at the testis mediastinum, after 45 days of transplant.

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Sperm maturation in dogs: enzymatic antioxidant activity and sperm functional changes

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Introduction

Sperm maturation occurs during the sperm transit through the epididymis. The morphological and functional changes in sperm cells provide, at the end of the process, progressive motility and fertilizing capacity (1). However, these structural changes originate an increased susceptibility to the attack of reactive oxygen species (ROS) present in the epididymal lumen. Therefore, an antioxidant defense mechanism is necessary to avoid sperm damage during the long progress and storage through the epididymis (1). Nevertheless, the epididymal environment content has not yet been precisely determined in the dog, an important experimental model for wild animals and humans (2). Thus, the aim of this study was to verify the functional changes in canine sperm along the caput, corpus and tail of the epididymis and to correlate such findings to the enzymatic antioxidant status.

Material and Methods

Epididymides from thirteen sexually mature dogs, aging from 2 to 7 years were used. Testicles were surgically removed and stored at 5°C for 24 hours prior to processing. Sperm was subsequently collected through small incisions (<1 mm) of the caput, corpus and tail, aspirated separately and maintained in 200 µl PBS extender. Experimental groups were then constituted of: sperm from the caput (CAP), corpus (COR) and tail (TAIL) of the epididymis. Sperm samples were evaluated for motility and vigor. Eosin/nigrosin stain was employed to evaluate the permeability of the plasma membrane and the spermatic morphologic defects; the simple stain (rose bengal/fast green) was used to assess acrossomal integrity. The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were determined as previously described (3). Data were analyzed by Tukey test and Pearson correlation ($p<0.05$).

Results and Discussion

As expected, tail sperm showed the highest motility and vigor, followed by the corpus and caput samples, with significant difference among them. A gradual and significant increase on membrane and acrosome integrities were found throughout the epididymis (CAP vs. COR vs. TAIL, respectively; $p<0.05$). This may suggest that the modifications in the spermatozoa, responsible for the acquisition of motility and fertilizing capacity, may involve changes in the plasma and acrosome membranes' permeability that allow the spermatozoa to endure the challenges following ejaculation (1). No detectable levels of CAT were found on the epididymal compartments, reinforcing the results of previous studies in man and rats, in which low levels of CAT were found in the reproductive tract. Nevertheless, the presence of CAT in human semen samples has been related to contamination with neutrophils (4). No differences on SOD and GPx levels were found between epididymal regions, similarly to studies performed in humans and rodents (4). However, a significant positive correlation between SOD and distal droplet ($r=0.45$, $p=0.038$) was found in the caput segment. On the other hand, a negative correlation ($r=-0.44$, $p=0.047$) was found between GPx and proximal droplets in the tail. In fact, previous study (5) suggests that, in sperm collected from the epididymis, proximal and distal droplets may play pro-oxidative and antioxidant roles, respectively. Also, a positive correlation between GPx and the percentage of sperm with intact acrosome ($r=0.67$, $p=0.0008$) was found in the COR. Thus, SOD and GPx may play important role to ensure the necessary protection for epididymal spermatozoa to adequately undergo the maturation process.

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Effects of *Euterpe edulis* extract in the seminiferous tubules of adult rats

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Introduction

Euterpe edulis (Arecaceae) fruits contain large amounts of anthocyanins, which are natural pigments that show great pharmacological potential, mainly because of its antioxidant properties (1). Commonly known worldwide as "açaí", this species is becoming popular in many countries, although there are not enough studies regarding its intake with benefits to the body functions, such as reproduction. Thus, it is essential to evaluate the efficacy and safety of such herbal products for the establishment of pharmacological evidence and toxicological evaluation (2). The present study aimed to evaluate the effects of the hydroalcoholic extract of açaí on the seminiferous tubules of adult Wistar rats.

Material and Methods

The animals were separated into four experimental groups (n=6): a control group (distilled water) and three treated groups that received 200, 400 and 600mg/kg/day of hydroalcoholic extract of açaí diluted in distilled water. The treatment was given by gavage, for 120 days. The animals were anesthetized, euthanized and weighed. Testicular fragments were immersed in Karnovsky's fixative, being routinely processed for light microscopy. Digital images of the testicular parenchyma were taken and analyzed with the software Image-Pro Plus. The average diameter of seminiferous tubules (DT) and the lumen diameter (LD) were obtained from 20 circular cross-sections in each animal. In the same sections, was also measured seminiferous epithelium height (EH), from the basal membrane to the luminal edge. The length of the seminiferous tubule was calculated using the volume formula for a cylinder, where the length is the volume divided by the base area. Thus, the total volume of seminiferous tubules divided by the area of the cross section corresponds to the total length of seminiferous tubules per testis (TL), which can also be converted to meters per gram of testis (TL/g). The tubulesomatic index (TSI) was calculated using the seminiferous tubule volume and total body weight. Results were statistically tested with ANOVA followed by Student Newman-Keuls test. The results were considered significant for $p<0.05$.

Results and Discussion

Significant increasing of DT (307 ± 8.03) and LD (101 ± 11.2) were observed in the animals receiving the lowest dose of açaí extract compared to control (291.96 ± 4.9 and 87.54 ± 3.01), although EH and TL did not vary among groups. There was significant reduction in TL/g (10.61 ± 0.68) in group II compared to control (12.24 ± 0.62). TSI, which is a parameter to measure the investment in tubules in relation to body weight, did not differ between experimental groups. Quantitative parameters directly related to the seminiferous tubule, such as DT, EH and TL, have a positive relationship with spermatogenic activity (3). Thus, the increase of DT observed in animals treated with the lower dose of açaí extract may suggest an increase in spermatogenic activity. The quantification of germ cells population in this group is essential to evaluate the possible stimulating effect of the extract on the spermatogenic cells. Since the other tubular parameters did not present significant changes after treatment, it is safe to infer that continuous intake of such açaí extracts was harmless for the spermatogenic process.

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Histopathological evaluation of seminiferous tubules in rats treated with lead acetate

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Introduction

Lead is an environmental pollutant with a wide variety of toxic effects with negative influence on reproduction (1). Histopathological changes have been used as important markers for the toxicity of environmental pollutants. It is possible to identify potential cellular targets for the toxic action of an agent through the evaluation of these changes (2). The aim of this study was to evaluate qualitative changes in the seminiferous tubules of rats intoxicated with lead acetate at different concentrations.

Material and Methods

The animals were weighed and separated in five experimental groups (n=5): a control group (I) which received an oral daily dose of 0.5mL acidified distilled water and four treated groups (II, III, IV and V) that received an oral daily dose of 0.5mL lead acetate dissolved in acidified distilled water at concentrations of 16, 32, 64 and 128mg/Kg b.w., respectively, by gavage, for 30 consecutive days. At the end of the experimental period, the rats were anesthetized and euthanized. Testicular fragments were processed for light and electron microscopic examination.

Results and Discussion

The examination by light and electron microscopy demonstrated variable adverse effects of lead acetate to the seminiferous epithelium, such as vacuolization, increase of intercellular space and detachment of germ cells, presence of apoptotic bodies, large lipid droplets and blood-testis barrier rupture. The vacuolization observed in the cytoplasm of Sertoli cells may be derived from the dilatation and vesiculation of smooth endoplasmic reticulum (3). Consequently, changes in Sertoli cell function result in loss of germ cells in the seminiferous epithelium. The occlusive junctions between the Sertoli cells and Sertoli cell/germ cell junctions are the initial target and the subsequent dissolution is likely the result of negative regulation of junctional proteins, leading to disruption of the blood-testis barrier (4). Thus, there is induction of cell loss from the germ epithelium detachment manifested by these cells. Changes on the increase and amount of lipid droplets may be associated with degeneration of germ cells in the seminiferous tubules of rats intoxicated with lead (5). We concluded that treatment with lead acetate negatively interfered in the seminiferous tubules in all concentrations, demonstrating their deleterious effects on spermatogenesis.

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Comparative influence of maternal obesity and postnatal overnutrition on testis structure and sperm production in rats

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Introduction

Overweight and obesity are the fifth leading risk for global deaths. The impact of obesity on male reproductive function and the underlying mechanisms are still poorly known. This study evaluates the effects of exposure to obesogenic environment in different periods of development on testis structure and sperm parameters in adult rats.

Material and Methods

Male Wistar rats with 18 weeks of age were subjected (n = 8 per group) to normal nutrition (control group, 4% lipid in diet) or to obesogenic environment (OE) in different periods of development, namely, from gestation to weaning (O1 group), from weaning to adulthood (O2 group) or from gestation to adulthood (O3 group). Obesogenic environment was induced to female rats before the pregnancy (group O1 and O3) and also to offspring of groups O2 by high-fat diet (20% saturated lipid) for 15 weeks. The obesity was evaluated by determination of body weight, adiposity index and plasma leptin levels. The total circulating testosterone was assayed. The testicular structure was examined using light microscopy and stereological analysis. In addition, it was also determined the sperm counts in the testis and epididymal regions, the daily sperm production (DSP) and the transit time of spermatozoa in the epididymis.

Results and Discussion

Regardless of the period of exposure, the OE led to obesity in adulthood. Obesity was more accentuated in groups subjected to OE during sexual maturation (O2 and O3 groups). The OE also reduced the testosterone levels in adulthood. This reduction was more pronounced (~ 70%) in the group exposed to OE at all stages of development. Testes histological analysis of obese rats demonstrated premature detachment of germ cells as demonstrated by the percentage of seminiferous tubules with cell detached (C: 3.0 ± 1%; O1: 19 ± 1.9%; O2: 21 ± 2.1%; O3: 41 ± 15.5%). It was also detected a significant increase in the seminiferous tubules diameter of the animals from all groups subjected to the OE (C: 312.9 ± 4.6; O1: 354.9 ± 3.6; O2: 331.1 ± 3.6; O3: 338.8 ± 4.5). Cells released from the germinal epithelium formed dense clusters in the tubular lumen, which probably contributes for the increase of the seminiferous tubules diameter. Furthermore, the number of spermatids in the testis and the DSP decreased about 26% in rats subjected to the OE. The number of sperm in the region caput/corpus epididymis decreased 27% for the O1 and O3, and 18% for group O2. This reduction was 29% for group O1, 20% for group O2 and 26% for group O3 in the cauda; however, it did not affect the transit time of spermatozoa through the caput/corpus and cauda of the epididymis. In conclusion, the OE reduces sperm production and reserves, regardless of the development period. Thus, the data indicate a positive association between the testosterone reduction and premature detachment of germ cells with sperm damage. Additionally, these findings indicate that exposure to OE during pregnancy/breastfeeding has an irreversible impact on adiposity in adulthood.

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Characterization of the stages of the seminiferous epithelium cycle and the duration of spermatogenesis in the spiny-rat, *Proechimys guyannensis* (Rodentia: Echimyidae)

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Introduction

The *Proechimys guyannensis*, known as "casiragua" in Brazil, is a spiny-rat species that lives in the Amazonian region. This species has received some attention as a natural host of infectious parasites (1) and has been used as an animal model for investigating resistance to epilepsy (2). Concerning reproductive biology, there are very few reports in the literature for *P. guyannensis*. Therefore, our aims in the present study were to investigate the basic aspects related to the testis function and spermatogenesis in this spiny-rat species, particularly the characterization of the stages of the seminiferous epithelium cycle, their frequencies and the duration of spermatogenesis. The investigation of these basic parameters will allow the determination of other crucial parameters such as Sertoli cell and spermatogenic efficiencies.

Materials and Methods

Ten sexually mature animals from the UFSJ vivarium were utilized. Testis were fixed by immersion in buffered glutaraldehyde and bouin, and routinely processed for histological, stereological and immunostaining analyses. Intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrDU; 150mg/kg) were performed to determine the duration of spermatogenesis. All data are presented as the mean ± SEM

Results and Discussion

The body weight and the testis weight were respectively 288 ± 11g and 1.63 ± 0.2g, resulting in a gonadosomatic index (GSI; total testis weight divided by body weight) of 1.15 ± 0.1%. In comparison to the mammalian species already investigated this GSI is very high. Based on the development of the acrosomic system, twelve stages of the seminiferous epithelium cycle were characterized. Stages VI and VII presented the highest frequencies (~16%), while the stages II, III, IV and V showed the lowest frequencies (~3 to ~4%). The most advanced germ cell types labeled at 1 hour and 20 days after BrDU injection were respectively preleptotene/leptotene spermatocytes at the stage VII and elongated spermatids at the stage III. Based on the stages frequencies and BrDU labeled germ cells, each spermatogenic cycle and the entire spermatogenic process lasted respectively 7.5 ± 0.01 and 33.7 ± 0.06 days. The seminiferous tubules occupied 97.4 ± 0.7% of the testis parenchyma, whereas Leydig cells comprised only 0.99 ± 0.4%. In contrast to the remarkably small value observe for Leydig cell volume density (%), these preliminary data suggest that *P. guyannensis* has one of the fastest spermatogenic cycle length and one of the greatest seminiferous tubule volume density among the mammalian species already investigated. In order to better characterize the testis structure and function in this species we are currently investigating several other parameters and, from the data already obtained, we could expect a very high spermatogenic efficiency for this rodent species.

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Expression of Caspase-3 in seminiferous tubular cells of Mus domesticus exposed to hypobaric hypoxia

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Introduction

There are a lot of changes in mice exposed to hypobaric chamber (1, 2, 3). However, quantification of spermatogenic cells damage related to hypoxia is unclear. The main objective of the present work is to look into effects in specific germ cells from combined stages in mice exposed to hypobaric hypoxia (HHC), that express caspase-3 as damage marker (combined to simplify the observation schedule).

Material and Methods

Nine mice (CF1 Mus domesticus) of 3 months of age were obtained from Laboratory of Reproductive Biology vivarium. All animal studies were conducted in accordance with the principles and procedures outlined by the Bioethics Committee of the School of Medicine, Universidad de Chile. Mice were separated in 3 groups. Animals were exposed to HHC by 0 (control group), 8.3 and 16.6 days. After exposure, they were sacrificed and one teste was dissected and fixed in Bouin. Paraffins sections cut at 4um and processed for immunohistochemistry (IHQ) using ABC (peroxidase) system. Primary antibody was Biocare anti caspase-3 cleaved (cp229).

Results and Discussion

See table below. Positive spermatogenic cells were counted by stages of the cycle (al least 5 tubules per stage).In control testis, no expression of caspase-3 was observed. In contrast, in the epithelium of mice subjected for 8.3 days, there was expression in mitotic cells, and preleptotene stages. At 16.6 days, depletion of the epithelium, sloughing of cells and cellular debris into the lumen, promotes non-specific detection of caspase-3. However, expression is seen throughout the epithelium, this being more intense in basal cells and sloughed round spermatids. This is the first information about specific effects of HHC on epithelial cell types. The level of expression of caspase-3 is selective, mainly in preleptotene spermatocytes, which could be correlated to a pachytene checkpoint. Futures studies will target checkpoint markers related to apoptosis, perhaps apoptosis could be triggered by another cascades of events. Caspase-3 positive cells are not necessarily related to physiological apoptosis in spermatogenesis.

Spermatogonium	Stages IX X XI	Stages XII I II	Stages III IV V	Stages VI VII VIII
IHQ 00dHHC A	0%	0%	0%	0%
IHQ 00dHHC B	0%	0%	0%	0%
IHQ 00dHHC C	0%	0%	0%	0%
IHQ 08dHHC A	74%	68%	73%	75%
IHQ 08dHHC B	69%	70%	77%	61%
IHQ 08dHHC C	71%	67%	71%	69%
IHQ 16dHHC A	83%	82%	79%	85%
Preleptotene	Stages IX X XI	Stages XII I II	Stages III IV V	Stages VI VII VIII
IHQ 00dHHC A	---	---	---	0%
IHQ 00dHHC B	---	---	---	0%
IHQ 00dHHC C	---	---	---	0%
IHQ 08dHHC A	---	---	---	81%
IHQ 08dHHC B	---	---	---	84%
IHQ 08dHHC C	---	---	---	80%
IHQ 16dHHC A	---	---	---	78%
Pachytene	Stages IX X XI	Stages XII I II	Stages III IV V	Stages VI VII VIII
IHQ 00dHHC A	0%	0%	0%	0%
IHQ 00dHHC B	0%	0%	0%	0%
IHQ 00dHHC C	0%	0%	0%	0%
IHQ 08dHHC A	2%	4%	3%	1%
IHQ 08dHHC B	1%	1%	3%	3%
IHQ 08dHHC C	1%	4%	2%	1%
IHQ 16dHHC A	11%	9%	13%	8%

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Gradients of distribution of Type A spermatogonia in the testis of sexually mature Nile-tilapia (Oreochromis niloticus)

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Introduction

Nile tilapia (*Oreochromis niloticus*) is a highly appreciated fish for human consumption worldwide. Spermatogonial stem cells (SSCs) are the germ stem cells in the testis with high biotechnological potential. In tilapia, as in most vertebrates, SSCs are a subpopulation of type A spermatogonia, which is a major part of the stem cell and transit amplifying pools in the seminiferous epithelium (1).

Materials and Methods

Our main objective was to study the distribution patterns of type A spermatogonia in the adult tilapia (n=5) testis. Unbiased stereological methods were used: Cavalieri method for volume estimation and Physical dissector for cell number estimation (2). Absolute numbers of A-spematogonia associated with specific cyst types (A_{und} and A_{diff}) were obtained for three regions in the longitudinal testis axis (cranial, middle, and caudal), and three regions in the dorsoventral axis (external/subalbugineal, intermediate, and internal/ductal). Statistical analysis was performed using one-way ANOVA and the results are presented as the mean±s.e.m. All calculations were performed using the software SPSS for Windows v17.0.

Results and Discussion

Isolated (A_{und}) and paired A_{diff} spermatogonia were uniformly distributed in both the longitudinal and dorsoventral axes, with a tendency to be more abundant in the caudal region. There was also a trend of higher isolated (A_{und}) and paired A_{diff} numbers in the subalbugineal region of the dorsoventral axis. Total A-spermatogonial number was significantly higher ($p<0.01$) in the subalbugineal region (Fig. 1), with A_{diff} cysts of >4cells representing approximately two-thirds of A-spermatogonia population in that region. In conclusion, our preliminary results suggest that there is a preferential location pattern of type A spermatogonia in the adult tilapia testis, suggesting the presence of active cell gradients probably involving SSCs and somatic cells (niche) in both the longitudinal and dorsoventral axes.

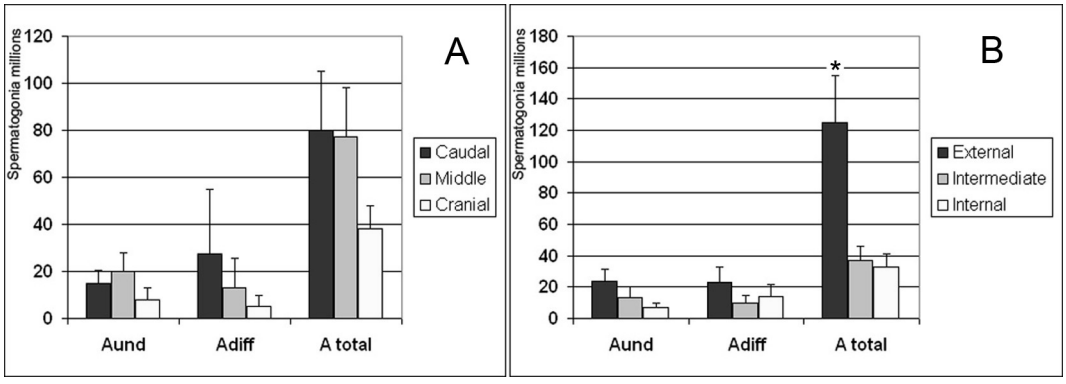

Figure 1. Distribution of two types of A spermatogonia in the testis of adult tilapia along the A) longitudinal (Caudal, Middle and Cranial regions) and B) dorsoventral axis (External, Intermediate and Internal regions). A_{und} are undifferentiated type A spermatogonia, allegedly spermatogonial single stem cells. A_{diff} are type A spermatogonia committed to differentiate, found in cysts of two (as counted in the present work) or up to eight cells. A_{total} are all type A spermatogonia (A_{und} and A_{diff} in pairs or cysts of more cells). (* $P < 0.01$).

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Differential effects of intratesticular injection of Bradykinin-Potentiating Peptides from *B. jararaca* venom on spermatogenesis in mice

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Introduction

BPP-10c, a potent selective C-domain inhibitor of sACE, modified spermatogenesis in male Swiss mice treated (i.p.) without affecting blood-testis barrier (BTB) permeability or the distribution of claudin-1, a protein found at the site of the BTB (1). Considering the structural and functional particularities of these bioactive peptides, the aim of the present study was to assess the effects of BPP-10c (<ENWPHQIPP), BPP-11e (<EARPPHPPIPP), BPP-AP (<EARPPHPPIPPAP) and (inv)BPP-10c [PPIQPHPWNE (containing the inverted BPP-10c sequence)]and Captopril on the seminiferous epithelium (SE) of mice following intratesticular (i.t.) injection.

Materials and Methods

Male adult mice (30-35 g) were assigned to groups (n=5, per group) and treated with Saline solution (0.91% w/v) into the right testicle (RT) and the samples (BPPs or Captopril) into the left testicle (LT) at a concentration of 120 nmol/dose per testicle. The animals were kept in the vivarium for one week, and then the RT and LT were collected for morphological and morphometric analysis of the SE. All data were presented as the mean ± SEM and the criteria for statistical significance was set at $P<0.05$.

Results and Discussion

BPP-AP promoted epithelium ruptures and the movement of the germinative cells to the lumen of the seminiferous tubules (ST) was more prominent than with BPP-10c (Fig.1), showing significant reduction ($P<0.001$) of round spermatids (7.75±1.50), spermatocytes (zygotene, 7.25±1.50; pachytene, 5.75±0.86) when compared to the right testis (29.5 ± 0.5, 15.2 ± 2.5 and 24.5±3.1, respectively). BPP-AP also led to a significant reduction in SC in stages I, V and VII/VIII and XII ($p<0.0001$) in comparison to the control and BPP-11e. Interestingly, no morphological or morphometric alterations were observed in animals treated with Captopril, (inv)BPP-10c and BPP-11e. These data suggest that the effects of BPPs on the SE are dependent on their primary structures and offer new perspectives for the study of possible mechanisms involved in the impairment of spermatogenesis.

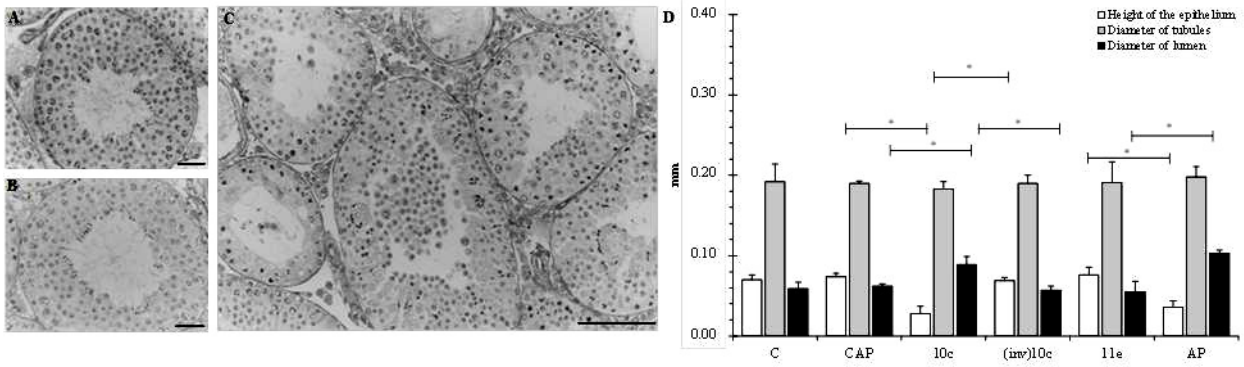

Figure 1. Effects of BPPs in the SE. (A) RT with vehicle (Control); (B) LT treated with BPP-11e; (C) LT treated with BPP-AP; (D) Morphometric aspects of ST (*$P<0.001$); Staining: Mallory's Trichome; Bar = 10 µm.

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Spermatogonial stem cell characterization and niche in the bullfrog (*Lithobates catesbeianus*)

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Introduction

Spermatogenesis is a very efficient process of gamete production that is fairly well conserved among vertebrates. Spermatogonial stem cells (SSCs) represent the fundamental basis of spermatogenesis maintaining the process through all the reproductive life. These cells resides in a specific microenvironment in the testes called "niche", which regulates through extrinsic and intrinsic factors the stem cell properties such as self-renew, differentiation and homing (1). Although SSCs are crucial for male reproduction, the regulatory molecular mechanisms of these cells are still not well characterized and there are few specific markers for SSCs, particularly in lower vertebrates. The bullfrog is an excellent experimental amphibian model for biology investigations including reproductive studies. To our knowledge there are no data regarding SSCs characterization and niche in bullfrog testes. Thus, our main objectives were to investigate the SSCs physiology/biology and niche in mature bullfrog testes by histomorphometric and immunohistochemistry analyses, using some specific markers already evaluated for mammals (2,3).

Material and Methods

Testes from eight mature bullfrogs were collected and routinely processed for histology and immunohistochemistry. For eventual expression of GFRα-1 and Oct4 in SSCs, were utilized antibodies raised against rat and fish proteins, respectively. The SSCs distribution in the testis parenchyma was evaluated in two different regions; i.e. near to the tunica albuginea (periphery) and in the center of the testis parenchyma, using ImageJ software. Besides that, in both regions, we investigated if SSCs were located adjacent to the interstitial compartment (tubule-interstitium) or in the areas where seminiferous tubules contacted each other (tubule-tubule). Data were analyzed using Graph Pad Prism 5.0 Software using t-test and the level for significance was $p<0.05$.

Results and Discussion

In comparison to the central area (0.53%), the results found indicated that SSCs were located preferentially near to the tunica albuginea (1.25%) ($p<0.05$). Moreover, in both regions investigated SSCs were found mainly in the areas of tubule-interstitium contact (~60%) ($p<0.05$). These results corroborate data in the literature for other vertebrate species investigated, suggesting that the SSC niche may be regulated across all vertebrate classes by the interstitial compartment elements, including blood vessels [1]. Regarding the molecular analysis, the SSCs in bullfrog testis were positive for both GFRα-1 and Oct4, demonstrating that these SSCs markers are highly conserved among vertebrates. Besides evaluating the distribution of GFRα-1 and Oct4 positive SSCs in the testis parenchyma, we are currently investigating other potential SSCs markers for these cells in bullfrogs, as well as performing western blotting analyses aiming to evaluate the presence of proteins for GFRα-1 and Oct4.

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Evaluation of testis structure and function in *Foxn1* adult mutant mice

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Introduction
Mice homozygous for the *nude* mutation are athymic and represent a very attractive immunodeficient experimental model, for instance they are adequate recipient for spermatogonial stem cell transplantation. It is already known that athymic male mice have reduced gonadotropins and testosterone in comparison to their normal littermates. In this context, considering that there very few data in the literature regarding reproductive biology in Foxn1 mutant mice, our main objectives were to investigate testis structure and function in wild type and *nude* adult male mice.

Materials and Methods
Nine *nude* and nine wild type BALB/c adult mice were utilized. They had their testes perfused-fixed with glutaraldehyde and processed for histological and morphometric analyses. Additionally, two mice from each group received intraperitonial injection of tritiated-thymidine and were sacrificed at two different time periods (1 hour and 9-10 days) after injection, to estimate the duration of one spermatogenic cycle and the total duration of spermatogenesis. Also, in order to estimate these parameters, the XII stages of the seminiferous epithelium cycle, previously characterized in mice according to the development of the acrosome over the spermatids nuclei, had their frequencies (%) evaluated. Student t-test was performed using GraphPad Prism 5.00.288 and the significance level was set as $p<0.05$.

Results and Discussion
Based on the most advanced germ cell labeled after thymidine injection (preleptotene/leptotenes in stage VIII at 1 hour and pachytene spermatocytes/early round spermatids in stages IX/I at 9-10 days after injection) and stages frequencies, the duration of one spermatogenic cycle and the total duration of spermatogenesis were very similar in *nude* and wild type mice (~9 and ~40days respectively). On the other hand, many parameters analyzed were significantly different. For instance, due to the lower Sertoli cell number per testis, several parameters such as testis weight, gonadosomatic index (total testis mass divided by body weight), total seminiferous tubules length, and daily sperm production were significantly reduced ($p<0.05$) in *nude* mice. These morphometric results are probably related to the decreased levels of gonadotropins (particularly FSH, known to regulate Sertoli cell proliferation) already cited in the literature for the athymic *nude* mice. Regarding Leydig cells, their nuclear, cytoplasmic and individual volumes were also decreased ($p<0.05$) in the athymic *nude* mice. On the other hand, the volume density and also the Leydig cell number per testis were significantly higher in this strain in comparison to the wild type. However, it remains to be investigated whether these findings are related or not to the lower testosterone levels also cited in the literature for the *nude* mice.

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Effect of chromatin integrity on bull sperm head morphometry

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Introduction

Variations in the size of bull sperm head could be an indication of damaged chromatin, because sperm head is mainly composed by chromatin (DNA and protamine). Both, sperm head morphometry (1) and chromatin integrity (2) are associated with bull fertility and a relationship between chromatin integrity and bull sperm head morphometry could be expected. The aim of this study was to evaluate the effect of chromatin integrity on the morphometric dimensions of the bull sperm head.

Material and Methods

Thawed semen from 15 ejaculates of 5 Brahman bulls was used. Semen was stained with toluidine blue (3) to determine the chromatin status and to morphometric analysis simultaneously. Effect of chromatin integrity on length, width, area, perimeter and ellipticity was determined with general linear model of SAS.

Results and Discussion

Length, width, area, perimeter and ellipticity were determined in 1599 heads with normal chromatin and 674 heads with damaged chromatin. Sperm with damaged chromatin had more width (4.32 ± 0.009 µm, $P < 0.05$) and area (29.91 ± 0.09µm^2, $P<0.05$) than sperm with normal chromatin (width: 4.22 ± 0.006 µm; area: 29.23±0.06µm^2). No significant differences in the length and perimeter were observed, while ellipticity were decreased in sperm with damaged chromatin (1.83±0.004, $P < 0.05$) in comparison with sperm with normal chromatin (1.87±0.003). Dimensions observed in this study were lower than those observed by Rubio-Guillen et al. (4), probably because a different stain was used. Results of this study demonstrate that damaged chromatin results in a subtle increase of area and width and a decrease in the ellipticity, making sperm head lightly more rounded. These results are in agree with Sailer et al. (5) who observed a positive correlation between the percentage of sperm susceptible to acid denaturalization and morphometric parameters. Nevertheless, a negative relation between chromatin decondensation with area and perimeter has been observed (3). Additionally, sperm with less packet chromatin has lower area than sperm with normal chromatin (6); in that studies *Bos taurus* semen was used while in the present study semen was from *Bos indicus* bulls, and differences between both genetic groups has been reported in morphometry and chromatin susceptibility to in vitro procedures (7,8).

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Evaluation of fertilizing capacity of frozen goat semen with or without addition of cholesterol

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Introduction

Cholesterol is the main steroid of the sperm (1), and it could be regulator of the spermatic function (2). *In vitro*, the addition of cholesterol to the medium prevents earlier acrosomic reaction (3). The perivitelline membrane of hen's yolk egg binding assay may be used to evaluate the fertilization capacity of goat spermatozoa (4). The aim of this study was to evaluate the binding capacity of frozen goat sperm in two different diluents with the addition of cyclodextrin-cholesterol complex (CCC) through the perivitelline membrane of hen´s yolk egg binding assay.

Material and Methods

Four semen samples were taken from four male goats, each ejaculate was diluted as follows: A1= Tris-glycerol + Soy Lecithin; A2 = Tris-glycerol + Soy Lecithin plus 1.0 mg mL^{-1} of CCC; B1 = Ethylene glycol + egg yolk; B2 = Ethylene glycol + egg yolk plus 1.0 mg mL^{-1} of CCC. The semen was submitted to cryopreservation process by cooling for one hour at 5 °C, followed by 15 min in nitrogen vapor and then immersed in liquid nitrogen at -196 °C.

Results and Discussion

There was no difference between treatments (Kruskal-Wallis test; P>0.05), demonstrating that the inclusion of CCC does not interfere in the binding of sperm to the perivitelline membrane of hen's yolk egg (tab. 1). The amount of sperm ($1x10^6$) incubated with the perivitelline membrane can explain the why no differences among treatments were observed. It seems that there is a plateau, on which the fertility rates remained constant, and it is not possible to see differences between treatments (5). Study of human semen showed that the percentage of sperm bound decreases on a dose-dependent basis, indicating that, starting from a plateau, an increase on sperm concentration reduces the sperm capacity to bind to the perivitelline membrane (6).

Table 1. Binding test of frozen goat sperm to perivitelina membrane of chicken egg yolk.

Treatment	Binding Test (Mean ± Standard Error of Mean)
A1	1147.81 ± 176.94
A2	1282.25 ± 240.59
B1	1028.50 ± 186.41
B2	1119.50 ± 256.99

P > 0.05.

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Use of L-carnitine in cooled stallion semen

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Introduction

Over the years, technology for cooling semen has developed. However, despite progress, pregnancy rates are still variable and relates to variable semen quality of stallions. L-carnitine (LC) and acetyl-L-carnitine (AC) modulate several sperm metabolic functions, such as fatty acids oxidation, acetyl-CoA/free CoA ratio and utilization of pyruvate and lactate as energy substrates (1,2,3). The LC has a powerful antioxidant effect by reducing the availability of lipids to peroxidation and increasing antioxidant enzymes activity as *superoxide dismutase* and *glutathione peroxidase* (4). Moreover, the presence of carnitines ensures the operation of oxidative pathways by reducing acetyl-CoA levels, and provides acetyl groups for sperm motility. Despite these findings, there are no reports about the use of LC in cooling diluents for equine semen. The aim of the study was to evaluate the effect of this substance on cooled sperm viability.

Material and Methods

Two ejaculates of 10 stallions were used. After collection, the ejaculates were evaluated for volume, motility and concentration, and then diluted with skim milk-based extender (Botu-Semen^{TM}) to a final concentration of $50x10^6$spermatozoa/mL. Ejaculates were divided into 4 groups: Control (no LC/AC), G1 (0.05mM/mL of LC), G2 (0.1mM/mL of LC) and G3 (0.15mM/mL of LC). After, the samples were placed at 5°C during 24 and 48 hours. Spermatozoal variables evaluated were: kinetics of movement through computerized system (Hamilton Thorne^{TM}), and plasma membrane integrity by association of fluorescent probes, carboxyfluorescein and propidium iodide, by epifluorescence microscopy. Evaluations were performed in fresh semen (0h), 24 hours (24h) and 48 hours (48h) of cooling. Statistical analyses were performed using ANOVA followed by Tukey test and significance was set at $p<0.05$.

Results and Discussion

After 24 hours of storage groups 2 and 3 yielded significantly higher percentages of PMI than control and G1 ($p<0.05$). Although parameters TM, PM and RAP did not significantly differ at 24h and 48h, it was noted that treated groups were numerically greater when compared to control, as well PMI at 48 hours of storage. Probably with a larger number of ejaculates, this numerical trend can be confirmed, differing significantly. Based on the results, it was concluded that the addition of L-carnitine gave better protection to plasma membrane after 24 hours of storage at 5°C.

Table 1. Mean values of Total Motility (TM), Progressive Motility (PM), Rapid Cells (RAP) and Plasma Membrane Integrity (PMI) analyzed at 0 (0h), 24 (24h) and 48 hours (48h).

	0h	24h				48h			
		C	G1	G2	G3	C	G1	G2	G3
TM (%)	73 ± 12	55 ± 21	62 ± 20	60 ± 19	57 ± 17	31 ± 19	42 ± 22	42 ± 18	43 ± 11
PM (%)	35 ± 14	25 ± 15	34 ± 16	34 ± 16	31 ± 16	10 ± 11	18 ± 13	17 ± 12	18 ± 12
RAP (%)	63 ± 16	44 ± 22	52 ± 21	51 ± 20	48 ± 19	22 ± 17	31 ± 20	31 ± 17	32 ± 18
PMI (%)	62 ± 10	53 ±1 5^{b}	59 ± 10^{ab}	63 ± 11^{a}	63 ± 10^{a}	50 ±1 1	57 ± 12	57 ± 13	57 ± 12

**Values in the same row with different superscript differ significantly ($p<0.05$).

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Validation of flow cytometry for assessment of membrane lipid peroxidation of equine spermatozoa

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Introduction

Flow cytometry is a quick, repeatable and objective method of evaluating compartments of spermatozoa. Equine sperm have a relative high content of polyunsaturated fatty acids in their plasma membrane (1), being susceptible to lipid peroxidation (2). This study aims was to validate a protocol to be used in the assessment of membrane lipid peroxidation of equine spermatozoa through flow cytometry.

Materials and Methods

Two ejaculates from five stallions were collected using an artificial vagina. Samples were diluted in modified Tyrode's medium (MTM) at concentration of 5 x 10^6 sperm/mL. For the assessment of sperm lipid peroxidation, two 499.5-µL aliquots of each ejaculate were added to 0.5 µL C11-BODIPY$^{581/591}$ (1 mg/mL, D-3861, Molecular Probes) at 37°C for 30 min. After this, the samples were centrifuged at 300 x *g* for 10 min, the negative control was ressuspended in 1 mL MTM and the positive control in 80 µM ferrous sulfate (Fe_2SO_4) and 5 µM cumene hidroperoxide (CuOOH) (Sigma Chemical Co.) for 15 min at room temperature in the dark. Then, 1 µL Hoechst 33342 (100 µg/mL, H-1399, Molecular Probes) was added and re-incubated at 37°C for 5 min. A total of 10 000 gated-events were analyzed per sample by flow cytometry (BD LSRFortessa™). The green fluorescence (FL1) was collected through a 580-nm bandpass filter and the red fluorescence (FL3) through a 635-nm bandpass filter. Statistical analysis was performed using GraphPad Prism Version 4.03 (2005). Linear regression was carried out to determine the ratio between treatments.

Results and Discussion

As seen in Figure 1, regression analysis indicated a high degree of correlation between the BODIPY fluorescence (FL1) and the percentage of sperm lipid peroxidation in the presence of Fe_2SO_4 and CuOOH, according the methodology described. Regression plots allowed simple visualization of the distribution of the data points around the line of equality (slope=1.0) by showing how close the slope of the regression line of the data (slope=0.8955) is to the line of equality. In conclusion, this protocol standardization assay was consistent, applicable and had a reliable measure of plasma membrane lipid peroxidation in equine spermatozoa through flow cytometry (BD LSRFortessa™).

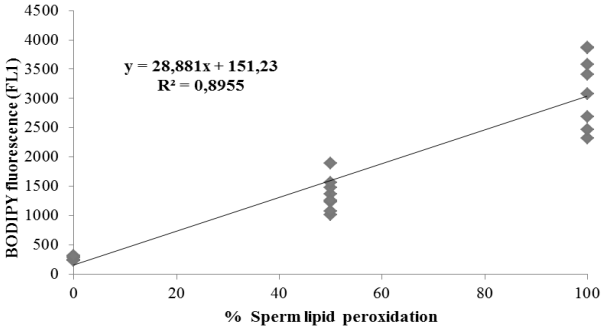

Figure 1. Green fluorescence of membrane lipid peroxidation detected by flow cytometry.

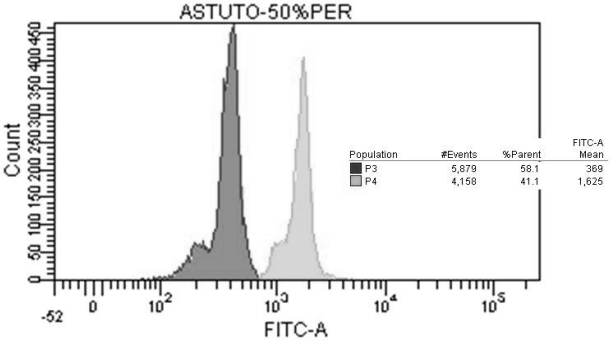

Figure 2. Representative histogram of BODIPY staining in membrane of equine spermatozoa.

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Breeding soundness evaluation in margay (*Leopardus wiedii* Schinz, 1821) males

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Introduction

Captive breeding has becoming an important aspect in the conservation of endangered species, where zoos and captive breeding centers are specifically managed to maintain biodiversity and genetic lineages (1). The breeding soundness evaluation is a tool of paramount importance for the identification of animals capable of reproducing and animals that have reproductive problems, making assisted reproduction more efficient.

Material and Methods

Margay males (n=6) were used from the Itaipu Wild Animals Captive Breeding Center, located in Foz do Iguassu, PR, Brazil. They were submitted to anesthesia and semen collection three times, with a 30 days interval. Electrical stimuli were administered, using a controlled voltage electrostimulator (PT Electronics, USA) in three separate series (30, 30 and 20 stimuli series) at incrementally higher voltages (range, 2-5 V), with 5 minutes intervals between series. For each collection procedure, a total of 80 stimuli were administered over a 15 minutes (2).

Results and Discussion

Two males (IDs 1883 and 1851) showed no sperm in any of the exams and were characterized as azoospermic. Another male (ID 2333) showed no sperm production in only one of the collections. The animal 1801 in one of the collections presented oligospermia. Mean values obtained were lower than shown by (3), particularly when comparing the total number of cells. Factors that possibly contributed to these results are advanced age, stress resulting from captive conditions and inadequate mineral supplementation, considering that all tested animals showed varying degrees of hair plucking. Using a linear correlation to determine the correlation between the mean of the total number of cells with body weight, age and testicular volume, showed a strong positive correlation with body weight (r= 0.83); considering testicular volume showed a moderate positive correlation (r= 0.60) and considering age showed a weak negative correlation (r= -0.36). This indicates that factors such as overweight and low testicular volume may contribute negatively in the production of sperm cells.

Table 1. Breeding soundness evaluation (BSE) results (mean ± EPM) in margays (*Leopardus wiedii*).

Identification	2333	1495	1801	1883	1851	2409	Mean ± SEM
Age (years)	5	16	12	12	12	3	10 ± 4.94
Weight (kg)	5.25	3.68	4.91	2.81	3.68	3.50	3,97 ± 0.93
Testicular volume (cm^3)	4.7	3.5	4.9	4.2	1,7	2.6	3,61 ± 1,24
pH	7.5	8.0	8.0	8.0	8.0	7.7	7,9 ± 0,2
Ejaculate volume (µl)	190.7	216	397.3	483.7	269	54.3	268.5 ± 153.35
Concentration ($x10^6$/ml)	150	41.7	48.3	0	0	73.3	78.33 ± 49.68
Total cells number ($x10^6$)	16.2	9.4	16,8	0	0	10.8	13.3 ± 3.7
Motile sperm ($x10^6$)	13.8	6.0	15.2	0	0	9.6	11.2 ± 4.2
Motility (%)	56.6	65	83.3	0	0	86.7	72.9 ± 14.45
Status (0-5)	2.8	2.8	3.8	0	0	3.8	3.3 ± 0.58
Teratospermia (%)	38	50.7	43.3	0	0	47.7	44.93 ± 5.53

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Cholesterol addition to sperm plasma membrane: an alternative for bad cooler stallions

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Introduction

Artificial insemination with cooled-shipped semen has been widely used in horse breeding. However, some stallions present abrupt fertility decrease when their semen is processed, cooled and transported. These animals are referred to as "Bad coolers" and have limited reproductive performance, resulting in economic losses. Alternatively to improve the reproductive function of these stallions is to increase the sperm membrane stability and fluidity at low temperatures by cholesterol incorporation (1). The aim of this study was to assess the effects of cholesterol incorporation into sperm membranes on cooled semen quality of "Good cooler" and "Bad cooler" stallions.

