The effects of IGF-I on zebrafish testis in tissue culture

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Introduction

IGF-I is a well-conserved growth factor that has important roles during pre and post-natal development in vertebrates, but it also controls tissue homeostasis throughout life via regulation of cell proliferation, differentiation and apoptosis. In vertebrate testis IGF-I can promote germ cell proliferation/differentiation, acting alone or as a permissive factor for androgens, for example. It is known that IGF-I has endocrine, paracrine and autocrine effects. Although some questions have been answered about the possible mechanisms of IGF-I action, many aspects of its function need to be clarified. Based on that, we decided to investigate the role(s) of IGF-I using zebrafish as a vertebrate model.

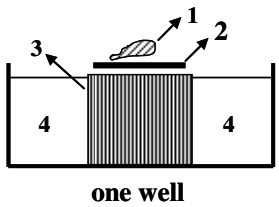
Materials and Methods

Fifteen zebrafish (Tabs) from our animal facilities were anesthetized, sacrificed, and fresh testes were removed and divided in two (half-testis was considered to be one fragment). For each IGF-I concentration, five or six replicates consisting of two fragments were placed on agarose blocks covered with a nitrocellulose membrane, and each agarose block was placed separately in 24-well plastic tissue-culture dishes (Costar) (Fig. 1). The basal culture medium consisted of Leibovitz-15 medium supplemented with 0.5% BSA and 10 mM HEPES (pH 7.4). Culture was carried out for 7 days with various IGF-I concentrations (0, 12.5, 25 and 50 ng/mL) in order to evaluate the *in vitro* effect(s) on zebrafish spermatogenesis. Medium was changed on the 4th day of culture. After one week, fragments were fixed by immersion with 4% glutaraldehyde and embedded in plastic (2-hydroxyethyl metacrylate), and routinely prepared for morphological and morphometric analysis (Fig. 2). Data are presented as mean ± SEM and analysed via ANOVA (Newman-Keuls test); p-values below 0.05 were considered statistically significant.

Results and Discussion

After one week all germ cell types were present in testis tissue cultured in all groups. Although not statistically significant, early spermatogonia tended to accumulate in tissue fragments cultured without IGF-I. A clear effect was visible with respect to an increase in the relative (%) amount of primary spermatocytes, reaching statistical significance in the presence of the highest IGF-I dose (50 ng/mL).

Also the relative quantity (%) of spermatids was significantly increased, while the apparently lower incidence of apoptosis at the higher doses of IGF-I (25 and 50 ng/mL) approached but did not reach significance ($p<0.08$ for 50 ng/mL). These data altogether suggest that in zebrafish IGF-I alone can stimulate spermatogonial differentiation into spermatocytes and spermatids. Our preliminary findings are different from the results obtained for the Japanese eel, where IGF-I rather acts as permissive factor, but similar to those for newt and trout.



1- ZF testis fragment
2- filter paper
3- agarose block
4- medium (1mL)

Fig. 1. The zebrafish testis culture system. The system consists of a pre-soaked agarose block (750 µL) and medium (1 mL) in a 24-well plate, covered with a filter paper containing two zebrafish testes fragments equal to one single testis.

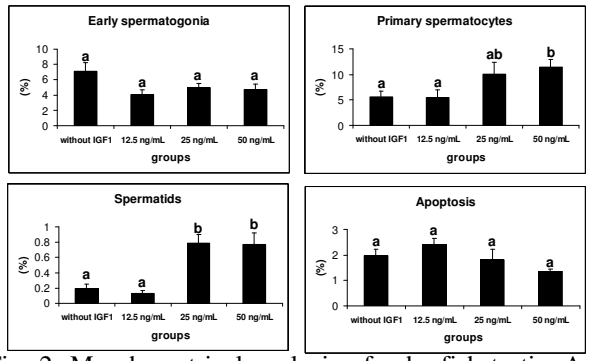


Fig. 2. Morphometrical analysis of zebrafish testis. A-D) Relative volume (%) of zebrafish testis components.

Conclusions

- This is the first report to show IGF-I effect(s) on zebrafish spermatogenesis in tissue culture;
- Zebrafish testis tissue culture is a suitable model to study IGF-I effect(s) on spermatogenesis;
- IGF-I alone promoted entry of spermatogonia into meiosis;
- Further studies will be necessary to elucidate, in more details, the mechanism(s) of IGF-I action in this vertebrate species.