Material and Methods

Two ejaculates from seventeen stallions were collected. Each ejaculate was extended to 30 x 10^6 sperm per mL with skim milk-based extender (Botu-Sêmen^{TM}) and splitted into two 10 mL aliquots. Then, for each ejaculate, one aliquot served as control while 75 µL of 6.1mM of cholesterol-loaded clyclodextrin was added in the other, consisting the treated semen. The stallions were classified in "Good coolers" (n=9) and "Bad coolers" (n=8) based on the ability to maintain sperm progressive motility after 24 hours of cooling at 5 ºC (2). After both treatments, the samples were cooled at 5 °C for 24 hours. Semen samples were evaluated before and after cooling by CASA for total motility (TM), progressive motility (PM), rapid sperm (RAP) and plasma membrane integrity (PMI) by epifluorescent microscopy. All parameters were analyzed by ANOVA, followed by TUKEY to identify the significant differences ($P < 0.05$). The statistical analyses were performed using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

For "Bad cooler" stallions, treated samples presented significant improvements in all parameters compared to control samples (Table 1). On the other hand, the "Good cooler" stallions showed similar sperm quality before and after cooling in the treated samples (Table 1). These results suggest the incorporation of cholesterol-loaded cyclodextrin to the plasma membrane is effective for maintaining sperm quality in "Good cooler" stallions and provides substantial resistance to sperm from "Bad cooler" stallions after storage for 24 hours at 5 ºC. In conclusion, the addition of cholesterol-loaded cyclodextrin may be an option to enable the utilization of cooled-shipped semen from "bad coolers" stallions for artificial insemination. Additional studies are being performed to elucidate the cholesterol treatment influence on sperm fertility efficiency.

Table 1. Mean (± S.D.) values of sperm parameters of TM, PM, RAP and PMI from "Bad cooler" and "Good cooler" stallions before cooling (Initial) and after storage at 5 °C for 24 h (Post-cooling) in Control and Treated groups.

	Bad coolers			Good coolers		
	Initial	Post-cooling		Initial	Post-cooling	
		Control	Treated		Control	Treated
MT (%)	73 ± 11^{a}	39 ± 17^{c}	60 ± 12^{b}	80 ± 7^{A}	65 ± 12^{B}	76 ± 6^{A}
MP (%)	39 ± 9^{a}	15 ± 10^{c}	30 ± 11^{b}	48 ± 8^{A}	36 ± 9^{B}	45 ± 9^{A}
RAP (%)	65 ± 15^{a}	30 ± 16^{c}	52 ± 15^{b}	73 ± 9^{A}	55 ± 14^{B}	66 ± 8^{A}
IMP (%)	69 ± 9^{a}	52 ± 12^{b}	63 ± 9^{a}	77 ± 10^{A}	65 ± 14^{B}	72 ± 10^{AB}

Values with different superscripts $^{(a, b, c)}$ in a row differ significantly ($P < 0.05$) for "Bad coolers". Values with different superscripts $^{(A, B, C)}$ in a row differ significantly ($P < 0.05$) for "Good coolers". Control (Botu-Sêmen^{TM}), Treated (cholesterol-loaded cyclodextrin).

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Decreased fertilizing ability of frozen/thawed rabbit sperm incubated with homologous seminal plasma

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Introduction

Rabbit sperm have a high sensitivity to cryopreservation manifested in changes on the plasma membrane (1) that affect their fertilizing capacity. Recent studies show that seminal plasma (SP) addition to frozen/thawed ram sperm improves their viability and fertility (2). In addition, there is evidence to suggest that components of SP enhance sperm survival within the female reproductive tract (3). The aim of this study was to evaluate the in vivo fertilizing capacity of frozen/thawed rabbit sperm of Californian breed incubated with SP.

Material and Methods

The ejaculates were obtained with artificial vagina and were diluted with Tris-egg yolk-DMSO-glycerol extender, and frozen in 0.25 ml straws in liquid nitrogen vapors. SP was obtained according to Evans (4) and it was storage at -18 ºC. Twenty semen straws were thawed and incubated for 5 minutes at 37ºC with (10% v/v) or without homologous SP (TSP and TC, respectively). Twenty rabbit does were synchronized with eCG (60 UI) and were artificial inseminated with TSP (n=10) or TC (n=10). Ninety six hours after insemination time, uterine horns were flushed for embryo recovery with phosphate buffer solution (PBS) plus BSA (2%). Collected structures were evaluated under stereomicroscope and classified in blastocyst, degenerated embryos and oocytes.

Results and Discussion

The embryo recovery rate was significantly higher in rabbits inseminated with sperm TC compared to those inseminated with sperm TSP (Fig. 1).The recovery rate of unfertilized oocytes was significantly lower in rabbits inseminated with sperm TC, compared with TSP. In addition, 62.5% of the embryos obtained in TSP were degenerate, while in TC was obtained only 14.3%. Based on these results, TSP has showed decreased fertilizing capacity compared to TC. It was suggested that rabbit seminal plasma has de-capacitating factors (5). In addition, the protocol that we used to obtain SP, could remove seminal droplets, which have been associated capacitation process and acrosome reaction (6). We conclude that incubation of frozen/thawed rabbit sperm with homologous SP decreased their fertilizing capacity.

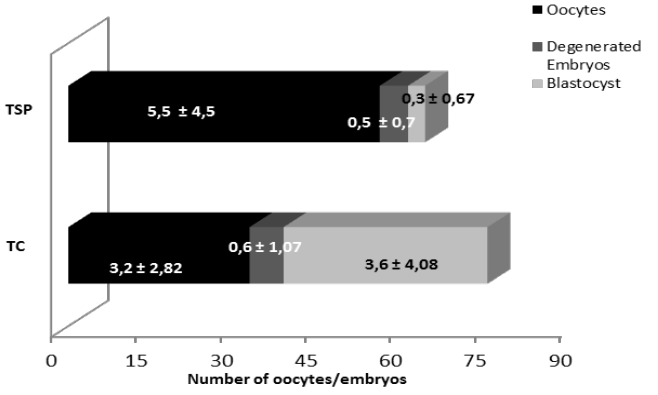

Figure 1: Oocytes and embryos (blastocyst and degenerated) recovery from Californian rabbit doe inseminated with frozen/thawed sperm, incubated (TSP) or not with seminal plasma (TC).

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Scrotal insulation effects on bovine embryo production: preliminary results

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Introduction

Scrotal insulation has frequently been used to study the dynamics of sperm defects (1) and DNA fragmentation (2) in bovine spermatozoa. Scrotal insulation increased the testicular temperature and metabolism, disrupt spermatogenesis with a consequent decrease in sperm production, semen quality (3) and fertilizing ability (1). The objectives of the present study were to assess the effects of induced testicular degeneration in *Bos taurus x Bos indicus* bulls on fertilizing ability of sperm.

Material and Methods

Scrotal insulation was performed in 2 bulls (*Bos taurus x Bos indicus*) for 72 hours. The scrotum was wrapped with a wool bag (kept in place with tape) and covered with nylon plastic. Semen was collected by an artificial vagina and then freezing. A single ejaculated was collected 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 weeks after insulation. For *in vitro* embryo production control was used semen of an animal with knowed fertility acquired in an Artificial Insemination Center. The semen was thawed and centrifugated in a Percoll gradient 45/90%. Recovered spermatozoa were used for fertilized oocytes *in vitro* maturated. Cleavage rate was assessed 48h after *in vitro* fertilization (IVF) and blastocyst rate after 7 days of IVF. The relative percentage of cleavage and blastocyst rates of scrotal insulated bulls was performed by comparison with cleavage and blastocyst rates of the 0 week of insulation. Qui-square was performed for statistical analysis with $p<0.05$.

Results and Discussion

Cleavage rate presented a reduction after 2 weeks pos-insulation, and became zero in 3, 4 e 5 weeks after testicular degeneration ($p<0.0001$). Seven weeks from scrotal insulation cleavage rate was increase; showed that spermatozoa are capable to fertilizing an oocyte. Blastocyst rate presented a reduction ($p<0001$). These results suggest that spermatozoa can fertilize an oocyte; however embryos are not capable to development until blastocyst stage. Testicular degeneration causes DNA fragmentation compromising embryo development (4). Embryo gene expression activation begins in 4-8 cells stage and sperm DNA damage became sensitive to embryo apoptosis mechanism and mitosis block interrupting embryo development, and evidenced by failure in blastocyst formation, nuclear fragmentation and failure meiotic fuse formation (5). The results suggest that testicular temperature increase compromise cleavage rate and blastocyst formation.

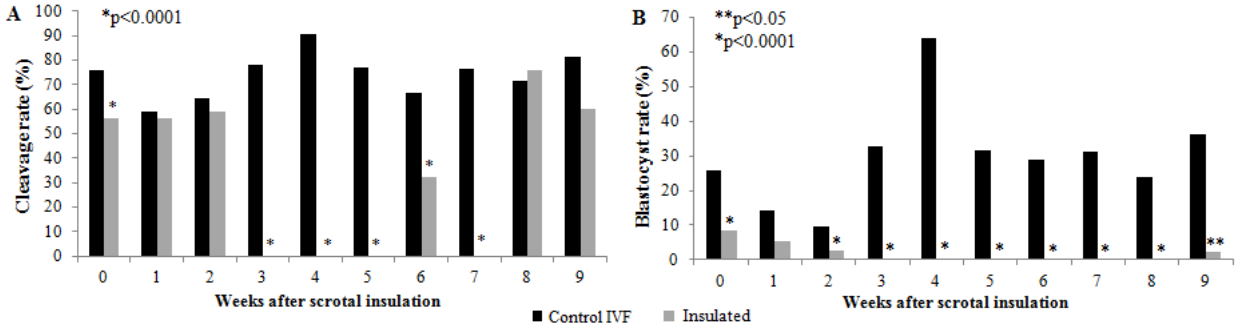

Figure 1. (A) Scrotal insulation effect on cleavage rate. (B) Scrotal insulation effect on blastocyst rate.

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Correlation between different incubation times in fluorescent probes for spermatic membrane integrity evaluation in Piau swine breed

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Introduction

The evaluation of the plasma membrane can be accomplished using fluorescent probes such as carboxyfluorescein diacetate and propidium iodide, which serve as markers. The propidium iodide is a marker of damaged cells and has lipophilic properties and thus penetrates the membrane lesions staining DNA with red fluorescence. Carboxyfluorescein diacetate has a low molecular weight and thus when the membrane has a damage it is rapidly lost, by staining the cell in green indicating that the membrane is intact (1). Incubation times of semen with fluorescent probes can influence the results of complementary tests (2), thus this study aimed to evaluate the integrity of the sperm plasma membrane of Piau swine pig breed using different incubation times.

Material and Methods

Twenty frozen semen samples were used from five males (four semen samples from each male) sound for reproduction by breeding soundness evaluation. To check post-thawing sperm quality were used sperm motility, and sperm morphology evaluation, and to check sperm viability were used the hipoosmotic swelling test, supra vital staining, and fluorescent assay using carboxyfluorescein diacetate and propidium iodide fluorescent probes. The experiment was performed by dividing the incubation times and temperatures in three treatments: 1= Incubation for 15 minutes at 25 °C (3); 2= Incubation for 8 minutes at 38.5 °C (4); 3= Incubation for 15 minutes at 38 °C (5).

Resultados and Discussion

For the physical assessment of semen (sperm motility, vigor) and sperm viability (supra vital staining and hipoosmotic swelling test), there was no difference (P>0.05) between matches. The results of these evaluations were 54.5±5.8%, 3.1±0.3, 53.1±5.6% and 12.8±6.3%, respectively. In relation to incubation time, there was no difference between treatments (P>0.05), yielding 25.0±10.7%, 28.8±13.1% and 26.9±13.1% of sperm membrane integrity, respectively, for treatments 1 to 3, not influencing the results and showing that there is no need to incubate the semen in a water bath, making the test using fluorescent probes more viable. Our results contrast with those found by Feitosa (2) who concluded that shorter incubation time yield higher motility and membrane integrity. Incubation times and temperatures did not influence the accuracy of tests in evaluation of sperm membrane integrity using fluorescent probes.

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Association of *nuclear annulus* and chromatin in bull sperm

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Introduction

Fertility problems have been associated with alterations of chromatin condensation in sperm cells . This feature can be modulated by interactions between chromatin and specific regions of nuclear matrix. The aim of this study was to characterize biochemically the composition of the nuclear annulus, a component of the sperm nuclear matrix, and how it associates with chromatin.

Materials and Methods

Samples of bovine semen were treated with Triton X-100® and dithiothreitol (DTT). Nuclear annuli, along with dispersed fibrillar material, were isolated by means of ultracentrifugation on sucrose and cesium chloride gradients , followed by observation under scanning electron microscopy. (Figure 1) Additionaly, the isolated material was smeared onto histological slides followed by staining with xylidine ponceau to identify protein components, PAS (periodic acid-Schiff) to identify carbohydrate moieties on proteins, and toluidine blue pH 4.0 to evidence DNA. The slides were observed under light microscopy. Purified samples were also subjected to SDS-PAGE before and after treatment with DNAse. After treatment with DNAse samples were applied to Superdex and reverse phase columns on HPLC.

Results and Discussion

The positivity of the nuclear annuli to all three cytochemical methods (Figure 2) evidenced that it is a glycoproteic structure (Figure 2 A, B) with heterogeneity of proteins. This proteic structure contained portions of DNA attached on it, which are continuous with the rest of chromatin (Figure 2C). SDS-PAGE analysis showed that treatment with DNAse fragmented the annuli in smaller subunits, evidencing that DNA may contribute to the maintenance and stability of its structure. We believe that this attachment is dependent on the presence of nuclear matrix attachment regions (MARs), which may have an important role in gene regulation in the early embryonic development.

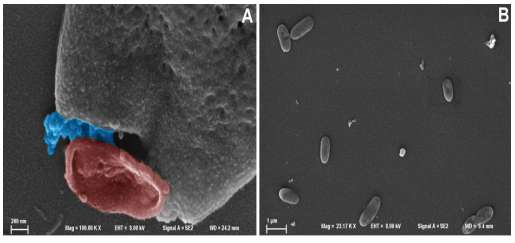

Figure 1. A-The Electron-nuclear annulus (pink) Still attached the sperm head by strands of DNA (blue). B-Electron of isolated nuclear annulus

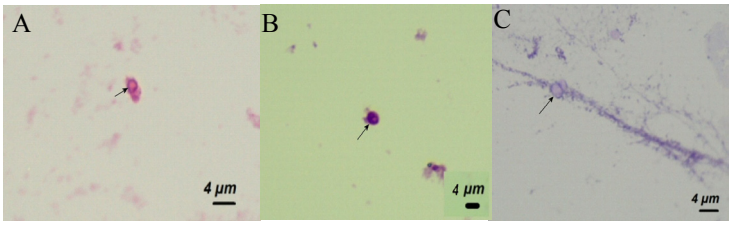

Figure 2. Cytochemical staining of nuclear annulus. A-Xylidine Ponceau, B- PAS and C-Toluidine blue.

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Description of ultrastructural characteristics of six-banded armadillo (*Euphractus sexcinctus*) sperm

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Introduction

In spite of the ecological importance of six-banded armadillos (*Euphractus sexcinctus*), knowledge on their reproductive physiology is scarce. The morphology of their sperm was previously studied by light microscopy (1), however, this method does not provide structural details on nanometer scales. Therefore, the objective of the present study is to describe, for the first time, the ultrastructural characteristics of *Euphractus sexcinctus* sperm using the Transmission Electron Microscopy (TEM).

Material and Methods

Semen from 04 adult male six-banded armadillos, bred under captivity at the Centre of Multiplication of Wild Animals (CEMAS – UFERSA, Mossoró, RN, Brasil), was collected by electroejaculation (1). Samples were pooled and processed for ultrastructural analysis (2). Randomly fields were examined by a transmission electronic microscope (Morgagni 268 D, FEI Company, Hillsboro, EUA) and photographed for further analysis and description.

Results and Discussion

The TEM revealed that six-banded armadillo sperm present a flattened head containing a nucleus of electron dense aspect, almost homogeneous, except by some electron lucent diffuse points. The acrosome takes two thirds of the nucleus. The electron dense contents of the acrosome are limited by internal and external acrosome membranes. The subacrosomal space, between the acrosome and the apex of the nucleus, was occupied by a material with variable electron density. The tail implantation fossa is covered by a thick layer of a very dense material, consisting in the basal plate or *capitulum*. This structure, in association to the proximal centriole and the threaded columns, comprises a connection piece. The tail is divided in mid, main and terminal pieces. In longitudinal sections, it is possible to view the mitochondria arranged in helix form around the outer dense fiber throughout the length of the midpiece. Approximately 45 mitochondrial spirals were identified in the mitochondrial sheath of the midpiece. Immediately caudal to the last mitochondrial spiral, a thick ring of dense material formed the *annulus* where there is a strong adhesion between the plasma membrane and flagellum. From the *annulus* begins the region corresponding to the main piece of the tail constituted by the outer dense fibers surrounded by the fibrous sheath. In cross section, there are electron-dense points that correspond to the dorsal and ventral longitudinal columns in the fibrous sheath. The axoneme consists on nine pairs of microtubules forming a bundle surrounding a cylindrical central pair of microtubules. It is possible to identify radial bridges that extend from the central pair of microtubules to the peripheral pairs. General ultrastructural characteristics of *Euphractus sexcinctus* sperm are similar to that previously described for other armadillos, except by the unilateral subacrosomal region verified only in the *Cabassous unicinctus* (3), and the bilaminar plate verified in the acrosome equatorial region in *Dasypus novemcinctus mexicanus* (4) sperm. In conclusion, the present study provides pioneer information regarding the ultrastructure of the *Euphractus sexcinctus* sperm. This information is valuable for future studies on the reproductive biology of this and other members of Dasypodidae family.

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Concentrations of ions and proteins present in plasma seminal of equine ejaculate

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Introduction

The presence of mineral elements in seminal plasma of horses is correlated with semen quality and their ability to survive the process of freeze-thaw (1). It also has in its composition around 10mg/ml protein, and its function may be related to preventing thermal shock, maintenance of membrane integrity, maintaining the total and progressive motility, the maturing process, control of sperm capacitation (2).

Material and methods

Semen was collected from seven stallions Mangalarga Marchador. Were quantified the concentrations of calcium (Ca), chlorine (Cl), phosphorus (P), magnesium (Mg), potassium (K) and Sodium (Sa) ions in seminal plasma, in two fractions, named sperm rich fraction (SRF) and a sperm poor fractiono (SPF), as well as the protein concentrations in the first and second fraction of the ejaculate.

Results and discussion

In SPF, the average concentration of calcium ions (3.06 ± 4.14), chlorine (126.29 ± 7.16) and magnesium (3.03 ± 3.12) showed no statistical difference (P = 0 Ca , 2704, P = 0.8974 Cl, Mg, P = 0.3517) compared with SRF (1.15 ± 0.38), (125.71 ± 8.94) (1.83 ± 0.68) respectively. There was no statistical difference (P = 0.9465) between the average about concentrations of potassium ion (21.13 ± 5.12), phosphorus (0.89 ± 0.20) and sodium (126.86 ± 5.45) in SRF , SLF (21.37 ± 7.80; 0.73 ± 0,17 and 121.43 ± 13.33) respectively. The high concentration of calcium ions in the seminal plasma can be harmful to sperm capacitation and for triggering the acrosome reaction prematurely (3), and this hypothesis is of great importance in the handling of their semen for cryopreservation. The SPF had average (14.5 ± 10.2 mg / ml) protein concentration less than SRF (17.0 ± 10.0 mg / ml), presenting a significant difference (P = 0.0418), which is important for the freezing procedure equine semen, because it was found that the seminal plasma of the studs with good heat resistance has a higher protein concentration over the seminal plasma of studs with low thermal resistance to freezing (4). In another study, it was observed that the protection sperm to thermal shock and freezing was higher in the group that was used in concentrated protein processing to yield a better integrity and motility after freezing membrane (5). It can be concluded that, due to the increased amount of protein in the ejaculate SRF, this has a better chance to freeze than the total ejaculate or sperm-poor fraction.

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Assessment of bovine sperm attributes by capillary cytometry at different stages of cryopreservation

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Introduction

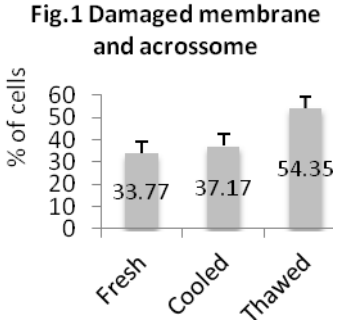
The process of bovine sperm cryopreservation has been widely used as a form of preservation and storage of gametes from genetically superior male. Despite its advantages, the biotechnical may have deleterious effects on sperm like the loss of membrane integrity and DNA lesions (1,2). The aim of this study was to evaluate the effect of cryopreservation on sperm cells during this process by capillary cytometry.

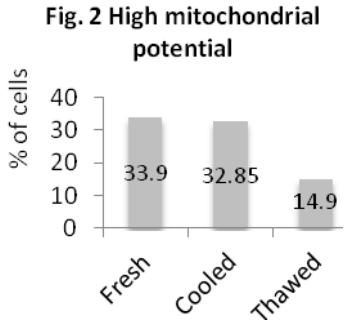
Materials and Methods

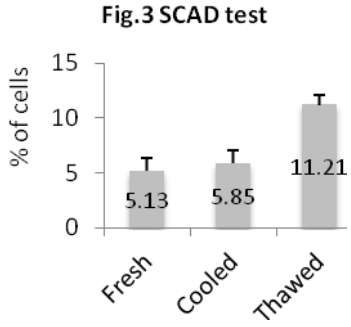
Semen samples were collected weekly from four Holstein bulls by artificial vagina during 7 weeks. Each semen sample was diluted with commercial diluting Botubov® and then separated in 3 treatments: 37°C semen (fresh), 5°C semen (cooled) and thawed at -196°C semen (thawed). The evaluations were made 0h and 2h after incubation in TALP semen medium. To measure the integrity of membranes and mitochondrial potential, sperm were stained with FITC-PI and JC-1 respectively. To evaluate the susceptibility of chromatin to acid denaturation (SCAD) sperm were stained with acridine orange. Each probes settings was performed in flow capillary where 10,000 events were analyzed.

Results and Discussion

As shown in Figure 1, there was an increase in the percentage of sperm with damaged acrosome and membrane at the thawed group (54.35 ± 5.18) when compared to fresh (33.77 ± 5.29) and cooled (37.17 ± 5.29) groups. For the JC-1 probe (figure 2), data presented as median and quartiles, there was a decrease in the percentage of cells with high mitochondrial potential in the group frozen [14.90 (5.64; 18.90)] compared to fresh [33.90 (20.35; 42.15)] and cooled [32.85 (12.80; 39.20)] treatment. In the SCAD (figure 3), the frozen group (11.21 ± 0.85), showed an increase in the percentage of sperm positive for the test when compared to fresh (5.13 ± 1.27) and cooled (5.85 ± 1.18) groups. There were also differences between the incubation times. The samples incubated for 2h showed a higher percentage of cells positive for the SCAD (9.57 ± 1.14) compared to the 0h group (5.70 ± 0.62). These data indicate loss of membrane integrity and cellular viability during the cryopreservation process. Besides these injuries, SCAD test suggest that cryopreservation induces increased susceptibility to fragmentation of the sperm chromatin.







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Effect of thawing methods on stallion semen

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Introduction

The collection of semen should be carried out with appropriate equipment and correctly, as well as the freezing-thawing process, avoiding injuries and preserving the fertilizing capacity. In the open collection, the glans stays discovery and the semen does not come into contact with the artificial vagina preventing its exposure at high temperatures, which makes this method more hygienic and less harmful. The objective of this study was compare the ultra-fast defrost method with the thawing at 37°C per 30 seconds, collected by the conventional.

Material and Methods

Semen was collected from five stallions of the breed Mangalarga Marchador aged 5 to 10, to this was used a conventional artificial vagina, Hannover model, to perform closed or conventional gathering, which has only a single container where the semen is deposited. The semen has been diluted, centrifuged and then resuspend in diluent to freezing getting a concentration of 100 x 10^6 sptz/ml, then it was potting and sealed into 0,5mL vanes. The vanes were cooled by 20 min at 5°C, and after this time were placed 4cm above the level of N_2 for 10 minutes and then immersed in N_2L. The vanes have been unfrozen at different temperatures. In treatment 1, the palette have been unfrozen in water at 37ºC ±1°C for 30 seconds and in treatment 2, the palette have been unfrozen at 98ºC (±2ºC) for 4 seconds, followed by a temperature about 37°C (±2 C) untilevaluation (1). The semen has been assessed on the total motility (TM), progressive motility (PM), plasma membrane integrity and acrosome integrity.

Results nd Discussion

In conventional collection, the MP and MT showed no statistical difference when compared with the methods of ultra-rapid and conventional thawing. Disagreeing with (1) who obtained better motility (41.2 ± 14.8%) when thawed at a temperature of 75ºC for 7 seconds. The average sperm with membrane damage thawed by the conventional method (52.70 ± 13.52%) was higher than the average ultra-rapid thawing (47.63 ± 7.44%), with statistical difference (P=0,0387). The acrosomal integrity analysis showed that the mean sperm acrosome reaction of true (4.16 ± 6.42%) was significantly higher (P=0.0440), when the semen was thawed in a conventional method when compared to ultra-fast method (1.89 ± 3.13%). The same fact was demonstrated in bovine semen, which yielded a mean of sperm with the membrane and acrosome intact superior when thawed ultra-fast method (2). Since the membrane and acrosome integrity proved superior in ultra-rapid thawing and knowing that these two parameters are of vital importance in the biological process of sperm cell (3), we recommend the use of this method of thawing.

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Aknowlegements: FAPERJ, PPGCA-UENF.

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Toroidal structures in sperm chromatin of bull, turkey and human: a morphological characterization

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Introduction

During spermiogenesis, spermatid histones are almost completely substituted for a filamentous protein known as protamine, altering the classical nucleosome pattern. Protamine is a basic and arginin rich protein which binds in DNA minor groove, neutralizing phosphate radicals, making it more stable and neutral, allowing DNA strains to pile in several layers. This stack forms a circular structure denominated toroid, also known as "doughnuts". This kind of chromatin intensely decrease de size of spermatozoa head and protects DNA from damage until it reaches oocyte (1; 2). The aim of this study is to characterize toroid morphometric and its relation with sperm chromatin in spermatozoa heads of bull, turkey, and human.

Material and Methods

For this research samples of each species were divided into two groups: control group and a group treated with sodium duodecyl sulfate (SDS) and dithiothreitol (DTT) to break dissulfide bounds. Sperm was then processed for transmission electron microscopy.

Results and Discussion

Control group presented a dense and homogenous chromatin conformation. In treated samples spermatozoa chromatin was disorganized, enabling visualization of circular structures (toroids), with a average diameter of 50.85 ± 6.71 nm in bull (Fig. 1A), 59.42 ± 7.24 nm in turkey (Fig. 1B) and 55.10 ± 7.02 nm in human (Fig. 1C), immersed in a fine filamentous network (nuclear matrix). The nuclear matrix is connected to internal nucleus membrane and toroids. Its biding site is called matrix attached regions (MARs) which is believed to be constituted by classical nucleosomes with an important function in epigenetic inheritance. The differences between toroid diameters could be due the DNA loops contained in each toroid or due to the greater chromatin packaging, which alters toroid susceptibility to DTT. Thus we could prove visually the toroid structure in sperm chromatin, its intimate relation with the nuclear matrix and the importance of disulfide bounds of protamines in is structural stability.

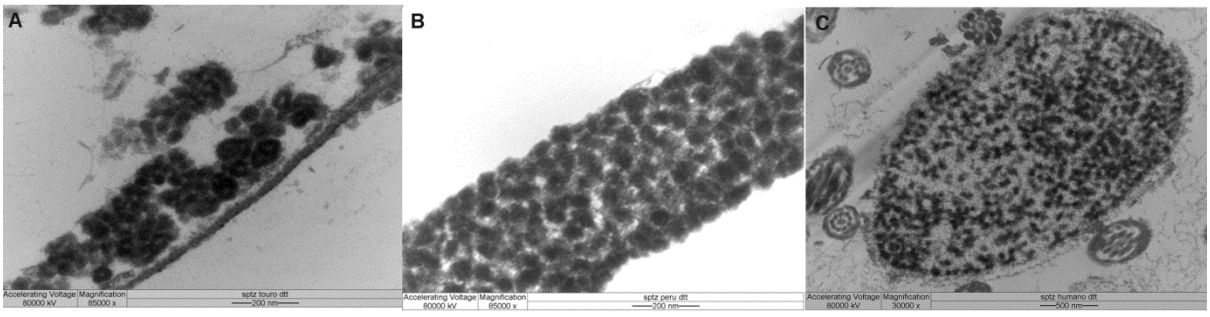

Figure 1. Electronmicrography of toridal structure of bull (A), turkey (B)and human (C) sperm.

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Flow cytometric analysis of fertile and subfertile frozen stallion spermatozoa

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Introduction:
Artificial insemination (AI) is a widely used technique in equine industry, the doses of semen are usually supplied as chilled or frozen, and both processes can cause irreversible damage to sperm cells. The identification of these damages by flow cytometric analysis may help in choosing the best technique to be used for artificial insemination and thus obtain better rates of pregnancies. The objective of this study was to identify the sperm damage by evaluation of motility, plasmatic and acrossomal membrane integrity and DNA fragmentation from fertile and subfertile frozen stallion spermatozoa.

Material and Methods:
Three fertile (> 60% conception rate) and three subfertile stallions (< 20% conception rate) aged 6 to 15 years were submitted to four semen collection each. Semen was diluted in a skim milk-based extender (v:v), centrifuged at 600 x *g* for 10 min, resuspended in BotuCrio™ freezing extender at concentration of 200 x 10^6 sperm/mL and then submitted to freezing process. Straws were thawed at 46°C for 20 seconds and evaluated by CASA for total motility (TM), progressive motility (PM) and rapid sperm (RAP). Samples were then diluted in modified Tyrode's medium at concentration of 25 x 10^6 sperm/mL. For the assessment of plasma membrane, a 200-µL aliquot was added to 2 µL of Hoechst 33342 (H33342; 40 µg/mL, Molecular Probes), 3 µL of Iodide Propidium (IP-0.5 mg/mL, L0770, Sigma-Aldrich) and 10 µL of FITC-PSA (100 µg/mL, L-0770, Sigma-Aldrich). Samples were incubated at 37°C for 15 minutes in the dark. For evaluation of DNA fragmentation, a 100-µL aliquot was added to 400 µL of acid-detergent solution (0.08 N HCl, 0.15 M NaCl, 0.1% Triton x 100; pH 1.4). Exactly 30 seconds later, 1.20 mL of acridine orange staining solution (0.037 M citric acid, 0.126 M Na_2HPO_4, 0.0011 M dissodium EDTA, 0.15 M NaCl; pH 6.0, 4uC) containing 6 mg/mL electrophoretically purified AO was added. Samples were analyzed after 30 sec in the dark. A total of 10 000 gated-events were analyzed per sample by flow cytometry (BD LSRFortessa). The green fluorescence (FL1) was collected through a 580-nm bandpass filter and the red fluorescence (FL3) through a 635-nm bandpass filter. The mean channel fluorescence was determined for both red and green.

Results and Discussion
Significant differences were observed on sperm parameters of TM (73.6±11.2[a] *vs* 55.8±9.7[b]), PM (38.4±9.6[a] *vs* 25.7±7.1[b]) and RAP (62.7±11.5[a] *vs* 48.5±14.2[b]) of fertile and subfertile sperm samples.

Table 1. Mean values and standard deviations of damage plasma membrane and intact acrosome (DPIA), damage plasma membrane and damage acrosome (DPDA), intact plasma membrane and intact acrosome (IPIA), intact plasma membrane and damage acrosome (IPDA) and DNA fragmentation index (DFI) evaluated after thawing in samples of fertile and subfertile sperm

	DPIA (%)	DPDA (%)	IPIA (%)	IPDA (%)	DFI (%)
Fertile	42 ± 6.3[b]	11.2 ± 6.8[b]	46.1 ± 11.9[a]	0.7 ± 0.5[b]	1.0 ± 0.4[b]
Subfertile	48 ± 8.2[a]	21.2 ± 6.6[a]	28.2 ± 8.2[b]	1.9 ± 1.0[a]	3.4 ± 4.1[a]

[a,b]Different letters in a column indicate differences ($P < 0.05$).

Sperm from subfertile stallions are more susceptible to damages in plasma and acrosomal membrane integrity, allowing the arrival of a few viable sperm at the site of fertilization, moreover, higher DNA fragmentation index can negatively affect embryo development, contributing to poor fertility in these animals.

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Goat Binder of SPerm (BSP) protein homologs bind to phospholipids of Andromed®

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Introduction

Andromed® is a commercial semen extender based on soybean phospholipids. It has been considered as one of the substitutes for egg yolk and milk-based extender due to its protective role against cold shock. Studies have shown that major proteins of the bovine seminal plasma, named Binder of SPerm (BSP) proteins, interact with phospholipids constituents of Andromed® and this interaction has been suggested to be the basis of sperm protection during storage. Recently, homologues of these BSP proteins were characterized in goat seminal plasma. Therefore, this study was designed to investigate the interaction between Andromed® components and goat BSP proteins.

Material and Methods

To evaluate the interaction between goat BSP proteins and Andromed® components, we performed ultracentrifugation and gel filtration analysis. The phospholipids vesicles were isolated from Andromed® extender by ultracentrifugation at 100 000 x *g* for 30 minutes. The pellet consisting of phospholipids vesicles was incubated with goat seminal plasma proteins and re-centrifuged. An equal volume of the pellet and the supernatant were subjected to 15% SDS-PAGE analysis. Additionally, soybean-based extender was incubated with goat seminal plasma proteins and loaded onto Sepharose CL-4B column previously equilibrated with Tris buffer (20 mM Tris, 150 mM NaCl, 0.02% sodium azide, pH 7.4). The proteins were eluted and fractions were analysed by immunoblotting. In parallel, goat seminal plasma proteins alone were subjected to gel filtration on the same column (control) and fractions were analyzed.

Results and Discussion

All goat BSP proteins bound to Andromed® extender lipid constituents, but with different affinities. Goat BSP proteins of 14 and 15 kDa (GSP-14 and GSP-15) showed a preferential binding to phospholipids of Andromed® as compared to goat BSP proteins of 20 and 22 kDa (GSP-20, GSP-22). These results suggest that GSP-14 and GSP-15 may differ in structural organization as compared to GSP-20 and GSP-22. The current findings are in agreement with previous studies (1, 2) and indicate that BSP proteins, in general, interact with phospholipids. In summary, our results indicate that the entire goat BSP proteins (GSP-14, GSP-15, GSP-20 and GSP-22) interact with phospholipids from Andromed® extender and this could play a significant role in protecting goat sperm by sequestrating all BSP proteins in semen. The improvement of sperm preservation requires in-depth knowledge of the interactions among sperm cells, seminal plasma proteins and extender components. Thus, these findings are crucial to understand the mechanisms of sperm protection by extender components and for the development of novel extenders

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Timed insemination efficiency following progesterone-based synchronization protocols associated to GnRH, estradiol and estradiol plus eCG in *Bos indicus* beef heifers

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Introduction

In countries where estrogen is forbidden to synchronize the ovulation time, the use of the 5-day CO-Synch + CIDR protocol is becoming each more popular in order to program the time of artificial insemination (TAI). Nevertheless, to be accepted in countries like Brazil this protocol must have a fertility performance similar to those obtained by estrogen-based protocols. The aim of this study was to evaluate the conception rates of Nelore (*Bos indicus)* heifers treated with the 5-day CO-Synch + CIDR protocol or the conventional 8-day progesterone-based synchronization protocol associated to estradiol with or without eCG.

Material and Methods

Pubertal Nelore heifers (n=90) were treated (Day 0) with a progesterone intravaginal device (Primer®, Tecnopec, São Paulo, Brazil) containing 1 g of progesterone and assigned randomly in three groups. Group 1 (n=30) was injected with GnRH (100μg i.m. Fertagyl®, Intervet) Groups 2 (n=30) and 3 (n=30) with estradiol benzoate (2mg EB, Estrogin, AUSA, Brazil). Primer was removed on Day 5 (Group 1) and Day 8 (Groups 2 and 3) and administered one injection of cloprostenol (125 mcg, Prolise®, Tecnopec, São Paulo, Brazil). At this time, Group 3 was injected im with 300 IU of eCG. Twenty-four hours later, Group 1 received a second injection of cloprostenol while Groups 2 and 3 received 2 mg EB. Heifers were TAI (semen from two sires) 72 (Group 1) or 54 (Groups 2 and 3) hours after Primer removal and GnRH was administered concurrent with TAI in Group 1. Estrus was monitoring after cloprostenol injection and pregnancy status was evaluated via transrectal ultrasonography on day 40 after TAI. Data were analyzed by Chi-square test.

Results and Discussion

The conception rates for the groups 1, 2 and 3 were 28.3, 17.9 and 57.7%, respectively ($P<0.05$). In a recent study, Other authors (1) did not find differences in the conception rate (~35%) of Nelore heifers treated with a 5-day Co-Synch + CIDR protocol or 8-day progesterone-based plus estradiol protocol. Results of the present study showed that eCG added to progesterone-based plus estradiol protocol can improve the fertility of the heifers. Because body condition was relatively homogenous (between 2,5 and 3 in a score of 0 to 5), it can be speculated that ovarian inactivity (not detected by rectal palpation) was present in the most of heifers. Indeed, in cows treated with the 5-day CO-Synch + CIDR protocol, Stevenson et al. (2) showed that cycling cows were 1.5 times (95% confidence intervals = 1.17 to 2.3) more ($P=0.02$) likely to become pregnant after the timed AI than noncycling cows. These results indicate that eCG is required to maximize timed AI pregnancy rates in the estradiol protocol. Further studies are needed to elucidate if eCG can also increase the pregnancy rate of heifers treated with the 5-day CO-Synch + CIDR protocol.

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Phosphoethanolamine effect on the quality of diluted and cooled equine semen

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Introduction

The equine cooled semen is the most widely used method for reproduction biotechnology. In the last years several protocols have been recommended using different cooling rates or the use of molecules which improve spermatozoa viability (1, 2). This study aimed to evaluate the effect of phosphoethanolamine and caffeine in equine cooled semen at 9°C diluted with Botu-semen®.

Materials and Methods

Ten ejaculates of a single throughbred Manga Larga Marchador stallion, 8 years old, were evaluated (n=10), from November up to December 2011, in Uberaba, Minas Gerais, Brasil. Collect was performed through a artificial vagina. The ejaculate was divided into four groups as described: Group 1 (ejaculate without diluting), Group 2 (ejaculate with diluting Botu-semen®), Group 3 (ejaculate with Botu-semen® plus caffeine) and at last Group 4 (ejaculate with Botu-semen® plus phosphoethanolamine). The groups were evaluated as for progressive motility, plasma membrane integrity (3% eosin stain), and membrane functionality by hypoosmotic test (HOST). These tests were evaluated by bright field microscopy at 10x, 40x, and 40x magnification, respectively. Samples in Group 2, 3 and 4 were submitted to a cooling rate for two and a half hours with the final temperature of 9 °C. These samples were evaluated after 8h, 24h, 48h of cooling, according to the aforementioned parameters. Non-parametric analysis (Kruskal-Wallis) was used for the results.

Results and Discussions

In evaluating progressive motility, plasma membrane integrity and membrane function of dilute semen, no statistical difference was verified when groups were evaluated before cooling, thus suggesting that the substances tested did not interfere in the medium osmolarity. In the evaluation of cooled semen for a period of 8 hours, it was observed that only the progressive motility parameter for groups 4 and 2 showed a positive statistical difference ($p < 0.05$) in relation to the group 3 (42.5% and 38% vs 28.5%). For the periods of 24 hours and 48hours, group 2 (30% and 23%) showed statistical difference ($p < 0.05$) when compared to group 3 (23.2% and 18.6%) and group 4 (21.6 % and 16.8%), for membrane integrity parameter. It is concluded that further studies should be performed with phosphoethanolamine, considering that the effect of molar concentration toxicity may have interfered in progressive motility and membrane integrity. This result is enhanced because the progressive motility enables the effect of the biochemical metabolism that at certain doses can interfere with the metabolism of plasma membrane itself (3).

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Evaluation of enthalpy response of germinative cells in the presence of distinct substrates

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Introduction

The search for a high efficiency in fecundation using frozen semen, brings the need of a wide study about many aspects related to the sperm cellular physiology, and the improvement of the tests applied to analyze the viability of the sperms under cryopreservation (1). The utilization of microcalorimetry techniques to assess cellular metabolism is greatly significant, since this it allows the determination of thermodynamics parameters involved in vital phenomena vital for both cell structure and function (2). The aim of this study is to evaluate the thermodynamic response of the cryopreserved bovine sperm metabolism in the presence of distinct carbohydrates.

Materials and Methods

Eight pallets of one ejaculate of a single bull (n=8) were used. The pallets were thawed following the Brazilian College of Animal Reproduction protocol. Before the microcalorimetric assays, sperm motility, viability and membrane integrity (eosin staining 3%) were evaluated. Four different treatments were carried out, where the samples were diluted in 1mL of buffer(PBS- 50 mM) pH 7.4 and submitted to microcalorimetric assays, which has determined the heat production of bovine spermatozoa from endogenous and exogenous (glucose, fructose and lactose 6 mM) source.

Results and Discussion

The supply of exogenous substrates for the sperm cells resulted in an increase in the values of enthalpy when compared with the endogenous ones, which represent just 10% of the value generated from the metabolism of carbohydrates (3) (Fig,1). Both processes give energy as final product, but in different amount. Among the evaluated carbohydrates, glucose (17463.35 μJ) showed the biggest heat production that could be associated with the functional ability of the structures responsible for the energy production, which induces a motility increase (Table 1), since the ATP is a molecule that establishes a connection between the sperm activity and the process of glycolysis. Thus, the use of microcalorimetry offered additional information for bovine sperm metabolism evaluation and was efficient in detecting important information from sperm cell metabolism.

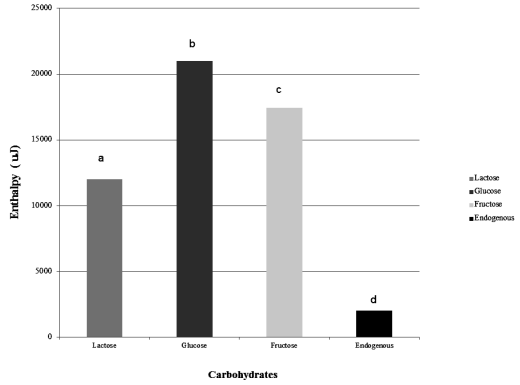

Figure 1. Enthalpy value involved in spermatozoa metabolism obtained by the conduction microcalimeter after thawed.

Table 1. Percentage of motility, viability, membrane integrity, and value of enthalpy, after thawing.

Substrate	Motility (%)	Viability (%)	Membrane Integrity (%)	Enthalpy (μJ)
Lactose	35	3	45	12031,375
Glucose	45	3	58	17563,35
Frutose	40	2	61	14985,46
Endogenous	40	3	53	983,54

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Study of correlation between sperm DNA integrity (SCSA) with parameters of *in vitro* fertilization

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Introduction

Studies for the evaluation of bovine semen have been carried out so as to establish the correlation of each laboratory test or its association with the rates *in vitro*, seeking this way to predict the fertility potential aggregated to semen samples (1.2). The aim of this study was to search for a correlation between the level of bovine sperm DNA integrity with the percentage of cleavage, and D8 cells.

Materials and Methods

Semen samples of 11 Nellore bulls were used. The semen collection was performed by the artificial vagina technique, followed by evaluation. Ejaculates containing a minimum of 60% of spermatozoa with progressive motility were used in this study. The semen was frozen according to Alta Genetics Reproduction Central protocol. Samples were thawed then evaluated for DNA fragmentation sperm chromatin structure assay (SCSA) (3), and *in vitro* fertilization rate (*Biovitro*). Comparison analysis test Pearson was performed between DNA fragmentation rate with cleavage rate and D8 cells percentage.

Results and Discussion

The results show no significant relationships between any pair of variables in the correlation table ($P > 0.050$) (%DNA integrity *vs* %Cleavage; %DNA integrity *vs* %D8; and %D8 *vs* %cleavage), as shown in Table 1. The data prove that there is no a specific correlation between sperm DNA damage and the in *vitro* fertilization percentage, as proposed by (2), suggesting that there are still other variables in sperm evaluating that can determine the best *in vitro* fertilization rate.

Table 1. Comparison between DNA fragmentation rate with cleavage rate and D8.

	Correlation coefficient
Parameter	r
DNA *vs* D8	- 0.307
DNA *vs* Cleavage	0.360
Cleavage *vs* D8	0.012

*Pearson Correlation ($P > 0.050$).

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Sperm-binding assay in the Piau swine breed using perivitelline membranes from hen's egg

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Introduction

The fertilizing capability of spermatozoa in pigs can be evaluated by binding to the oocyte. The in vitro penetration test in oocytes which simulates the processes involved in the fertilization mechanism (1), may also be based on the sperm-binding capability to hen's egg perivitelline membrane (PVM), simulating the interaction of gametes, which is a more efficient test to check the ability of sperm to undergo the acrosome reaction (2). According Barbato et al. (3), similarities between the zona pellucida glycoproteins and hen's egg PVM allows the binding of sperm from other species to this membrane. The objective of this study was to verify the use of sperm-binding assay in Piau swine breeds to the hen's egg PVM as an alternative to predict semen post-thawing fertilizing capability.

Material and Methods

Twenty five frozen semen samples from five boars were used. To check post-thawing sperm viability were used sperm motility, sperm vigor, and the sperm binding test to hen's egg PVM. It were used 20 µL of sperm with the concentration of 10 x 10^6 spermatozoa/mL and incubated with the PVM using Beltsville thawing solution (BTS) and Bull TALP media (B-TALP), beyond Hoechst (33342) fluorescent probe to visualize bound sperm to the PVM.

Results and Discussion

Post-thawing sperm motility and sperm vigor were 39.4±1.2 and 3.0±0.5, with no difference between animals and samples ($p>0.05$). The average number of sperm-binding/mm^2 was 339.3±42.9 and 275.4±42.9, and percentage of bound sperm of 0.34±0.4 and 0.27±0.4, respectively for BTS and B-TALP media, with no difference between themselves ($p>0.05$). There was also no difference in the sperm-egg binding assay between animals and post-thawed samples ($p>0.05$). The variables studied showed no significant correlation, while Barbato et al. (3) reported correlations of the binding assay with some semen characteristics such as, sperm concentration (0.54) and fertility rate (0.83). These same authors also claim that the results range from relatively high fertility in cases of animals with high binding potential may occur probably because the maximum potential fertilization requires many attributes as well as motility and binding. However, sperm populations with a low potential binding, probably have less bound sperm.

Conclusions

The results obtained indicate that there was no variability between the animals frozen semen and that they have the same fertilizing capability. In addition, the characteristics of analyzed animals showed the same pattern to others pure breed and commercial lines of swine.

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Cumulus expansion and nuclear maturation of sheep oocytes cultured *in vitro* with meiosis inhibitors

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Introduction

Despite of recent scientific advances, the efficiency of *in vitro* embryo production is still low (1). In this context, the *in vitro* pre-maturation of *cumulus*-oocyte complexes (COCs) with physiological or pharmacological inhibitors of meiosis has been studied in several animal species in order to increase the development potential of oocytes and embryos (2). However, there is a lack of information about the effects of meiosis inhibitors in the ovine. Thus, the aim of this studied was to evaluate the effect of roscovitine (a specific inhibitor of cyclin-dependent kinases) and cycloheximide (an inhibitor of protein synthesis) on the *cumulus* expansion and nuclear maturation in sheep COCs.

Materials and Methods

COCs grade 1 and 2 from slaughterhouse ovaries were cultured in maturation medium consisting of TCM199, fetal bovine serum, cysteamine, pyruvate, penicillin, LH and FSH (control group) containing 100μM roscotivine or 1ug/mL cycloheximide (treatment group) for 24 h at 38.5ºC in a 5% CO_2 saturated humidity air atmosphere. The concentrations of meiosis inhibitors were based on previous studies and literature information (3,4). At the end of 24h, the degree of *cumulus* expansion was evaluated under a stereomicroscope. After this, COCs were denuded, stained with Hoechst 33342 and the chromosomal configuration was evaluated under a fluorescence microscope. The experimental design was completely randomized with three experimental groups and 6 replicates with 150 oocytes for each group. A total of 450 oocytes were evaluated. The results were submitted to analysis of variance and means were compared by the Scheffé test at 5% probability.

Results and Discussion

As expected, high proportion of COCs in control group had full *cumulus* expansion (72.60%) and 79,9%, reached the metaphase II stage (MII) whereas only 8.1% were at metaphase I (MI), 6% at germinal vesicle breakdown (GVBD) an 6% degenerated. This demonstrates the conditions of *in vitro* culture were adequate and allowed *cumulus* expansion and meiosis progression. In contrast, there was no *cumulus* expansion in 85.9% of COCs treated with roscovitine and 55.6% of those treated with cycloheximide had partial expansion. These data indicate that both meiosis inhibitors interfered in the *cumulus* expansion, but the action of cycloheximide was less intense. In the treatment with roscovitine was also observed that 15.9% e 37.1% of COCs were at GVBD and MI, respectively, whereas 23.5% reached the MII stage and the same proportion degenerate. In contrast, 5.6% of COCs treated with cycloheximide remained at GVBD while 84.1% were at MI and 10.3% degenerate. These results indicate that the studied concentration of both inhibitors promoted meiosis delay but were not adequate to keep the oocytes in the germinal vesicle stage. Further studies have been developed in order to assess different concentrations, the reversibility of action of theses meiosis inhibitors and the subsequent *in vitro* development potential of oocyte and embryo in sheep.

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Nutritional supplementation of Nelore heifers with polyunsaturated fatty acids do not interfere in the development and cryotolerance of *in vitro* produced embryos

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Introduction

The supply of polyunsaturated fatty acids (PUFAs) in cattle diet resulted in improved development potential of oocytes to the blastocyst stage, as well as the quality of embryos in high-yielding dairy cows (1). The positive effects of feeding supplementation of cows with fat on reproduction may be mediated by changes in composition of follicular fluid, which is the microenvironment that surrounds the oocytes during its development and maturation (2, 3). This study was conducted to assess the effects of dietary supplementation of Nelore heifers, with a source of rumen protected fat enriched with Omega-6 (n-6) and 3 (n-3) PUFAs, on the development and cryotolerance of *in vitro* produced embryos.

Material and Methods

Sixteen heifers were randomly divided in two groups: CONTR (n=8, maintenance diet) and FAT (n=8, maintenance diet containing 100 g/animal/day Megalac-E® in its composition). The groups were evaluated in a "cross-over" experiment and were used isoenergetic and isoproteic diets. After 60 days of feeding, ovum pick-up sessions were performed (total of 6 sessions), and the follicular population was also evalueted. Cumulus-oocyte complexes obtained from these animals (n=1255) were matured during 24h in B199 medium (Tissue Culture Medium-199 supplemented with bicarbonate, hormones and 10% fetal calf serum - FCS). After fertilization, zygotes were *in vitro* cultured (IVC) in Synthetic Oviduct Fluid (SOF) medium (5% bovine serum albumin + 2.5% FCS) in 5% CO_2 atmosphere. Cleavage was evaluated at 48 hours post insemination (hpi), and blastocysts at 168 and 192 hpi, when they were vitrified by Vitri-Ingá® protocol (Ingámed ®, Maringá-PR, Brazil). After, they were heated (n=77) and embryo survival (re-expansion rates) were evaluated after 3 and 24h of post-devitrification IVC. Means (least squares±SEM) were analyzed by GLIMMIX procedure of SAS, and the embryo re-expansion rates were analyzed by Chi-square test.

Results and Discussion

There was no significant difference (P>0.05) on the number of aspirated follicles (17.9±1.0 *vs* 15.8±1.0), total oocytes retrieved (14.4±1.4 *vs* 14.5±1.3), and number of viable oocytes (12.1±1.2 *vs* 12.5±1.1), respectively for groups CONTR and FAT. The recovery rate (total number of recovered oocytes/total number of aspirated follicles: 78.3%±4.7 *vs* 86.8%±4.5), and the oocytes utilization (total number of viable oocytes/total number of recovered oocytes: 82.8%±2.1 *vs* 85.4%±2.0) did not differ (P>0.05) between CONTR and FAT groups, respectively. Similarly, the cleavage (68.7%±3.2 *vs* 61.3%±3.0), embryonic development (47.0%±3.7 *vs* 39.4%±3.6), and embryo re-expansion rates did not differ (P>0.05) between FAT and CONTR treatments (3h: 77.5 *vs* 78.4%; 24h: 77.5 *vs* 81.1%). In conclusion, dietary supplementation of Nelore heifers with a source of rumen protected fat, rich in n-6 and n-3 PUFAs, did not affected the recovery and oocyte quality, *in vitro* development and embryo survival after cryopreservation.

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Techniques for evaluation of physical integrity of bovine sperm membrane

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Introduction

The aim of this work was to evaluate the integrity of plasma membrane using three distinct evaluation techniques: (a) optical microscopy with eosin stain (3% w/v) (1); (b) flow cytometry (2); and (c) epifluorescence microscopy (3). The plasma membrane evaluation is greatly significant for determining both the viability and quality of sperm cell (4).

Materials and Methods

Two ejaculates of 10 Nellore cattle (n=20) were used. Microscopic evaluation of semen *in natura* (progressive motility, vigor) was performed. All the parameters of frozen samples previously described were evaluated; and for the evaluation of plasma membrane physical integrity, the optical microscopy with eosin stain (3% w/v) was carried out; epifluorescence microscopy with dye carboxyfluorescein diacetate (CFDA) and flow cytometry (FACS Can, Becton Dickinson®) with the stain propidium iodide (PI). The data collected by flow cytometry were analyzed by using Flow Jo software (Becton Dickinson). The averages of all values were compared following nonparametric statistical analysis (Kruskal-Wallis).

Results and Discussion

After evaluation of both raw and thawed semen, progressive motility (62.5% vs 40.5%, $p<0.05$), and vigor (4.7 vs 3.6, $p<0.05$) were considered as normal, in accordance with the Manual of the Brazilian College of Animal Reproduction (4), which recommends after freezing a progressive motility $\geq 30\%$ and vigor ≥ 3. In the evaluation of membrane integrity the results suggest significant points regarding the use of the techniques. In the comparative evaluation between the averages of optical microscopy (eosin) and flow cytometry techniques, the results showed no statistical difference (49.4% vs 42.7%, $p<0.05$). Nevertheless, in comparing both the techniques to that of epifluorescence microscopy, it was observed that the results showed statistical differences (49.4% e 42.7% vs 62.9%; $p<0.05$). This fact can be caused by non-specificity of the chromophore CFDA and also by the appearance of the variables involved in the technique, such as incubation temperature and reaction time that can interfere directly with the test quality (5).

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Fertility of frozen-thawed ram sperm: effect of catalase and pre-freezing equilibration during semen cryopreservation

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Introduction

In the last few years, our laboratory has done studies to evaluate the effects of antioxidants on semen cryopreservation. Recently, it was shown that catalase and pre-freezing equilibration may be beneficial to frozen-thawed ram sperm (1). Thus, the aim of this study was to evaluate the effects of catalase and pre-freezing equilibration during semen cryopreservation on the *in vivo* fertility of thawed ram sperm.

Material and Methods

Pooled semen from four rams was split into two equal aliquots and diluted with Tris-egg yolk base extender, with 6% glycerol, without antioxidant (control-C) or supplemented with catalase (20 U/mL-CAT). Extended semen from C and CAT treatments were packed into 0.25 mL straws (100×10^6 sperm/mL) and maintained in a water bath (5 °C) for 90 min. Following, half of the doses of both treatments were placed in a programmable freezer (TK-3000, Uberaba, MG, Brazil), previously stabilized at 5 °C. Semen was cooled from to -120 °C at a rate of 12.5 °C/min. Once straws reached -120 °C, they were plunged into liquid. The procedure was repeated with remaining samples from C and CAT treatments after 12 h of equilibration at 5 °C (C12 and CAT12). Immediately and 1 h after thawing, membrane and acrosomal integrity were detected using fluorescence probes, and kinematics of sperm were detected by CASA. Treatments were executed in triplicate. In a second time, estrus was synchronized in 85 ewes, and fixed time laparoscopic insemination (LAI) with frozen-thawed semen from C and CAT12 treatments was performed. Pregnancy rates were ultrasonographically assessed 45 d after LAI. Differences of sperm parameters between treatments were assessed using ANOVA, followed by Tukey's test. Pregnancy rates were analyzed by χ^2 test.

Results and Discussion

Immediately after thawing, there were no differences ($P > 0.05$) among treatments for *in vitro* parameters. In contrast, 1 h after thawing total motility (TM) and plasma membrane integrity (iPM) was better preserved ($P < 0.05$) in CAT12 in comparison to the C treatment (Table 1). Thus, semen samples from C and CAT12 were used to LAI in 41 and 44 ewes, respectively, and there was no difference ($P > 0.05$) in the pregnancy rate between C (31.7%) and CAT12 (34.1%). *In vitro* parameters do not necessarily reflect *in vivo* fertility (2), since the higher stability of TM and iAC of ram sperm cryopreserved after 12 h pre-freezing equilibration in a Tris-egg yolk base extender supplemented with catalase did not improve pregnancy rate after LAI.

Table 1. Mean ± SE percentages of total motility (TM), intact plasma membranes (iPM), and intact acrosome (iAC) of frozen-thawed ram sperm following dilution in Tris-egg yolk extender with no antioxidant (C) or supplemented with 20 U/mL of catalase (CAT), and cryopreserved after thermal equilibrium was reached at 5 °C; or 12 h after equilibration (C12 and CAT12, respectively). Evaluations done 0 h and 1 h after thawing.

Treatment	*In vitro* parameters					
	TM (%)		iPM (%)		IAC (%)	
	Post-thaw 0h	Post-thaw 1h	Post-thaw 0h	Post-thaw 1h	Post-thaw 0h	Post-thaw 1h
C	$41,7 \pm 4.4^{a}$	25.0 ± 5.8^{b}	49.7 ± 8.1^{a}	25.7 ± 4.1^{a}	62.7 ± 1.4^{a}	60.0 ± 2.0^{a}
CAT	45.0 ± 5.8^{a}	38.3 ± 4.4^{ab}	48.0 ± 7.6^{a}	29.3 ± 2.3^{ab}	61.0 ± 2.0^{a}	58.3 ± 2.0^{a}
C12	48.3 ± 1.7^{a}	38.3 ± 1.7^{ab}	61.7 ± 7.8^{a}	33.0 ± 3.1^{ab}	68.3 ± 2.3^{a}	67.0 ± 0.6^{a}
CAT12	55.0 ± 2.9^{a}	46.7 ± 3.3^{a}	54.7 ± 8.0^{a}	38.7 ± 0.9^{a}	66.7 ± 2.4^{a}	63.0 ± 2.6^{a}

[a-b]Within a column, means without a common superscript differed ($P < 0.05$).

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Molecular detection of Bovine Herpesvirus 1 in ovarian structures of naturally infected animals

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Introduction

Brazil has actually considerable prominence as producer and exporter of *in vitro* producer embryos (1). However, although more secure compared to the transport of live animals, trade of bovine embryos must meet sanitary requirements of importing countries. This is because the bovine embryos may be sources of contamination of infectious diseases such as Rinotraqueite infectious bovine (IBR) caused by Bovine Herpesvirus 1 (BoHV-1) (2). This virus causes negative effects on the development of embryos and the main sources of contamination of *in vitro* produced embryos are the oocytes, spermatozoa, fetal bovine serum and cell culture (3). The aim of this study was to evaluate the presence of BoHV-1DNA in ovarian tissue, oocyte, follicular fluid and blood of slaughter-derived naturally infected cows by polymerase chain reaction (PCR). Also was evaluated the serological profile of this animals.

Materials and Methods

Serum samples, blood, ovarian tissue, follicular fluid and cumulus-oocyte complex were collected from 147 never-vaccinated animals against BoHV-1. Toxic or insufficient samples were discarded. Serological tests allowed the detection of seropositive animals and was performed according of Manual of Standards for Diagnostic Tests and Vacines" (4). The PCR and sequence of primers was performed as described by (5), using a total volume of 25 µl.

Results and Discussion

The PCR reaction detected the presence of viral DNA in 0.9% (1/115) of oocytes, at 4.3% (5/117) of ovarian tissue, 2.8% (3/108) of blood and any of follicular fluid sample. Sorological samples had 83.6% (117/140) of serum-positive for BoHV-1. All samples PCR + was from serum-positive animals. PCR test has proved to be effective to detection of BoHV-1in samples of oocytes (6), follicular fluid (7,8), blood (9) and bovine ovarian tissue (10). However, these experiments are performed with animals experimentally infected or infected *in vitro*, different from method of infection of animals evaluated in this work (naturally infected). Thus, animals showed high prevalence of antibodies against BoHV-1 and molecular tests detected the presence of viral DNA in genital samples from cows, suggesting that these structures could be infectious sites of this virus.

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Effect of the Growth Factor Iinsulin-like-I (IGF-I) on the *in vitro* culture of caprine preantral follicles

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Introduction

The development of biotechnology of reproduction has been made in recent years aimed to a better use of genetic material of animals of high value zootechinical and endangered species. Thus, the manipulation of preantral follicle-oocytes enclosed (MOEPF) has contributed to provide the large number of oocytes of same animal and reducing the interval between generations. The preparation of efficient cultive media to the *in vitro* development of preantral follicles is based on studies of substances involved in the control of foliculogenese as is the case of insulin-like growth factor-I (IGF-I). Considering the above, this study aimed to evaluated the effect of IGF-I on the *in vitro* cultivation of preantral follicles goats.

Materials and Methods

We collect in the sector of Caprinocultura of the Federal University of Viçosa 16 ovaries of goats (n= 8) Pardo-Alpine, which were transported to the laboratory at 4 °C, within 1 hour. After this period, the ovarian córtex was fragmented (n=7) which is one of the fragments immediately fixed for classical histology (fresh control) and the other cultured in α-MEM supplemented with Insulin-Transferrin-Selenium (ITS), glutamine, hypoxanthine and bovine serum albumin (BSA), designated α-MEM+. For the experimental conditions tested, the medium (α-MEM+) was supplemented with IGF-I concentrations of 50ng/mL (I50), 100ng/mL (I100) by 1 (D1) and 7 days (D7). The culture medium was completely replaced every 2 days. After the cultivation, the fragments were fixed for histological analysis and stained with periodic acid Schiff´s (PAS) + hematoxylin. Data Analysis Using Chi-Square Test ($P<0.01$).

Results and Discussion

As for the follicular survival, on D1 of cultivation the treatments α-MEM+, I50 and I100 ng/mL showed a lower percentage of follicular survival ($P<0.01$) when compared to fresh control (Fig. 1). However, on D7 cultivation there was a reduction in the rate of follicular survival in all treatments. This fact indicates that the cultivation time, from D1 to D7 interfered negatively in survival *in vitro* of primordial follicles, increasing numbers of atresic follicles. The treatment I100 on D1 of cultivation had the best result (86.7 %) when compared to control fresh (96.7 %) and the other treatments. The result found on D1 I100 was similar to that found by (1), which after 1 day of cultivation, found the highest percentage of normal follicles (90.1 %) in the treatment of 100 ng/mL IGF-I. However, in the present study the α-MEM+ and 100 ng/mL IGF-I reduced the follicular viability after seven days of cultivation, compared with the follicular viability in the concentration of 50 ng/mL IGF-I on D7. Thus, the concentration of 100 ng/mL IGF-I keeps the follicular survival in vitro only on first day of cultivation, inducing atresia of this treatment until D7. The concentration of 50 ng/mL IGF-I was able to maintain the follicular survival after 7 days of culture.

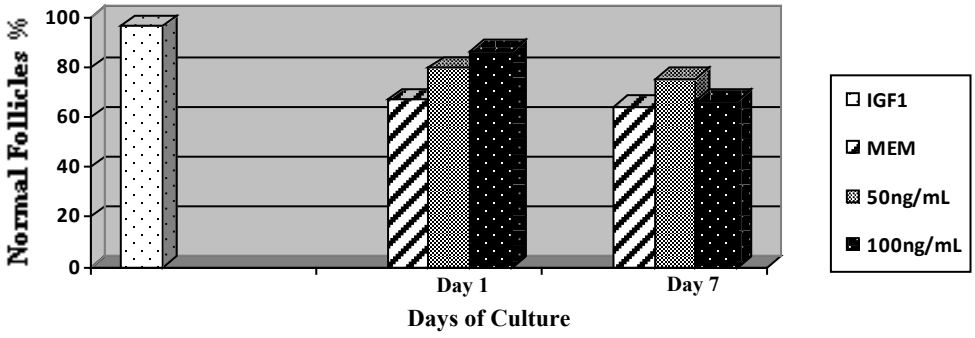

Figure 1. Percentage of normal pre-antral follicles on tissue non-cultured or cultured by 1 and 7 days in α-MEM$^+$ supplemented or not with differents concentrations of IGF-1.

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Testicular transcutaneous ultrasound in Japanese quail: a new approach for selection of breeders

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Introduction

Genetically superior males with higher reproductive potential can produce more offspring. Nowadays, there is no reliable method for comparison and selection of Japanese quail, probably because of the intra-abdominal location of the gonads. Ultrasound examination is considered quick, safe and non-invasive, and may be used to indirectly estimate sperm production, but has been considered impractical in birds. The main obstacle to the realization of testicular transcutaneous ultrasonography in birds is the air sacs, which limit access to the coelomic cavity to small acoustic windows (1). Ultrasound examination has been restricted to clinical investigations in "pet" birds (2). Therefore, the purpose with the present study was to evaluate the viability of different approaches with ultrasound as a tool for measurement and evaluation of the testes.

Materials and Methods

After plucking feathers, coupling gel was applied to the skin and transcutaneous ultrasound examination was performed in ten male Japanese quails at 60 days old. Linear and cardiac pediatric sectorial transducers, at frequencies of 7.5 and 10MHz were evaluated by both the flank and the parasternal acoustic window. Qualitative analysis and measurement of testes were performed in B-mode. Soon after, birds were slaughtered by cervical displacement and testes were removed, weighted and measured with an electronic digital caliper. The length and width obtained by both methods were compared with Student's t test and correlated using Pearson's correlation at 1% level of significance. The change in cloacal temperature was also recorded.

Results and Discussion

The testes were identified as oval structures with low to medium echogenicity, surrounded by a thin hyperechoic rim. The testicular length was significantly lower at the ultrasound exam for both testes (Table 1), presumably by the interference of the air sacs in the conduction of the sound waves at the cranial pole. The width was similar between the techniques, with linear correlation coefficient (r) of 0.72 for the left testicle and 0.75 for the right one, suggesting that it can be used to compare the reproductive potential between males of Japanese quail, since the width is highly correlated with testis weight. The best approach was flank ultrasonography, with linear transducer and frequency of 10MHz, which allowed rapid assessment of the gonads in all birds. Cloacal temperature dropped from 40.6 to 38.7ºC, suggesting the need to preheat the coupling gel to prevent hypothermia. We conclude that testicular transcutaneous ultrasonography in Japanese quail, although limited, can contribute to the evaluation and selection of breeders.

Table 1. Testicular lenght and width obtained by ultrasound and caliper for both testes in Japanese quails (mean values ± standard deviation).

	Left testis		Right testis	
	Lenght (mm)	Width (mm)	Lenght (mm)	Width (mm)
Ultrasound	12.60 ± 1.93 a	12.79 ± 1.47	14.15 ± 1.56 a	11.39 ± 1.41
Caliper	20.88 ± 1.62 b	14.52 ± 1.64	23.77 ± 1.69 b	12.48 ± 1.39

Different letters in the same column indicate statistical difference in Student's t test ($P < 0.01$).

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Association of cryoprotectants and ascorbic acid to cool and vitrify preantral bovine follicles

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Introduction

The mammalian ovaries contain thousands of oocytes, most being enclosed in preantral follicles (PF), and only a small proportion will ovulate. Therefore, the biotech manipulation of oocytes enclosed in preantral follicles (MOEPF) is necessary to rescue PF of the ovarian environment (1), their cryopreservation (2) and/or *in vitro* culture until the maturation (3), preventing the follicular death which normally occurs *in vivo*. Cell damage may occur during ovarian cryopreservation procedure, mostly toxic and osmotic damages (4) caused by reactive oxygen species (ROS). Ascorbic acid (AA) is a hydrosoluble vitamin that converts ROS in inactive forms (5), it is found in ovary, granulose cells, internal teca cells and oocytes (6).

Material and Methods

Ovaries were collected (n=10) from five crossbred heifers at 14 months age. In laboratory 22 ovarian fragments were taken from the cortical region and distributed in: two fragments to fresh control (0 hour) submitted to histological analysis and 20 fragments cooled at 4 ºC for the periods of 4 and 24 hours in TCM-199+HEPES medium. From the 20 fragments cooled, four were fixed as control 4 and 24 hours (two piece each) and the remaining 16 fragments were distributed in four vitrification treatments for each cooling time, respectively: Treatments V4a and V24a: TCM-199 + dimethyl sulfoxide (DMSO) 1.5M + Ethylene glycol (EG) 1.5M; Treatments V4b and V24b: TCM-199 + DMSO 1.5M + EG 1.5M + sucrose (SUC) 0.5M; Treatments V4c and V24c: TCM-199 + DMSO 1.5M + EG 1.5M + Ascorbic Acid (AA) 0.1 mM/L and treatments V4d and V24d: TCM-199 + DMSO 1.5M + EG 1.5M + SUC 0.5M + AA 0.1mM/L. Fragments were heated in solution of decreasing SUC and fixed for histology. Qualitative variables for morphology were analyzed by Kruskal-Wallis test.

Results and Discussion

There was no difference in percentage of morphologically normal follicles between fresh control group and control group cooled for 4 h (99.3% and 96.0% respectively; $P>0.05$), nevertheless, there was reduction of PF morphological integrity of control cooled for 24 h (86%; $P<0.05$). After vitrification, there was reduction ($P<0.05$) of normal morphology in all treatments when being compared with cooled control groups. On the other hand, treatments V4c and V24c showed higher capacity of morphological preservation of PF than other treatments ($P<0.05$). *In vitro* culture of bovine PF shows that AA increases morphological maintenance (7). High survival capacity was verified in mouse embryos cryopreserved with AA (8). Results of this study indicate that AA can reduce toxic and osmotic damages caused by cryopreservation procedure.

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Effect of electrolyte profile on oocyte quality and nuclear maturation from heifers and lactating cows

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Introduction

The metabolic changes during negative energy balance (NEB) lead to a decline in dairy cows fertility (1). Such alterations reflect on biochemical follicular fluid composition (2). The aim of this study was evaluate the differences between lactating cows and heifers in blood and follicular fluid electrolytes levels (Na, K and Ca) in association with oocyte quality and rate of nuclear maturation.

Material and Methods

Girolando heifers (n=17) and cows (n=162), on third lactation order, were submitted to transvaginal follicular aspiration and their oocytes were classified according to the quality (3) and forwarded to in vitro maturation for 24 hours. Next, they were evaluated for the presence of the first polar body which indicates nuclear maturation degree. Samples of blood, largest follicular fluid (LF; > 8 mm) and smaller follicular fluid (SF; 2 - 8 mm) were collected and Na, K and Ca concentrations (mmol/L) determined by the system AVL-9180 Electrolyte Analizer®. The data were expressed as percentage and mean ± S.E.M. Electrolytes average levels on serum, LF and SF between cows and heifers, were submitted to Kruskal-Wallis test. The Pearson correlation coefficient analyzed the relationship between electrolytes levels, amount and quality of oocytes from cows and heifers.

Results and Discussion

The analyzes showed no difference in serum Na, K and Ca between cows (137.86±1.9, 4.62±0.1 and 0.73±0.1) and heifers (136.9±6.6, 4.97±0.3 and 0.78±0.3). In both categories, the SF follicular fluid presented higher rates of Na and Ca ($p<0.05$). The concentration of Na and K were lower ($p<0.05$) for cows compared to heifers in LF follicular fluid (220.8±10.9 *vs* 309.1±21.4 and 4.5±0.1 *vs* 6.0±0.3) and SF (382.6±17.1 *vs* 483.8±29.1 and 6.5±0.4 *vs* 8.52±1.1). Heifers had higher ($p<0.01$) proportion of good quality oocytes (61.2%) and better in vitro maturation rate (84.1%) (Fig.1). It was observed a positive correlation between K amount in SF follicular fluid and total number of oocytes ($r = 0.2712$; $p=0.05$) in heifers. In general, there is a decrease in Na, K and Ca levels on smallest to largest follicles, although, did not observe a link between these ions and oocytes quality. The heifers K follicular fluid concentrations kept above standard serum which may be an indicator of dairy bovine fertility.

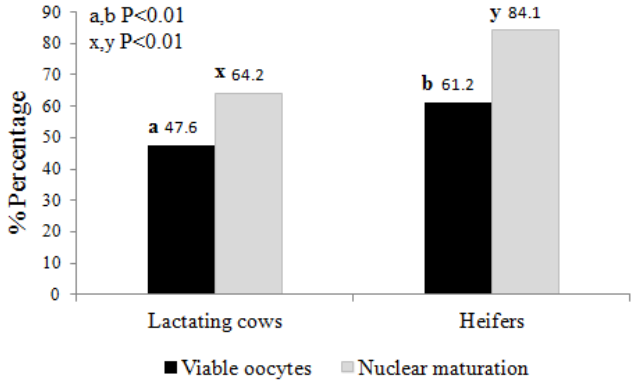

Figure 1. Oocyte quality and nuclear maturation of heifers and lactating cows.

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Vitrification of collared peccaries (*Tayassu tajacu*) ovarian tissue using various cryoprotectants: preliminary results

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Introduction

In Latin America, collared peccaries (*Tayassu tajacu*) are among the most hunted species owing to the local as well as international appreciation of their pelt and meat (1). The use of reproductive biotechnologies, especially those related to gametes preservation, would allow the maintenance and the exchange of genetic source from the animals (2). However, the preservation of female gametes from this species was described only for short time by chilling (3) and there is no information about cryopreservation of ovarian tissue in collared peccaries. Thus, the aim of this study was to evaluate the effect of different cryoprotectants on *Tayassu tajacu* preantral follicles after ovarian tissue vitrification.

Material and Methods

Three pairs of ovaries derived from adult females that belonged to the Center of Multiplication of Wild Animals – UFERSA – were used in the experiment. Each ovarian pair was cut into 7 fragments (~ 3 x 3 x 1 mm) from which one was immediately fixed in Carnoy's solution for 5 h for histological analysis (control group). The other fragments were randomly allocated for each treatment that consisted of 3 or 6 M of Dimethylsulfoxide (DMSO), Ethylene Glycol (EG) or Dimethylformamide (DMF), and then were submitted to solid surface vitrification procedure (4). After 1 week samples were rewarmed (4) and submitted to histological analysis as described for control group. Morphologically normal follicles contained an intact oocyte and granulosa cells, whereas degenerated follicles were an oocyte with a pyknotic nucleus, ooplasma shrinkage and/or granulosa cell layers that were disorganized and detached from the basement membrane (5). The percentages of morphologically normal follicles were compared among treatments. Data were arcsine transformed and analyzed by ANOVA followed by Scheffe test ($P < 0.05$).

Results and Discussion

A total of 1050 preantral follicles were evaluated. As it is shown at table 1, there were no differences between fresh control and vitrified preantral follicles, thus no differences were obtained among treatments after vitrification procedure in relation to morphological integrity of follicles ($P > 0.05$). The results were divergent of those obtained for domestic swine (6) in which vitrification procedure drastically decreased the number of follicles. Although the method of vitrification used was the same (Solid Surface), authors used a combination of EG and DMSO as cryoprotectants, while in the present work the cryoprotectants were individually used. The cryopreservation of ovarian tissues has potential for preserving female germ cells of animals and the vitrification protocol has greatly simplified cryopreservation procedures, representing a tool for conservation of wild animals such as *Tayassu tayassu.*

Table 1. Percentage of morphologically normal preantral follicles of *Tayassu tayassu*, in fresh ovarian tissue (control) and after vitrification procedure, using different cryoprotectants.

		Cryoprotectants					
	Control	DMSO		EG		DMF	
Morphological integrity (%)	93.3 ± 2.7	3 M	6 M	3 M	6 M	3 M	6 M
		76.0 ± 2.5	72.6 ± 3.5	80.3 ± 9.1	75.3 ± 3.4	72.7 ± 3.3	75.00 ± 2.5

No differences were observed among treatments. $P > 0.05$.

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Individual ejaculates and *pooled* semen in dog: are there differences under experimental conditions?

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Introduction

Seeking alternatives to increase chilled dog semen longevity Verstegen et al. (2005) observed that extender renewal after long-time storage at 5°C apparently increased sperm motility. In their work, as well as in others concerning chilled dog semen, the authors used as experimental unites pooled dog semen, in order to increase volume, reduce the effect of individual variations and improve statistical analyses. The aim with this paper is to compare the results obtained when we submit individual ejaculates and pooled semen to the same extender renewal treatment.

Material and Methods

Semen was collected from six dogs and processed as individuals samples (n=6) or as *pool*s (n=6). After collection the semen was diluted in a 1:1 ratio in Tris-Yolk extender, centrifuged at 500g/10min and ressuspended to final concentration of 50 x 10^6 sptz/mL. Semen was cooled at rates of 0.26 °C /min between 37 and 16 °C, and 0.08/min from 16 to 8 °C, and then kept in a refrigerator at 5 °C for 14 days. In Treatement 1 the extender was renewed every six days and in Treatment 2 after 12 days. Cooled semen was evaluated every 48 hours, and before and after extender renewal for sperm motility, using the Sperm Class Analyser® (SCA). Data were analyzed using ANOVA and means values were compared using Tukey-Kramer test.

Results and Discussion

As seen in Tab.1 individual ejaculates and pooled semen react similarly to extender renewal: there was a decrease in the non-renewed treatment (2), associated with sperm centrifugation and a conservation of sperm motility on the renewed treatment (1). Following up sperm motility over the 14 days we observed that after the first extender renewal sperm motility was higher on Treatment 1, both in individuals ejaculates and in the pooled semen. Though individual ejaculates and pooled semen react similarly to extender renewal, as seen in Tab. 1, the other results for individual ejaculates showed statistical differences that were missing in the pooled semen.

Table 1. Chilled dog semen motility at days 6 and 12, *before and **after extender renewal, in individual ejaculates (I) and in pooled semen (P) in treatments 1 and 2. The results are presented as average and standard deviation. Averages followed by letters in the same line indicates statistical differences ($P < 0.05$).

	Progressive motility				**Total motility**			
Days	I-1	I-2	P-1	P-2	I-1	I-2	P-1	P-2
*6**	34,87 ± 14,72	34,87 ± 14,72	64,97 ± 7,29	64,97 ± 7,29	57,04 ± 15,07	57,04 ± 15,07	74,89 ± 7,47	74,89 ± 7,47
*6***	$33,59^{A}$ ± 10,11	$21,11^{B}$ ± 10,93	57,53 ± 14,91	52,31 ± 15,13	$55,40^{A}$ ± 14,18	$36,25^{B}$ ± 10,18	70,66 ± 13,55	62,26 ± 13,86
*12**	4,11 ± 7,91	2,24 ± 4,56	9,01 ± 10,64	5,02 ± 5,60	9,53 ± 13,29	4,08 ± 6,95	15,41 ± 14,50	12,95 ± 13,59
*12***	3,34 ± 4,52	4,66 ± 9,12	6,67 ± 4,46	7,83 ± 5,22	9,84 ± 8,02	8,59 ± 14,76	13,83 ± 9,24	17,02 ± 12,42

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Effect of stabilization time on cryopreservation of spermatozoa obtained from bull epididymis

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Introduction

Epididymal sperm cryopreservation is an alternative to genetic material preservation from animals with high livestock value killed early. It is known that the stabilization period in bovine specie has been studied in order to reduce the time spent in this cryopreservation stage (1). Thus, the aim of this work was to evaluate the effect of different stabilization times on cryopreservation of spermatozoa obtained from bull epididymis.

Material and Methods

Sixteen pairs of testes and epididymis, obtained from Nelore bulls two hours after slaughter, were transported to the Andrology Laboratory at room temperature (28 ºC). Spermatozoa were recovered by flotation technique (2), after epididymis slicing, and immediately placed for immersion in 5.0 mL of Tris-egg yolk (3.605g Tris; 2.024g citric acid; 1.488g fructose; 100mL distilled water; 20% yolk egg; 7% glycerol; pH 7). Doses were standardized at a 100 x 10^6 spermatozoa/mL concentration, packaged in straws (0.25 mL) and frozen in automated system (TK-3000®, TK Tecnologia em congelação LTD, Uberaba, Brazil). Spermatozoa samples were evaluated for total and progressive motility by CASA system (SCA®,Microptic,Barcelona, Spain) and plasma membrane integrity by immunofluorescence system (PI/CFD) in fresh and after 0, 2 and 4h stabilization/cooling times, and after thawing (37 °C/ 30''). Data were analyzed by ANOVA and Tukey-Kramer, with 5% significance level.

Results and Discussion

No significant differences were observed among the stabilization times for parameters of total and progressive motility. In plasma membrane evaluation, it was observed that stabilization of 0h (47.87±17.29) and 2h (52.50±16.82) determined a lower ($P<0.05$) integrity when compared with 4h stabilization time (65.87±14.04), which did not differ ($P>0.05$) among fresh (90.87±1.18) and cooled samples (T0= 88.37±1.97; T2= 86.87±5.07; T4= 89.50±1.68). These results can be explained because changes between extra and intracellular environment are required for cell adaptation under temperature reduction, then, a few time under refrigeration before to freezing curve is insufficient for its changes and the plasma membrane undergoes more lesions than a cell exposed more time at cryopreservation extender. Thus, it can be concluded that stabilization times used in cryopreservation process affects plasma membrane integrity of bull spermatozoa obtained from epididymis.

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Embryo recovery and pregnancy rates in different age mares in a equine embryo transfer program

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Introduction

The efficacy of a embryo transfer program (ET) is determined by the rates of embryo recovery and pregnancy. The age of the mares influences in the rates of embryo recovery and pregnancy. By comparing the effect of age in Quarter Horse and Paint Horse mares according their ages: 2 - 4 years, 4 - 16 years and older than 16 years, the embryo recovery rate was 81.1% (69/85), 73, 2% (478/653) and 53.3% (79/148), respectively (1). In Quarter Horse mares and Mangalarga mares, the embryo recovery rate was 62% (431/699) for young mares (≤ 12 years) compared with 51% (215/423) for older mares (≥ 18 years) (2). The pregnancy rate in mares between 6 and 8 years, 9 and 11 years and above 11 years was 80%, 76.5% and 38.5%, respectively (3). The study aimed to assess the rates of embryo recovery and pregnancy in mares of different ages.

Material and Methods

To evaluate the embryo recovery rate were used 70 donor mares and a total of 347 embryo collections, while for the pregnancy rate were used 100 recipient mares in 100 embryo transfers. All mares Mangalarga Marchador breed. The mares were separated into three groups according their age: Donors: DI young mares aged between 3 and 6 years (37 mares – 181 embryo collections), DII adult mares aged between 7 and 17 years (24 mares – 133 embryo collections) and DIII ≥ mares older than 18 years (9 mares – 33 embryo collections); Recipient: RI (3 to 6 years – 34 mares), RII (7 to 10 years – 39mares), RIII ≥ 11 years – 27 mares. The mares were examined by transrectal palpation and ultrasonography until a follicle ≥ 35 mm was detected when it was made the ovulation induction using 1000UI hCG (Chorulon ®). Twenty-four hours after induction, the donor mares were inseminated. The embryo collections were performed through the transcervical method (4) between eight and nine days after ovulation. Only grade I and II embryos were transferred to recipient mares previously synchronized between three and eight days after ovulation. Pregnancy diagnosis was performed by ultrasound at 15 days of embryo old. Data were statistically analyzed using Chi-Square ($\chi 2$) with a significance level of 5%.

Results and Discussion

When we evaluated effect of age on embryo recovery rate, the results for groups DI, DII and DIII were: 54.7% (99/181), 48.9% (65/133) and 33.3% (11/33) respectively. Statisticall significant difference ($P < 0.05$) were obtained between groups DI and DIII, corroborating with previous results where embryo recovery rate was higher in young and adults mares (81.1% e 73.2%) when compared to older mares 53.3% ($P < 0.05$) (1). The results for pregnancy rates were: RI 50% (17/34), RII 61.5% (24/39) and RIII 33.3% (9/27), there was significant difference ($P < 0.05$) between groups RII and RIII, corroborating with results obtained in a previous study (3) where the pregnancy rate was lower in mares over 11 years old (38.5%) compared to the group of mares between 6 and 8 years old (80 %) and 9 and 11 years (76.5%) ($P < 0.05$). These results shows that the embryo recovery and pregnancy rates are influenced by the age of the mare.

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Influence of cloprostenol in endometrial cytology of crossbred dairy cows

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Introduction
The cytological endometritis is diagnosed by increased number of neutrophils in cytology specimens obtained by the endometrial cytobrush method (1), leading to infertility when the uterine infection is present and sub fertility even after completion of successful treatments (2). Therefore, the objective was to evaluate the effect of treatment with cloprostenol in endometrial cytology at 43 days postpartum and thus evaluate its interference in the inflammatory process of the uterine environment.

Material and Methods
Two groups of crossbred dairy cows; G1-control group (n = 29) and G2-cloprostenol group (n = 25) were clinically evaluated by trans-rectal ultrasound examination at 7, 14, 21, 28 and 43 days postpartum (dpp). Group 2 received two doses of 0.526 mg of cloprostenol until 12 hours and 72 hours postpartum. On 43 dpp an endometrial cytology by cytobrush technique was carried out (3) and the presence of more than 10% neutrophils was considered as endometritis cytological. Cows from G1 with uterine infections at 10 dpp were removed and treated representing 10.3% (3/29) . The Minitab Release 15 statistical analysis program was used showing a significance level of 5% (4).

Results and Discussion
There was no difference (p = 0,303) in the incidence of endometritis cytological between G1 and G2, 20% and 34% respectively. In an experiment with Holstein cows 18.7% of endometritis cytology with 35 ± 3 days postpartum was found (5). There was no difference in the incidence of metritis (P = 0289) and clinical endometritis (p = 0 931) according to the Williams et al. classification (6). Therefore, there was no effect of the proposed protocol with cloprostenol on the incidence of metritis, clinical endometritis and endometritis cytology. It is concluded that crossbred dairy cows have endometritis cytological and so further studies are needed to assess ways of prevention and their effects on reproductive efficiency.

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Prior evaluation of the viability of cryopreserved *Pseudoplatystoma reticulatum* semen

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Introduction

The preservation of fish semen has become an important tool for breeding programs and biodiversity conservation (1). Sperm cells often suffer cryoinjuries, then cryoprotectant solutions should provide an ideal balance between toxicity and ability to protect cells from freezing injury (2). The cachara (*Pseudoplatystoma reticulatum*) is a large migratory species, with high biological importance and a high commercial value in South America (3). The study aimed to evaluate the seminal parameters after cryopreservation with different cryoprotectant solutions and its efficacy in maintaining the ability to fertilize the oocytes.

Material and Methods

Semen of two individuals were diluted in eight different cryoprotectant solutions: 1 (10% methanol (MeOH) + 5% glucose (GLU) + 20% egg yolk (EY) + distilled water (DW)), 2 (10% DMSO + 5% GLU + 20% EY + DW), 3 (10% MeOH + 5% GLU + 20% EY + saline solution (SS)), 4 (10% DMSO + 5% GLU + 20% EY + SS), 5 (10% MeOH + SS), 6 (10% DMSO + SS), 7 (10% MeOH + 15% milk powder + SS), 8 (10% DMSO + 15% milk powder + SS). The semen was diluted, drawn, kept in the cryogenic shipper for 6 hours until transfer to cryogenic shipper stored , where it was stored in liquid nitrogen for 2 hours. The evaluation of progressive motility and spermatic vigor, according to (1), was performed immediately after dilution of semen and after thawing. Then the fertilization and hatching rates for each treatment was evaluated.