Culture of bovine spermatogonial stem cells in agar medium

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Introduction

Spermatogonial stem cells (SSC) are a subset of type A spermatogonia capable of self-renewal in the testis. In vitro, these cells proliferate and form colonies [1-3]. When cultured in common liquid media some SSC will move during culture [4]. When isolated and cultured, SSC approach to each other, tend to aggregate, and doing so they also tend to differentiate (Kanatsu-Shinohara, M., personal communication) . One way to override this problem would be to take precautions to evenly distribute cells in the culture wells [1]. An alternative approach would be to culture SSC in semisolid media. Immobilization of SSC would allow doing quantitative research on SSC behavior in vitro. In the present work we cultured bovine SSC in agar medium in order to validate this methodology.

Materials and Methods

Bovine spermatogonial cultures were grown in agar medium as well as regular culture medium (MEM). General proliferation was evaluated through the WST1 assay. Colonies appearing in culture were morphologically evaluated and quantified.

Results and Discussion

During the first 10 days of culture in agar, the number of viable cells tended to drop, as measured by WST1 (Fig 1). At day 15 these values significantly raised ($P < 0.05$) in the non-refreshed cultures (a treatment in which all nutrients were dissolved in agar at the starting point), (Fig. 1), but most likely due to agar dehydratation and subsequent concentration of solutes, as the morphology of individual SSC began to deteriorate. Nevertheless, at day 15 cultures which had been periodically refreshed showed better SSC morphology compared to non-refreshed cultures. Colony numbers were significantly higher while culturing in agar than in liquid medium (MEM) (17 ± 1.4 vs 5 ± 2.0, $P < 0.01$), but colonies appearing under MEM were bigger and cells looked healthier, suggesting agar was able to immobilize SSC but prevented important cell-to-cell contacts and differentiation during culture.

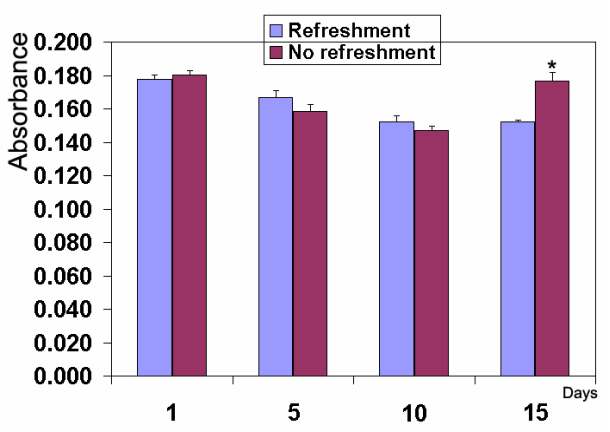

Fig. 1. General proliferation of bovine spermatogonial cultures (WST1 assay).*, $P < 0.05$

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Spermatogonial transplantation in the Nile-tilapia

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Introduction

Germ cell transplantation is a fascinating and powerful technique developed recently, aiming to investigate spermatogenesis and the stem cell biology. This technique also offers great potential for studies involving biotechnology, transgenic animals, and the preservation of the genetic stock of valuable animals or endangered species. Although germ cell transplantation is well characterized for mammals, there is no study utilizing this approach for fish. Tilapias (*Oreochromis niloticus*) are an excellent experimental model due to their fast growth, relative small size when they reach sexual maturity, good adaptability to different environmental conditions and economical importance. Therefore, our main goal in the present study was to investigate the viability of adult tilapias as an experimental model for germ cells transplantation in fishes.

Materials and Methods

The depletion of endogenous spermatogenesis was performed successfully with two busulfan injections [18mg/kg/BW and 15mg/kg/BW (two weeks after the first injection)] in tilapias kept at 35ºC. The germ cells to be transplanted were obtained from fifteen sexually mature tilapias that had their testes enzymatically digested with collagenase, trypsin, and DNAse. Spermatogonial germ cells were selected and enriched utilizing Percoll gradient. These cells were labeled with 3.6 X 10^{-5} M of PKH26 (Red Fluorescent Cell Linker, Sigma) and mixed with a trypan blue solution to check the transplantation efficiency. Eighteen busulfan treated adult tilapias received, into the testis, a total of ~10^7 donor germ cells. Recipient fish had their testes analyzed by light and fluorescence microscopy, 15 minutes, 14 hours and 2, 4, 5, 6, 7, and 8 weeks after transplantation, in order to investigate the eventual presence of PKH26 labeled germ cells in the seminiferous tubules