Results and Discussion

Progressive motility and spermatic vigor fresh semen was 100% and 5 points, respectively. The semen diluted in solution 5 resulted in better rates of progressive motility and spermatic vigor (95 e 5, respectively), and the worst rate was observed in solution 3 (50 e 3, respectively), as seen in Tab.1. After thawing, the best values of progressive motility and spermatic vigor were recorded in a solution 1 (40 e 3, respectively), and the worst in the solutions 3, 5 and 7 (5 e 2, respectively). The highest fertilization rate (36% of viable eggs) was observed in solution 6 and hatching rate in the solution 8 (80%). According to (4), countless cryoinjuries in sperm can be caused by low temperature. (5) have suggested that sperm quality, when they are efficient in fertilization of oocytes.

Table 1. Progressive motility, spermatic vigor, fertilization rate and hatching rate using cachara semen cryopreserved with different cryoprotectant solutions.

Cryoprotectant solution	Progressive motility (%)		Spermatic vigor (points)		Fertilization rate (%viable)	Hatching rate (%)
	diluted	thawed	diluted	thawed		
1	90	40	4	3	8	50
2	70	40	3	2	4	20
3	50	5	3	2	0	0
4	80	20	4	3	0	0
5	95	5	5	2	7	30
6	85	15	4	2	36	40
7	85	5	4	2	7	70
8	85	10	4	2	25	80

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Climate condition influences kinematic parameters of cryopreserved ovine spermatozoa

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Introduction

It is known that climate condition interferes on animal reproduction, perhaps because of the high luminosity during summer or the food demand be limited by fewer rainfall incidences, causing dry soil and poor food offer, respectively. Other factors as thermal stress determines lower food ingestion and higher release of stress thermal protein that negatively interferes on male reproduction (1). Indeed, plasma seminal production undergoes decrease on the energetic components and motility sperm, resulting in lower fertility during dry season (2). Then, our objective was to study the kinematic parameters after ovine sperm cryopreservation during different climates conditions.

Material and Methods

This study was realized in Paraiba state, Brazil (06°29'18"S and 35°38'14"W) at an altitude of 168 m, with a tropical semi-arid climate and mean annual precipitation of 431.8 mm^3. Evaluations were performed during one year period (2009), divided in two climate conditions: dry and rainy season, depending on the rainfall index for this region (dry period: lower than 50 mm3; rainy period: higher than this index). Five rams previously tested were used; six ejaculates for each animal and for each season were obtained by artificial vagina and diluted with Tris-egg yolk extender (3.605g Tris; 2.024g citric acid; 1.488g fructose; 100mL bidistilled water; 20% yolk egg; 7% glycerol). Doses were standardized at a 240 x 10^6 spermatozoa/mL concentration, packaged in straws (0.25 mL) and cooled/frozen in automated system (TK-3000®, TK Tecnologia em congelação LTD, Uberaba, Brazil). After thawing (37 °C/ 30 sec), sperm samples were evaluated for kinematic parameters by CASA system (Sperm Class Analyzer/SCA; Microptics, S.L., Barcelona, Spain). Data were analyzed by ANOVA and Tukey-Kramer, with 5% significance level.

Results and Discussion

Kinematic parameters as total motility (TM), linearity (LIN), straightness (STR), wobble (WOB), curvilinear velocity (CLV), straight line velocity (SLV), average path velocity (APV), amplitude lateral of head displacement (ALH) and beat cross frequency (BCF) were analyzed. Data were expressed as mean and standard deviation (mean±sd), for dry and rainy seasons, respectively. TM (55.01±1.97^b; 74.28±1.64^a), LIN (36.63±0.79^b; 41.28±1.02^a), STR (62.68±0.86^a; 59.68±0.77^b) and WOB (65.64±0.80^a; 61.27±0.60^b) were better during rainy season compared to dry season. No significant differences were observed for CLV (51.69±3.77; 59.97±4.61), SLV (21.41±1.83; 21.93±1.72), APV (33.96±1.83; 36.72±2.77), and ALH (3.18±0.13; 3.67±0.10) and BCF (6.73±0.26; 6.47±0.22). Parameters as linearity are directly involved in fertility capacity of spermatozoa (3), thus, higher values in this evaluation may be used as efficient signaling for male selection and, consequently, ovine reproduction. At rainy climate condition, spermatozoa and plasma seminal productions are favorable, probably, by better food offer as well as the thermal comfort, resulting in lower stress condition and release of thermal stress proteins. These findings indicate that climate conditions interfere on the kinematic parameters for ram sperm cryopreservation.

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In vitro pre-maturation of bovine oocytes with cAMP modulators in the presence of serum impairs meiotic progression

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Introduction

Oocyte maturation *in vivo* is a highly orchestrated induced process. However, when this event is performed *in vitro*, oocytes irrevocably reinitiate meiosis spontaneously compromising developmental competence. A novel *ex vivo* simulated physiological oocyte maturation (SPOM) strategy was recently described (1), in which cAMP modulators are used during *in vitro* maturation (IVM) aiming to increase oocyte-*cumulus* cell gap-junctional communication and to slow meiotic progression. Recently, we evaluated the nuclear dynamics after use of this system in serum-free IVM. Despite of meiotic progression attenuation caused by the modulators, the results of embryonic development were unsatisfactory. Then, in the present study we assessed the nuclear behavior of bovine oocytes pre-matured with SPOM system in IVM medium containing FBS as protein source.

Materials and Methods

Oocytes were cultured in TCM 199 supplemented with 10% FBS, 1.0 µg/mL FSH, 50 µg/mL hCG, 1.0 µg/mL estradiol, 0.20 mM sodium pyruvate and 83.4 µg/mL amikacin. In treated group, 150 µM forskolin (adenylate cyclase activator) and 750 µM IBMX (nonspecific phosphodiesterase inhibitor) were applied in the first 2 h of culture. Subsequently, oocytes were transferred to IVM base medium supplemented with 20 µM cilostamide (oocyte-specific phosphodiesterase inhibitor) in groups of 15-20, at 38.5° C in 5% CO_2 in air atmosphere. Samples were obtained 20, 24 and 28 h after maturation and treatments, and oocytes were stripped from *cumulus* cells with 2% hyaluronidase, stained with 10 µg/mL Hoechst 33342 for 15 min and evaluated regarding meiotic progression. Nuclear maturation rates were assessed by chi-square (χ^2) or, when appropriate, by Fisher's exact test, in SAS v.8.2.

Results and Discussion

After 20 h IVM, 75.5% (37/49) of oocytes from control group were considered mature, i. e., in metaphase II with extrusion of the 1st polar body, whereas only 38.3% (28/73; $p<0.05$) of oocytes from treated group were in the same stage. In the evaluation after 24 h IVM, the maturation rates were significantly different between control (83.5%; 66/79) and treated (65.5%; 59/90) groups. In contrast to previous results from serum-free IVM medium, in this study we verified that FBS interfered in the meiotic progression, since after 28 h IVM the treated group still maintained inferior maturation rates (62.5% - 25/40) compared to control group (83.9% - 26/31). Several studies (2, 3, 4) report that serum has a biphasic effect, inhibiting the first cleavage divisions and accelerating embryonic development to the blastocyst stage. Therefore, it is possible that serum has shown a synergistic effect with cAMP modulators, causing a higher delay in meiotic progression during IVM. Further experiments for detection of this combined effect on embryonic development are required.

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The use of forskolin to produce *in vitro* bovine embryos

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Introduction

The oocyte *in vitro* maturation (IVM) is an important reproductive technology that generates mature oocytes that are capable of supporting embryo pre-implantation development and full development to term (1). However, one of the most widely used techniques in maturation is inhibition or delay in the nuclear meiotic of oocytes, which would allow a more regulated and appropriate transition from prophase I to metaphase II. When the cAMP concentrations are decreased, there is a stimulation of the nuclear maturation process but when the intracellular concentrations are high, the maturation is inhibited (2, 3). In this matter, the forskolin (Sigma-Aldrich, St. Louis) is an efficient inhibitor of nuclear maturation because of its ability in raising the levels of intracellular cAMP. This study aimed to show if the use of forskolin alone is able to inhibit meiosis in bovine oocytes and produce a higher rate of *in vitro* produced embryos.

Material and Methods

Nelore oocytes were matured in TCM-199 with Earle's salt + 10% FCS, FSH and LH, in 5% CO_2 in air atmosphere. To delay meiosis, the oocytes were maintained for 6 hours in medium in presence of 1mM forskolin. Then the oocytes were cultured for 18 hours in agent-free medium to resume meiosis, completing 24 hours of maturation. After resume of meiosis, the oocytes were stained with Hoechst 33342 (0.01 mg/mL) to evaluate in which state the nucleus was: germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), metaphase II (MII), degenerated or unidentified (D/U). Then (day 0), oocytes were fertilized in human tubal fluid (HTF – Irvine, New Zeland) under the same condition above. Semen was selected through Percoll gradient and the concentration adjusted to 2 x 10^6 sperm/mL. The presumably zygotes were culture in 90µL droplets of SOFaa + 0.6% BSA + 2.5% FCS in 5% CO_2, 5% O_2, 90% N_2 atmosphere until day 7, when blastocyst rate was evaluated. Eight replicates were made. Data were analyzed by ANOVA, followed by Tukey test using the general linear model (PROC GLM) of SAS. The level of significance adopted was 5%.

Results and Discussion

There were found statistical differences in MI in the groups: Control: 8.3 ± 6.2^{a}; Forskolin 1mM: 34.1 ± 6.7^{b} – P<0.05 (Control: GV: 0^{ab}, GVBD: 0.8 ± 0.9^{ab}, MII: 67.6 ± 9.6^{ab}, D/U: 7.3 ± 3.8^{ab}; Forskolin 1mM: GV: 0^{ab}, GVBD: 1.0 ± 0.9^{ab}, MII: 50.2 ± 10.4^{ab}, D/U: 14.1 ± 4.1^{ab}). No statistical differences were observed in blastocyst production rate: Control: 36.7% ± 3.7; Forskolin 1mM: 25.1% ± 3.7 – P=0.168. The dose of forskolin was effective to achieve an MII equivalent to Control group. However, if we reduce to half of the forskolin dose, maybe we can decrease the rate of oocytes in MI. Forskolin was able to produce embryos without degeneration and with similar qualities to the agent-free group.

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Replacing TCM with BARC medium supplemented with fetal calf serum for in vitro oocyte maturation did not support subsequent blastocyst production

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Introduction

Oocyte in vitro maturation has a crucial importance in the in vitro production of embryos. Tissue culture medium (TCM 199), a complex base medium, is the most common medium available for oocyte maturation in vitro. However, synthetic oviductal fluid (SOF), a well- known embryo culture medium, has also been examined for maturation of bovine oocytes (1,2). On the other hand, use of Beltsville Agriculture Research Center (BARC) medium has been restricted to the in vitro culture of cloned (3) and not cloned bovine embryos (4), and there are, to our knowledge, no published studies about its use for in vitro maturation of oocytes. The purpose of this is study was to investigate the effects of BARC supplemented with different concentrations of fetal calf serum (FCS) on in vitro bovine oocyte maturation, as evidenced by subsequent cleavage rate and blastocyst formation.

Materials and Methods

Oocytes were washed and selected following removal from bovine ovarian follicles (2-7 mm in diameter). Four treatment groups (15-20 oocytes/drop) were assessed, as follows: control, TCM 199 (SigmaAldrich, USA) plus 10% (v/v) FCS, and three BARC treatments at concentrations of 0, 5 and 10% FCS. All of media were supplemented with 0.5 µg/ml FSH (Folltropin, Bioniche Inc., Canada), 50 µg/ml LH (Lutropin- V, Bioniche Inc., Canada) for 24 h at 38.7º C in an environment of 5% CO_2 in air. Then, semen was thawed and the sperm selected by centrifugation in discontinuous Percoll gradient. The resulting pellet was diluted in TALP (with PHE and heparin) medium and used for in vitro fertilization (IVF). After 20 h post-insemination (h pi) the cumulus cells were partially removed and presumptive zygotes were transferred to drops of modified SOF. Results of four replicates are reported as percentages of oocytes that cleaved (C) following IVF, and reached blastocyst (B), expanded blastocyst (EB) and hatching blastocyst (HB) stages at 72 and 168 h pi, respectively. ANOVA and Bonferroni *t* test were used to determine differences among groups. Differences of $P < 0.05$ were taken as significant.

Results and Discussion

Differences were found ($P < 0.05$) among treatments for cleavage rates with group control yielding the best result. Higher ($P < 0.05$) percentage of blastocysts at different developmental stages (B to HB) were observed for TCM (49.3%) than for all BARC treatments, but more ($P < 0.05$) embryos developed to the blastocysts stages in BARC supplemented with 5 (11.3%) or 10% (19.7%) FCS compared to 0% FCS (4.1%) with similar results for 5 and 10% FCS. Lower percentage of blastocysts in BARC medium is in agreement with reports of (5) that used SOF and TCM for bovine oocyte maturation. In contrast, (1) found similar results for embryo production when TCM was compared to SOF plus bovine serum albumin or FCS. The lack of beneficial effect of BARC medium could be due to its simplex composition which can compromise the oocyte competence for continuing in vitro development, and therefore, profoundly alter subsequent embryonic development even if cleavage rate had not been affected. Cytoplasmic oocyte maturation involves molecular events and development of appropriate metabolic pathways which are essential for embryonic development (6). In conclusion, BARC medium either with or without FCS supplementation had no minimal requirements to be capable of promoting effective oocyte competence, leading to poor blastocyst production.

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Aloe vera sp. was an acceptable alternative to egg yolk for preserving goat semen at 4°C

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Introduction

Diluents containing egg yolk are the most practical for preserving semen in low temperatures. However, due to the recent requirements for disease control and security in biological processes, it has been suggested the elimination of animal products in diluents for semen conservation (1). Though a few studies have been performed on the effect of *Aloe vera sp*. in ram semen (2), to date in vitro evaluation of goat semen after cooling with use of *Aloe vera sp.* has not been studied. Therefore, this study assessed the effect of 5 or 10% (wt/vol) *Aloe vera sp.* (T4 and T5) or 5 or 10% (v/v) egg yolk (T2 and T3) in a coconut water powder extender (ACP-101®) for preservation of goat semen at 4°C.

Material and Methods

For this, a *pool* of ejaculates from 4 male goats (1 ejaculate/goat) was used. Control group was ACP-101® only (T1). Kinetic parameters and sperm viability were assessed using the computer-assisted sperm analyzer (CASA; SCA®, Microptics SL, Spain) and Eosin-nigrosine test, respectively, at 0h (fresh and diluted semen before cooling) and at 6, 12, 24 e 48 h after cooling. The data were subjected to the tests of Kolmogorov-Smirnov and Bartlett for confirmation of normal distribution and homogeneity of variance between treatments, respectively. Confirmed fulfillment of the requirements for performing ANOVA, this was performed by GLM procedure of SAS (2002) and Student-Newman-Keuls test was used to compare mean variables corresponding to the MP, VSL and VAP. The other variables were analyzed by using the nonparametric Kruskall Wallis test. Results were expressed as mean ± standard deviation and differences were considered significant when $p < 0.05$.

Results and Discussion

The best results in terms of sperm motility was observed with 5% egg yolk at 0 and 6h ($P<0.05$). Nevertheless from 24 to 48 h of evaluation, 5% *Aloe vera sp.* revealed the best averages for total motility (70.3% ± 4.4 e 71.6% ± 4.1, respectively) ($P<0.05$). In relation to progressive motility, in the first 24 h, 5% *Aloe vera sp*. presented significant higher averages ($P<0.05$) when compared to treatments with egg yolk, but was similar to control group and T5. Regarding sperm viability it was observed significant higher percentages for 5% egg yolk (87.5% ± 2) at 0h ($P<0.05$). During the storage of semen (24 and 48h) higher viability was observed in T3 (63.8% ± 6.2 e 60% ± 5.7, respectively) when compared to 5 (37.3% ± 8.6 e 21.6% ± 7.8) or 10% egg yolk (37.0% ± 8.1 e 22.5% ± 8.8) ($P<0.05$). There were no significant differences when comparing the groups using *Aloe vera sp.* parameters as the MT, MP and Viability. Regarding the parameters curvilinear velocity (VCL), linear velocity (VSL), mean velocity (VAP) and linear coefficient (LIN), only the control group and the treatments used *Aloe vera sp.* maintained their average constant during all times analyzed. By comparing the treatments that used egg yolk, the T2 was similar to the T1 and T4 to the analysis time of 12 hours after that, their average decreased significantly. However, T3 was significantly lower since the beginning of the analysis. To our knowledge, this is the first report regarding the use of *Aloe vera* sp. as a substitution for egg yolk in water coconut powder extender (ACP-101®) for preserving chilled goat semen. According to our results, we suggest that the optimal concentration of *Aloe vera sp*. is 5% in the ACP-101® extender. In addition, we conclude that *Aloe vera sp.* can be used as a substitute for egg yolk in ACP-101® extender for preserving goat semen for 48h at 4ºC.

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Ultrasound diameter of embryonic vesicle during early gestation in mares inseminated with stallion or donkey semen

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Introduction

Pregnancy is detected by transrectal ultrasonography as early as 9-14 days postovulation in mares (1,2). Increasing demand of mules during the last decade in Brazil resulted in need for an early pregnancy diagnose of mares mated with donkeys. However, it is not known if similar uterine ultrasound characteristics are detected in mares with an equine or mule embryo. This study aimed to compare the diameter of embryonic vesicle detected by ultrasound evaluation from 11 to 14 days postovulation in mares mated with stallion or donkey.

Material and Methods

Mares with ovarian follicle diameter of 30-35 mm were inseminated with diluted raw semen every 48 h until ovulation was detected. Pregnant mares inseminated with stallion (n = 38) or donkey (n = 22) semen were scanned by transrectal ultrasonography on days 11, 12, 13 or 14 postovulation to evaluate the diameter of embryonic vesicle. For the ultrasound scanning, a B-mode (gray-scale) ultrasound instrument (Aloka SSD-500; Aloka America, USA) equipped with a 5.0-MHz sector was used. The diameter of each embryonic vesicle was determined using a B-mode still image and the tracing function.

Results and Discussion

There was no difference in the diameter of embryonic vesicle on days 11, 12, 13 or 14 postovulation between mares with stallion and donkey semen ($P>0.05$; Table 1). The diameter and growth rate (0.35 cm/day) from day 11-14 of the equine or mule embryo in the present study were similar to the values reported (3) in mares with an equine embryo. However, the present results were greater than the values reported (4) during 10-13 days postovulation in jennies with an asinine embryo. The results indicated that there is no influence of the donkey's genes on embryo growth during early gestation in mares. Thus, the early pregnancy diagnoses and the evaluation of early embryo development by transrectal ultrasonography is a practical method to identify either mare pregnant of stallion or donkey semen. This allows a better management of the animals in the breeding farms of mules.

Table 1. Means ± standard deviation of diameter (cm) of embryonic vesicle on days 11, 12, 13 and 14 postovulation in mares inseminated with stallion or donkey semen.

	Days postovulation			
Breeder	11	12	13	14
Stallion	0.87 ± 0.17 (n = 6)	1.30 ± 0.27 (n = 11)	1.62 ± 023 (n = 8)	2.01 ± 0.52 (n = 13)
Donkey	0.92 ± 0.28 (n = 5)	1.10 ± 0.42 (n = 3)	1.51 ± 0.43 (n = 9)	1.76 ± 0.85 (n = 5)
Total	0.89 ± 0.19^{C} (n = 11)	1.26 ± 0.30^{C} (n = 14)	1.56 ± 0.38^{B} (n = 17)	1.94 ± 0.61^{A} (n = 18)

Means followed by a different superscript letter in the same row differ by LSD test (α = 5% of probability).

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Use of intracytoplasmic sperm injection to produce a foal from a mare with severe scarring of the posterior reproductive tract and a stallion with limited quantities of frozen semen

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Introduction

Traumatic injuries following complicated parturitions are common causes of reproductive tract abnormalities in the mare. Improper healing of these wounds can have serious and deleterious effects on the mare's reproductive future (1). Advanced assisted reproductive techniques, such as oocyte transfer and intracytoplasmic sperm injection (ICSI), are options for mares unable to produce offspring because of physical compromise of the reproductive tract, although they have normal folliculogenesis (2).

Material and Methods

A 4-year, Quarter Horse mare was referred to the Equine Reproduction Laboratory at Colorado State University to obtain an offspring after severe injuries of her perineum, vestibule and vagina during a prior parturition. Perineal examination showed a chronic second-degree perineal laceration including most of the vulva, with the absence of both vulvar lips and extensive areas of fibrosis (Figure 1). A fibrotic ring was present between the vestibule and vagina with an approximately 4-cm opening. Scarring was present in the vagina, and the cervix appeared incompetent. During a vaginoscopic exam, a pool of purulent fluid and hyperemia of the mucosa was observed in the anterior vagina. Upon ultrasound examination, the uterus contained lumenal fluid, approximately 2 cm in depth, with hyperechoic reflections indicative of pneumouterus. The ovaries had normal follicular development. Follicular maturation was initiated when the mare obtained a >35 mm follicle during estrus. Oocyte collection was attempted using a trochar placed in the mares left flank and ipsilateral to the preovulatory follicle (3). However, an enlarged spleen prevented optimal manipulation of the ovary. Therefore, the mare's vaginal was lavaged, and transvaginal ultrasound-guided follicular aspiration was performed (4). For the oocyte collection, a linear ultrasound transducer, housed in a casing with a needle guide, was placed into the anterior vagina. The ovary was positioned per rectum to align the preovulatory follicle with the transducer and needle guide, and the follicular antrum was punctured and follicular contents were suctioned to retrieve the oocyte. Limited quantities of frozen semen were available for ICSI; therefore, a small section of a 0.5-ml straw was cut under liquid nitrogen and thawed. A sperm was selected using a swim-up procedure and microscopic evaluation. Sperm injection was done using a micromanipulator and a Piezo injection system. After ICSI, the oocyte was incubated at 38.5 ^{o}C in 5% CO_2, 5% O_2 and 90% N_2 in DMEM/12 containing 10% fetal calf serum. The presumptive zygote was cultured for approximately 36 h before evaluation of embryonic development.

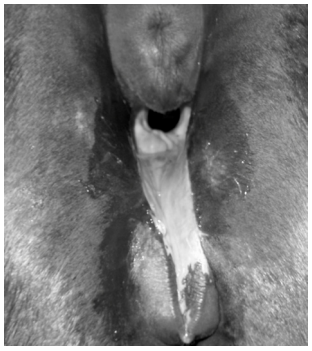

Figure 1. Perineal view.

Results and Discussion

Approximately 1.5 d after ICSI, an 8-cell embryo was transferred into the oviduct of a recipient mare through a standing flank laparotomy; the recipient mare ovulated 2 d prior. For transfer, the embryo was loaded into a sterile, fire-polished glass pipette with a small volume of medium and deposited 3 to 4 cm into the infundibulum. An ultrasound exam for pregnancy was performed 12 d after ICSI, and an embryonic vesicle, 5-mm in diameter, was imaged. A normal gestation of 343 d ended in birth of a foal. In conclusion, the use ICSI allowed the production of an offspring from a mare with severe reproductive tract injuries and a stallion with limited frozen sperm. ICSI is an example of an assisted reproductive technique that can help in the preservation of valuable genetics and the production of offspring that would not be possible using standard breeding procedures or embryo transfer.

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Do ewe preovulatory follicles decrease their diameters before ovulation?

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Introduction

Knowledge of physiology and follicular dynamics in sheep are essential to develop protocols of ovulation synchronization. The evaluation of the follicular stage in ewes is generally made by US exams and usually requires technical knowledge and practical experience of the operator. Follicular grow, statics and regression phases are well described in literature (1,2). Reduction of follicular diameter was observed close to the ovulation period when Santa Ines ewes were subjected to a protocol of ovulation synchronization. The present study aims to characterize the rate of follicular growth and the rate of retraction of preovulatory follicles.

Materials and Methods

Ten fertile cyclic Santa Inês ewes were kept at the ruminant´s sector of the veterinary hospital from the FMVZ/USP. US examinations were performed using ALOKA (SSD-500, Berger, Brazil) with a linear probe 5.0 MHz attached to a handle to allow safe intra rectal manipulation. The exams were performed at a 24 hour intervals from three days (D-3) before ewes were treated with an intravaginal progesterone device (D0) to the day of withdrawal (D9). On D9, 30 µg of prostaglandin (*d*-cloprostenol - Prolise®-Tecnopec, Brazil) was administered. From D9 to ovulation, US exams were performed every 12 hours. Ovulation was assessed by the disappearance of the growing larger follicles present in previous examinations. To study the growth rate of follicular phases after the vaginal dispositive removal, different variables were created (Figure) based on follicular size measured by US exam at different time intervals (-48, -24, 0, 6, 18, 30, 42, 54, 66, 78, 90 and 102 h). Data were analyzed using the MIXED SAS procedure.

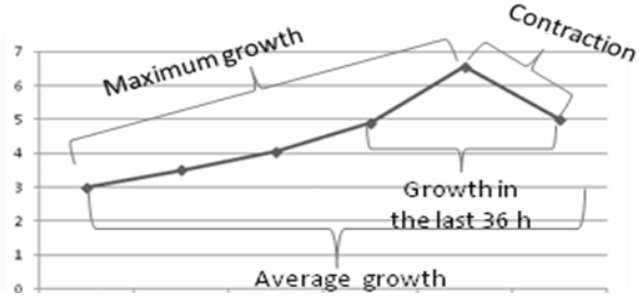

Figure. Follicular growth rate of ewes after treatment with a protocol of ovulation synchronization.

Results and Discussion

Ovulation occurred after 73 ±14.38h dispositive withdrawal. The initial and maximum follicle diameters were 3.48±0.28mm and 5.63 ±0.66mm, respectively. The mean growth rate of the follicle during the present study was 0.73 ± 0.43mm/day. The maximum growth rate was 1.14 ± 0.73 mm/day; and the mean growth rate from the last 36 hours before ovulation was 0.34 ± 0.92 mm/day. The follicular regression rate was -1,37 ± 1,04mm/day, leading to the decrease of preovulatory follicle diameter to 5.22 ± 0.72 mm.

Cyclic changes of leucocytes population on the ovaries may occur during ovulation. These types of cell actively participate on the functional and structural changes of follicle and corpus luteum (3). Some factors responsible for the cascade of events that drives ovulation in sheep may cause this regression of follicular diameter, such as the local PGF2α release which causes smooth muscle contraction on the ovaries (4). Accordingly, this study reports the decrease of follicular diameter before ovulation in ewes.

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Comparison between different straw size and thawing temperatures used for the cryopreservation of collared peccaries (*Tayassu tajacu*) semen

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Introduction

It was recently demonstrated that collared peccaries (*Tayassu tajacu*) semen could be efficiently preserved by using a coconut water based (ACP®-116C) extender (1). As important as the cryopreservation is the thawing method that is influenced by the straw size and the temperature (2). Thus, we aimed to compare different straw sizes and thawing temperatures used during the collared peccary semen cryopreservation.

Materials and Methods

A total of 12 ejaculates were obtained by electroejaculation from 08 adult male collared peccaries. Samples were evaluated and extended in ACP®-116C plus 20% egg yolk and 3% glycerol. After dilution, the samples were chilled by using a rapid curve (3), packed in 0.25- or 0.50-mL plastic straws and stored in liquid nitrogen. After one week, samples were thawed at 37 °C/1min or 70ºC/8s and reevaluated for sperm motility, vigor, viability, and structural membrane integrity by using the fluorescent probes 6-carboxy- fluorescein diacetate (C-FDA) and propidium iodide (PI). The effect of straw size and thawing temperature on sperm characteristics was analyzed by ANOVA followed by Scheffe test ($P<0.05$).

Results and Discussion

All the values for fresh semen characteristics were in the normal range for the species (4). A marked negative effect of the thawing at 70ºC/8s on semen characteristics was verified ($P<0.05$) (Table 1.). Due to this fact, fluorescence analysis was not conducted in such treatment. In addition, no differences were observed between straws thawed at 37 ºC ($P>0.05$) (Table 1.). The results confirm the statement that thawing at 37 ºC is safer than higher temperatures because the time spent in high temperatures is always critical and could have a lethal influence on the sperm viability (5) (Table 1.). Therefore, we recommend that collared peccary semen cryopreserved in ACP-106C® should be packed in 0.25- or 0.50 mL straws and thawed at 37 ºC/1 min.

Table 1. Mean values (±SEM) for sperm characteristics (n=12 ejaculates) in collared peccaries (Tayassu tajacu) frozen/thawed semen by using different straws and thawing temperatures.

Straw size	0.25 mL		0.50 mL	
Thawing temperature	37 ºC / 1 min	70 °C / 8 s	37ºC / 1 min	70 °C / 8 s
Sperm Motility (%)	42.1 ± 8.1[a]	0.9 ± 0.2[b]	41.7 ± 6.6[a]	20.1 ± 8.2[c]
Vigor (0-5)	2.8 ± 0.3[a]	0.3 ± 0.3[b]	2.8 ± 0.2[a]	1.4 ± 0.4[b]
Sperm viability (%)	35.6 ± 5.4[a]	19.84 ± 4.0[b]	37.75 ± 5.6[a]	34.5 ± 5.67[a]
Sperm membrane integrity (%)	22.8 ± 4.2[a]	*	15.8 ± 3.5[a]	*
Normal morphology (%)	58.2 ± 8.1[a]	65.8 ± 6.8[a]	64.4 ± 5.6[a]	65.8 ± 6.4[a]

[a,b]Within a row, values without a common superscript differ ($P < 0.05$).*Fluorescence not performed.

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Effect of time of eCG administration and utilization or not of hCG on pregnancy rates of inseminated in wooless ewes

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Introduction

The growing demand for ovine meat has encouraged the use of biotechnologies focused on animal reproduction in order to raise and standardize the production of these animals. The artificial insemination has attracted more and more interest from breeders. However, conventional protocols for synchronization of oestrus and ovulation aren't, in most cases, producing the expected results, observing low percentages in the pregnancy rate. Thus, the objective was to compares six protocols of short duration for fixed-time artificial insemination (TAI), evaluating the time of application of gonadotropins on synchronization of ovulation and increase pregnancy rate.

Materials and Methods

Sixty-six ewes received intravaginal dispositive containing 60 mg of medroxyprogesterone acetate (day 0) and were randomly divided into 6 groups. On day 4 of the protocol each animal was treated with 37,5 μg of the cloprostenol. Were administered 400 UI of eCG at different times: at G1 (control, n = 11) and G4 (n = 11) were on day 6; at G2 and G5 were on day 5; and in G3 and G6 were administrated on day 4 of the protocol. The sponge was removed (day 6) and after 34 hours were given 200 UI of hCG in G4, G5 and G6. Ewes were inseminated by laparoscopy, 52 – 55 hours after sponge withdrawal, using cryopreserved semen. Thirty five days after inseminations, females were submitted to ultrasound examination to detect pregnancy.

Results and Discussion

In the present study we observed no difference in pregnancy rates between groups G1 (27.3%) and G2 (63.3%), but G1 differed from G3 (90.9%) ($p < 0.05$). This result can be obtained by reducing the number of smaller follicles and the anticipation of emergence of medium and large follicles, improving pregnancy rates in TAI programs (1). The pregnancy rate was better in groups G4, (90.9%), G5 (81.8%) and G6 (81.8%) than in G1 ($p < 0.05$). The use of hCG on TAI protocols induces ovulation and increases plasma levels of progesterone, increasing fertility in female (2). An earlier administration of eCG and/or use of hCG have been beneficial for ovulation induction, allowing higher pregnancy rates in TAI programs, but more studies are needed to confirm the luteotrophic ability of hCG in ewes.

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Fertility after fixed-time artificial insemination using bovine sperm rediluted in powdered coconut water (PCW-111)

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Introduction

Studies of seminal diluents are the aim at researchers of worldwide. An effective natural semen diluent in ruminants called coconut water *in natura* (CWN) was developed by researchers in the Northeast of Brazil (1). The success of the CWN brought the need for standardization of constituents found in coconut water, and subsequently the development of thinner water-based powdered coconut water called PCW ® (2). The present study aimed to evaluate the effect of redilution of the frozen bovine sperm and citrate - egg yolk-glycerol solution-based coconut powdered water (ACP-111®).

Material and Methods

The fixed-time artificial insemination (FTAI) was performed using doses of semen (n = 50) in frozen commercial extender (CE) citrate - egg yolk - glycerol. A total of fifty cows Girolanda were submitted to FTAI. Twenty-five were inseminated with semen bovine frozen-thawed in comercial extender (Group I: CE), twenty-five remaining females were inseminated after thawing and re-dilution based solution of coconut water (Group II: CE + PCW -111®). The fertility was analysed by ultrasonography and conception rates were analyzed by the Chi-square test, $P < 0.05$.

Results and Discussion

As can be seen in **Tab.1** the benefit effect of redilution bovine sperm under fertility and conception rates were observed ($P < 0.05$). The increase in fertility indices is probably due to three factors: (1) the increased in volume after redilution, which increased the space for the moviment of sperm cells, (2) dilution of ROS produced during cryopreservation by redilution, and (3) the supply of extra nutrients in the ACP extender (carbohydrates, proteins, lipids, vitamins), especially carbohydrates served as a source of energy for sperm cells with intact plasma membrane and normal metabolism reach the site of fertilization in the fallopian tube in female.

Table 1. Fertility of Girolanda cows inseminated with frozen-thawed semen diluted with commercial extender (CE) only, or rediluted in powdered coconut water based solution (CE + PCW-111®).

Treatments	Fertility rates (%)		Conception rates (%)
	40 days	64 days	
CE	36%[a] (9/25)	24%[a] (6/25)	20%[a] (5/25)
CE + PCW-111	52%[b] (13/25)	44%[b] (11/25)	44%[b] (11/25)
Total	44%[a] (22/50)	34%[a] (17/50)	32%[a,b] (16/50)

[a,b]Different superscripts indicate significant differences between treatments (Chi-square test, $P < 0.05$).CE: commercial extender (citrate - egg yolk - glycerol); PCW-111: extender based on powder coconut water.

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Cryopreservation of semen goats in periods low and high precipitation index in Northeast Brazil

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Introduction

In cryopreservation of semen in domestic animal species occurring biochemical changes in spermatozoa Thus, the success of sperm freezing depends on the maintenance of various functions that will enable the fertilization of the oocyte, such as the preservation of the plasma membrane structure, integrity of the acrosome, functions of the mitochondria and axoneme as well as the integrity of the DNA (1). The objective of this study verifies that the period of absence of rain can also be used for cryopreservation of semen.

Materials and Methods

The experiment was conducted in the State of Pernambuco, Brazil 8 ° 21 ' 35 ° 45' (Lat. S, Long.W) and climate tropical, with a minimum temperature of 10 °C and a maximum of 26 ºC; relative humidity ranges from 40.0 to 70.0%. Semen samples were collected in two periods of the year (2005 and 2006), classified based on the precipitation index as low (LRI) 0 mm; 22.7 °C, and high (HRI) 210 mm; 18.8 °C. Were collected 96 samples of semen by the method of artificial vagina from three buck Alpine. The samples were evaluated progressive motility, sperm vigor and acrosomes, packed in straws (0.25 mL) and cryopreserved in machine TK3000. After thawing (37 °C, 30s) reassessed,. The statistical analyses were performed using ANOVA, Student's t-test and Kruskal-Wallis on the SPSS (version 13). The level of significance was set at 5%.

Results and Discussion

Analyses of fresh semen samples showed that the volume (mL) and sperm concentration during the LRI and HRI was not significantly different ($P>0.05$) between animals, animals and interaction year x years. There was no significant difference ($P>0.05$) in sperm motility and vigor of fresh semen between animals and between periods LRI (79.82%; 4.17) and HRI (81.25%; 4.00) respectively. But we observed a greater ($P<0.05$) percentage of sperm with intact acrosomes in fresh semen collected during HRI (90.25%) than in the LRI (80.26%). However, there were significant differences ($P <0.05$) between the years evaluated (2005 and 2006) in both periods (LRI and HRI). The percentage of sperm with intact acrosomes differed ($P<0.05$) between the years evaluated during LRI, while during HRI there was no significant difference ($P>0.05$) between animals, and years, animal x year interaction. After thawing showed a significant difference ($P<0.05$) in progressive motility samples collected during periods of LRI (60.00%) and HRI (71.30%) and nonsignificant ($P>0.05$) in the percentage of cells with acrosomes in LRI (67.98%) and HRI (66.83%). Post-thaw motility was observed in a significant difference ($P<0.05$) between the years studied, with smaller percentage of cells with progressive motility in the thawed samples in 2006 compared to 2005. The percentage of sperm with intact acrosomes post-thaw, showed a significant difference ($P<0.05$) between animals and the year of assessment during LRI. It is concluded that the period of low rainfall is not ideal for cryopreservation of semen.

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Influence of hCG on the vascular perfusion of preovulatory follicle

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Introduction

Doppler ultrasonography is a real time noninvasive technique recently used to study the blood flow of reproductive tract in large animals, and is considered one of the best techniques to the study of ovarian hemodynamics in vivo of mares [1]. Assessment of the hemodynamic follicular wall provides important information about estrous cycle. Additionally, a relationship between greater preovulatory follicle vascularity and higher pregnancy rate was recently reported in mares [2]. Differences in the follicular vascular perfusion of mares treated or not treated with hCG was observed moments near ovulation [3]. However, changes have not been detected moments after administration of hCG probably because of the low evaluations frequencies. The purpose of this study was to evaluate the effect of hCG on vascular perfusion of preovulatory follicles in mares.

Materials and Methods

Total of 10 cycling mares, 5-13 years of age, were used in this study. The follicles ≥25mm were scanned daily using B-mode ultrasound. When the largest follicle reached 35mm and evident uterine edema was detected mares were randomly distributed into two groups (n=5 mares/ group): control (0.9% NaCl 1mL) and hCG (hCG 2500UI) groups. Power-flow Doppler ultrasonography evaluation of the preovulatory follicle was performed immediately before and every 6 hours after treatment (H0 = treatment) until detection of ovulation in hCG group, and from H0 to H48 in control group. Vascular perfusion of the preovulatory follicle was subjectively scored by estimating the percentage of the follicular wall with color Doppler signals during a real-time imaging of 1 minute continuous scans. F-test was used to determine the t-test. The t- test to equivalent variances was used to test the hypothesis of equality between the variables. Differences between groups within time were reported using probability of $P \leq 0.05$ to indicate significance. Data are presented as mean±S.E.M.

Results and Discussion

All mares from hCG group ovulated between 30 and 36 hours post-treatment, while no ovulation occurred during the first 48 hours in control mares. Preovulatory follicle vascular perfusion was low and statistically similar ($P>0.05$) in control and hCG groups at H0 (18.8%±5.4 and 31.3%±6.7, respectively) and higher for hCG groups ($P<0.05$) from H6 to H30. Similar to the described previously [3], dominant follicles showed a progressive increase on vascular perfusion until ovulation in both groups. According to Gastal [3], the initial increase on follicle vascular perfusion of mares treated with saline or hCG is similar during the first 36 hours after treatment, however, when normalizing data to moment of ovulation, differences between groups and among moments are detected and followed by a decrease during the 4h period before ovulation. Ours findings indicate a more intensive increase on vascular perfusion after hCG treatment and no decrease previous to ovulation, probably due to the high interval of ultrasonography exam used in this study. In conclusion, early hemodynamics changes in the dominant follicle are observed after the induction of ovulation with hCG in mares that ovulated until 36h post-treatment.

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In vitro maturation of bovine oocytes under chemically defined or serum-containing media

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Introduction

It has been demonstrated that *in vitro* produced bovine embryos have lower developmental competence than *in vivo* produced embryos. Oocyte and embryo culture conditions can affect *in vitro* preimplantation embryonic development. Although serum or bovine serum albumin (BSA) are classically used as protein supplement to improve culture efficiency, different lots of this macromolecules can produce highly variable results. Moreover, serum supplementation has been shown to negatively affect embryo morphology and kinetics. Therefore, the objective of this study was to evaluate the effect of fetal calf serum (FCS), polyvinyl alcohol (PVA) and BSA during *in vitro* maturation (IVM) of bovine oocytes.

Materials and Methods

Slaughterhouse derived cumulus-oocyte complexes (COCs) were subjected to IVM in maturation medium [Tissue Culture Medium-199 (TCM-199) – Bicarbonate containing 50 μg/mL gentamicin, 0.2 mM sodium pyruvate, 2 μg/mL estradiol and 20 μg/mL FSH] supplemented with 10% (v/v) FCS, 6 mg/mL essentially fatty-acid free BSA[1] or 1 mg/mL PVA[2] for 22 hours at 38.5°C in 5% CO_2. Following IVM oocytes were subjected to *in vitro* fertilization and culture at 38.5°C in 5% CO_2. Cleavage rate was evaluated at day 3 and development to the blastocyst stage was determined at day 9 after fertilization. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS.

Results and Discussion

There was no difference (P> 0.05) on the proportion of oocytes that cleaved (FCS 75 $\pm$ 1.9%; BSA 71 $\pm$ 1.9% and PVA 67% $\pm$ 1.9%) and developed to the blastocyst stage (FCS 21 $\pm$ 3.5%; BSA 23 $\pm$ 3.5% and PVA 18 $\pm$ 3.5%). These results indicated that BSA and PVA could be used as maturation medium supplements to replace FCS without compromising oocyte developmental competence. The establishment of a chemically defined *in vitro* maturation system allows a better understanding of the requirements for development of immature bovine oocytes.

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Evaluation of different freezing curves and centrifugations in Piau swine breed semen criopreservation

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Introduction

Current protocols for boar semen cryopreservation require the centrifugation of semen in order to separate sperm cells from the seminal plasma, but very little attention has been paid to the centrifugation step. The present study aimed to investigate the effect of centrifugation protocol and freezing curve in semen freezing ability of the Piau swine breed, an important endangered Brazilian naturalized breed (1).

Material and Methods

Thirty collections of semen from five males were made and submitted to freezing, according Bianchi et al. (2), using lactose-egg yolk media composed by the cooling extender (80% of β-lactose solution, 20% of egg yolk and 100 µg/mL of canamycin sulfate) and freezing extender (72.5% lactose at 11%, 20% egg yolk, 6% glycerol and 1.5% of Orvus-es-paste). For each collection, sperm-rich fraction was diluted and cooled to 15 ºC before centrifugation and then divided into four treatments: T1 - centrifugation at 2400G for 3 minutes and freezing curve controlled by programmed machine (3); T2 - centrifugation at 800 G for 10 minutes and freezing curve controlled by programmed machine (3); T3 - centrifugation at 2400G for 3 minutes and freezing curve in nitrogen vapor, in which the samples were placed 5 cm above the liquid nitrogen, undergoing approximately -120ºC; and T4 - centrifugation at 800G for 10 minutes and freezing curve in nitrogen vapor. To check post-thawing sperm viability sperm motility, sperm vigor, hyposmotic swelling test, supravital staining, fluorescent assay using carboxifluorescein diacetate and iodidium propide fluorescent probes, sperm morphology evaluation, and sperm binding test to hen's egg perivitelline membrane were assessed. The statistical analysis was made using the statistical program SAEG 9.1. The quantitative data were evaluated by ANOVA, and the means compared by Tukey test, with 5% of error probability. Parameters that did not meet the premises of ANOVA were assessed by non-parametric analysis, with mean comparison by the Kruskal Wallis test.

Results and Discussion

The mean total motility and spermatic vigor were: 44.0 ± 10.3 and 3.1 ± 0.4 for T1, 44.3 ± 7.7 and 3.0 ± 0.3 for T2, 39.2 ± 10.2 and 2.9 ± 0.4 for T3 and 38.0 ± 9.2 and 2.9 ± 0.4 for T4, without difference between them ($p > 0.05$). In the analysis by fluorescent probes, T2 (20.5 ± 10.4) and T4 (19.8 ± 10.1) were higher ($p < 0.05$) than T3 (12.8 ± 6.1), but T1 (15.7 ± 8.3) did not differ from the other treatments ($p > 0.05$). There was no difference between treatments in the other complementary tests performed ($p > 0.05$). The time and centrifugation speed and freezing curves used in this study did not affect sperm viability.

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Use of the essential oil of *Myrrhinium atropurpureum* Schott on stallion cooled semen

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Introduction

Cooled-shipped semen is a routine method in horse breeding management. However, when stored over more than 24 h at 5°C, sperm motion characteristics decline significantly (1). Loss of motility and fertilizing ability has in part been attributed to lipid peroxidation of the sperm plasma membrane caused by the generation of reactive oxygen species during cold storage. Essential oils are natural antioxidants extracted from plants containing primary phenolic agents with reduction capacity; thus, show a positive effect on sperm viability (2). Also, some plant oil extracts have antimicrobial properties. The objective of this study was to evaluate the influence of *Myrrhinium atropurpureum* Schott essential oil in stallion semen cooled at 5°C.

Material and Methods

Essential oil was obtained by hydrodistillation of leaves from *Myrrhinium atropurpureum* Schott. After boiling, vapor was collected and treated with Na_2SO_4 for water removal and stored at 5°C until use. For semen assay, eleven ejaculates from four stallions were used for cooling at 5°C using INRA-96 as extender with or without essential oil. Five concentrations (v/v) were tested: 1%, 0.1%, 0.01%, 0.001% and 0.0001%. Samples without essential oil was used as negative control. The following parameters were evaluated: total and progressive motility, sperm membrane integrity (CFDA+PI) and sperm membrane functionality (HOST). Data were analyzed by repeated measures ANOVA with Bonferroni as post-test, with a significance of 5%.

Results and Discussion

A spermicidal effect of *M. atropurpureum* essential oil was observed when extender with 1%, 0.1% and 0.01 % (data not shown) of oil was used. However, no differences were observed between the lower concentrations (0.001 to 0.0001%) and the negative control (table 1).

Table 1. Seminal parameters of stallion semen preserved with or without *M. atropurpureum* essential oil

	1 hour			2 hours			24 hours		
	Control	0.001%	0.0001%	Control	0.001%	0.0001%	Control	0.001%	0.0001%
TM	59.1 ± 27.0	46.5 ± 32.6	59.5 ± 26.8	56.8 ± 27.1	36.8 ± 29.9	58.6 ± 26.1	41.4 ± 26.7	28.6 ± 26.1	41.8 ± 30.3
PM	46.9 ± 23.4	32.5 ± 18.9	44.4 ± 22.3	34.4 ± 21.1	19.4 ± 17.2	36.9 ± 20.2	20.0 ± 14.9	18.1 ± 15.3	25.0 ± 14.1
MI	81.0 ± 7.3	78.7 ± 9.0	81.0 ± 6.1	78.0 ± 6.8	73.2 ± 9.7	79.6 ± 5.1	67.6 ± 16.2	65.6 ± 24.4	72.9 ± 20.4
MF	48.6 ± 13.2	44.5 ± 17.6	51.7 ± 15.4	46.5 ± 16.2	40.3 ± 17.5	51.0 ± 14.2	40.9 ± 18.6	36.3 ± 19.4	48.1 ± 19.2

TM – total motility; PM – Progressive motility; MI – membrane integrity; MF – membrane functionality.

The present results show that concentrations under 0.001% of *M. atropurpureum* essential oil in semen extender do not affect sperm quality in vitro. This data is important to permit further experiments to assess the antimicrobial properties of the *M. atropurpureum* essential oil in semen doses. Use of commercial antibiotics in cooled and frozen semen is criticized due to problems of drug resistance of bacterial infections in female reproductive tract. Therefore, other antimicrobial additives to extenders like plant extracts may be a valuable tool in semen technology.

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Effect of Trolox on *in vitro* viability of frozen goat sperm

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Introduction

Trolox is a non enzymatic antioxidant that acts preventing formation (1) and scavenging of ROS (reactive oxygen species) formed during lipoperoxidation (2). Then, the aim of this study was to evaluate the effect of Trolox on *in vitro* viability of frozen goat sperm.

Material and Methods

Six goat semen *pools* were collected by artificial vagina from five matured breeders, divided in four aliquots and diluted with skim milk based-extender, added with antioxidant (T0= 0mM; T100= 100mM; T200= 200mM; T300= 300mM of Trolox). After, the samples were packed in straws, frozen in automatic system (TK 3000) and stored in liquid nitrogen (-196 °C). Semen samples were thawed (37 ºC/ 30 sec) and analyzed to motility in automatic system (CASA), plasma membrane integrity (PMi) using double staining with carboxyfluorescein diacetate and propidium iodide (3) and mitochondrial membrane potential (MMP) with JC-1 (4). All results were statistically analyzed by ANOVA, with significant level of 0.05.

Results and Discussion

Data are shown in the table below. After evaluation of post-thawed goat sperm, were not observed significant differences among experimental groups to all kinematic parameters, as well as PMi and MMP. These results can be the consequence of the compromising of Trolox effect as result of factors as storage conditions, membrane lipid composition, antioxidant concentrations on the semen (5) and on the extender (6). In conclusion, the results demonstrate that Trolox (100, 200 and 300mM) did no protect the goat sperm against the deleterious effects of cryopreservation.

Table 1. Mean and standard deviation of post-thawed goat sperm frozen with Trolox.

	Experimental groups			
Parameters	T0	T100	T200	T300
MT (%)	71.92 ± 25.82	78.05 ± 14.51	85.80 ± 6.45	76.52 ± 18.19
VCL (μm/s)	114.94 ± 5.92	117.74 ± 9.43	124.01 ± 6.14	125.85 ± 10.12
VSL (μm/s)	68.27 ± 6.28	66.78 ± 4.72	66.25 ± 3.30	66.92 ± 6.23
VAP (μm/s)	81.54 ± 7.97	83.83 ± 4.68	84.11 ± 2.12	86.20 ± 4.49
LIN (%)	59.34 ± 3.40	57.13 ± 7.21	53.60 ± 4.82	53.53 ± 7.02
STR (%)	83,77 ± 7.97	79.89 ± 7.54	78.82 ± 4.76	77.54 ± 4.23
WOB (%)	71.09 ± 3.74	71.38 ± 3.79	67.96 ± 3.74	68.79 ± 5.46
ALH (μm)	3.67 ± 0.37	3.62 ± 0.55	4.08 ± 0.68	3.92 ± 0.44
BCF (Hz)	12.22 ± 1.99	12.03 ± 1.52	10.88 ± 0.45	11.23 ± 1.54
iMP (%)	38.24 ± 7.90	42.50 ± 8.17	39.86 ± 9.96	44.08 ± 3.80
aPMM (%)	8.58 ± 6.96	8.71 ± 7.28	15.53 ± 15,17	15.13 ± 14.26

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Evaluation of cooling time of canine semen diluted in Tris-egg yolk containing or not glycerol and antioxidant

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Introduction

The cooling process lowers costs and damages sperm cells. The production of Reactive Oxygen Species (ROS) results from the normal metabolism of cells submitted to aerobic conditions and causes a type of injury to sperm cells; therefore, extracellular antioxidants are supplementary under *in vitro* conditions (1). Furthermore, the addition of glycerol to the extender aims to provide osmolality of the medium to protect the cell against thermal damage, but how cryoprotectants work is still not fully elucidated (2).

Material and Methods

Semen was collected from twelve dogs and a pool of semen was originated from three animals. Volume, spermatic motility, vigor and concentration were determined before each pool was divided. Each pool was allocated in three treatments: Tris- egg yolk (3) (Control); control extender containing 7% glycerol (G), control extender and antioxidant (Trolox, 0.25 mMol) (T) or control extender and glycerol and Trolox (GT), submitted to morphological evaluation, hyposmotic and fluorescence test, lipid peroxidation and eosin-nigrosin staining technique. Then, the samples were chilled at 4°C for 48, 72 and 96 hours. The morphology spermatic and additional tests were replicated for each extender every cooling time.

Results and Discussion

The treatments Control and T differed from G and GT and showed, respectively, the following parameters of spermatic motility (56% and 47.7% vs 34.7% and 31.2%), sperm vitality by eosin-nigrosin staining (36.8% and 36.9% vs 18.2% and 15.2%) and measurement of free radicals (2.4 and 1.6 vs 0.6 and 0.7nMolTBARS). However, the integrity of the plasma membrane by hyposmotic and fluorescence tests did not differ ($p>0.05$) among treatments neither along cooling time nor did the total spermatic defects. No difference ($p>0.05$) was found among treatments in fresh semen for all tests. Some parameters differ for cooling time. For spermatic motility chilled after 48 hours, Control and T treatments differed from G and GT (59% and 55.3% vs 28.2% and 28 %; $p<0.05$); after 72 hours, the Control and T treatments differed from GT (48.4% and 35.9% vs 15.6%; $p< 0.05$); and G differed only from T treatment (22.2%; $p<0.05$); after 96 hours, there was a difference among Control and G and GT treatments (37.4% vs 11.9% and 6.9%; $p<0.05$), which did not differ from T (23.4%; $p>0.05$). For eosin-nigrosin staining, Control and T treatments differed from G and GT, respectively (40.3% and 41.7% vs16.9%and 13.7%) after 48 hours, (35% and 32.6% vs 13.9% and 8.3%) after 72 hours, and (21.5% and 26.4% vs 6% and 7.1; $p<0.05$%) after 96 hours cooling. There was a difference for the production of free radicals among Control, G and GT treatments (1.63 vs 0.54 and 0.52n/Mol TBARS; $p<0.05$), and T treatment did not differ (1.36n/Mol TBARS; $p>0.05$) after 48 hours of cooling. After72 hours, Control differed from GT (1.8vs 0.6n /Mol TBARS; $p<0.05$), but both did not differ ($p>0.05$) from T and G (1.6 and 0.8n/Mol TBARS), and after 96 hours, T differed from G (1.79 vs 0.56n/Mol TBARS; $p<0.05$), and both did not differ from Control and GT (1.4 and 0.8n/Mol TBARS). Antioxidants did not have an effect on sperm quality, since the extender T did not differ from Control in all tests over cooling time. G and GT treatments decreased sperm quality to motility and eosin-nigrosin staining parameters. Glycerol may induce cellular damage through two distinct mechanisms, either by an osmotic effect and/or by a biochemical one (4). According (5), the toxicity of glycerol as a cryoprotective agent in semen diluents reduces sperm motility and alters the acrosome integrity by interfering with the permeability of the sperm membrane. (6) reported that glycerol induced cellular damage, since a significant decrease of the intact membrane percentage was observed in samples of stallion semen incubated in the presence of 5% glycerol. The treatments with addition of glycerol showed a lower production of free radicals and this can be explained by the ability of glycerol to scavenge the hydroxyl radical (7). It was concluded that the addition of an antioxidant to the extender did not improve sperm quality in relation to the Control in all additional tests, and the addition of 7% glycerol associated with antioxidant reduced canine semen quality in spermatic motility and eosin-nigrosin staining test, even though fluorescent and hyposmotic tests show the same results.

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The effects of the uterine biopsy, uterine washing and follicular aspiration procedures performed six days after TAI on pregnancy rates of nelore cows

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Introduction

In cattle, estrous cycle events that surround ovulation exert direct influence on the uterine capacity to produce, secrete and metabolize molecules of different nature, implicating in obvious consequences to the establishment and maintenance of pregnancy. Identifying a molecule or group of molecules associated with probability of pregnancy success, may potentially contribute to the decision-making process during selection of recipient cows prior to embryo transfer. Having access to reproductive tissues that are responsive to the endocrine fluctuations of the reproductive axis and, therefore, may represent the local molecular and/or biochemical profile associated with uterine receptivity, without disturbing pregnancy is essential. However little is known about the implications of such invasive techniques on pregnancy maintenance. In order to identify such molecules, reliable sampling techniques need to be developed and tested. The objective was to evaluate the impact of uterine biopsy, uterine washing and follicular aspiration procedures performed on D6 of pregnancy on pregnancy rates measured on 30 and 60 days after TAI.

Material and Methods

On experiment 1, 155 cows (Nelore and Guzera) were randomly inseminated, at fixed-time, with semen from 7 different bulls of proven fertility. On D6 post-TAI, cows were divided into control (n=50) endometrial biopsy (n=55) or uterine washing (n=50) groups. Biopsies were performed using a Yomann biopsy forceps and endometrial fragments from the uterine horn contralateral to the ovary containing the corpus luteum (CL) were collected. The uterine horn, contralateral to the CL, was washed with 20 ml of PBS using a Foley catheter. After massage of the uterine horn, washing was collected in a serynge by suction. On experiment 2, 82 Nelore cows were inseminated at fixed-time. On D6 post-TAI animals were divided into control (n=41) and aspirated (n=41; aspiration of the dominant follicle) groups. Pregnancy diagnosis was performed by ultrasonography on days 30 (Exp. 1 and 2) and 60 (Exp. 1) post-TAI.

Results and Discussion

On experiment 1, uterine biopsy and washing caused a decrease of 30.8% and 32.4% on pregnancy rates at D30 post-TAI, respectively, in comparison to control group (37%). Effects of both procedures on pregnancy rates at D60 were more pronounced (42.7% and 38.5% for the biopsy and washing groups, respectively). A comparison between pregnancy rates on D30 and D60, per group, showed a decrease of 12%, 27.3% and 20% in control, biopsy and washing groups, respectively. The decrease in these rates is likely due to embryonic loss, commonly observed during this period of gestation. The overall reduction in pregnancy rates from D30 to D60 might be explained by the influence of the sampling procedures on placentation, which takes place at around 45 days of gestation. As for experiment 2, pregnancy rates measured on D30 post-TAI were 53% and 56% for the aspirate and control groups, respectively, suggesting no harmful effect of the procedure on pregnancy maintenance. In conclusion, although uterine biopsy and washing affect negatively pregnancy rates, neither procedure can be considered incompatible with pregnancy maintenance. Additionally, follicular aspiration was shown to be an innocuous method for sampling a pregnant reproductive tract in cattle.

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Short and long protocols to estrus synchronization and timed-AI in Dorper and White Dorper purebred ewes

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Introduction

The estrus synchronization protocols for seasonal ewes use a 14 days progesterone priming that turns viable the timed-AI with frozen semen by laparoscopy (1). However, breeds that are less seasonal or not seasonal could get a good response and results on timed-AI using short protocols. The aim of this work was to compare three estrus synchronization protocols with different progesterone primings and timed artificial insemination (TAI) in purebred Dorper (D; n=207), and White Dorper (WD; n=289) sheep.

Materials and Methods

In November 2011 (n=244) and March 2012 (n=252), sheep were given an intravaginal insert containing 330mg progesterone (Eazi-breed CIDR , Pfizer, Brazil) for 14 (CIDR14; n=165), 9 (CIDR9; n=164) or 6 days (CIDR6; n=167). Of these animals 264 were yearling lambs (D= 138; WD= 126) and 232 ewes (D= 69; WD= 163). At CIDR withdrawal every animal received 400IU of eCG (Folligon, Intervet, Brazil) and, in addition, 10mg of Dinoprost (Lutalyse, Pfizer, Brazil) in the groups CIDR6 and CIDR9. Teasers were used for sexual stimulation, but estrus behavior was not recorded. Forty-eight hours after CIDR withdrawal inseminations were performed by laparoscopy with 0.4 ml of fresh semen diluted in PBS (1:5), with an average concentration of 150 x 10^9 spermatozoa per uterine horn from seven White Dorper and six Dorper rams. To avoid variability in the moment of TAI among treatments, all the inseminations were random performed between 7:00am and 3:00pm, which was 420 in the morning (M) and 76 in the afternoon (A), with an average of 2.3 minutes per animal. Pregnancy was detected by ultrasound (Honda HS1500, Japan) with a 5MHz linear array probe forty-one days after the inseminations. The analyses were conducted with a generalized linear model for binary data for DG with a logit link function (Software SAS® -SAS Institute, 1999)(2). The model included the fixed effects of treatment (6, 9 or 14 days), period of insemination (M or A), animal category (yearling lamb or ewe), breed (WD or D), beyond the doubles and triple interactions between the factors treatment, category and breed. The average of all data was corrected by List square means methodology.

Results and Discussion

No effect of treatment (58,2% CIDR 6; 66,4% CIDR 9; 69,5 CIDR 14; p=0,18) was verified over pregnancy rates, as well as TAI period effect (M=64,7%; A= 64,4; p=0,77), which an pregnancy rate average of 64,7% (321/496). There was a difference between breeds (D=56,8% vs. WD= 74,0%; p<0.01) and between ewes and yearling lambs (61,3% vs. 70,3% respectively; p<007), evidencing higher fertility rate by the synchronization and TAI for White Dorper than Dorper, and for yearling lambs than ewes. Although all the rams have been evaluated previously, the lower result for Dorper was mainly due to four rams, which fertility rates were lower than 55%, while on White Dorper the result was among 60 e 90% in 6 of 7 rams used. High fertility rates obtained for yearling lambs support the use of TAI by laparoscopy in this animal category due to the lower fertility rates following vaginal insemination Compared to the 14-day protocol, both short protocols resulted in adequate pregnancy rates, thus becoming an alternative for TAI programs using fresh semen by laparoscopy in Dorper and White Dorper sheep, that optimize the use of good and high genetic value rams on herd.

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Xenograft as a tool to develop equid chimeric testis

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Introduction

In most species investigated, grafting of testis fragments and cell aggregates under the dorsal skin of an immunodeficient host mouse have resulted in full spermatogenesis and the production of fertile sperm (1). Particularly, *de novo* testis morphogenesis shows the ability of isolated testicular cells to recognize and to interact with each other. Although spermatogenesis does not advance beyond primary spermatocytes in mules, previous studies from our laboratory suggested that their testicular somatic cells and the testis environment are functional. In this context, the present study aimed to investigate the capacity of mule and equine testicular cells to functionally interact after enzymatic dissociation, and also whether the equine germ cells could colonize the mule testis fragment after the co-grafting (testis graft plus isolated equine germ cells) approach.

Material and Methods

After the testicular enzymatic dissociation, xenografts were performed according to the following experimental protocols: i) a single-donor cell suspension (mule); ii) a mixed cell suspension [equine germ cells and testicular mule somatic cells (Sertoli, Leydig, peritubular myoid and others)]; and iii) mule testis fragment + isolated equine germ cells. The evaluation of *de novo* testis formation and the development/status of the recovered xenografted fragments were performed at 3, 5 and 7 months after grafting.

Results and Discussion

Three months after grafting, the samples evaluated derived from mule single-donor cell suspensions presented a typical testicular arrangement, with "*rete testis*" and seminiferous cords/tubules containing Sertoli and spermatogonial cells. At 5 months a noticeable mule *de novo* testis formation was observed and primary spermatocytes were the most advanced germ cell type found. Considering the mixed cell suspensions, round spermatids were observed in the seminiferous tubules at 5 months after xenografting. At 7 months post-grafting, a similar result was obtained (presence of round spermatids) from mule testis fragments mixed with equine germ cells. Because postmeiotic germ cells are rarely found in the mule testis, our results strongly suggest that these haploid cells were originated from equine germ cells. Altogether, these very promising results indicate that mule and horse testicular cells were able to functionally interact providing a valuable tool to investigate the regulatory mechanisms involved in the testis development and cell interactions in equids. However, it still remains to be investigated whether horse spermatogenesis will result in the production of viable sperm. (CETEA/UFMG/Protocol#56/2011).

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Effect of nutritional flushing on *in vivo* development of demi-embryos from superovulated Dairy Gir cows

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Introduction

The diffusion of embryo bisection technique would increase the offspring of genetically superior animals and maximize the efficiency of commercial programs of embryo transfer (ET) and would make viable the experiments with monozygotic twins.

Material and Methods

The experiment was carried out with 26 primiparous healthy Dairy Gir cows, reproductively good, body weight (BW) over 350kg, with 45-90 days postpartum, body condition score 2.0-2.5 on the scale of 1 to 5 (1), aged 36-48 months, which were distributed randomly into two treatments with (TF) and without nutritional flushing (TC). In both, the cows were submitted to the same superovulation protocol (SP). In TF the cows received energetic supplementation with 75% corn, 23% soybean meal and 2% of mineral salt, in a percentage of 1% BW, from six days before the beginning of SP to 16 days after, twice daily. Ultrasonographic evaluations were done 7 days and 1 day before the beginning of SP and at the embryo recovery day. Superovulation response was based on the corpus luteum (CL) numbers at recovery day, which was performed seven days after AI by trans-cervical technique using the Foley's catheter. The recovered structures were washed 10 times in maintenance medium (TQC® - Holding) and classified (2, 3). Only embryos classified as compact morulae, morulae and blastocyst, and morphologically excellent were selected. Half were transferred intact (IE) to the recipients and half was submitted to bipartition (DE) and classified (4). The fresh IE and DE selected were transferred at random at the collection day to the recipients, previously synchronized for embryo transference at fixed time (FTET). Crossbreed heifers (78) were synchronized for embryo receptors (ER). The pregnancy diagnosis was made 35 days after embryo transference. The results were analysed by descriptive statistics.

Results and Discussion

Five embryo donors in TC and 3 in TF were discarded for some reason. The TF did not increase the CL numbers nor the collected structures. But viable embryos were 18% more frequent in TF donors than TC ones. Only 48 of 78 ER synchronized heifers showed CL of good quality for embryo transfer. Twenty four ER received intact embryos (12 from TC and 12 from TF) and 24 ER received demi-embryos (12 from TC and 12 from TF). The pregnancy rate was 20/48 of ER. The treatment did not affect the percentage of ER pregnants, but its pregnancy frequency was 10% greater in ER that received intact embryo than those that received demi-embryos.

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Use of vitamin E analog (Trolox) in Tris egg-yolk extender for canine epididymal sperm cells freezing

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Introduction

The cryopreservation process evolves the production of reactive oxygen species (ROS), which may cause serious damages to sperm cells submitted to this method. Thus, the aim of the present study was to evaluate effects of the addition of 5, 2.5 or 0.5 mMol of vitamin E analog (Trolox) to the Tris egg-yolk extender on frozen canine epididymal sperm.

Material and Methods

Fifteen experimental units (EU), each one comprised by a pool of sperm cells recovered from testis of five healthy dogs, previously submitted to elective orchiectomy were used in this study. The EU were equally divided into three experimental groups (GI, GII, GIII). Additionally, in each group, the EU were divided into two aliquots of equal volume that were immediately centrifuged. The pellets formed were resuspended in Tris egg-yolk extender and in one of the aliquots, 5 (GI), 2.5 (GII) or 0.5 mMol (GIII) of Trolox were added to the extender, however in the remaining aliquot no Trolox was added (Control). The aliquots were freezed in 0.5 mL straws, with $40x10^6$ spermatozoa per straw. The straws were chilled to 5°C for 60 min and frozen at a distance of 6 cm from the liquid nitrogen vapor for 20 min, and then immersed and stored in a cryogenic container. Thawing was performed at 70°C for 8 sec[6] Sperm morphology, progressive motility, vigor, vital test (yellow eosin), membrane integrity (hypoosmotic swelling test and fluorescent probes), mitochondrial activity (3,3 '- diaminobenzidine - DAB) and measurement of lipid peroxidation were assessed immediately after the collection and after freezing/thawing. The data were analyzed by Friedman's test (nonparametric) and Tukey's test (parametric) with $P < 0.05$.

Results and Discussion

In the fresh sperm cell analyses no significant difference ($P > 0.05$) in sperm parameters was noticed among the control and the treated aliquots of each group. However, after thawing the addition of 0.5 mMol or 2.5 mMol of Trolox promoted a slight, although not significant increase in progressive motility and vigor, corroborating with the findings performed on canine post-thawed semen using 0.6 mMol of vitamin E (1). Additionally, despite the lack of significance ($P > 0.05$) the addition of 5 mMol of vitamin E analog was deleterious to the epididymal sperm cells. Similar results were described for canine semen (2) and it is believed that the decrease in sperm viability is due to the fact that the vitamin E in high concentrations inhibits the lipid peroxidation by removing peroxyl radicals (3, 4). Differently from the findings obtained from feline epididymal sperm cells that maintained membrane and DNA integrity after the addition of Trolox ($P > 0.05$) (5), in the present study no significance was noticed among the control and treatments aliquots when assessing membrane integrity by the hyposmotic swelling test, fluorescent probes stain and yellow eosin stain. The mitochondrial activity obtained by the difference of fresh and post-thawed epididymal sperm cells presented percentage of GI: 38% (C) x 44% (T); GII: 29% (C) x 26% (T); GIII: 33% (C) x 14% (T). These values show that 0.5 mMol of Trolox significantly maintained the mitochondrial activity after thawing while 2.5 mMol and 5 mMol decreased this parameter. Statistically, the lipid peroxidation test did not present significance among treatments. Thus, we conclude that despite the similarity to the control aliquots the concentration of 0.5 mMol showed better results than the 2.5 and 5 mMol of Trolox in canine sperm cells after thawing.

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Isolation and characterization of fibroblasts and adipose derived stem cells from rabbits

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Introduction

Fibroblasts and adipose derived stem cells (ADSCs) are easily collected cells, isolated by a procedure that is simple and safe for the patient or animal. From these, it is expected to produce induced pluripotent stem cells (iPS) by the introduction of four transcription factors (Oct3/4, Sox2, c-Myc, and KLF4).

Materials and Methods

The research is being judged by the ethics committee of FMVZ / USP under Protocol No. 2560/2012. The gathering of samples was performed at the slaughterhouse for rabbits; fibroblasts were isolated from cartilage tissue from the ears and ADSCs from inguinal subcutaneous adipose tissue. So far, the cells were grown and frozen in several passages, they were morphologically analyzed and the cell growth curve and colony has been realized with 3 x 10^4 cells cultured in 60 mm culture dishes. Cells were counted each 48 hours. The cultures shall be immunologically characterized by immunocytochemistry and flow cytometry further on.

Results and Discussion

The isolated ADSCs showed morphology characteristic of mesenchymal stem cells, that being, fibroblastoid, stretched, refringent and with a high proliferative potential. Cells proliferate until twelfth passage in the study of growth curve. With the production of iPS, we expect to develop a Brazilian technology still unpublished in rabbits, and to create a lineage of iPS cells for future application in cellular therapy.

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Characterization and culture of Nile-tilapia spermatogonial stem cells

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Introduction

Spermatogonial stem cells (SSCs) continuously undergo self-renewal division to support spermatogenesis and are exceptional adult stem cells capable of transfer genes to new generations. Recently, we established a novel SSC transplantation technique in adult Nile-tilapia (*Oreochromis niloticus*) that successfully resulted in donor-derived offspring (1). Besides being the only functional approach available to study SSC biology this technique have the potential to be a very powerful tool for producing transgenic animals through transplantation of genetically modified SSCs. Nevertheless, there is no available technique to prospectively isolate SSCs prior to transplantation in fish. Here, some potential molecular markers, which could be properly used to characterize and select SSCs were evaluated. In addition, we investigated the effective long-term culture conditions for propagating tilapia SSCs *in vitro*.

Material and Methods

First, adult tilapia testes were evaluated for the presence of specific markers for undifferentiated spermatogonia in mammals (GFRA1, NOTCH1, POU5F1, Nanos1 and Nanos2) through immunohistochemistry, western blot and flow cytometry. Additionally, testicular cell suspensions containing tilapia SSCs were enriched by density gradient centrifugation and were cultured in the presence of Epidermal Growth Factor, basic Fibroblastic Growth Factor, fish serum from adult tilapia and fish embryo extract from 7-day-old tilapia embryos. Cultured SSCs were assayed for viability and proliferation using Alamar Blue and by BrdU incorporation. The DNA content (cell ploidy) of cultured spermatogonia and freshly isolated tilapia testis cells was determined by the propidium iodide method. After long term-culture the expression of specific SSCs markers was analyzed by immunofluorescence, RT-PCR and western blotting.

Results and Discussion

Immunolocalization revealed that GFRA1, NOTCH1 and POU5F1 are expressed exclusively in single type-A spermatogonia (presumptive SSCs) preferentially in those located at the blind ending of the seminiferous tubules. Likewise, the expression of Nanos1 and Nanos2 proteins was observed in single type-A spermatogonia at the seminiferous tubules blind bottom; however Nanos1 and Nanos2 positive spermatogonia have also been identified in cysts with two to eight germ cells. In the specific *in vitro* conditions here investigated tilapia SSCs were able to be cultured and actively proliferate for at least one month with a doubling time between 3 and 4 days, as observed by Alamar Blue assay and BrDU incorporation. Also, they remained euploid (unchanged DNA content) after at least 10 passages. Importantly, immunostaining revealed that cultured tilapia SSCs retained the expression of Vasa, GFRA1, POU5F1, Nanos1, Nanos2 and Neurogenin3. Moreover, the number of positive cells for these markers noticeably increased during the entire culture period. Therefore, besides allowing a better knowledge on fish SSCs biology, these findings provide the first step in establishing a system that will allow fish SSCs expansion *in vitro*, representing an important progress toward the development of new biotechnologies in aquaculture, specially the genetic manipulation and modification of SSCs for the production of transgenic fish.

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Profile SDS-PAGE of seminal plasma in sambar deer (*Cervus unicolor*)

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Introduction

Seminal plasma is a complex of secretions of the male accessory reproductive organs and appears to exert important effects on sperm function (1). The protein quality of the seminal plasma may affect positively the animal fertility (2). Peptides of 55 and 66 kDa were present in excellent spermatic conditions for example motility and vigor (3). On the other hand, 13 and 33 kDa peptides were observed in association with unfavourable spermatic conditions (3). The objective of this study was to determine the profile SDS-PAGE of seminal plasma and evaluate the semen characteristics in sambar deer, *Cervus unicolor*.

Material and Methods

Semen from four sambar deer, 36 months old, was collected by electroejaculation (Autoejac®, Neovet) during spring (november). From each male a total of 5 samples were collected on days: 0, 7, 14, 21 and 28, totalizing 20 ejaculates. Semen characteristics were evaluated according to (6) and subsequently compared regarding the composition of seminal plasma proteins. Samples of seminal plasma were centrifuged (1500g/15min) and conditioned in criotubes and stored at –20ºC. Proteins were extracted from 200 µL of each sample in 2 mL of extraction buffer composed by 0.625 M Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol and 20% of glycerol. Proteins were quantified according to (4) and electrophoresis was performed according to (5). Gels were fixed with isopropanol: acetic acid: water (4:1:5 v/v) for 30 minutes, and stained in the same solution containing 2% of Comassie Blue R250. Each semen collection was used in duplicate. The concentration of proteins was measured using a spectrophotometer PF-901(Chemistry Analyser Labsystems). Gels were submitted to a photodocumentation system (Bio Doc-IT and Visidoc-IT Gel Documentation systems, UVP) and analysed by Doc-IT-LS 6.0 software. GLM from SAS, version 6, was used in order to evaluate possible variations of seminal variables and protein molecular mass. Statistical significance was accepted from $P<0.05\%$.

Results and Discussion

The means of semen variables were: volume (0.75±0.19 mL), motility (78.5±6.2%), vigor (4±0), concentration (980±210x10^6/mL), major defects (8.3±2.2%) and minor defects (14.5±4.8%). The results of analyses of gels revealed a variety of proteins in each animal. There were 30 different major polypeptides, ranging from 16 to 22 bands in each individual deer. In two animals the presence of low molecular weight (LMW 13kDa and 33kDa) proteins was not associated with low motility differing of (3) in bulls. In all animals, 55kDa, 66kDa or 80kDa proteins were present and associated with a satisfactory semen condition in accordance with (3). In cattle, the 55, 66 and 80kDa proteins are associated positively with camp-dependent progressive motility (2). Consistently, in the present experiment, there was a positive relationship of presence of seminal plasma proteins 55kDa, 66kDa and 80kDa and semen quality (motility and major defects). The presence of these proteins suggests an increase in semen quality.

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Exposition to low concentrations of endosulfan did not affect the epididymal morphometry in *Artibeus lituratus*

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Introduction

Evidence has been accumulating indicating that humans and wildlife species have suffered adverse health consequences from exposure to environmental chemicals that interact with the endocrine system (1). Endosulfan is a broad-spectrum cyclodiene insecticide that may act as an endocrine disruptors (2). Considering that the epididymis is an androgen-dependent organ, and its function on the sperm maturation and storage can be potencially affected by the deleterious effects of endosulfan, we aimed to evaluate the effect of different concentrations of endosulfan on epididymal morphometric parameters in neotropical fruit bats.

Material and Methods

Twelve male bats (*Artibeus lituratus*) from the Atlantic Forest (MG, Brasil) were collected and divided into four groups. Animals from G1 (control group; n=3) were fed with papaya, and the other three groups received papaya embedded with adhesive spreader Wil Fix (G2; n=3), and 0,3% (G3; n=3) and 0,6% of endosulfan AG (G4; n=3). Bats were maintained in individual cages while submitted to those treatments during five weeks. They were euthanized and their epididymis sectioned to obtain a fragment of each epididymal region, caput and cauda. The fragments were fixed in Karnovsky solution (24 h) and embedded in methacrylate plastic (Historesin®). The following measurements were performed: tubular (TD; µm) and luminal diameters (LD; µm), epithelium height (EH; µm), and volumetric proportion (%), considering tubular (epithelium, basal lamina, lumen with spermatozoa – LWS, and lumen without spermatozoa- L) and intertubular compartments (blood vessels and connective tissue). All the measurements were estimated using the Image Pro-plus software, and the results were analyzed by ANOVA and Newman Keuls tests ($P < 0.05$)

Results and Discussion

There were no differences among experimental groups for tubular and luminal diameters and epithelium height, considering both epididymal regions ($P > 0.05$). Regarding the volumetric proportion, differences among treatments were observed only in the caput region for one parameter of tubular compartment (Tab 1).

Table 1. Volumetric proportion of tubular compartments (%) in the caput region of epididymis from fruit bats (*Artibeus lituratus*).

	G1	G2	G3	G4
Epithelium	55.63 ± 12.89[a]	56.23 ± 6.97[a]	52.90 ± 2.21[a]	48.82 ± 3.64[a]
Basal lamina	32.87 ± 9.89[a]	31.59 ± 7.36[a]	36.18 ± 7.12[a]	38.0 ± 5.69[a]
LWS	3.94 ± 3.5[a]	3.77 ± 2.17[a]	6.61 ± 6.06[a]	9.97 ± 4.26[a]
L	7.56 ± 3.72[a b]	8.41 ± 1.94[a]	4.31 ± 1.11[b]	3.21 ± 0.01[b]

Mean ± SD; [a,b] Within a row, means without a common superscript differed ($P < 0.05$).

Our results are in agreement with others which also showed no testicular alterations in response to the exposition to low endosulfan concentrations in rats, as well as similar values of sperm concentration in epididymal lumen and seric testosterone following treatment (3). We conclude that a 5-weeks exposition to low concentrations of endosulfan did not cause alterations on the epididymal histomorphometry of fruit bats.

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Semen cryopreservation in ocelots (*Leopardus pardalis* Linnaeus, 1758): comparison of two methods

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Introduction

The cryopreservation of wild animals' semen has been used for AI and also in building genome resource banking of endangered species. Described techniques are not still applicable to all felid species, increasing the need in developing specie-specific protocols.

Material and Methods

This research was approved by the Ethic Committee (CEUA, UFPR – Campus Palotina, protocol 26/2010). Two curves were tested for cryopreservation of ocelot semen samples (n=20) in four males collected five times each, cryopreserved with commercial diluent (Test Yolk Buffer®, Irvine Scientific, Santa Ana, CA, USA): A curve (GEL) using conventional refrigerator and ice chest with 5 cm of liquid nitrogen, ranging from 20ºC to 5°C in an hour, stabilized at 5°C during 1h: 30min and frozen from 5°C to -196°C in 4 min. Another freezing curve (TK) performed by the cryopreservation equipment (TK-3000, TK - *Tecnologia em Congelação Uberlandia-MG*, Brazil) decreased from 20°C to 5°C in an hour and stabilized at 5°C at 1h:30min, with a cooling rate of -20°C/min to reach a temperature of -120°C for immersion in liquid nitrogen. Samples were thawed and analyzed for motility (0-100%), status (0-5) and sperm motility index (SMI), morphology and percentage of acrosome lesions using POPE staining (Fast green and Rose Bengal).

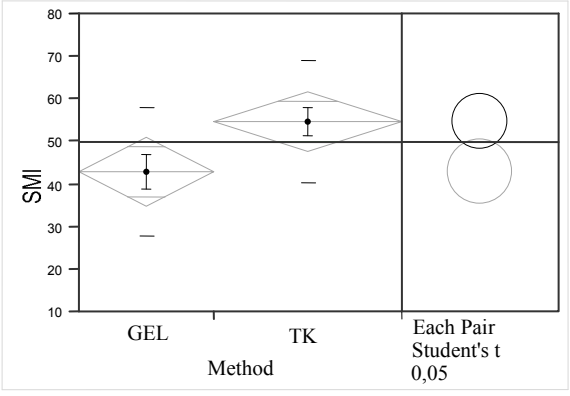

Figure 1. Freezing curves (GEL and TK) SMI results using ocelot semen.

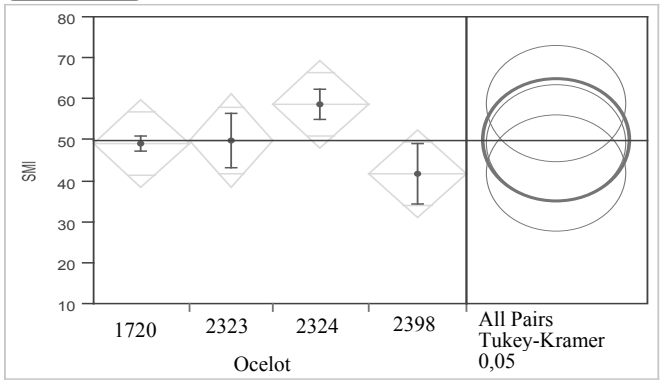

Figure 2. Post thawing SMI in semen of ocelots (n = 4).

Results and Discussion

Semen frozen using TK curve showed an SMI mean (54.86±3.49) higher (p=0.038) than GEL (43.15±4.10) curve (Fig. 1), using Student *t* test. There was no significant difference (p=0.193) for SMI between male ocelots (Fig. 2), using ANOVA with Tukey-Kramer test. The percentage of acrosome lesions presented in TK and GEL curves were 47.2% and 52.7%, respectively (p=0.354), using Student *t* test. These sperm samples showed a mean of 20.85±11.09% of morphological abnormalities. The use of automatic equipment, with temperature programming, improved cryopreservation results of ocelot semen.

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Short-term preservation at 5ºC of Armadillo's (*Euphractus sexcinctus*) semen

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Introduction

In spite of the ecological importance of six-banded armadillos (*Euphractus sexcinctus*), knowledge on their reproductive aspects is scarce. Recently, semen collection (1) and evaluation (2) was demonstrated in this species; however, there is no report on its semen preservation. In domestic species, extenders based in Tris (3) or coconut water (4) have been shown as effective for semen preservation under low temperatures. Thus, we aimed to evaluate the short-term preservation at 5 ºC of armadillo's semen using different extenders.

Material and Methods

The ejaculates from 04 adult male six-banded armadillos, bred under captivity at the Centre of Multiplication of Wild Animals (CEMAS – UFERSA, Mossoró, RN, Brasil), were collected by electroejaculation (3). Semen was evaluated for sperm motility (%) and viability (%). Samples were divided in aliquots that were diluted in Tris at 310 mOsm/L, powdered coconut water (ACP®) at 300 mOsm/L or ACP® at 380 mOsm/L, which is the physiological osmolarity of the armadillo ejaculate. Dilutions were conducted on a proportion of one part semen to one part extender. Samples were stored in a biological oxygen demand incubator (Q315M®, Quimis, Diadema, Brazil) at 5 ºC and evaluated at 3 h intervals during 12 h. The effect of time on semen preservation was evaluated by repeated measures ANOVA ($P < 0.05$). The efficiency of the extenders on the preservation of sperm motility and viability was compared by Scheffe'stest ($P < 0.05$).

Results and Discussion

A total of 9 ejaculates were obtained from 4 individuals. Semen characteristics evaluated during storage are reported in Table 1. No immediate effect of extension in Tris or ACP® was verified on sperm motility or viability ($P > 0.05$); however, sperm motility drastically reduced after 3 h storage ($P < 0.05$). By this moment, no sperm motility was verified for ACP® at 300 mOsm/L, but viability test revealed that there were viable sperm in the sample. In addition, there were no differences among extenders during all the experiment ($P > 0.05$). In general, sperm quality in armadillos was more affected by short-term preservation than that in domestic animals such as ovine (3) and canine (4) that presented higher values for sperm motility even after 24 h storage at 5 ºC. In conclusion, we demonstrate that armadillo's sperm seems to be very sensitive to short-term preservation under low temperature independently of using Tris or ACP® extenders.

Table 1. Values (Mean ± SEM) for sperm motility (%) and viability (%) of armadillo (*Euphractus sexcinctus*) semen preserved at 5 ºC during 12 h, after dilution in Tris or powdered coconut water at 300 (ACP-300) and 380 mOsm/L (ACP-380).

Semen characteristics	Extender*	Fresh semen	After extension	Storing time			
				3 h	6h	9 h	12 h
Motility (%)	Tris	56.6 ± 9^{a}	38.9 ± 11.1^{a}	16.7 ± 8^{b}	18.9 ± 9.6^{bc}	11.7 ± 6.2^{cd}	3.3 ± 1.7^{d}
	ACP-300		30.6 ± 8.1^{a}	0^{b}	0^{b}	0^{b}	0^{b}
	ACP-380		37.2 ± 10.5^{a}	7.8 ± 3.9^{b}	10.9 ± 8.8^{bc}	5 ± 3.5^{bc}	4.2 ± 2.7^{c}
Viability (%)	Tris	48.2 ± 6.7^{a}	44.2 ± 7.5^{a}	28.2 ± 5.3^{b}	24 ± 8.4^{c}	14.1 ± 6.6^{d}	3.2 ± 1.4^{e}
	ACP-300		36.7 ± 8.3^{ab}	22.6 ± 5.8^{b}	20.7 ± 6.2^{b}	8.4 ± 4.9^{c}	4.5 ± 1.7^{c}
	ACP-380		37.4 ± 7.8^{a}	17.6 ± 4.2^{b}	26 ± 8.5^{ab}	14 ± 5.7^{c}	6.5 ± 3.2^{d}

[a,b,c,d,e] Within a row, means for sperm motility or vigor without a common superscript differ ($P < 0.05$). * No differences were verified among extenders in any time ($P > 0.05$).

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Comparison between square-wave and sine-wave electrical stimuli for electroejaculation in captive agoutis (*Dasyprocta aguti*): Preliminary results

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Introduction:
Agoutis (*Dasyprocta aguti*) are Neotropical terrestrial rodents that are distributed from Southern Mexico to Argentina, including throughout Brazil (1). They are currently hunted by the local population that is interested in consuming their meat (2). The use of semen technology associated to artificial insemination could contribute for their conservation and multiplication. However, literature is scarce on this subject and the unique electroejaculation protocol reported for agoutis used sine-wave stimuli (3). It is known that the probes used to stimulate emission, erection and ejaculation in domestic animals transmit an oscillating current of either a sine-wave or pulse-wave (square-wave) form (4). Thus, we proposed to compare the sine-wave and square-wave stimuli for electroejaculation in agoutis.

Material and Methods:
A total of 08 adult male agoutis, bred at the Centre of Multiplication of Wilde Animals (CEMAS-UFERSA), were used in the experiment. For the electroejaculation, animals were pre-medicated with intramuscle administration of ketamine (7mg/Kg) and anesthetized with propofol (5 mg/Kg) in bolus. Each animal was randomly subjected to two electroejaculation protocols at 15 days interval. The first protocol consisted in the use of a rectal probe with ring electrode emitting sine-wave stimuli (Autojac®, Neovet, Campinas, SP, Brazil) from 5 to 12 V during 10 min. The second protocol used a probe presenting longitudinal electrodes and emitting a square-wave stimuli (Eletrojet®, Eletrovet, São Paulo, SP, Brazil) varying from 2 to 10 V for 10 min. The occurrence of erection during the procedures was noted. Ejaculates were collected in plastic tubes and immediately evaluated. The efficiency of the protocols for ejaculate collection was compared by the Fisher's test ($P < 0.05$).

Results and Discussion:
Erection occurred in 75% procedures using sine-wave stimuli and 100% using square wave stimuli. Both protocols presented 100% efficiency for electroejaculation ($P > 0.05$); however, no sperm was identified in the ejaculates that were constituted only by seminal plasma and epithelial cells. Volumes of 0.8±0.4 mL and 0.8±0.2 mL seminal plasma were obtained for sine-wave and square-wave stimuli, respectively ($P > 0.05$). In a previous research (3), the presence of sperm in ejaculates obtained by electroejaculation using sine-wave stimuli was verified in 41% of the samples; however, 50 male agoutis were collected. We conclude that both sine-wave and square-wave stimuli are efficient for seminal plasma collection by electroejaculation in agoutis; however, the protocols should be improved in order to achieve the sperm recovery.

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Hormonal induction of reproduction of *Mylossoma duriventre* with follicle stimulate hormone, luteinizing hormone and pituitary carp extract

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Introduction

Mylossoma duriventre reaches up to 25 cm in length and 500g weight and is found in upper Paraguay, being ecologically important for subsistence fishing and socioeconomic market (1). For fry production in captivity is needed hormonal induction, for production larvaes in laboratory, but the problem is that this specie doesn't reproduct, because they are migratory fish and have a low reproductive efficience due to the inappropriate gonadal maturation, which are determined by the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (2).

Material and Methods

Six females showing bulging belly, hyperemic urogenital papilla and abdominal tenderness were submitted to hormonal treatments. Three hormones were used, each with two applications, initially being applied a priming dose of 0.5 mg / kg PCE (pituitary carp extract - EB -2 animals), 0.0125 mg / kg LH (LH-2 animals) and FSH 0125 (FSH-2 animals) and a stimulatory dose after 6 hours, 5.0 mg / kg PCE, 0.125mg/kg LH and 1.25 mg / kg of FSH. Blood was collected for serum analysis of FSH and LH before and after induction. Due to the low number of animals the statistical analysis was descriptive.

Results

This is the first report of induced reproduction using porcine FSH and LH in migratory teleost fish. Two females responded to hormonal treatment, one with PCE and another with LH as presented on table 1. The end of vitellogenesis is caused by LH, causing migration of the germinal vesicle of oocytes and release of them from follicles (3). All females used in this study had FSH values ranging from 0.78 to 0.84 IU / ml (table 1). Females that received only FSH did not spawned, probably because of the lack of LH to stimulate the final maturation of vitellogenic follicles (4). The female EB showed a weight of 24 g of spawning with 5 oocytes displaying a peripheral position of the germinal vesicle (PPVG) (table 1), while the female treated with LH, has no oocyte with PPVG, indicating that the oocytes were not able to fertilization (5). The LH apparently had a positive effect on maturation, triggering the spawning of *M. duriventre*. However more studies are needed to standardize the technique of induction with this hormone.

Table 1. values of seric LH and FSH in fish submitted to different hormonal treatments for induction of reproduction of *Mylossoma duriventre*.

Treatment	Identification	Induction	LH	FSH	Reproduction	PPVG	Weight of eggs
PCE	EB 1	1	0.05	0.78	negative	-	-
		2	0.31	0.78		-	-
	EB2	1	0.21	0.83	positive	5	24 g
		2	0.21	0.81		-	-
LH	LH1	1	0.55	0.82	negative	-	-
		2	0.54	0,78		-	-
	LH2	1	0.62	0.79	positive	0	26 g
		2	1.20	0.74		-	-
FSH	FSH1	1	0.04	0.79	negative	-	-
		2	1.29	0.84		-	-
	FSH1	1	1.11	0.49	negative	-	-
		2	0.67	1.02		-	-

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Macro and microscopic aspects of the genital organs of free-living female giant anteater (*Myrmecophaga tridactyla*, Linnaeus, 1758)

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Introduction

The giant anteater (*Myrmecophaga tridactyla*) is one of the main victims of habitat loss and environment modification caused by man. Thus, it is one of the most endangered animals of the Brazilian fauna, listed in the "Vulnerable" (VU) category, at both global and national levels (1), along with other 496 species worldwide. The study of morphological descriptions, macro and micro-structures of the female genital organs is essential to help managing this species, considering that extremely demanding environmental and behavioral aspects are in question, representing a great challenge for reproduction in captivity.

Material and Method

Five *Myrmecophaga tridactyla* provided by the São Paulo state Environmental Police Department and sent to the Division of Clinical and surgical procedures of Wild animals (SACCAS), Veterinary Hospital "Dr. Halim Atique "in Sao José do Rio Preto - SP, Brazil, were used in the present study. Fragments of the *Myrmecophaga tridactyla* female genital system were removed, fixed in 10% formaldehyde for 24 hours and submitted to routine paraffin histology processing. Histological sections were stained by hematoxylin-eosin (HE), Masson's trichrome, blue toluidine and PAS, and examined under a light microscope. The study was approved by the Animal Ethics Committee at the Faculty of Veterinary Medicine, University of São Paulo (process number 2583/2012). The Nomina Anatomica Veterinaria (2) was used for the description of anatomical structures

Results and Discussion

The giant anteater (*Myrmecophaga tridactyla*) presents a singular female genital system compared to other mammals (3), such as thick lips and vulvas located in the perineal region. The neck presents a rather modest uterus and cervix compared to other edentatas (2,4), and does not exhibit uterine horns. The uterus has a pear shape, differing from other mammalian species (5). The oviduct is the union of the uterus to the ovaries (5) and the ovaries show other shapes similar to edentatas (4). The ovaries have a serous simple cuboid epithelium, albuginea and a medulla surrounded by the cortex. The cortex is composed of a stroma that contains ovarian follicles at different stages of development (6,7). The fallopian tubes are rolled and filiform, with three parts: infundibulum, ampulla and isthmus. Histologically, the wall consists of the following layers: mucosa, tunica albuginea, and tunica serosa muscle. The mucosa is characterized by a ciliated columnar epithelium. Ciliated epithelial cells are more frequent in the infundibulum. The ampoule is characterized by mucosa folds; while the isthmus has a few folds and more muscle tunica (6,7).

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Effects of ACTH challenge on fecal corticoid and sex steroid metabolites in maned wolves (*Chrysocyon brachyurus*) and domestic dogs (*Canis lupus familiaris*)

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Introduction

Despite conservationist efforts, breeding success for the maned wolf (*Chrysocyon brachyurus*), the largest canid of South America, is still very low. Among several factors, low reproductive efficiency may be related to stressful captive environment. It is know from domestic cats that activation of hypothalamic-pituitary-adrenal axis (HPA) increases plasmatic cortisol as well as sex steroid hormones of adrenal origin (1). Together this could increase susceptibility to diseases and impairment of gonadal function. In this study we aimed to evaluate the effects of ACTH administration on corticoid and sex steroid metabolite concentrations in feces of intact captive maned wolves and domestic dogs.

Material and Methods

Fecal samples were collected daily from intact adult maned wolves (2 males and 2 females) and domestic dogs (3 male and 3 females) from the day before until 5 days after ACTH administration (Synacthen®, 0,25mg/mL/animal; IM, Novartis, Berlin). Fecal steroids were extracted in ethanol (80%) and corticoid, androgen, progestin and estrogen metabolites were quantified by enzyme immunoassay (2). For each species and gender data are presented as percentage of change after ACTH administration, considering the mean steroid concentration on the day of the corticoid peak in relation to the mean concentration found on the day before ACTH (*student* t-test). Due to the reduced sample size (*n*) and the individual variability, the statistical analyses was only possible to be performed for fecal corticoids, considering genders of each species together to increase *n*.

Results and Discussion

The ACTH challenge stimulated the HPA (Fig.1). An increase on fecal corticoids metabolites was found at 24h (males) or 48h (females) after ACTH administration for both species ($p<0.05$) and was higher in maned wolves than in domestic dogs ($p<0.05$), with the highest peak found in female wolves. Furthermore, ACTH also increased sex steroid metabolites in feces of male and females of both species, except for a decrease on androgen levels in maned wolf males. Although the statistical significance of these changes after ACTH challenge was not possible to be proved, our data indicated that during stressful conditions, sex steroids of adrenal origin may contribute to hormonal imbalances and compromise gonadal function in canids.

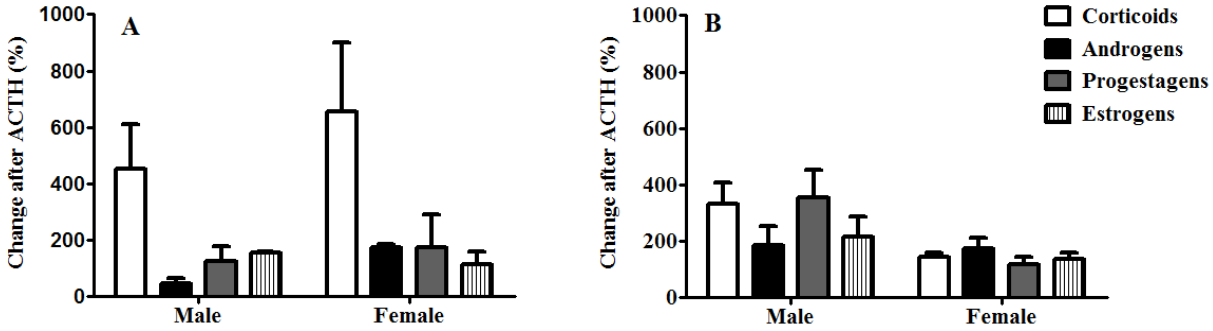

Figure1. Percentage of change on fecal steroid metabolites concentrations in male and female maned wolves (A) and domestic dogs (B), on the day of corticoid peak in relation to the day before ACTH administration.

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Protein profile of seminal plasma in collared peccaries (*Tayassu tajacu*)

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Introduction

The characterization and identification of seminal proteins will enable us to understand their function and relationship with the fertility (1). Proteomics is a critical approach to identify large numbers of proteins in complex media, such as those from the male reproductive tract (2). Studies regarding seminal plasma protein characterization are abundant, but only a few are related to wild species. The present research was conducted in order to characterize the proteins present in the seminal plasma and study their associations with semen quality in collared peccaries (*Tayassu tajacu*).

Material and Methods

Ejaculates from 9 adult male collared peccaries were obtained by electroejaculation and immediately evaluated. Aliquots of seminal plasma containing 400 μg of total protein were electrofocused (13 cm IPG strips; pH 4-7), and subjected to SDS-PAGE. The gels were stained with colloidal Coomassie blue and scanned. The images were analyzed with PDQUEST software, version 7.3.0 (Bio-Rad, Rockville Centre, NY, USA). Catalase, peroxidase activity, ascorbato peroxidase activity and superoxide dismutase activities in seminal plasma were also evaluated (3). Correlation coefficients between spot intensities in the protein maps and semen criteria were also calculated ($P < 0.05$).

Results

The ejaculates of collared peccaries presented a concentration of 207 ± 160.75 x10^6 sperm/mL, 83 ± 20.9% motile sperm, with vigor score of 4.2 ± 1.1, and 72.6 ± 10.4% viable sperm. The two-dimensional maps had, on average, 177.56 ± 22.33 spots, ranging from 207 to 136 spots per gel (Figure 1). The most abundant spots were 4001(16.86 kDa, pI 5.49), 4002 (15.4 kDa, pI 5.5), 7005 (15.63kDa, pI 6.31), 7001 (17.24kDa, pI 6.31) and 4105 (18.72kDa, pI 5.49), representing, together, 27.93 % of the total spot intensities. Some protein spots were correlated with semen characteristics and superoxide dismutase (SOD) activity in seminal plasma (Table 1). To the best of our knowledge, this is the first description of the protein profile of seminal plasma in collared peccaries, and their association with semen criteria. Identification of these proteins is the next step and will bring important insights on how these proteins affect sperm function in this species.

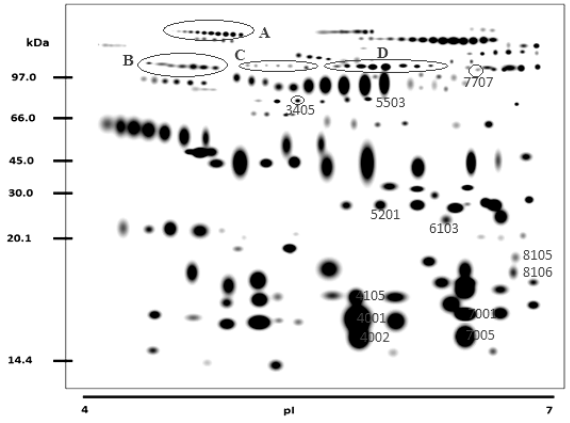

Figure 1. Protein profile of seminal plasma of peccaries (*Tayassu tajacu*). Proteins were separated by 2D-PAGE.

Table 1. Correlation between spots and semen characteristics collected from adult *Tayassu tajacu* by electroejaculation (n = 9 ejaculates).

Characteristics	Spot/Train	Pearson'/Spearman" Correlation coefficients*
Vigor	3405	-0.8367"
Viability	5201	-0.7414'
Viability	5503	-0.8698'
Viability	6103	-0.9881'
Viability	7707	-0.8698'
SOD	8105	0.8358'
SOD	8106	0.8621'
Viability	A	-0.6892'
SOD	B	0.7811'
Concentration	C	0.7840'
SOD	D	0.7137"

* $P < 0.05$; SOD – superoxide dismutase activity.

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Duration of spermatogenesis in the Atlantic Forest tree rat, *Phyllomys* sp (Rodentia: Echimyidae)

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Introduction

The Atlantic Forest tree rat of the genus *Phyllomys* (Rodentia: Echimyidae) is geographically restricted to the forest habitats from Ceará to Rio Grande do Sul. This rodent is arboreal and nocturnal and is characterized by spiny to soft fur, small feet, large eyes, squarish nose, small and round ears and long vibrissae. Rodents of this genus in general are difficult to collect using conventional traps and some species of this genus are listed as threatened. They present a karyotype with 56 chromosomes and the fundamental number (FN) is 102. This karyotype was never reported for any *Phyllomys* species, indicating that this tree rat could represent a new species. Concerning reproductive biology, there are very few reports in the literature for the genus *Phyllomys*. Therefore, our objectives were to investigate the basic aspects related to testis function and duration of spermatogenesis in this Atlantic Forest tree rat species.

Materials and Methods

The *Phyllomys* sp were collected in Araçuaí, Minas Gerais state, Brazil. Testis of nine adult males were perfused-fixed by gravity through the left ventricle with 0.9% saline and 4% buffered glutaraldehyde and routinely processed for histological and stereological analyses. Intraperitoneal injections of tritiated thymidine were performed 1 hour and 14 days before the sacrifice in order to determine the duration of spermatogenesis. All data are presented as the mean ± SEM.

Results and Discussion

The mean body and testis weights for the *Phyllomys* sp were respectively 182 ± 1.02g and 0.46 ± 0.04g, providing a gonadosomatic index (GSI) (testes mass divided by body weight) of 0.5 ± 0.04%. Based on the development of the acrosomic system, twelve stages of the seminiferous epithelium cycle were characterized. Stages VI and VII presented the highest frequencies (~17-18%), while the stages III, IV and V showed the lowest frequencies (~3-5%). The most advanced germ cell types labeled at 1 hour and 14 days after tritiated thymidine injections were respectively zygotene spermatocytes at the stage XI and round spermatids at the stage II. Based on the stages frequencies and most advanced labeled germ cells, each spermatogenic cycle and the entire spermatogenic process lasted respectively 11.3 ± 0.5 and 50.8 ± 2.1 days. These data indicate that this species present a longer duration of spermatogenesis when compared to other member of the Echimyidae rodents already investigated (1). In order to investigate if this *Phyllomys* sp is a new species, we are currently performing cytogenetic and genomic studies. Also, we are evaluating several other parameters in order to better characterize testis structure and spermatogenic efficiency in this Atlantic Forest tree rat species.

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Scrotal infrared temperature in beef bulls (*Bos taurus indicus*)

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Introduction

Infrared thermography is a noninvasive method of assessing scrotal surface temperature (SST) by infrared emissions (radiated heat energy) with an accuracy of 0.10ºC (1,2). Researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature (1,3). There was no significant effect of diurnal variation on SST (4). The aim of the study was to determine the scrotal surface temperature measured with infrared thermometry in Nellore bulls under field conditions.

Material and Methods

Semen from five Nellore bulls, 35 months old, was collected by electroejaculation (Autoejac®, Neovet) during autumn (april-may) at ambient temperatures (25 to 27ºC) under field conditions. From each male a total of 7 samples were collected on days: 0, 7, 14, 21, 28, 35 and 42. Semen characteristics were evaluated according to (5). The infrared thermometry was conducted before and after electroejaculation. An infrared thermometer (Incoterm®) was positioned 2cm behind the scrotum to measure SST on spermatic cord (T1), at the top of the testicle (T2), at the middle of the testicle (T3), at the bottom of the testicle (T4) and at the cauda of the epididimides (T5) according to (6).

Results and Discussion

The means of variables: volume (6.02±1.75 mL), motility (77.5±4.5%), vigor (4±0), concentration (950±240x10^6/mL), major defects (5.3±3.2%) and minor defects (12.7±4.2%) showed a good semen quality.
The testes in the scrotum must be maintained at a temperature 2 to 5ºC lower than body temperature (3). The area of increased SST (T3 and T4) was attributed to a localized increase in SST over the cauda epididimides (Table 1).

Table 1. Scrotal surface temperature (ºC) and body temperature-BT (ºC) before and after electroejaculation.

	BT	T1	T2	T3	T4	T5
before	38.5 ± 0.5	33.9 ± 0.4	33.3 ± 0.3	32.7 ± 0.3	31.5 ± 0.2	30.3 ± 0.4
after	38.9 ± 0.4	34.1 ± 0.3	33.6 ± 0.4	33.6 ± 0.2	32.4 ± 0.3	31.5 ± 0.3
difference	0.4	0.2	0.3	0.9	0.9	1.2

Contraction of the epididimides during ejaculation produces heat that increases surface temperature of the overlying scrotum (4). In conclusion, infrared thermometry should be performed before semen collection and can be made under field conditions. Further researchers are needed to improve methods for interpretation of scrotal temperature patterns in zebu bulls.

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Effect of chloprostenol on uterine involution and fluid accumulation of postpartum buffaloes

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Introduction

Prostaglandin F2alfa (PGF2alfa) induces uterine involution in females during puerperium (1). Administration of PGF2alfa analogues, as chloprostenol, has been shown similar effect (2). The object of this study was to evaluate the effect of sodium chloprostenol, applied at different puerperium periods, on uterine involution and fluid accumulation of postpartum buffaloes.

Material and Methods

For that, 44 pluriparous buffaloes were used, with body condition score equal or greater than 3.5 (1 to 5 scale) in "El Cangre" Livestock Genetics Company, Cuba. Animals were divided into three treatment groups: CON (control, n = 16) – application of 2 ml saline intramuscular (i.m.) at days 2, 5, 15 and 20 postpartum; CLO2 (n = 15) – application of 2 ml chloprostenol (0.530mg de Ciosin®, Schering Plough Coopers, São Paulo) i.m. at days 2 and 5 postpartum and 2 ml saline i.m. at days 15 and 20 postpartum; CLO15 (n = 13) – application of 2 ml saline i.m. at days 2 and 5 postpartum and 2 ml of chloprostenol i.m. at days 15 and 20 postpartum. Buffaloes were submitted to gynecological examinations on days 7, 14, 21, 28 e 35 postpartum (considering parturition as day 0). During gynecological exams, the degree of uterine involution was evaluated by transrectal palpation (1 to 3 score) and fluid accumulation by ultrasonography (0 to 3 score). For statistical analysis, data were submited to ANOVA and Tukey test at 5% probability.

Results and Discussion

Mean uterine involution of CON, CLO2 and CLO15 groups were, respectively: 1.1±0.2, 1.1±0.3 and 1.1±0.3 at 7 days; 1.5±0.6, 1.8±0.9 and 2.0±0.8 at 14 days; 2.6±0.5, 2.5±0.6 and 2.7±0.5 at 21 days; 2.9±0.3, 2.9±0.3 and 3.0±0.0 at 28 days; 3.0±0.0, 3.0±0.0 and 3.0±0.0 at 35 days. Mean fluid accumulation were: 2.87±0.34, 2.07±0.88 and 2.23±0.72 at 7 days; 1.50±0.73, 1.20±0.86 and 1.08±0.95 at 14 days; 0.44±0.63, 0.07±0.26 and 0.15±0.37 at 21 days; 0.19±0.40, 0.07±0.26 and 0.08±0.28 at 28 days; 0.06±0.25, 0.00±0.00 and 0.00±0.00 at 35 days, for groups CON, CLO2 and CLO15, respectively. There was significant difference in uterine involution degree only at day 14 postpartum between groups that received chloprostenol and control group. Fluid accumulation differed significantly at days 7, 14 and 21 postpartum. At this period, it was observed difference between chloprostenol groups and control group. These data suggest that prostaglandin analogue application at first or third weeks postpartum did not accelerate buffalo uterine involution, despite it decreased intrauterine fluid accumulation at puerperium inicial weeks.

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Link between agonistic behaviour and reproductive patterns in captive male Iberian ibex (*Capra pyrenaica*)

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Introduction

In several ungulates there is a positive relation between testosterone and aggressive behaviour. Therefore, the study of agonistic behaviour in hierarchical groups might provide information about individual reproductive patterns without the application of invasive techniques. The aim of this trial was to determine if there is a positive relationship between the frequency of dominant agonistic behaviours and the reproductive patterns (testosterone concentrations, scrotal circumference, and seminal parameters) in captive male Iberian ibex.

Material and Methods

Two groups of 5 and 6 adult males were housed in 250m^2 sandy-floored stable adapted for this species, with a partial roof cover. Animals were supplemented with barley grain, barley straw and dry alfalfa, with free access to water and vitamin/mineral blocks. Spontaneous dominant agonistic behaviours were recorded during 1h/week for one year in each group. A total of 662 and 1328 agonistic behaviours were recorded in each group, from which 81 and 147 had physical contact, and 581 and 1181 without physical contact. The frequency of dominant agonistic behaviours (number of dominances/h) with and without physical contact was calculated for each animal. All animals were anesthetized (intra-muscular 270 µg/kg of detomidine, plus 1.4 mg/kg of ketamine hydrochloride) every 30-60 days during one year, and the scrotal circumference was measured, blood samples for testosterone measurement by RIA were obtained, and animals were electroejaculated to collect semen. Basic seminal parameters (total spermatozoa in the ejaculate, score, percentage of motile, alive spermatozoa and abnormal spermatozoa) were determined for each male. The data were analyzed by simple linear regression.

Results and Discussion

The frequency of dominant agonistic behaviours with physical contact was positively related to serum testosterone ($r=0.07$; $P=0.02$). The frequency of total dominant agonistic behaviours was positively related to semen score ($r=0.06$; $P=0.03$) and tended to be related to percentage of motile spermatozoa ($r=0.05$; $P=0.06$). The frequency of dominant agonistic behaviour without physical contact was positively related to semen score ($r=0.06$; $P=0.03$) and tended to be related to scrotal circumference ($r=0.03$; $P=0.1$) and percentage of motile spermatozoa ($r=0.04$; $P=0.07$). No other parameter was related with the frequency of dominant agonistic behaviour. Though testosterone and agonistic interactions were related, there was not a clear link between agonistic interactions and reproductive patterns. This is probably due to the low relationship it has with testosterone levels. The low r value does not allow to use the aggressive behaviour as a predictor of reproductive patterns.

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Effect of chloprostenol sodium administration on dairy cattle early puerperium

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Introduction

At early puerperium, the dominant endocrine phenomenon is release of high quantity of prostaglandins. High $PGF_{2\alpha}$ level intensifies uterine involution process (1), by stimulating uterine muscular activity (myometrium) after parturition (2). This study aimed to evaluate the effect of two doses of chloprostenol sodium, a PGF2α analogue, at first week postpartum of dairy cows, on degree of uterine involution, parturition-first estrus interval and service period, and to correlate uterine involution speed and return of ovarian activity.

Material and Methods

Seventy six cows were evaluated at 13 farms in Coronel Xavier Chaves - MG, Brazil, all presenting normal parturition without retained placenta. After parturition, animals were randomly divided into two groups: Group 1 (control): applications of 2 ml saline intramuscular (i.m.) at days 2 and 5 postpartum (parturition = day 0); Group 2: 2 ml (0.530 mg, Jofadel, Varginha) chloprostenol sodium i.m. at days 2 and 5 postpartum. After treatment, the degree of uterine involution was evaluated via rectal palpation between the 4^{th} and the 5^{th} week postpartum. Evaluation of uterine involution was based on a 1 to 3 scale: 1 was the least and 3 the most involuted uterus. Reproductive occurrences, such as estruses and inseminations, were recorded in forms. Pregnancies were diagnosed by rectal palpation.

Results and Discussion

Postpartum rectal palpation revealed that animals of group 2 presented more involuted uterus than those of control group (1.34 ± 0.57B and 1.94 $\pm$ 0.87A for groups 1 and 2, respectively; different letters mean significant difference between groups). The return of postpartum ovarian activity was more rapid in group that received two doses of chloprostenol. On average, group 2 showed a shortest parturition-first estrus interval than group 1: 87.66 $\pm$ 28.85A and 69.06 $\pm$ 25.35B days for groups 1 and 2, respectively. Due to a shorter parturition-first estrus interval, group 2 presented shorter service period than control group (97.51 ± 34.31A and 78.09 $\pm$ 33.70B for groups 1 and 2, respectively). The number of services necessary for conception was not significantly different in the groups. Data suggest that chloprostenol sodium interferes with uterine involution degree and provides a more rapid return to postpartum reproductive activity. Administration of two 0.530mg chloprostenol sodium doses improves dairy cows' reproductive performance.

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Loss of pregnancy and calve mortality in Holstein-Zebu dairy cows

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Introduction

The objective of this study was to diagnose and quantify losses of pregnancy and calve mortality in dairy cattle. For that, ten herds were monitored from January to December, in Coronel Xavier Chaves, south Minas Gerais, Brazil.

Material and Methods

Reproductive failures were studied in 380 adult Holstein-Zebu cows with body condition score over than 3 (1 to 5 scale) and average daily milk production of 15 liters per cow. Animals fed on *Brachiaria decumbens*, 22% protein ration and mineral mixture in semi-confined system. All pregnant cows and newborn calves were submitted to clinical and reproductive examinations every fifteen days, when losses and mortality were diagnosed and recorded. Clinical and reproductive examinations consisted of diagnosing: estrus repetition (animal that, even after natural breeding or artificial insemination, manifests estrus again after 18-23 days; estrus repetition was evaluated because it can be confused with early embryonic loss), abortion (interruption of pregnancy, previously diagnosed by rectal palpation, with fetus expulsion between 45 and 260 days), fetal mummification (presence of a dark colored mass with no bacterial contamination in uterus, corresponding to a dehydrated dead fetus, diagnosed by rectal palpation), perinatal mortality (death of calf found until 72 hours after parturition) and neonatal mortality (death of calf found between 72 hours and four weeks after parturition). Data of reproductive failures occurrence were evaluated by Chi-square test ($P< 0.05$).

Results and Discussion

During the study, were observed 9.21% of estrus repetition, 2.10% of abortion, 0.26% of fetal mummification, 1.58% of neonatal mortality and 0.79% of perinatal mortality. The farms did not differ significantly for variables studied. Occurrences of abortion, fetal mummification, neonatal and perinatal mortality were not alarming. Estrus repetition was more frequent probably due to its many causes, as wrong procedures during insemination, low quality semen, reproductive tract-related diseases and bad nutrition.

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Occurrence of ovarian dysfunctions and disorders of fertilization in Holstein-Zebu dairy cows

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Introduction

Bovine must have adequate nutritional and health management to present a good reproductive performance (1). Nutritional deficiences, contamination by infectious agents and incorrect animal management can lead to the development of reproductive failures (2, 3, 4). This study aimed to verify occurrence of dairy cattle ovarian dysfunctions and disorders of fertilization during a year in Coronel Xavier Chaves, south Minas Gerais, Brazil.

Material and Methods

Reproductive failures were studied in 380 adult Holstein-Zebu cows from 10 farms, with body condition score over than 3 (1 to 5 scale) and average daily milk production of 15 liters per cow. Animals fed on *Brachiaria decumbens*, 22% protein ration and mineral mixture in semi-confined system. Observations and exams were performed in alternated weeks, when occurrences were diagnosed and recorded in forms. During reproductive examinations, were diagnosed anestrous (absence of estrous signs more than 30 days, with no ovarian activity detected) and ovarian cysts (persistent anovulatory follicular structure for more than 10 days with interruption of ovarian cyclicity, in corpus luteum absence). Also was observed nymphomania (exacerbated signs of estrus), early insemination (performed before 11 hours after estrous detection), errors in estrous detection (mistakes in estrous signs observation by the employee, when animal is misdiagnosed in estrous) and silent heat (absence of estrous behavior). Data of ovarian dysfunctions and disorders of fertilization occurrence were analyzed by Chi-square test at 5% probability.

Results and Discussion

During the study, were observed 6.32% of anestrous, 0.79% of ovarian cysts, 3.68% of nymphomania, 0.53% of early insemination, 2.63% of errors in estrous detection and 1.84% of silent heat. Farms did not differ significantly for variables studied. In general, reproductive failures rates were low. Major index was anestrous, which is much common in dairy cattle, due to postpartum negative energetic balance.

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The influence of environmental factors on sperm quality of bulls

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Introduction

The homeothermy is maintained by the processes of heat production and loss with a critical upper limit between 24°C and 27°C. When the environmental temperature exceeds this limit the animal comes into thermal stress (1). In order to have a perfect spermatogenesis, testicles should be at a temperature between 2°C to 6°C below the body temperature (2). In consequence of climate changes, extreme events like heat waves will occur more frequently in the summer time with negative effects in fertility. The purpose of this abstract was to associate the climatic variables in different seasons with the sperm quality in Braford bulls.

Materials and Methods

This study was conducted in the city of Uruguaiana (Rio Grande do Sul state – Brazil) in spring (10/09/2011), summer (02/03/2012) and autumn (04/28/2012). Bulls Breeding Soundness Evaluation (BBSE) was performed in Braford bulls (n=17) with 30 months of age. The scrotal circumference (SC) was confirmed and semen was collected by electroejaculation. The meteorological data were obtained by an automatic weather station from National Meteorology Institute (INMET) located at 29° 84" 35' South and 57° 08" 23' West, for the period of 62 days before the BBSE (equal to the spermatogenic cycle). In order to calculate the Temperature and Humidity Index (THI) we applied a mathematical equation (3), using a total of 1488 hours of observations at each season. We adopted THI equal or greater than 72 as a warning value for thermal stress. Statistical analysis was performed using the Statistical Analysis System (SAS Institute, Cary, NC). Tukey test was performed to compare the physiologic and the climatic variables.

Results and Discussion

We found THI ≥72 in 97 h of spring, 982 h of summer and 626 h of autumn with a positive correlation between sperm pathologies (primary, secondary and total) and THI and a negative correlation between THI and motility and vigor (Table 1). Our results agree with insulation experiments that found the same correlations (4; 5), with the difference that in this study the stress source was represented by the environment temperature and humidity (Table 2).

Therefore we concluded that climatic factors as temperature and humidity interfere on Braford bull's spermatogenesis mainly in the summer time (THI ≥72 in 65% of days) affecting directly the sperm quality.

Table 1. Sperm quality.

Variables	SEASON		
	Spring	Summer	Autumn
S.C. (cm)	34.26[a]	34.32[a]	33.77[a]
MOT. (%)	61.47 [ab]	49.41[b]	75.00[a]
VIGOR	3.41[ab]	2.70[b]	3.76[a]
P.P. (%)	4.29[a]	40.58[b]	15.26[a]
S.P. (%)	7.00[a]	19.70[b]	9.82[a]
T.P. (%)	11.29[a]	62.58[b]	21.38[a]

Different letters in lines means ($p < 0.05\%$).

Table 2. Correlations between THI and sperm quality.

	THI	MOT.	VIGOR	P.P.	S.P.	T.P.
THI		-0.16396	-0.21946	0.6837	0.40669	0.7229
MOT	-0.16396		0.93420	-0.3254	-0.22081	-0.38056
VIGOR	-0.21946	0.93420		-0.35498	0.19016	-0.39320
P.P.	0.6837	-0.32517	-0.35498		0.33057	0.89759
S.P.	0.40669	-0.22081	-0.19066	0.33057		0.59338
T.P.	0.7229	-0.38056	-0.39320	0.89759	0.93338	

S.C. - Scrotal Circumference, MOT. - motility; P.P. - primary pathologies; S. P. - secondary pathologies; T. P. - total pathologies; THI - Temperature and Humidity Index

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Effect of heat stress in Holstein cow fertility

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Introduction

The air temperature´s conditions, relative humidity and thermal radiation present in tropical and subtropical environments are extremely uncomfortable for dairy cow, reducing their production and reproductive performance. Dairy cattle present increased body temperature when the environmental temperature reaches 26°C (3). Heat stress leads to a reduction in the length and intensity of estrus (low circulating estradiol), changes in follicular dynamics and hormone levels, embryo death (low protein synthesis and apoptosis), increased pregnancy loss (inadequate uterine ambient, low embryo implantation and blood redistribution to the peripheral regions of the body) (1,2,6). Thus, this research was to evaluate the effect of heat stress on the reproduction performance of Holstein cows managed in a tropical region under high environmental temperatures.

Material and Methods

Twenty Holstein cows were monitored every 15 days, through 12 months in a tropical climate region (northern Mato Grosso - Sinop / MT - Brazil). The environmental annual average temperature was 22-36°C, with a rainfall corresponding to 1160 mm. The rainy season is from October to March with average temperatures of 30°C (period I) and the dry season months from April to September with average temperature of 35°C (period II) (4). The animals were managed on pasture of Tanzania (*Panicum maximum*) with supplementation with silage and concentrate, under natural shade, maintaining a body condition score between 3 and 4 throughout the research. At each oestrous cycle the occurrence of estrus was observed (teaser bull and visual observation). The characteristics of the reproductive system and pregnancy of the animals 50 days after artificial insemination (AI) were observed by ultrasonography.

Results and Discussion

On average, the observations were similar within period I and II. The animals that calved in period I had normal estrus with the first clinical observation at 77 days postpartum, and with 40 days intervals between the AI and four AI´s were necessary for pregnancy. During this period the ovaries showed few structures of small diameter and flaccid uterus with endometrial atrophy. The animals that calved in period II showed very weak expression of estrus with this first clinical observation at 95 days postpartum, and with 22 days intervals between the AI and nine AI´s were necessary for pregnancy. During this period the ovaries do not have structures detected and uterus with endometrial atrophy. The results show that heat stress affects follicular and uterine development, hormone synthesis and embryo survival, which is similar to what was observed in other researchers (1, 2, 3 and 6). There was also a longer estrus interval in high humidity period and a low expression of estrus and changes in follicular growth, especially in periods of low humidity, wich shows similar characteristics to others reported (2,5). Heat stress acts in a multifactorial manner in the reproduction of Holstein cows, affecting the development of estrus, follicular dynamics, embryo and uterine ambient.

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Seasonal reproductive patterns and effectiveness as teasers (ram effect) of Corriedale and Milchschaf rams

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Introduction

Terminal crossbreeding with rams from meat producing breeds have developed great interest among sheep producers in Uruguay. Out-of-season crossbreeding, which may be induced with simple management strategies such as the ram effect, can be of interest for producers pursuing high meat prices for off season supply. As male sexual behaviour is a determinant of teaser' effectiveness, it would be important to compare seasonal reproductive pattern of potential meat producer-breeds with Corriedale rams, which is the predominant breed. The first objective was to compare the seasonal reproductive pattern of Corriedale and Milchschaf rams. As seasonal patterns were similar, a second objective was to compare the effectiveness of rams from both breeds to induce oestrus in seasonal anoestrous Corriedale ewes with the ram effect.

Material and Methods

In the first experiment, body weight, scrotal circumference, testosterone concentrations, sexual behaviour toward oestrous ewes (ano-genital sniffing, lateral approaches, flehmen, mount attempts, mounts, and mates (mount with ejaculation), and semen characteristics (total spermatozoa in the ejaculate, mass motility, and percentage of alive and normal spermatozoa) were recorded during 13 months in 1-y-old Corriedale (n=5) and Milchschaf (n=7) rams. Experiment 2 was performed during the non-breeding season with 169 Corriedale and Polwarth mulitparous ewes. All ewes were isolated from the rams so that they could not see, hear, or smell them (minimum distance: 1000 m) from Day –40 (Day 0=introduction of the rams), and primed during 8 days with an intravaginal sponge impregnated with 50 mg of medroxyprogeterone. Four Corriedale rams were introduced to 83 ewes, and 4 Milchschaf rams to the remaining 86 ewes at sponge withdrawal, and oestrus was recorded for 5 days.

Results and Discussion

Body weight increased with age ($P<0.0001$), and there was an interaction ($P=0.005$): Corriedale rams had a greater weight gain than Milchschaf rams. Scrotal circumference increased with age ($P<0.0001$), and tended to be greater in Milchschaf than Corriedale rams ($P=0.1$). Testosterone concentration varied with age ($P<0.0001$), with similar patterns in both breeds. All sexual behaviours and semen parameters were affected by season ($P<0.0001$) but there was no effect of breed. Maximum reproductive patterns were observed during summer and autumn simultaneously for both breeds. In Experiment 2, on Day 5 no ewe of the group bred with Milchschaf rams was marked. In contrast 46/83 (55.4%) of the ewes bred with Corriedale rams were marked ($P<0.0001$). It was concluded that it was concluded that reproductive seasonal pattern of Corriedale and Milchschaf rams including scrotal circumference, testosterone concentrations, sexual behaviour, and semen characteristics was similar. However, Corriedale rams were more effective than Milchschaf rams in inducing oestrus in anoestrus ewes (the ram effect). We should however be cautious in assuming that knowledge of a breed of rams' general reproductive pattern alone is adequate to predict its effectiveness as teasers.

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Pregnancy rate in an equine embryo transfer program under summer climate conditions in Baixada Fluminense, RJ, Brazil

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Introduction

In species other than horses, it is Known that periods of high environmental temperature and humidity can cause heat stress it lead to negative effects on reproductive performance by influencing the function of the hypothalamic-pituitary-gonadal axis. Thus it alters ovarian steroidogenic activity and follicular dynamic (1), commits the luteolytic mechanisms (2) and influences the ability of the endometrium in to produce and to secrete prostaglandins, which can lead to premature luteolysis, embryo loss and embryonic death in cows (3)

Materials and Methods

We evaluated 155 transcervicals embryo transfers during summer of breeding seasons 2008/2009, 2009/2010 and 2010/2011 in Mangalarga Marchador mares in Seropédica-RJ. It was used recipients mares with a uterine and cervical tone consistent with diestrus, absence of endometrial folds or uterine secretion and degrees of synchrony varying between +1 to -6 (recipient ovulated 1 day before through 6 days after the donor) (4). The pregnancy diagnosis (PD) was performed by transrectal ultrasonography at 15 days of embryo old. Climatological data during summer of each breeding season were obtained from the National Institute of Meteorology website (Automatic Station - A601) in Seropédica, RJ, Brazil (Lat. -22° 44 '38"; Long. -43° 42' 27 "). It has been compiled daily data about environment temperature (T ° C) and humidity (H %) (daily mean), which were used to obtain the daily values of Temperature Humidity Index (THI) for measurement of thermal comfort (5) . To evaluate the relationship between pregnancy rate (PR) and weather conditions, it were taken to mean climatic values from *n* days in the period between administration of PGF2α (beginning of the new cycle of the recipient) to PD date on an individual basis for each transfer. So, it were compared the average climate with positive or negative PD. The data were treated by the non-parametric Chi-square (χ^2) and Fisher's test to compare means with significance level at 5% ($p < 0.05$).

Results and Discussion

Higher PR was obtained at room temperature of 24 °C (81.5%), there was a decrease in the PR when the temperature rose to 27 to 28 °C (35.3%, 40%) ($p < 0.05$). It is important to explain that temperature values were taken using the calculation: TM = (T 9:00 + T max + T min + 2 x T 21:00) / 5, that considers environment temperature values of nine a.m. end nine p.m. to obtain a medium value and not only maximum and minimum values. The same it was observed for the PR relative to THI, where higher PR was obtained by THI between 74 and 75 units (68.3%) reducing as the THI increased, and lower PR was obtained at UTI ≥ 78 units (45%) ($p < 0.05$). Humidity alone did not show significance relative to PR ($p > 0.05$). The results suggest a involvement of the luteolytic mechanism, as in cows between 8 and 16 days gestation under heat stress, a greater amount of circulating PGF2α and its metabolites in relation to comfort thermal group (6), as well as, *in vitro*, endometrial cells of sows secreted larger amount of PGF2 α compared to control group (7). Moreover, in mice under heat stress at 34 °C and humidity of 65% it was obtained less uterine implantation sites (8) and higher embryonic loss (9) than mice maintained under thermal comfort at 21 °C.

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Seasonality of rectal temperature and conception rate in crossbred dairy cows

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Introduction

The heat stress is one of the factors that reduce the reproductive efficiency in dairy cows, due to the negative effects on oocytes and embryo development (1). Crossbred dairy cows Holstein/Gyr were more adapted to hot environments, thus they have less negative effects of heat stress on reproductive performance. The aim of this study was to evaluate effects of seasonality on rectal temperature (RT) and conception rate (CR) in crossbred dairy cows.

Material e Methods

Were analyzed 1219 inseminations of dairy cows from a commercial farm with 480 lactating cows, located in hot climate in Minas Gerais State, in Brazil, maintained in pasture during the rainy season and at lousing house at dry season, producing 18.75 kg of milk/day. The herd was routinely subjected to a fixed-time artificial insemination protocol (TAI) and early pregnancy diagnosis by ultrasound. The rectal temperature was measured at AI time by digital thermometer. The effects of season (Autumn/Winter vs. Spring/Summer) and moment of AI (morning vs. afternoon) on RT was analyzed by ANOVA using the SAS program. The effects of season, moment of AI and adjusted RT (TR below vs. above average) on CR were analyzed by logistic regression using the SAS program.

Results and Discussion

The average RT was 39.35°C. The RT and CR was affected ($P < 0.01$) by season, at hot season crossbred dairy cows had higher RT and lower CR (Table 1). The negative effects of hot season on CR in crossbred dairy cows were previous reported (2). Cows with RT > 39.35 °C had lower ($P = 0.001$) conception rate (25.78 vs. 32.54%) than cows with RT < 39.35°C. It was previous reported that higher RT reduces the CR in dairy cows (3). The moment of AI affects ($P < 0.01$) the RT and CR, cows inseminated at morning had lower RT (38.96 ± 0.49 vs. 39.60 ± 0.50 ^{O}C) and higher CR (32.86 vs. 26.06%) than cows inseminated at afternoon. In conclusion crossbred dairy cows also had higher rectal temperature and lower conception rate during the hot season, and the insemination should preferential be performed at the morning time in TAI programs.

Table 1. Effects of season on rectal temperature and conception rate in crossbred dairy cows.

Season of the Year (n)	Rectal Temperature (^{O}C)	Conception Rate (%)
Autumn/Winter (652)	39.27 ± 0.59	31.75
Spring/Summer (567)	39.44 ± 0.56	25.49
P Value	0.01	0.01

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Effect of heat stress on oocyte production from crossbred dairy cows

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Introduction

Reduced competence of bovine oocytes for fertilization and its subsequent development can be explained by several factors but the most studied is heat stress (HS) (1). *Bos indicus* cattle are less subject to extremes of temperature effects in tropical and subtropical regions when compared to *Bos taurus* cattle. Thus they have better reproductive performance when they are exposed to high ambient temperatures (2). The objective of this experiment was to evaluate the effect of HS on reproductive efficiency by production of total (PTO) and viable oocytes (PVO) in crossbred dairy cows (Holstein/Gir) submitted to ovum pick-up (OPU).

Material and Methods

Oocytes were collected from crossbred dairy cows (n = 45) by OPU at 7, 15, 30, 45, 70 and 90 days in milk (DIM) during two seasons (Autumn/Winter. vs. Spring/Summer). On the day of the OPU measurements of rectal temperature (RT) by analog thermometer, Respiratory Rate (RR) counting breaths/minute, Body Condition Score (BCS), Milk Production (MP), Index Temperature - Humidity (ITH) and Hours of Insolation (HI) were performed. The oocytes were evaluated according to Palma (3). The effects of the variables collected on PTO and PVO were analyzed by GLM using the MINITAB program.

Results and Discussion

The effects of DIM on PTO and PVO were not detected, so another analysis was done without this variable. No difference (P>0.10) was found between seasons of year in the RT (38.22 ± 1.002ºC in the Autumn/Winter vs. 38.50 ± 0.728ºC in the Spring/Summer). There was no detectable effect of adjusted RT (> or < average = 38.35°C) in PTO, but tendency (P = 0.081) of RT affects on PVO was detected. Cows with RT <38.35 °C produce more viable oocytes (3.952 ± 0.686) than cows with TR> 38.35 °C (2.526 ± 0.410) and this could probably be because of the effects of HS on oocyte nuclear maturation ability (4). No effect of the season on the PTO was detected, but hot season tended (P = 0.056) to increase PVO (Autumn/Winter: 2.512 ± 0.309 vs. Spring/Summer: 4.077 ± 0.774). This is in according with data that shows that *Bos indicus* cattle are less subject to HS since their immature oocytes express less Hsp 70.1 than *Bos taurus* (2). No effects of MP, RR, BCS, ITH and HI were detected in PVO and PTO. It is concluded that PVO in crossbred cows may be damaged by high RT but not by seasonal heat.

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Effects of LPS on *Rhinella schneideri* spermatogenesis

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Introduction

Pathogens that spread by water can contaminate anurans. Environments that receive sewage effluents are frequently contaminated with *Escherichia coli*. In mice, the lipopolysaccharide from this bacterium causes transient testicular dysfunction and apoptosis of testicular germ cells (1). Thus, we believe that *E. coli* LPS can affect the reproduction of *Rhinella schneideri*, interfering in spermatogenesis.

Material and Methods

We analysed the response of testicular germ cells to LPS exposure for 24h (LPS24h group) and 48h (LPS48h group). We used 10 adult males of *R. schneideri*, intraperitoneally administered with a single dose of 18 mg/kg of LPS and 5 animals administered with sterile saline adjusted to amphibians osmolarity (60% of mammals). The testes were submitted to histological routine and stained with hematoxylin-eosin for analysis.

Results and Discussion

The LPS treatment result in changes in germ cells occurrence (Fig.1). The LPS24h group showed an increase in bundled spermatozoids area (F=40,49; P=0,0001) compared to control group. There weren't changes in another germ cells areas either in locular and intersticial areas of this group. On the other hand, the LPS48h group had a decrease in the areas of spermatogonia (F=6,275; P=0,0019), spermatids I (F=28,5; P=0,0001), bundled spermatozoids (F=40,49; P=0,0001) and lumen spermatozoids (F=5,14; P=0,0043) as well as in locular area (F=6,489; P=0,0113). The spermatocytes I (F=3,132; P=0,05) and II (F=2,978; P=0,0521) and spermatids II (F=1,917; P=0,149) areas were not significantly different between control and experimental groups. These results indicates that LPS can interfere in spermatogenesis of *Rhinella schneideri* and after 48h of exposure the germ cells response is more evident. Thus, the reproductive output of these animals is compromised.

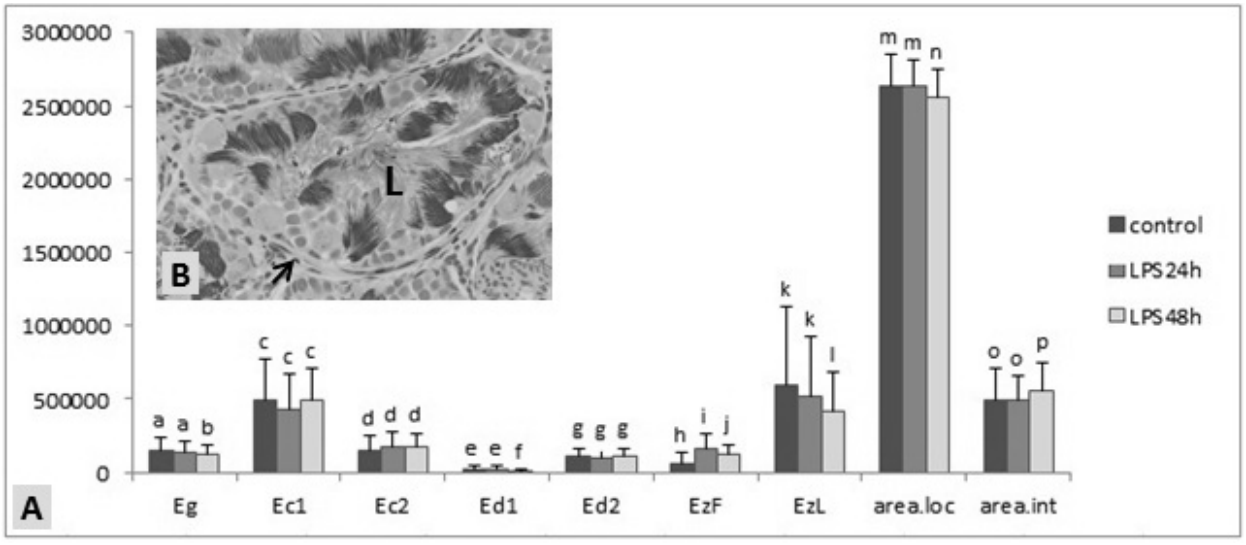

Figure 1. **(A)** Corresponding areas of spermatogonia (Eg), spermatocytes I (Ec1) and II (Ec2), spermatids I (Ed1) and II (Ed2), bundled spermatozoids (EzF), lumen spermatozoids (EzL) and locular (area.loc) and intersticial (area.int) areas of control, LPS24h and LPS48h groups. Different letters indicates statistical difference. **(B)** Microscopic photo of the seminiferous locule (L) and locular wall (arrow).

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Performance of young Braford bulls in libido tests on corral

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Introduction

Several authors have suggested the use of libido tests as an important selection tool for animals with better reproductive performance, in an attempt to increased the fertility rates in cattle herds. According to the interest in the utilization of libido test was increased when it became known that the differences between bulls are influenced by breed, age, and individual characteristics, in addition to facilitate the diagnosis problems associated with mating (1). The purpose of this study was to evaluate the behavior and sex-drive of young Braford bulls and the effect of the observation time of libido test within a corral.

Materials and Methods

The study was done in a private farm located within the city of Londrina – PR – Brazil, during April 2011. Thirty-six Braford bulls, with reproductive capacity and mean age of 21 months, were submitted to sexual behavior evaluation in a corral. The bulls were raised on pasture (Brachiaria hybrid CIAT 36061 cv mulato) and received commercial mineral salt feed *ad libitum*. All bulls were observed individually for 15 minutes, within a 50m² corral, and were mated with five mature cows (three in standing estrus and two out of estrus). The cows in estrus were replaced after being serviced by three bulls tested or when it necessary to prevent refusal of service by the accepting cow. Thereafter, all bulls were classified based on physiological events, and classified for libido, according on proposed scale (2): questionable (score 0-3); good (score 4-6); very good (score 7-8); and excellent (score 9-10). The bulls were classified individually during five minutes of observation and later cumulatively, considering all physiological events during a period of 15 minutes of observation. The score of libido was evaluated by using regression analysis as a function of the time of observation.

Results and Discussion

When the non-cumulative method of classification was used, the score attributed to the bulls, was considered as low, with averages of 6.08, 5.78, and 4.68 during the 0-5, 5-10, and 10-15 minutes periods respectively. The libido score did not change proportionally with time of observation ($p>0.05$) ($Y = -0{,}4x^2 + 0.9x + 5{,}58$, $R^2 = 1$); however, it demonstrated a slight reduction after the initial five minutes, and the bulls were classified as good at the end of the observation. When the bulls were classified by the cumulative method, an increased in score was observed, and bulls were classified as very good at the end. The libido score varied with the time of observation ($p<0.05$) ($Y = -0{,}4865x^2 + 2{,}973x + 3{,}5946$, $R^2 = 1$), with an average of 6.08, 7.59, and 8.14 during the 0-5, 5-10, and 10-15 minutes periods, respectively. The increase in the score might have occurred due to a constant number of a full services (mounts) made by the bulls during the evaluation period. Based in these results, it can be conclude that the method was easy to execute and the time used for observations was adequate to effectively evaluate and classify the bulls.

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Pentoxifylline effect on kinetics of post-thawed ram spermatozoa

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Introduction

Pentoxifylline is a metilxanthine derived from an hemorrhagic agent, possibly able to increase motility and viability of cooled and frozen spermatozoa when added to. Pentoxifylline acts as phosphodiesther inhibitor which may increase AMPc intracellular concentrations, and consequently, preserve motility (1). Thus, the goal of this work was to evaluate the addition of Pentoxifylline to post-thawed ram spermatozoa on kinetic parameters using a Computer-Assisted Semen Analyses – CASA.

Material and Methods

Sperm straws of five ram were used and thawed at 37°C/30 seconds. After thawing, samples were pooled and divided into two groups treatments, control and pentoxifylline, that received 0,5 mg/mL. Spermatozoa samples were evaluated for kinetics parameters by CASA system immediately post-thaw and 30' after the first moment (2). Samples were maintained in water bath at 37 °C during this time. Data were analyzed by ANOVA and Tukey-Kramer, with 5% significance level.

Results and Discussion

Data were expressed as (mean ± SD) and are showed on the table 1. Significant difference was observed for curvilinear motility and lateral amplitude of sperm head parameters, indicatinge that after incubation time, sperm kinetics of control group decrease these parameters when compared to pentoxifylline group, where motility increased after the same time.

Table 1. Kinetics parameters (mean ± SD) of thawed ram sperm with or without Pentoxifylline.

	Experimental groups			
	Control		Pentoxifylline	
Parameters	0h Time	30' Time	0h Time	30' Time
PM (%)	47.14 ± 15.04	40.17 ± 11.78	46.94 ± 8.66	45.08 ± 14.48
CLV (µm/s)	105.60 ± 11.01^{ab}	99.17 ± 17.29^{b}	117.22 ± 17.38^{ab}	129.78 ± 18.79^{a}
SLV (µm/s)	68.25 ± 19.60	58.62 ± 16.13	68.45 ± 19.48	74.63 ± 8.42
AVP (µm/s)	81.73 ± 14.59	74.67 ± 15.88	81.93 ± 14.82	89.27 ± 8.77
LIN (%)	63.77 ± 14.38	58.58 ± 11.29	57.83 ± 14.02	57.87 ± 4.95
STR (%)	81.93 ± 11.79	77.55 ± 7.06	82.03 ± 13.66	83.58 ± 3.47
WOB (%)	76.92 ± 8.55	75.03 ± 7.82	69.68 ± 6.41	69.15 ± 4.02
ALH (µm)	3.33 ± 0.67^{ab}	2.83 ± 0.57^{b}	3.93 ± 0.40^{a}	3.95 ± 0.41^{a}
BCF (Hz)	9.98 ± 1.71	11.42 ± 2.33	10.25 ± 3 .22	13.48 ± 1.13

PM= progressive motility; CLV= curvilinear velocity; SLV= stain line velocity; AVP= average velocity path; LIN= linearity; STR= straightness; WOB= wobble; ALH= amplitude lateral of sperm head; BCF= beat cross frequency. Different letters in the same line denote $P<0.05$.

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Bacterial compounds affect germs cells production of *Eupemphix nattereri* (Anura:Leiuperidae)

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Introduction

Amphibians are good bioindicators of environmental quality. They lives in environments polluted by sewage effluents that are often contaminated with *Escherichia coli*. Lipopolysaccharide from *Escherichia coli* causes apoptosis of testicular germ cells in mice (1) and that has been reported to directly influence the reproductive output of anurans (2). Thus, we evaluated the effects of LPS from *E. coli* in germ cells production of *Eupemphix nattereri*.

Material and Methods

We used 15 adult males of *Eupemphix nattereri* and analyzed the amount of germinative cells 2 hours (LPS 2h) and 24 hours (LPS 24h) after LPS administration. Ten adult males were intraperitoneally administered with a single dose of 18 mg/kg of LPS and 5 animals administered with sterile saline adjusted to anphibians osmolarity (60% of mammals). The testes were submitted to histological routine, stained with hematoxylin-eosin for analysis.

Results and Discussion

Spermatogonia decreased after 2hours of LPS administration (F= 12.33, P<0.00001). However, spermatocytes II, and spermatozoa decreased after 24 hours of administration (F= 8.41, P<0.00001; F= 8.46, P<0.00001, respectively). Spermatozoa decreased by 30% after 24hours of LPS administration, demonstrating that this compound has a profound action on species reproduction. However, some frog species have pigment cells in the testicle that protect germ cells from LPS, due to the bactericidal role of melanin (2). Therefore, sewage effluents may severely decrease anurans' fertility by decreasing the productions of germinative cells and directly influencing the reproductive output of species.

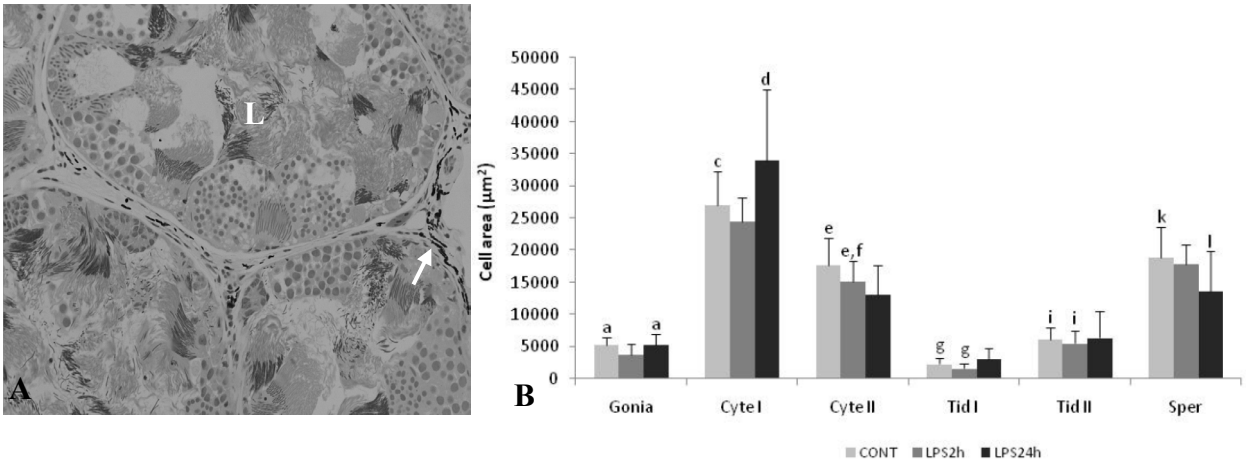

Figure 1: **A:**Histological section of *Eupemphix nattereri* testes showing seminiferous locule (L) with germ cells and pigmented cells in interstitial area (arrow). **B:** Variation of cell types with LPS administration. Different letters represent significant differences among the same cell type of the different experimental groups (P≤0.05). CONT: Control group; LPS 2h and 24h: animals analyzed 2 and 24 hours after LPS administration, respectively.

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Study of the involvement of protein kinase C (PKC) on the estradiol-stimulated synthesis of prostaglandin F2α in the endometrium

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Introduction

Endometrial explants from cows injected with estradiol-17β (E_2) as well as bovine endometrial cells (BEND) pre-exposed to E_2, when treated with calcium ionophore (CI) had a marked increase in the release of prostaglandin F2α (PGF2α) in comparison to explants or cells treated with E_2 or CI only. The mechanisms by which such an event occurs are unknown, and may involve E_2-stimulation of PKC activity, an enzyme that is essential for PGF2α synthesis. The hypotheses are that (1) inhibition of PKC activity in endometrial cells reduces the synthesis of PGF2α stimulated by E_2 and that (2) an increase in the synthesis of PKC occurs in endometrial explants of heifers treated with E_2. Objective was to measure the production of PGF2α in BEND cells treated or not with E_2 and CI in the presence or absence of a specific inhibitor of PKC (Experiment 1), and evaluate the expression of PKC protein in the endometrium of heifers treated or not with E_2 (Experiment 2).

Material and Methods

In Experiment 1, BEND cells were suspended in complete culture medium – CCM (40% HAM F-12, 40% MEM, 200 UI insulin/L, 10% Fetal Bovine Serum - FBS, 10% horse serum – HS and 1% solution of antibiotic and antimycotic). Were seeded at a density of 4 x 10^4 cells/well in 1.5mL of CCM and cultured at 38.5°C and 5% CO_2. After reaching 90% confluence, were washed twice in CCM with no serum (no serum medium; NSM) and incubated for 24 hours in NSM, and then exposed to treatment: NSM (Control group); NSM supplemented with 10μM of Bisindolimaleide I – BIS I (Group PKC inhibitor); NSM supplemented with 10μM of BIS I, 10^{-6}M CI to 10^{-13}M E_2 (Group PKC inhibitor + E_2 + CI); NSM supplemented with 10^{-6}M CI to 10^{-13}M E_2 (Group E_2 + CI). Treatments were performed in a total of nine wells per group. Cells were pre-incubated for 60 minutes with 1mL of NSM with or without BIS I. After pre-incubation, an aliquot of 100μL of medium was collected (T0) and, subsequently, 100μL of NSM with or without E_2 + CI was immediately added to the remaining 900μL. Twelve hours later, an aliquot of 100μL of culture medium was collected from each well (T12). Concentrations of PGF2α in the medium were measured by RIA. The total amount of PGF2α produced in 12 hours was obtained by subtracting the concentration of PGF2α at T0 from concentration PGF2α at T12 (DIF 12). In Experiment 2, non-pregnant Nelore heifers were paired on day 17 of a synchronized estrous cycle, treated or not with E_2 and slaughtered two hours after treatment. Endometrial tissue was isolated and subjected to protein extraction. Protein concentration was quantified by the Bradford method and subjected to western blotting. The total amount of protein used in each sample was 50μg. For PKC primary antibody was used at a dilution of 1:500 (# 93775, phopho-PKC theta (T538), rabbit Ab, Cell Signaling) whereas the secondary anti-rabbit IgG antibody was used at a dilution of 1:3000 (# 7074, anti-rabbit IgG-HRP linked antibody, Cell Signaling), with X-ray film exposure time of 3 minutes. Data from both experiments were analyzed by ANOVA using the proc GLM of program SAS.

Results and Discussion

In Experiment 1, DIF 12 was 17.36 ± 86.22 pg/mL (Control group), -2.41 ± 99.56 pg/mL (Group PKC inhibitor), 72.81 ± 111.32 pg/mL (Group PKC inhibitor + E_2 + CI) and 131.33 ± 93.13 pg/mL (Group E_2 + CI). No effect of the presence of CI + E_2 ($p = 0.3472$), the PKC inhibitor ($p = 0.6943$) and PKC inhibitor + CI + E_2 ($p = 0.8455$). There was a large variability in responses between the replicates of the same treatment. Although remarkable stimulatory effect of E_2 + CI compared to control group and inhibition of the effect of E_2 + CI in the presence of the PKC inhibitor, none of these differences were significant. The PKC inhibitor did not inhibit the synthesis of PGF2α induced E_2.In Experiment 2, the mean optical densities for PKC protein were 1.00 ± 0.18 (Untreated group) and 0.96 ± 0.14 (Group treated with E_2). There was no significant effect of E_2 in PKC protein expression ($p = 0.88$). The hypothesis of this study was not confirmed.

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Disruption of Sertoli cell intercellular junctions after exposure to Mono-(2-ethylhexyl) phthalate (MEHP): a potential role of oxidative stress

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Introduction

Active metabolites of di-(2-ethylhexyl) phthalate, such as MEHP, are recognized as reproductive toxicants, causing testicular atrophy and disruption of cellular redox state. The objective of this study was to evaluate the effects of MEHP on the integrity of intercellular junctions on Sertoli cell (SC) primary cultures and potential role of glutathione (GSH) and oxidative stress on the junctions' proteins induced changes.

Material and Methods

SC were isolated from 20-days-old male rats and cultivated for 5 days in chemically defined medium (1). Cells were exposed to MEHP (200 μM) alone or to MEHP (200 μM) and N-Acetyl-cysteine (NAC - 1mM) during the last 24 h of culture for junctions' proteins and GSH assays. For lipoperoxide (LP) assays SC were exposed to MEHP (200 μM) only during the last hour. Intracellular GSH levels were determined by spectrophotometry (1) and LP levels were quantified by the FOX2 method (2). The localization and expression of intercellular tight (occludin, claudin-11, zonula occludens-1(ZO-1)), adherens (N-cadherin, α and β-catenin) and gap (connexin 43 (Cx-43)) junctions' proteins were evaluated by immunofluorescence (IF) and Western blot (Wb; 3).

Results and Discussion.

A linear IF pattern of protein distribution was found at the level of intercellular junctions for all proteins in all treatments. However, for N-cadherin, α-catenin and ZO-1 exposition to MEHP induced a delocalization of the IF signal from membrane to the cytoplasm (Fig.1A). MEHP also increased N-cadherin, α-catenin and ZO-1 expression and reduced Cx-43 expression. No changes in claudin-11 and occludin expression were found. Exposure to MEHP reduced GSH levels in relation to controls. NAC treatment was able to prevent MEHP effects and, as a substrate for GSH synthesis, NAC alone or in combination with MEHP increased GSH levels in comparison to controls (Fig.1B). As an indicative of oxidative stress, LP concentrations were higher in SC exposed to MEHP (Fig. 1C) than in controls. Our data suggest that disruption of intercellular junctions induced by MEHP in SC may be mediated through oxidative stress signaling.

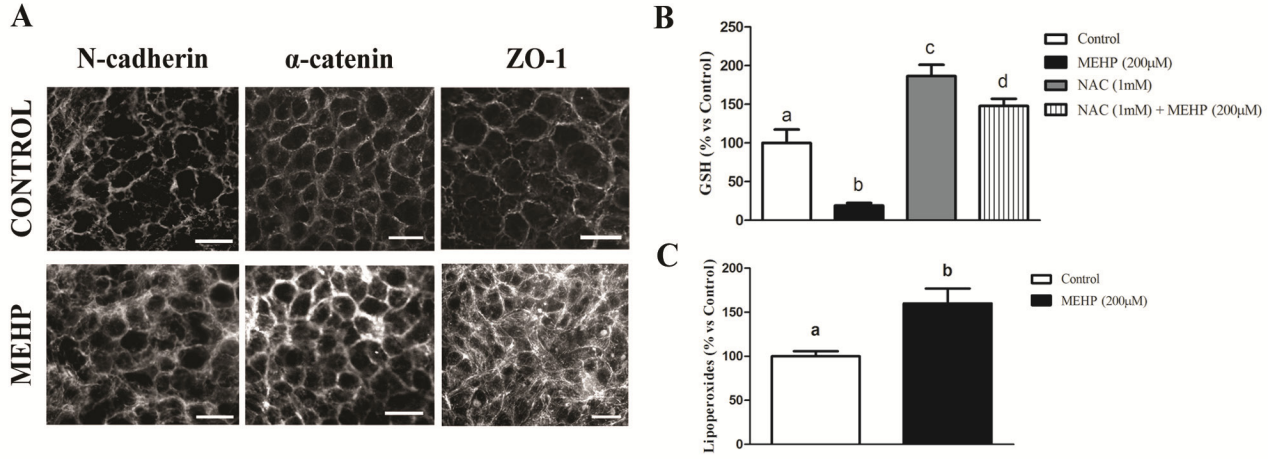

Figure.1. Expression and localization of SC intercellular junctions' proteins by IF (A; Bars: 25 μm) and levels of GSH (B) and lipoperoxides (C) in MEHP exposed *versus* control cells ($P<0.01$).

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Effects of progesterone replacement on gerbil (*Meriones unguiculatus*) prostate

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Introduction

The prostate is an accessory gland of reproductive system that originates from urogenital sinus branching, and can be found in males and females of several mammalian species (1, 2, 3, 4). The female prostate of gerbil is a functional gland with a paraurethral location homologous to the ventral prostate of male rodents (4). In both sexes the prostatic physiology is regulated by steroid hormones, especially androgens and estrogens. The progesterone hormone, though less studied, it has also proven be an important physiological regulator of prostate gland, mainly during the estrous cycle (5). In this work it was evaluated the potential effects of progesterone replacement on the gerbils prostate gland, observing their morphology and physiology.

Materials and Methods

Males and females of Mongolian gerbils (*Meriones unguiculatus*) were castrated at puberty, with 45 days of age. After reaching 90 days of age, some animals received progesterone subcutaneous doses (3 mg/kg b.w) for 14 days every 48 hours (CaP group), while another part only received subcutaneous doses of dilution vehicle (CaC group). After this period, the gerbils were killed by CO_2 inhalation followed by decapitation. Adult intact animals were sacrificed and used as normal control (NC group). The prostate glands were removed and fixed in Karnovsky solution. For general analyzes the histological sections were stained with hematoxylin-eosin (H & E) and P.A.S (Periodic acid-Schiff). Morphometric and stereological analysis were also made, seeking to evaluate, quantitatively, the prostatic tissue compartments. The quantitative data were analyzed by using One Way ANOVA and Tukey test for multiple comparisons. Values were considered statistically significant when $P \leq 0.05$.

Results and Discussion

The surgical castration triggered an intense glandular regression, being observed acinis with small lumen and a decrease in the epithelial cells height (28.51% for female and 58.68% for male), acquiring a cuboidal aspect. The withdrawal of main steroid hormones source also caused a reduction in secretory activity of the gland. This glandular reduction can be explained by a possible decrease in the amount of serum testosterone and estrogen levels due to castration, since the prostate is highly sensitive to imbalances between these two steroid hormones. The progesterone administration led to a regeneration of the gland in relation to castrated animals. This is evidenced by an increase in epithelial (13.27% for female and 23.17% for male) and stromal compartment height (35.17% for female and 12.74% for male). It was also observed some foci of abnormal growth in the epithelium, cellular debris within the lumen and intraepithelial neoplasia. The group of animals treated with progesterone also showed a great amount of glandular secretion in the lumen when compared to castrated and normal gerbils. Thus, progesterone appears to have anabolic characteristics on the prostatic gland, resembling the other steroid hormones like testosterone and estrogen, but with moderate intensity even at high concentrations.

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Biometry and testicular stereology of Wistar rats intoxicated with cadmium chloride

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Introduction

Environmental pollution and occupational exposure to heavy metals contribute to major chronic and malignant diseases, consequences to all tissues of the body [1]. Cadmium (Cd), a well-known environmental hazard, exerts a number of toxic effects on humans and animals. Tobacco smoke, contaminated food, environmental and industrial pollution are the main sources of Cd as potential hazards for humans and animals [2]. Industrial activities, such as smelting and refining of metals, and municipal waste incineration also release Cd into the atmosphere [3]. Cd toxicity is associated with severe damage in various organs, particularly the testes, in both humans and animals [3]. The present study was undertaken to evaluate stereological changes of testicular components caused by cadmium chloride ($CdCl_2$).

Material and Methods

In this study ten adult male Wistar rats (90 days old) were used. The animals were divided into two groups of five animals each: the control group (C) received sucrose solution 10% and the group cadmium (Cd) was treated with a 10% sucrose solution plus 75 mg/L of $CdCl_2$. After 30 days, the animals were euthanized and perfusion-fixed with 5% glutaraldehyde in sodium caccodylate buffer 0.05 M (pH 7.4) for 25-30 min. The testes were weighed, embedded in historesin (Leica), stained with 1% toluidine blue / sodium borate. The stereological analysis was performed with 10 images using a grid of 200 points each, to determine the proportion of testicular components (epithelium, lumen and interstitium).The mean values for each experimental group were subjected to statistical variance analysis (one-way ANOVA) followed by Tukey test, where $p<0.05$ was considered statistically significant.

Results

The body weight of animals in group C and Cd did not change during the treatment. There was no significant difference in the relative weight of the reproductive organs. The proportion of epithelium, interstitium and the lumen were also without significant difference between group C and group treated with cadmium chloride. These data are preliminary, but suggest that this dosage administered for 30 days is not very damaging to the testis nor to the general health of the rats. Biochemical and ultrastructural analyses will be performed to confirm these results.

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Effect of urea and tannin on the removal of egg adhesiveness in *Pseudoplatystoma fasciatum* (*Pisces, Siluriformes, Pimelodidae*)

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Introduction

The reproduction of hatchery-raised *Pseudoplatystoma fasciatum* is limited by an unusual problem in migratory fish, the adhesiveness of the eggs, forming clusters that result in a high mortality rate caused by asphyxiation and fungal growth (1). Therefore, the production of "pure" fish has been replaced by hybrid fish, bringing risk to the environment. With this in mind, in order to develop strategies for the production of "pure" *P. fasciatum*, the goal of this study was to evaluate the use of different protocols for removal of egg athesiveness and correlate with their viability.

Material and Methods

Two females and two males were induced with carp pituitary extract (0.5mg.kg-1 and 5.5mg.kg-1 with an interval of 12 hours between doses for females and 2.0mg.kg-1 for males at the time of second dose of females) (2). After stripping the fish, an oocyte pool was made and fertilized with a pool of semen. The oocytes were distributed by the following design: treatment 1 (T1), T2 and T3 with thirty, sixty and ninety minutes respectively of exposure to a urea 0.03% and NaCl 0.04% solution (in water); and T4 that was exposed for ten minutes in urea 0.03% and NaCl 0.04% (in water), followed by wash in a tannic acid solution 0.05% (in water) (2). Control (C) was not exposed to any treatment. Three replications were used per any Ts and C, by incubating 4 ml of eggs in 2.5 liters incubators (15 total), with constant flow of 0.5 liters per minute, at the average temperature of 26.0°C. The fertilization and hatching rates were determined 5 and 15 hours respectively after fertilization.

Results and Discussion

The fertilization mean rates were similar among T1, T2, T3 and C ($p < 0.05$). The hatching rates were higher in controls than all treatments ($p < 0.05$). T4 showed the lowest fertility and hatching rates ($p < 0.05$), however we observed in this treatment the most efficiency for the removal of the eggs adhesiveness. Thus the results showed no relationship between the loss of adhesion (with the used agents) and viability on the eggs of this species. The results point to the implementation of new approaches to establish the relationship between the ideal number of eggs and the area available for its adhesion in incubators.

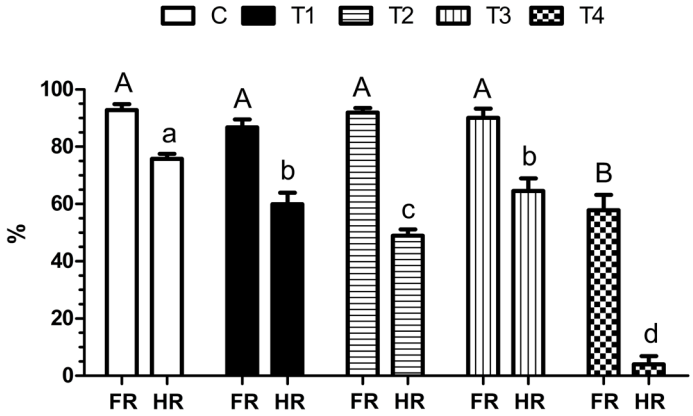

Figure 1. Average fertilization (FR) and hatching rates (HR). Capital letters indicate significative hatching rate difference ($P<0.05$) and lowercase letters indicate significative hatching rate difference ($P<0.05$) between treatments. Bars represent the standard error.

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The lack of heat shock and insulin-like growth factor-I effect on bovine oocyte caspase activity

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Introduction

Summer heat stress depression in fertility is a multifactorial problem that affects physiological and cellular functions in several tissues. Exposure of bovine oocytes to elevated temperature reduced oocyte developmental competence and triggered the apoptotic cascade. It has been demonstrated that insulin-like growth factor-I (IGF-I) plays a termoprotector role in the bovine oocyte. Therefore, the objective of this study was to evaluate the effect of heat shock on group II caspase activity (caspase-2, -3 and -7) and determine the role of IGF-I in this context.

Materials and Methods

Cumulus-oocyte complexes (COCs) collected from slaughterhouse-derived ovaries were subjected to Control (38.5°C for 14 h) or heat shock (41°C for 14 h) treatments in the presence of 0 or 100 ng/mL IGF-I during *in vitro* maturation (IVM). Immediately after 14 h treatment COCs were denuded by repeated pipetting and subjected to *in situ* caspase activity evaluation using specific caspase substrate PhiPhiLux-G_1D_2. Caspase activity was determined immediately after the end of heat shock using an Olympus IX81 fluorescence microscope. Digital images of each oocyte were acquired and stored as tiff files. Fluorescence intensity was analyzed and quantified using the software Image J version 1.43. The experiment was replicated 5 times using 50-58 COCs per treatment. Data were analyzed by Kruskal-Wallis e Wilcoxon procedure of SAS.

Results and Discussion

Exposure of bovine oocytes to 41°C heat shock during the first 14 hours IVM did not affect the percentage of oocytes with high caspase activity regardless of IGF-I. This result differed from a study conducted by Roth and Hansen (2004). In this experiment, heat-induced caspase activity was evaluated after 24 hours IVM, while in the present study caspase activity was determined immediately after 14-hour heat shock. It is possible that the period of 14 hours used in the current study was insufficient to cause the heat-induced peak on group II caspase activity.

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Characterization of the endometrial gene expression profile on day 7 post-estrus in response to a bovine model for modulation of the periovulatory endocrine milieu

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Introduction

Studies have demonstrated the positive effects of progesterone (P4) on early embryonic development as well as its influence on endometrial gene expression during the first week post-estrus in cattle. However, current experimental approaches used to assess the endometrial response to P4 during early diestrus have been based on exogenous supplementation. Modulation of the follicle growth to ovulate larger follicles induces the formation of larger corpora lutea (CL) and higher P4 production, and has been associated with higher probability of conception. The resulting periovulatory endocrine profiles of such an approach depend on the physiological capability of response of each animal. Additionally, distinct rates of follicle growth and luteal functional capacity of producing P4 lead to a profile of endocrine fluctuations that involve estradiol concentrations (proestrus and estrus) as well as a gradual post-ovulatory increase in P4 concentrations (metaestrus and diestrus), hereby denominated periovulatory endocrine milieu. Objective was to characterize the endometrial response to distinct periovulatory endocrine milieus according to the gene expression of genes previously identified as responsive to P4, CCN2, LTF, FST and TIMP2, as well as genes of the CCN family, WISP1, WISP2, WISP3 and NOV.

Material and Methods

Twenty-two cyclic, non-lactating Nelore cows received a P4-releasing device along with estradiol benzoate on day -10 (D-10). Animals were divided to receive a prostaglandin analog (PGF; HP group; N=11) or not (LP group, N=11) on D-10. Progesterone devices were removed and PGF injected on D-2.5 on cows from HP group, and on D-1.5 on cows from LP group. Ovulation was induced with GnRH on D0. Plasma P4 concentrations were measured daily from D0 to D7, when animals were slaughtered and endometrial fragments collected. Complementary cDNA was synthesized from endometrial total RNA extracts. Relative gene expression between experimental groups, determined by qPCR, was normalized by the cyclophilin gene and calculated by the ΔΔCt method. Means were compared by the student's t test.

Results and Discussion

No differential gene expression between HP and LP groups was observed for candidate genes FST, LTF, WISP1, WISP3, TIMP2 and CCN2. However, the expression of CCN family members NOV and WISP2 was reduced in the HP group (mean ± standard error of the mean; 0.8±0.1 vs. 1.1±0.1; 0.8±0.1 vs. 1.2±0.1, respectively; $P<0.1$). The lack of P4 in modulation on the expression of FST, LTF, TIMP2 and CTGF reported here is in contrast to previous reports (1, 2, 3). This suggests that the uterine biological response to P4 is different when the experimental approach used to modulate post-ovulatory P4 concentrations is through exogenous P4 supplementation (1, 2, 3) vs. modulation of the follicle growth (current abstract). Additionally, we identified NOV and WISP2 as P4-responsive genes. Their biological function associated with senescence and arrest of cell growth in addition to our previous data showing increased expression of the PCNA gene in the HP group, suggests an increase in the proliferative activity, and consequently a potential increase in cellular density and secretory capacity of the endometrial tissue.

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Analysis of the Sertoli cells and total number of testicular germ cells from the frugivorous bats *Artibeus lituratus* (Olfers, 1818) after acute exposure to the fungicide mancozeb

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Introduction

Fruit bats have been constantly feeding on fruits obtained from orchards sprayed with pesticides, wich offer an alternative source of food, especially in strongly altered areas. Once these animals act in maintaining the ecosystem and the regeneration of degraded environments (1), the effect of pesticides on them can endanger not only their survival and adaptation, but also the maintenance of forests. Thus, as no studies were found assessing the impact of ingestion of these substances on testicular morphophysiology in these animals, the purpose of this study was to analyze the acute effect of the fungicide mancozeb on concentrations established for its use in the field on morphophysiology of Sertoli cells and on the total number of germi cells from the frugivorous bat *Artibeus lituratus*'s testis.

Materials and Methods

Fruit Bats were divided into three groups: control 1 (animals fed with fruits without fungicide and without adhesive spreader for seven days, n=5); control 2 (animals fed with fruit containing adhesive spreader for seven days, n=5); and treated: animals fed fruits with mancozeb 2g/L and adhesive spreader for seven days (n=6). The experiment was approved by the ethics committee (CEUA/ UFV - number: 02/2012). On day 8th they were euthanized and their testis fixed in Karnovsky solution (24 h) and embedded in methacrylate plastic (Historesin®). Histological sections (3μm) were stained with toluidine blue and 1% sodium borates. The Sertoli cell index and the total support capacity of the Sertoli cell were determined using a light microscope and the analytical program of image Image Pro Plus 4.5. The total number of germ cells of the seminiferous tubules, as well as the number of Sertoli cell per testis and per gram of testis was calculated. The results were analyzed by ANOVA and Newman-Keuls *post hoc* test. Differences were considered significant at $p < 0.05$.

Results and Discussion

There were no differences between the means referring to the parameters of Sertoli cells and number of germ cells of the testis ($P > 0.05$). The mean of the all groups to Sertoli cell index, which measures the efficiency of that cell, and to total support capacity of the Sertoli cell was 12.14 ± 5.67 and 18.86 ± 6.86, respectively. In relation to the total number of germ cells present in a section of the seminiferous tubules and number of Sertoli cell per testis and per gram of testis, the means of the all groups was 89.01 ± 18.85, $6.65 \times 10^6 \pm 1.19 \times 10^6$ and $5.03 \times 10^7 \pm 1.46 \times 10^7$, respectively. Although studies using higher concentrations and longer exposure have shown testicular changes in rats exposed to this fungicide (2,3,4), we observed that the acute exposure (7 days) to mancozeb was not enough to interfere on morphometric parameters of Sertoli cells and on the number of germ cell of the testis. Therefore, we concluded that the concentration established for using this fungicide in the field, which are the same tested here, have low acute toxicity.

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Temperature and humidity index, body temperature at the time of artificial insemination and conception rates in Holstein cows

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Introduction

High yielding dairy cows have increased metabolism and heat production under influence of lactation and high energy feed intake. These factors, combined with high environmental temperature can result in heat stress which affects milk production and reproductive performance. The temperature and humidity index (THI) is one of the most commonly used to indicate heat stress in dairy cows (1, 2). The aim of this study was to investigate the relationship between THI, body temperature at the time of artificial insemination and conception rate of Holstein cows at different times of the year.

Material and Methods

262 Holstein cows had body temperature assessed at the time of artificial insemination using conventional semen after spontaneous or induced estrus. 375 inseminations were performed, 169 from April to September and 206 from October to March. Pregnancy diagnosis was performed by ultrasonography at 30 and 60 days after artificial insemination. Data from the average daily temperature (ADT) and relative humidity (RH) were obtained from the meteorological station near the experimental site. The Temperature and Humidity Index (THI) was calculated by THI = 0.8ADT + RH (ADT - 14.3) / 100 + 46.3 (1).

Results and Discussion

It was found values of less than 72 THI during the experimental period (Table 1). Higher values of THI indicate heat stress for dairy cows and reduce milk production. However, decreasing of conception rate can occur at lower THI values (1). The average of daily THI differed ($P < 0.05$) between seasons but did not differ ($P > 0.05$) between pregnant and non-pregnant cows. Possibly, the calculation of THI at different times of day is the most correct way to evaluate the duration of exposure of animals to THI over 72. Maybe, this index may not be optimal to evaluate the influence of heat stress on fertility of dairy cows because only consider the relative humidity and temperature, neglecting radiation, which is directly related to the heat exchange between different bodies (3). Rectal temperature in non-pregnant cows was higher from April to September, which it was not expected. There was no difference in conception rates between season of the year, 23.7% from October to March and 18.4% from April to September. In conclusion, there was no relationship between THI and the body temperature at the time of artificial insemination with the conception rate of Holstein cows at different times of the year.

Table 1. Relationship between the temperature and humidity index (THI), body temperature and the number of pregnant and non-pregnant cows, at different times of the year

Category	n		THI			Body Temperature (ºC)		
	Aut/Win	Spg/Sum	Aut/Win	Spg/Sum	General	Aut/Win	Spg/Sum	General
Pregnant cows	40	38	63.7 ± 3.9^a	70.4 ± 3.3^b	66.9 ± 4.9	38.9 ± 0.5^a	39.0 ± 0.5^a	38.9 ± 0.5
Non-pregnant cows	129	168	64.4 ± 4.3^a	70.2 ± 2.9^b	67.7 ± 4.2	39.0 ± 0.5^a	38.8 ± 0.6^b	39.0 ± 0.6
Total	169	206	64.2 ± 4.2^a	70.2 ± 3.0^b		38.8 ± 0.6^a	39.0 ± 0.5^b	

Means followed by different lowercase letters in the same row differ by t test or Mann-Whitney test ($P < 0.05$). Aut, Win, Spg and Sum means, respectively autumn, winter, spring and summer seasons.

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Factors associated with the occurrence of assisted births in Holstein cattle

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Introduction

Assisted birth has been associated with dystocia due to incompatibility between fetus and pelvis and it is related to following predisposing factors: parity, twins, stillbirths, calf's sex and body condition score. Early intervention at calving increases the incidence of unnecessary assisted births, and it has been implicated in negative effects on reproduction and milk production (1, 2, 3). This study aimed to assess the factors associated of assisted births in Holstein herd.

Material and Methods

During two consecutive years, it was studied 492 calvings (from 215 heifers and 277 cows) in Holstein cattle maintained in free-stall barns. Category, type of birth (normal or assisted), stillbirths, body condition scores (BCS), twins, calf's sex, time of day (6-18h, day; 18-6h, night) and time of year of births (April-September, autumn/winter; October-March, spring/summer) were considered in analysis. The means of the body condition score and body weight at birth were 3.6 and 3.8, 540 and 623kg for heifers and cows, respectively.

Results and Discussion

12.6% (62/492) of calvings had human intervention, with higher incidence of assisted births and stillbirths in heifers ($P < 0.05$). Undesirable BCS at calving (< 3.0 and > 3.5; scale of 1 to 5) and the birth of male were higher in cows (Tables 1 and 2). Most of assisted calvings occurred during the day and in the spring/summer (Table 3). The incidence of assisted births was higher than reported in other studies: 5.3% (1) and 7.2% to 8.4% (2), and it was mainly associated with stillbirth and heifers. Early intervention in calving occurred mostly during the day and increased the incidence of assisted births because it was related to the presence of employees in the free stall barn. Heat stress can be related to higher occurrence of assisted birth in the spring/summer. Early intervention at calving can result in lower calf viability due to higher incidence of assisted births and stillbirths in heifers and has negative impact on reproductive performance.

Table 1. Events related to calving in Holstein cattle according to animal category.

Category	Stillbirths	Twins	Male calves	BCS <3 e > 3,5
Heifers	17.7% (38/215)^{A}	2.8% (5/177)^{A}	48.3% (83/172)^{A}	30.5% (65/213)^{A}
Cows	8.7% (24/277)^{B}	5.1% (13/253)^{A}	57.9% (139/240)^{B}	54.4% (147/270)^{B}
Total	12.6% (62/492)	4.2% (18/430)	53.9% (222/412)	43.9% (212/483)

^{A,B} Different letters within columns are statistically different ($P < 0.05$) by Fisher test.

Table 2. Occurrence of assisted births according to category and risk factors for dystocia.

Category	Parity	Stillbirths	Twins	Male calves	BCS<3 and >3.5
Heifers	20.0% (43/215)^{A}	47.4% (18/38)^{A}	40.0% (2/5)	14.5% (12/83)	7.0% (15/213)
Cows	6.9% (19/277)^{B}	12.5% (3/24)^{B}	23.1% (3/13)	6.5% (9/139)	4.4% (12/270)

^{A,B} Different letters within columns are statistically different ($P < 0.05$) by Fisher test.

Table 3. Ocurrence of assisted births according to the category, time of day and time of year of births.

Category	Day	Night	Autumn/Winter	Spring/Summer
Heifers	26.8% (33/123)^{Aa}	13.9% (10/72)^{Ab}	16.4% (19/116)^{Aa}	30.4% (24/79)^{Ab}
Cows	14.7% (13/157)^{Ba}	2.9% (3/103)^{Aa}	6.2% (10/161)^{Aa}	6.5% (6/92)^{Aa}
Total	17.0% (46/270)^{a}	7.4% (13/175)^{b}	10.5% (29/277)^{a}	17.5% (30/171)^{b}

Different letters within lines (a,b) and columns (A,B) are statistically different ($P < 0.05$) by Fisher test.

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Chronic exposition of aluminum chloride on fertility of Wistar rats

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Introduction

Aluminum is the most widely distributed metal in the environment and is extensively used in daily life that provides easy exposure to human beings. Some studies showed that the aluminum chloride ($AlCl_3$) caused deterioration in sperm quality, enhancement of free radicals and alterations in antioxidants enzymes (1,2,3). The present work aimed to evaluate the effects of different concentrations of aluminum on sperm parameters of rats chronically exposed to $AlCl_3$.

Material and Methods

Thirty adult Wistar male rats were randomly distributed into five experimental groups (G; six per group). G1 group received 1.0 mL of distilled water by gavage (Gv), while animals of groups G2, G3, G4, and G5 received, respectively, 0.02 mg/L (4), 0.1 mg/L (4), 50 mg/Kg (1,2), and 200 mg/Kg of aluminum (1, 2; $AlCl_3$; Gv; 1 mL/ group) during 111 days. On day 112th treated males and non-treated females were allowed to mate (1 male: 2 females) for 5 days; thereafter they were maintained separated. Males were euthanized three days later and sperm were collected from the epididymal cauda. The sperm motility and kinetic parameters (VAP, VSL, VCL, LIN, and STR) were analyzed with computer assisted semen analysis (CASA). The structural integrity of plasma and acrosomal membranes were assessed by epifluorescence microscopy (PI/CFDA). Females were euthanized 15 days later, and the pregnancy rate was evaluated. The experiment received the approval of the Ethics Committee (number 19/2011).

Results and Discussion

There were no differences among experimental groups for the kinetic parameters of the sperm (ANOVA; $P > 0.05$). Results of the epifluorescence analysis are shown in Table 1. The number of pregnant females was lower in G5 (3/8) than G1 (10/12), G2 (10/12), G3 (10/12), and G4 (7/12; $P < 0.05$). Two males from G5 died during the treatment. In conclusion, a decrease in the integrity of the sperm membrane was observed already in the low dose group (0.1 mg/L) and a drop in the fertility was observed only in the higher dose group (200 mg/ Kg). The absence of a decrease in the fertility in groups receiving less than 200 mg/ Kg can be due to the fact that the damage caused by aluminum may be a compensatory defect.

Table 1. Percentual (Mean ± SD) of spermatozoa having an intact plasma membrane ($CFDA^+/PI^-$) and a damaged plasma membrane ($CFDA^-/PI^+$) analyzed by propidium iodide (PI) and 6-carboxifluorescein diacetate (CFDA) fluorescent dyes.

	G1	G2	G3	G4	G5
$CFDA^+/PI^-$	40.7 ± 10.5^c	24.3 ± 7.6^b	10.8 ± 8.9^a	6 ± 6.9^a	8.5 ± 8.7^a
$CFDA^-/PI^+$	48 ± 13.4^c	70.5 ± 7.8^b	89.2 ± 8.9^a	94 ± 6.9^a	88 ± 15^a

[a,b,c]Within row, values without common superscripts differ ($P < 0.05$) by ANOVA and Newman-Keuls test.

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Effects of heat stress in the quality of Santa Ines sheep oocytes and cell death by apoptosis

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Introduction

The sheep raising is a primary sector activity with significant socio-economic development across all regions of Brazil. However, the reproduction of these animals seems to suffer influence of climatic factors, due to stress caused by high temperatures and air humidity. The current study has the objective of evaluating the effects of heat stress in the quality and cell death of the oocytes of Santa Ines breed.

Material and Methods

Fourteen Santa Ines ewes were used, seven for each treatment, and submitted to two climatic conditions in a climatic chamber: comfort (25° C and 70% relative umidity (RH) and heat (35° C and 70% RH). After 20 days of acclimation, the ewes were treated with intra vaginal impregnated sponges (60 mg of medroxyprogesterone acetate), the beginning of the treatment was considered D0. On D7 75μg of D-cloprostenol was administered intramuscularly. The sponge was replaced on D10 (1st day of aspiration) and maintained up to the day of the last follicular aspiration. Each ewe was submitted to follicular aspiration on D10, D17 and D24. Forty eight hours before each follicular aspiration ewes received simultaneously a dose of FSH (70 mg NIH-FSH-P1) and eCG (300 IU). Follicular puncture guided by laparoscopy (LOPU) was performed in all ewes. Retrieved COCs were evaluated and ranked (G1- high quality, to G5 – low quality) using a stereoscopic microscope at 40x magnification. Ranking was based on the aspects of the cumulus and the oocyte cytoplasm (1). COCs classified as GI and GII were submitted to the test of DNA fragmentation (TUNEL) to evaluate occurrence of apoptosis.

Results and Discussion

No effect of treatments could be observed on the ranking of COCs quality. Results are shown in Table 1.

Table 1. Quality of COCs aspirated by laparoscopy of Santa Ines sheep kept in a climatic chamber with two environmental conditions - comfort (25°C/ 70%RH) and heat (35°C/70%RH).

COCs ranked	Treatments	
	Comfort (%)	Heat (%)
Degree – I	22 (22.45)[a]	21 (17.07)[a]
Degree – II	47 (47.96)[a]	63 (51.22)[a]
Degree – III	15 (15.31)[a]	22 (17.89)[a]
Degree – IV	4 (4.08)[a]	5 (4.06)[a]
Degenerate	10 (10.20)[a]	12 (9.76)[a]

Likewise, the percentage of oocytes with fragmentation of the DNA evaluated by the TUNEL method was not affected by climatic treatments ($P > 0.05$), 34% of the COCs collected from the comfort ewe group and 24% of those collected from ewes submitted to high temperature and humidity showed signs of apoptosis. It can be concluded that oocytes of Santa Ines ewes do not suffer from heat stress when environment conditions do not surpass 35°C and 70% RH. According to some studies (2), the cumulus cells appear to have a key role in the protection of the oocytes against cell death by apoptosis induced by stress

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Influence of maternal obesity on neonatal development of rat testis and gonocyte population

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Introduction

The gonocytes are the precursors of male germ cells found in fetal and neonatal period [1]. There are strong evidences about the role of androgens in the development of gonocytes during the fetal period [2]. It is also known that obesity alters the levels of sex hormones leading to an increase of androgens in women [3]. However, little is known about the effects of maternal obesity on the male genital tract development at neonatal period. This study evaluated if maternal obesity induced by high fat diet interferes in neonatal development of testis and gonocyte population.

Material and Methods

Obesity was induced in female rats by feeding diet containing 20% of saturated lipids for 15 weeks whereas control animals received balanced murine diet (4% lipids). Male offspring of normal and obese mothers at ages 0.5 and 4.5 days post partum (dpp) were killed (n = 6 per group) and the testis were processed for light microscopy and serially sectioned. The histological sections were submitted to immunocytochemical reaction for androgen receptor (AR) and Anti-Müllerian hormone (AMH). The number of gonocytes per testis was estimated based on procedures described by Delbès et al. (2007) [4]. We used ANOVA and Tukey HSD; $p \leq 0.005$ was considered statistically significant.

Results and Discussion

Maternal obesity induced by high fat diet decreased lightly the body weight and increased the testis weight at birth. Although these alterations were not statistically significant they resulted in an increase of 41% ($p = 0.03$) of the gonadosomatic index of newborn pups. The number of gonocytes at birth was unaffected in neonates born from obese mother. In contrast, the testicular weight and gonocyte number had a reduction of 23% ($p = 0.03$) and ~35% ($p = 0.001$) respectively at 4.5 dpp in relation to control group. Thus, maternal obesity probably did not affect the processes of gonocyte differentiation during fetal life but severely interfered in their postnatal development. Neonatal gonocytes showed no immunoreactivity to androgen receptor suggesting that the consequences of maternal obesity on these cells occurred independently of AR route.

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Positive effects of supplementation on reproductive performance of zebu cattle along breeding season

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Introduction

From 2007 to 2011, Brazil has produced an average of 49 million calves per year (1). The growth period of the calves it's very important in the production, it provides the raw material: the calf (3). This phase of the production often does not receive the necessary attention by some producers; they believe that this is an extremely laborious and low financial return. To increase the profit in this period, the producers should adopt measures that demonstrably increase the reproductive rates, such as the use of a good mineral mix, good body condition score (BCS), adoption of rational managements and implementation of the breeding season (BS) in order to anticipate the heat return for the subsequent BS (2, 4), thus providing the ideal financial model of any farm: a calf / cow / year. These actions taken pertinently reflect directly on any beef segment (3). This study aims to evaluate the effects of amino acid supplementation on the ovulation and early conception rate of non pregnant cows with good BCS.

Material and Methods

Forty six cows and two *Bos indicus* sires were used with the relationship: 1: 23. The cows were homogeneously distributed relating the age and BCS in two groups: G1 - cows supplemented with iodized salt, and G2 - cows supplemented with amino acid salt. The cows were placed into two paddocks and every 10 days the treatments were rotated between them. The early diagnosis of pregnancy and ovulation rate was performed 45 days after the beginning of the BS by transrectal ultrasound. The BCS was assessed by the method described by Nicholson & Butterworth (1986). The degree of ovulation of the animals was measured using a scale designed for this study, using the condition of the follicles and ovaries. The scale ranges from 1 to 6 points: 1) Bad Ovary= few follicles with small size; 2) = Middle Ovary: follicle beginning the dominant process; 3) Good Ovary = presence of dominant follicle; 4) = Left Corpus Luteum Ovary: presence of corpus luteum indicating a probable ovulation; 5) = Right Corpus Luteum Ovary: presence of corpus luteum indicating probable ovulation and 6) = Positive Pregnancy: presence of a fetus identified by ultrasound. To verify the existence of statistical differences a nonparametric test (Wilcoxon) was performed with a 95% confidence level (6).

Results and Discussion

A good BCS at birth is very important for the reproductive management, because these cows have a high probability of becoming pregnant at the start of next BS, so they won't face the effects of the postpartum anestrus. Both treatments showed good BCS 45 days after the beginning of the BS. The average of the BCS from G1 was 6.43 ± 0.15, whereas the BCS of the cows from G2 was 6.48 ± 0.16, no significance difference was observed ($z = -0.12$ $p = 0.90$). The ovulation rate for G1 was 3.78 ± 0.28 and 5.30 ± 0.22 for G2, no difference between treatments was observed ($z = -3.14$ $p = 0.001$). About the pregnancy rate, the animals of G2 had statistically higher rate of early pregnancy than G1 ($z = 2.02$ $0 = 0.04$). G1 obtained a 26% pregnancy rate, while the pregnancy rate of G2 was 61%. It can be concluded that the amino acid mineral supplementation stimulates the early conception, since the pasture and the BCS were the same for G1 and G2, differing only by the type of supplementation.

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Acknowledgments: Premix – Nutrição de Resultados.

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Correlations between consumption, corporal and seminal features of water buffaloes (*Bubalus bubalis*)

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Introduction

The recent utilization of agro-industrial byproducts available in the Eastern Amazon, such as coconut meal (CM) and palm kernel cake (PKC), to compose animal rations may constitute a bioeconomic alternative to animal production, especially in critical periods of the year (1). Some of these byproducts are rich in lipids and may provide relevant zootechnical gains, but their effects on reproductive aspects need to be elucidated. Thus, this study aimed to evaluate correlations between lipid intake, body weight, scrotal circumference and semen quality in buffaloes supplemented with experimental diets containing CM and PKC-based.

Material and Methods

Fifteen buffaloes were divided into groups Control (n=5; conventional concentrate mixture for termination; 3.64% fat), CM-Base (n=5; coconut meal-based ration; 8.87% fat), and PKC-Base (n=5; palm kernel cake-based ration; 11.82% fat). Animals were maintained on grazing and received isoproteic supplementation during 252 days (~18% CP; 1% BW). Body weight and scrotal circumference were evaluated each 28 days. Semen collections were weekly realized and sperm evaluations (n=173) were performed according CBRA (2). Pearson´s correlations were calculated using the *Statistical Analysis System* (SAS), with $P<0.05$.

Results and Discussion

The correlation between body weight and scrotal circumference (Fig. 1) was positive and highly significant ($r=0.88$; $P<0.0001$), in accordance with previous published data (3,4). The scrotal circumference also was positively correlated with sperm concentration (Fig. 2). Positive correlation was found between crude protein intake and sperm motility ($r=0.20$; $P<0.0068$). According to (5), who evaluated higher (14.45%) and lesser concentration (8.51%) of crude protein in the concentrate, there is significant increase in sperm motility in bulls supplemented with higher protein level. Correlations between lipid intake and sperm motility ($r=0.34$; $P<0.0001$) and lipid intake and plasma membrane integrity ($r=0.35$; $P<0.0001$) were also relevant. Diet can alter the composition of semen, plasma membrane integrity and fertilizing sperm (6). Therefore, higher levels of lipid consumption could explain both the better motility observed and the higher levels of sperm membrane integrity.

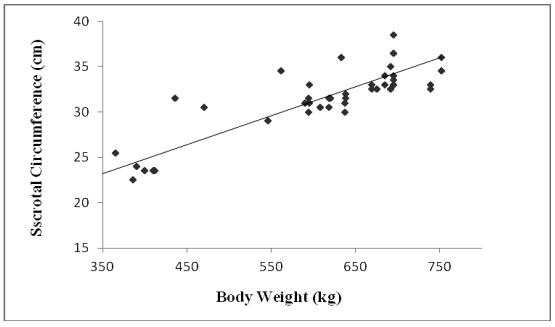

Figure 1. Correlation between body weight and scrotal circumference.

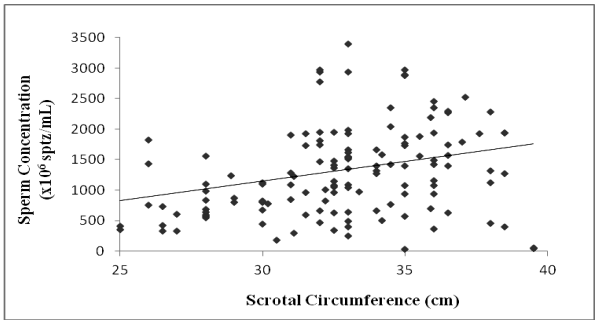

Figure 2. Correlation between scrotal circumference and sperm concentration.

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Seminal features of buffalos supplemented with rations based on coconut meal or palm kernel cake

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Introduction

Modern animal production requires biotechnical solutions to increase animal performance, and profitable alternatives that conjoin economic gains and environmental protection. Some agrindustrial byproducts are largely available in the Eastern Amazon, such as the residues of coconut (*Cocos nucifera*) and palm oil (*Elaeis guineensis* Jacq.) processing, which have potential to be incorporated in animal nutrition. Thus, the objective of this work was to compare the seminal features of buffaloes supplemented with conventional concentrate and concentrates based on coconut meal or palm kernel cake.

Material and Methods

Fifteen buffaloes (3.2±1.3 years, 578.6±101.9 kg) raised on pasture (*Panicum maximum*) at Embrapa Eastern Amazon were daily supplemented with isoproteic concentrates (1% BW) during 252 days, into three groups: Control (n=5; conventional concentrated for buffaloes in termination), CM-Base (n=5; ration with coconut meal base) and PKC-Base (n=5; ration with palm kernel cake base). The daily consumption of concentrates was individually calculated (kg) immediately after ingestion. Semen samples were collected weekly (173 samples). Evaluation considered the semen volume (mL), pH, mass activity (0-5), spermatic vigor (0-5), progressive sperm motility (%), integrity of spermatozoa plasma membrane (%) and sperm morphology (%), as CBRA (1) and (2). Data were submitted to analysis of variance and means were compared by t Test ($P<0.05$), using SAS statistical software (3).

Results and Discussion

The average consumption of concentrates was 4.778±1.233 kg in Control, 3.112 ± 0.693 kg in CM-Base and 4.558±1.077 kg in PKC-Base ($P>0.05$). The semen volume (Control=6.9±0.4, CM-Base=7.0±0.6 and PKC-Base=6.8±0.5), the mass activity (Control=2.9±1.9, CM-Base=3.4±1.8 and PKC-Base=3.1±1.5), the spermatic vigor (Control=3.6±1.0, CM-Base=3.7±1.1 and PKC-Base=3.9±1.0) did not differ ($P>0.05$). The pH of the ejaculates ranged from 6 to 8, but it did not differ (Control=6.9±0.4, CM-Base=7.0±0.6 and PKC-Base=6.8±0.5, $P>0.05$). Sperm concentration of Control (1326.3±893.8) did not differ of CM-Base (1698.1±1023.0) and PKC-Base (1003.2±569.0). The plasma membrane integrity was 68.0±19.5, 72.0±22.6 and 82.1±12.2 for Control, CM-Base and Base-PKC, respectively ($P<0.05$). Similarly, motility was higher ($P<0.05$) in PKC-Base (71.7±15.1) when compared to Control (59.3±20.5) and CM-Base (56 7 ± 24.8). The motility of the PKC-Base is in accordance to the variation from 70 and 80%, presented in excellent buffalos ejaculated (4). The rate of major defects (Control=29.6±18.9, CM-Base=27.8±15.3 and PKC-Base=30.1±21.4), the minor defects (Control=14.6±7.8, CM-Base=13.8±7.1 and PKC-Base=14.0±7.6) and the total defects (Control=44.2±18.5; CM-Base=41.3±16.1 and PKC-Base=44.3±19.2) did not differ ($P>0.05$). These results allows us infer that any increases in defects are not related to the addition of coconut meal and palm kernel cake in the diet. Buffaloes fed with PKC-Base ration showed a better nutrient use of experimental concentrates, which improved the sperm quality, especially in relation to higher motility and higher levels of sperm with plasma membrane integrity. Therefore, the palm kernel cake and coconut meal may be indicated for using in the buffalo bulls' diet, without compromising their sperm quality. Positive effects of palm kernel cake use were observed on two relevant seminal parameters for fertility.

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Effect of açaí pulp (*Euterpes edullis*) on the epididymal cauda region morphometry of Wistar rats exposed to cadmium chloride

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Introduction

Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life of 10-30 years. Its metabolism and excretion depends on the presence of antioxidants (1,2). The fruit of *Euterpes edullis*, known as açaí, contains significant amounts of a distinct class of flavonoids, the anthocyanis, which gives antioxidants properties to this fruit (3). This work aimed to evaluate the effect of açaí pulp on the action of cadmium chloride ($CdCl_2$) on morphometric parameters on cauda of epididymis when given before or after the açaí pulp.

Material and Methods

Adult rats (n=25) were exposed by $CdCl_2$ (single dose; 1.2 mg/kg/BW/intraperitoneal – Ip) and açaí pulp (1.5 ml/animal/gavage – Gv), according to experimental groups (G). The G1 (n=3) received saline (Ip and GV) during 56 days. The animals from G2 (n=6) received $CdCl_2$ (Ip) and saline (Gv), and were euthanized after 48h. Group G3 (n=4) was provided with saline (Ip) and açaí pulp (Gv, 56 days). Animals of G4 (preventive action, n=6) were treated with the açaí (Gv, 56 days) and afterward 56 days received $CdCl_2$, being euthanized 48h later. The G5 (curative action, n=6) received $CdCl_2$ and was treated with açaí (Gv, 56 days). Sections of cauda region were fixed in Karnovsky solution and embedded in methacrylate plastic (Historesin®). The following measurements were performed: tubular (TD) and luminal diameters (LD), and volumetric proportion (%), considering tubular (epithelium, basal lamina, lumen with spermatozoa and lumen without spermatozoa) and intertubular compartments (blood vessels, connective tissue, and smooth muscle). The data were obtained from 10 random microscopic fields per segment at 100x magnitude using a 266 point-grid test system. All the measurements were estimated using the Image Pro-plus software, and the results were analyzed by ANOVA and Newman Keuls tests. Differences were considered significant at $P < 0.05$.

Results and Discussion

The animals of G2 and G4 showed lower values for LD (96.9 ± 56.6 and 119.6 ± 92.4 µm, respectively) and TD (217.8 ± 24 µm and 209.7 ± 76.4 µm, respectively) when compared to G1 (LD - 303.9 ± 17.1 µm; TD - 353.9 ± 76.4 µm). The percentual of epithelium at G2 (38.4 ± 3.2), G3 (27.2 ± 1.1), G4 (38.1 ± 4.5) and G5 (31.6 ± 4.2) was significantly higher than G1 (21.3 ± 2.7). Moreover, the percentual of basal lamina at G2 (16.4 ± 3.2) was higher than G1 (6.9 ± 2.1), and the percentual of lumen with spermatozoa at G2 and G4 was lower than G1. The percentual of connective tissue at G2 (30.6 ± 9.3) and G4 (32.6 ± 12) was higher when compared to the control group (7.3 ± 6; $P < 0.05$). There were no differences among treatments for the percentual of blood vessels and lumen without spermatozoa. Therefore, we concluded that the administration of cadmium chloride resulted in significant alteration of mophometric parameters on the epididymal cauda region and the açaí pulp had no preventive and curative actions on the observed alterations.

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Effects of macro and micronutrients on buffalo semen quality

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Introduction

The quantity of minerals in the bull ejaculated is lower than in the organic tissues and it depends on nutritional elements offered in diets. In young males, macronutrients have an indirect effect on reproduction, while unbalance in micronutrient impairs spermatogenesis, reduces libido and, consequently, affects the fertility. Supplemental feeding strategies can increase productive performance, and effects of minerals offered in the diets can not be neglected (1). Therefore, this study evaluated the correlations between consumption of nutrients contained in concentrates, sperm motility and integrity of plasma membrane (IPM).

Material and Methods

Fifteen adult buffalo bulls (*Bubalus bubalis*) were divided in three groups according to: (Control, n=5; Base-CM, n=5; Base-PKC, n=5). Buffaloes were maintained on grazing and daily supplemented with experimental rations during 252 days (1%Body Weight). Macro and micronutrients consumed quantities were calculated as a function of consumption of experimental rations (kg/DM) and mineral concentration in dry matter base. Macro (Ca, Na, Mg) and micronutrients (Co, Se, Fe, Mn, Cr, Cu, Mo, Zn) were measured according (2, 3) and the individual consumption was daily controlled. In order to assess nutrients effect on seminal quality, ejaculates were weekly evaluated (4). Pearson´s correlations were calculated using *SAS* ($P<0.05$).

Results and Discussion

Correlations between minerals offered and seminal features are demonstrated in Table 1. There was significant and positive correlation, medium intensity, between Ca, Mg, Na and sperm motility, explained by direct involvement of these elements in regulation of cellular energy production and nutrient transport, essential for spermatozoa reaching the *locus* of fertilization. Lower correlations between Ca, Mg and IPM were observed, although these elements play a role in maintenance of osmolarity and stability of biological membranes (5). Sperm motility and IPM presented positive correlation, medium intensity, with all micronutrients, surprisingly the lower with zinc. Certain elements, in adequate concentrations, act as antioxidants and cofactors in capacitation, acrosome reaction and oocyte penetration (6). There was higher intake of macro and micronutrients and significant improve in sperm motility and IPM in Base-PKC animals (data not showed), that becomes palm kernel cake an efficient alternative feedstuff for production and reproduction.

Table 1. Correlations between minerals consumption, progressive sperm motility and integrity of plasma membrane (IPM) in buffalo semen.

Semen Features	Macronutrients			Micronutrients							
	Ca	Mg	Na	Co	Se	Fe	Mn	Cr	Cu	Mo	Zn
Motility (%)	0.338	0.301	0.363	0.376	0.366	0.367	0.339	0.347	0.331	0.319	0.171
IPM (%)	0.244	0.174	0.343	-	0.331	0.329	0.348	0.326	0.219	-	-
P Value	*	*	*	*	*	*	*	*	*	*	**

*P<0.0001; **P<0.001

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Oxidative status in heat stressed *Bos taurus* bulls submitted to polyunsaturated fatty acids oral supplementation.

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Introduction

A higher susceptibility to the heat stress has been observed in *Bos taurus* when compared to the *Bos indicus* bulls. A mechanism that may explain such finding is an increase on oxidative stress and consequent impairment on sperm quality (1). This may occur since sperm are highly susceptible to the attack of the reactive oxygen species (ROS), especially due to the high content of polyunsaturated fatty acids (PUFA) in the sperm membrane, known to positively influence important sperm functional attributes such as motility and fertilizing capacity (2,3). However, the double bounds (insaturations) present in the PUFAs are more easily oxidized by the attack of ROS (1). On the other hand, different ROS may induce damages in different degrees. In order to determine the most adequate antioxidant therapy, studies should be performed aiming to detect the most deleterious ROS. Therefore, the aim of the present study was to evaluate if the PUFA supplementation of heat stressed *Bos taurus* bulls would improve sperm quality and if such supplementation would also negatively influence the susceptibility of the spermatozoa to the different oxidative agents.

Material and Methods

Four sexually mature Bos taurus bulls were submitted to scrotal warming (testicles covered in a thermal bag) during four days. Animals were then submitted to PUFA supplementation (Megalac®) for 60 days. Semen samples, collected by electroejaculation, were divided into 4 aliquots which were incubated with 4 different pro-oxidative agents: xanthine/xanthine oxidase (O_2^-; superoxide anion generating system), hydrogen peroxide (H_2O_2), ascorbate and ferrous sulphate (OH; hydrodyl radical generating system), and malondyaldehyde (MDA; product of lipid peroxidation). Samples were then evaluated for motility by computer assisted sperm analysis (CASA, Ivos), membrane and acrosomal integrities (eosin/nigrosin and fast green/bengal rose stain, respectively), mitochondrial activity (diaminobenzidine stain; DABI: full mitochondrial activity, DABIV: no mitochondrial activity), sperm chromatin structure assay (SCSA) and tiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation. Statistical analysis was performed by the SAS System for Windows. Comparisons were performed using the Least Significant Differences (LSD) test. Significant differences were considered when $p<0.05$, and indicated in the text by different letters.

Results and Discussion

A higher susceptibility to the MDA and OH was found on mitochondrial activity (DABIV; O_2^-: 49.7 ± 19.2^{a}; H_2O_2: 49.8 ± 25.0^{a}; MDA: 88.8 ± 9.3^{b}; OH: 73.3 ± 15.4^{ab}). Also, sperm DNA susceptibility to acid denaturation was higher in MDA and OH incubation samples (SCSA; O_2^-: 48.9 ± 12.9^{b}; H_2O_2: 54.6 ± 8.9^{ab}; MDA: 78.9 ± 8.0^{a}; OH: 74.0 ± 8.6^{ab}). This higher susceptibility to the OH may have been due to the PUFA supplementation; with a higher amount of PUFA in the membrane, more substrate would be available to the attack of OH, the most reactive ROS. On the other hand, the increased susceptibility to MDA may have been exacerbated by the testicular warming. Previous studies indicate that MDA may induce cytotoxic effects by forming adducts with thiol and amino protein groups (e.g., glutathione, lysine, histidine), or by bonding with biomacromolecules such as functional and structural proteins and nucleic acids, affecting several cellular mechanisms (4). Therefore, an alternative to improve sperm quality in heat stressed bulls is the treatment with non-enzymatic antioxidants such as vitamin E, aiming to avoid the beginning of the oxidative chain reaction and the formation of cytotoxic products such as the MDA.

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Influence of sod concentrations on fertility of Nelore cows

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Introduction

Oxidation and free radical production is an integral part of aerobic metabolism (Halliwell, 2007). A variety of reactive oxygen species (ROS) are produced by normal metabolic processes and compromising animal health and welfare reproductive performance. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, and an antioxidant defense important for oxidative stress (Sordillo, 2007). Innovative approaches are needed to understand the physiological factors that affect reproductive rates, to resort to new strategies that contribute to the improvement of fertility. The objective of this study was to evaluate the fertility of beef suckled Nelore cows with different concentrations of superoxide dismutase at the time of artificial insemination.

Materials and Methods

we used 532 Nellore beef suckled cows, submitted to fixed-time artificial insemination (FTAI) protocol, which consisted of placing an intravaginal progesterone device (Sincrogest ®) plus the IM application of 2 mg of estradiol benzoate (Sincrodiol ®), 8 days after the implant was removed and given 0.5 mg of cloprostenol sodium (Sincrocio ®) and 300 IU of equine chorionic gonadotropin (Folligon ®), on the 9 th day was administered 1 mg of estradiol benzoate, and where the TAI 30 hours later. Blood samples for determination of oxidative stress were measured at the beginning of the protocol and at the time of FTAI. To evaluate the effects of oxidative stress were determined on blood concentrations of the enzyme superoxide dismutase (SOD). Pregnancy diagnosis was performed 35 days and 60 days after FTAI to determine the pregnancy rate. High SOD experimental groups (HS) and low SOD (LS) were determined retrospectively. The pregnancy rate and was analyzed by logistic regression using PROC LOGISTIC of SAS.

Results and Conclusions

There was a significant difference in plasma SOD ($P < 0.05$) between HS (2592 U / g Hb) and LS (1694 U / g Hb). There were differences in pregnancy rate to 30 (55.8 vs. 67.1 LS HS) and 60 days (54.9 vs. 67.1 LS HS). Cows with lower concentrations of SOD has a worse fertility, which may be related to the effects of free radicals in oocyte quality and ovulatory capacity in beef suckled Nellore cows, which reflects the importance of an adequate nutritional management and use supplements such as vitamin E and selenium, which may help to reduce oxidative stress and also of equine chorionic gonadotropin (eCG) to improve the ovulatory response. Oxidative stress adversely affects the fertility of beef suckled cows in tropical countries and this may be related mainly to the stress responsiveness of these animals, which can be measured by the concentration of the enzyme superoxide dismutase. However more studies are needed to reveal the mechanisms related to these processes.

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Assessment of the effects of heat stress on the reproductive performance of heifers during breeding season

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Introduction

Heat stress is a factor that affects the reproductive performance of cows, which is associated with pregnancy loss and impaired steroidogenesis, thus impairing the animal's estrous cycle duration and sexual behavior (Nebel et al, 1997). The objective of this study was to evaluate the effects of heat stress on reproductive performance of *Bos indicus* beef heifers during the breeding season.

Materials and Methods

The experiment was conducted from November 2009 to January 2010. We used 101 Nellore heifers at 24 months. The animals were maintained on Brachiaria brizantha with mineral and water ad libitum. At the beginning of the protocol parameters were evaluated body condition score (BCS also evaluated at the time of TAI and pregnancy diagnosis (DG). Rectal temperature (RT) and temperature and humidity index (THI) were evaluated at the time of TAI. The ITU is calculated from the model defined by Thom (1959) being: ITU: (0.8 x T ° C + (RH (%) / 100) x (T-14, 4) + 46.4) where: T = temperature ° C and RH = relative humidity. The climate data used were obtained from the Meteorological Station of USP. The following protocol hormone was used: D0 = implant insertion vaginal P4 + application of 2 mg EB; D8 = implant removal vaginal P4 + application of 300 IU of eCG, according to the body score + 25 mg of D- cloprostenol sodium, 1mg BE = D9, D10 = TAI. The heifers were retrospectively separated into groups according to the ITU at the time of TAI, equivalent to: Class 1 (69-74), Class 2 (75-79) and Class 3 (80-84), the latter being characterized by conditions more extreme temperature and humidity, and through the rectal temperature: Class 1 (37 to 39.4˚ C) and Class 2 (39.5 to 41.5 C). The pregnancy rate (PR) was evaluated by ultrasonography (Aloka 500 with 5.0 MHz linear probe) on days 30, 60 and 90 days after TAI, and separated in accordance with the ITU and TR classes. The data were subjected to frequency analysis using PROC FREQ and logistic regression analysis using PROC LOGISTIC, using the Statistical Analysis System (SAS 2.9) by adopting a significance level of 5%.

Results and Conclusions

There was a reduction ($p < 0.01$) in pregnancy rate according to the class of ITU class 2 and rectal temperature ($p < 0.05$). Beef heifers are therefore sensitive to hot weather, showing a heat stress. This study suggests that the pregnancy rate in beef heifers is affected by weather and body temperature at the time of insemination. The UR is of great importance in heat dissipation, since the more saturated, the greater difficulty losing heat, exacerbating heat stress. Further studies are needed to show the effects of heat stress in *Bos indicus* heifers that effective management strategies can be developed, aiming to increase the fertility of cattle in tropical countries.

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Effect of subcutaneous administration of a vitamin-mineral mix on boar semen quality

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Introduction

Minerals and vitamins play roles in the regulation of a wide range of functions, including homeostasis and reproductive physiology. Supplementation with a vitamin-mineral mix improves boar semen production and quality (1). However, long-term effects of vitamin-mineral administration on semen quality of boars are not known. Therefore, the purpose of this work is to assess the effects of subcutaneous (SC) injection of vitamin-mineral mix on boar semen criteria over the course of 60 days.

Material and Methods

Twelve sexually mature Topigs boars, routinely used for artificial insemination, received SC injection of vitamin and mineral mix (Selen-Fos® and Zimag®, FormilVet LTDA, Brazil), according to manufacturer's directions. Semen samples were collected before supplementation (D0) and every 20 days thereafter (D20, D40 and D60). Semen was evaluated for sperm concentration, motility and vigor, percentage of live and morphologically normal cells and acrosome status (2). Sperm membrane functionality was assessed using Hypo-Osmotic Swelling Test (HOST) (3). Parametric variables were compared ($p<0.05$) by Student's t test. Non-parametric variables were evaluated by Friedman's test.

Results and Discussion

Vitamin-mineral mix had positive long-term effects on all semen criteria by D60, compared to D0 (Table 1). Most semen parameters improved gradually, such as the number of sperm with functional membranes (HOST) and the amount of live sperm with intact acrosomes. The percentages of dead cells and those with damaged acrosome decreased as a function of supplementation. Those findings suggest that vitamin-mineral treatment has a positive effect on membrane integrity aspects, both plasma and acrosomal, associated with epididymal maturation. Vitamin E protects sperm membrane against lipid peroxidation by scavenging reactive oxygen species (ROS). Selenium is a co-factor of glutathione-peroxidase and zinc and cooper are co-factor of superoxide-dismutase, antioxidant enzymes that also mitigate oxidative stress effects. Reductions in the levels of ROS in seminal plasma are consistent with overall improvements in semen quality, as shown in our study, given that boar sperm are particularly sensitive to oxidative stress (4). In conclusion, our findings show that vitamin-mineral SC injection results in better semen quality, but such improvement is only evident with time. Our next objective is to investigate if such enhancement is related to changes in seminal plasma proteins.

Table 1. Means and standard errors of evaluated semen traits during the evaluated periods.

Period	Motility	Vigor	Normal	Droplets (%)		HOST	Vitality/Acrosome Integrity		
	(%)	(0-5)	Sperm (%)	Proximal	Distal	(%)	LIA (%)	LDA (%)	Dead (%)
D0	76.7 ± 4.4^{b}	3.6 ± 0.3^{b}	71.8 ± 7.5^{b}	5.2 ± 3.1^{a}	5.8 ± 4.6^{a}	57.1 ± 6.4^{b}	21.9 ± 6.6^{b}	49.5 ± 3.7^{a}	28.6 ± 3.6^{a}
D20	78.3 ± 2.5^{b}	3.8 ± 0.2^{b}	72.8 ± 4.3^{b}	3.2 ± 0.8^{a}	7.5 ± 2.8^{a}	68.0 ± 2.4^{ab}	27.6 ± 8.5^{b}	37.8 ± 15.4^{ab}	34.6 ± 23.9^{ab}
D40	80.8 ± 2.0^{b}	3.9 ± 0.1^{b}	77.9 ± 4.4^{ab}	2.5 ± 0.8^{ab}	3.4 ± 1.3^{a}	70.2 ± 3.7^{ab}	41.9 ± 8.7^{b}	44.2 ± 9.7^{ab}	13.9 ± 2.9^{bc}
D60	87.5 ± 1.7^{a}	4.3 ± 0.1^{a}	82.6 ± 5.1^{a}	1.3 ± 1.2^{b}	1.2 ± 1.1^{b}	77.8 ± 3.0^{a}	69.9 ± 2.1^{a}	20.9 ± 2.5^{b}	9.2 ± 1.3^{c}

Means with different superscript are significant ($p<0.05$). HOST: reactive cells in the HOST test, LIA: live cells with intact acrosome, LDA: live cells with damaged acrosome, Dead: number of dead cells.

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Effects of scrotal insulation on ram seminal plasma proteome

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Introduction

Heat stress negatively affects male reproductive efficiency, hampering spermatogenesis and epididymal maturation. Scrotal insulation (SI) is a common method for the study of testicular heat stress. In rams, SI for 8 days decreased semen quality (1). In this case, reproductive recovery was gradual, with the first ejaculated sperm being detected 63 days after the end of insulation and semen reaching normal values only 21 days later (2). Given that SP proteins affect a wide range of functions related to sperm physiology (3), the objective of our work is to describe the effects of SI on the proteome of the ram SP.

Material and Methods

Six adult and reproductively sound Morada Nova rams had their testes insulated for 8 days. Semen samples were obtained 7 days before, during SI and weekly until 134 days after insulation. SP was obtained by semen centrifugation and subjected to 2D electrophoresis. Coomassie-stained gels were evaluated using PDQuest software (Bio-Rad, USA). Differentially expressed protein spots, were cut from gels, digested and identified by tandem mass spectrometry (ESI-Q-ToF), as previously described (3). Variables were compared, before and after SI, by Student's t and Friedman's tests ($p<0.05$), according to their distribution.

Results and Discussion

Before SI, protein maps had an average of 269 ± 25 spots per gel, with 132 spots consistently present in all gels. Twenty-four days after SI, when semen was compromised and most rams were azoospermic, only 107 ± 13 spots per map were detected, and 57 were present on every gel of the match set. Even after 113 days, when semen criteria were similar to pre-insulation, there were only 158 ± 12 spots per gel, and 82 were seen on all gels. Scrotal insulation increased the amount of actin, albumin, heat shock protein 70 kDa (HSP70), DJ-1, and one isoform of bodhesin 2 (BDH2) in the SP. These proteins were maximally expressed 29 days after insulation, when most animals were azoospermic or when semen quality was severely damaged. Isoforms of the ram seminal vesicles protein 22 kDa (RSVP22) increased until 29 days, reaching their peak intensity, then decreased until 113 days after SI. Other proteins were decreased as a result of SI, including matrix metalloproteinase 2 and isoforms of BDH2, reaching their nadir at 113 days as well. HSP70 is a chaperone and secreted in response to several types of injury in the testis and epididymis (4). Albumin and DJ-1 act in protection against oxidative stress and prevention of apoptosis, with chaperone-like activity (5,6). Acidic seminal fluid protein (aSFP) is a bovine spermadhesin with antioxidant activity (7), exerted through the CUB domain. BDH2 has 90% sequence homology with aSFP, and also bears a CUB domain. Thus, it seems that secretion of BDH2 is changed as a response to the thermal injury caused by SI. Also, binder of sperm proteins (BSPs) has been shown to exert chaperone activity under heat stress in the bull SP (8). Given that RSVP22 also belongs to the BSP family, and its peak intensity coincided with the period of worst semen quality, this protein could be secreted as a response to the thermal injury in the Morada Nova rams. Although actin has no known protective function, its presence in the seminal plasma denotes leakage from dead cells. Therefore, its increase in the SP after insulation suggests an increase in cell death and outflow of cellular contents in the ejaculate, as shown by the increase in sperm morphological defects and decrease in the number of live sperm (1). In conclusion, we show that scrotal insulation leads to testicular damage and degradation of semen quality. For the first time, we present evidence that these changes were followed by alterations in the protein composition of seminal plasma. Although semen criteria returned to reference values 113 days after SI, more time was required for full recovery of the seminal plasma proteome.

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Evaluation of heat stress in beef cows during breeding season

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Introduction

Some studies have been developedto investigate the effects of heat stresson the abilityof embryonic development and the ability to thermo-tolerance in*Bos indicus* (Paula-Lopes et al. 2003), however, the relevance of these factors on fertility in terms of field hasbeen evaluated. Some hormonal mechanismsaimed atreproduction and thermoregulatory capacity, and reduce the tonic release of LH and cortisol synthesis (Tammingaetal., 1997). Under these conditions the cows also may have changed their thermo-tolerance and their reproductive performance compromised, due to changes in follicular dynamics and oocyte quality and consequently in the fertilization and cleavage. The aim of this study was to estimate the degree of thermal comfort of Nelore cows (Bos indicus) by THI index and rectal temperature at the time of artificial insemination, and the parameters relating to fertility during the breeding season in Brazil (during the summer months).

Materials and Methods

The experiment was conducted at the University of São Paulo in Pirassununga campus. Were used 404 cows and 102 heifers, previously evaluated for body condition score and ovarian condition. The animals were divided into classes according to THI values: A – THI valued from 69 to 74; B - between 75 and 79; and C - between 80 and 84. The values of rectal temperature were also divided into classes: a - between 37.0ºC and 39.4 ºC; and b - between 39.5ºC and 41.5 ºC. Data were analyzed by logistic regression to determine the pregnancy rate between the classes.

Results and Conclusions

For cows there was a significant difference (p=0.002) between THI classes A and C, and between B and C, ie a significant difference between pregnancy rates of animals that showed the lower THI (classes A and B) and the higher THI (class C). For rectal temperature, was observed a significant difference (p=0.002) between the two classes (a and b). Regarding to heifers there was also significant difference (p=0.01) between groups with lower and higher THI (classes A and B compared to C), but there was no significant difference (p=0.49) between classes of rectal temperature. We can conclude that there is a relationship between heat stress and fertility in Bos indicus, however, more studies are needed to elucidate all factors involved in this relationship.

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