Results and Discussion

Fifteen minutes after transplantation, PKH26 labeled germ cells were observed in the seminiferous tubule lumen of the recipient testes and at fourteen hours, these cells had established contact with the recipient Sertoli cells. At two to six weeks after transplantation, labeled germ cells formed evident spermatogenic cysts showing varied sizes and different stages of development. They were located mainly in the testis regions near to the urogenital papillae. The PKH26 labeled cysts surrounded by Sertoli cells were apparently bigger and more frequent at four to six weeks, compared with those observed in tilapias sacrificed two weeks earlier. Finally, seven to eight weeks after transplantation, round and small PKH26 labeled cells were eventually detected into the seminiferous tubule lumen. Moreover, these cells showed scarce cytoplasm (undetectable anti-actin labeling) suggesting that they were released spermatozoa originated from the transplanted spermatogonia.Thus, our results showed for the first time in fish, that spermatogonial germ cells can be successfully transplanted directly into the testes and are capable of colonization, proliferation and differentiation in the recipient testes. In this regard, tilapias can be utilized as an experimental model to investigate the germ cell biology and the testis function in teleosts. Moreover, these findings also provides a new approach for fish bioengineering, preservation of genetic resources of endangered fish species or even fish strains carrying commercially valuable traits.

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Effects of postnatal treatment with leuprolide and propyl-thiouracil (PTU) on Sertoli cells proliferation in wistar rats

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Introduction

The FSH is considered the main mitogenic factor for Sertoli cell (SC) whose proliferative period in rats ends between two to three weeks after birth. Because SC has a finite support capacity for germ cells, the total number of SC per testis, established before puberty, determines the final testis size and the sperm production in the adult animals. The period of SC mitotic activity is extended by approximately two weeks in rats made transiently hypothyroidic by PTU treatment during the neonatal period, leading therefore to significant increase in testis size, SC population, and sperm production in adult rats. Leuprolide is a very potent GnRH agonist/antogonist utilized to suppress gonadotropic hormones (LH and FSH). Unexpectedly, studies developed in our laboratory showed that the combination of PTU+leuprolide resulted in similar testis size and sperm production in adult rats in comparison to animals treated only with PTU. As FSH plasma levels rise markedly in leuprolide treated rats 5 weeks after the drug injection, a period when SC is probably still immature in PTU-treated rats, in the present work we investigated the effects of leuprolide+PTU (L+PTU) treatment on SC proliferation in rats sacrificed from 30 to 43 days of age.

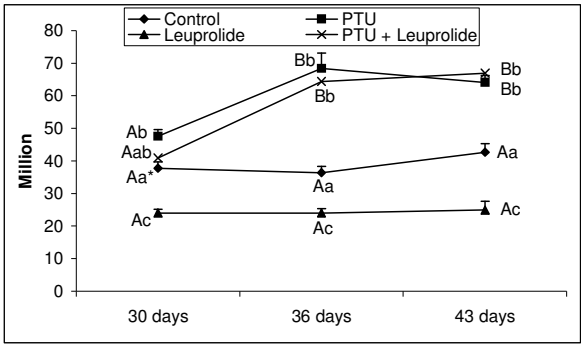
Materials and Methods

Randomly selected newborn male Wistar rats were treated with leuprolide (TAP Pharmaceuticals Inc.) (n=13), PTU (6-propyl-2-thiouracil, Sigma) (n=19), L+PTU (n=17), whereas the control rats (n=12) received only the vehicle. These rats were sacrificed at 30, 36, and 43 days after the beginning of the treatments. At least 4 rats were utilized for each experimental group at each age investigated. Testis tissue from all rats was fixed in 4% buffered glutaraldehyde, embedded in glycol methacrylate, and routinely prepared for histological and morphometric evaluation.

Results and Discussion

The total number of SC per testis is shown in Fig.1. Because little SC proliferation occurs after three weeks of age in rats, as expected the number of these cells in the control group did not change ($p>0.05$) in the different ages investigated. Although at a lower magnitude ($p<0.05$), in comparison to the controls, SC number per testis also did not change ($p>0.05$) from 30 to 43 days in leuprolide-only treated rats. However, compared to both groups cited above, the total number of these important somatic cells was significantly higher ($p<0.05$) in the animals treated with L+PTU and PTU. Particularly at 36 and 43 days of age, the figures obtained for SC were very similar in L+PTU and PTU treated animals. However, comparing only these two experimental groups, at 30 days of age SC number was still significantly reduced ($p<0.02$) in L+PTU treated rats. Taken together, these results show that the association of the SC immature status and high FSH plasma levels in L+PTU treated rats resulted in higher SC proliferation rate, and similar testis size and sperm production already observed for adult rats treated neonatally with PTU and L+PTU.

Figure 1. Total number of Sertoli cells per testis.



* Different capital letters in the same line and small letters for the same age mean significant differences ($p<0.05$).

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Effects of the neonatal administration of antide, PTU (6-propyl-2-thiouracil) and leuprolide on adult Leydig cells and macrophages, in wistar rats

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Introduction

The neonatal period is critical for the establishment of the total number of Sertoli cells, which ultimately dictates the testis size and the magnitude of sperm production in adult animals (1). The FSH (follicle-stimulating hormone) and thyroid hormones (T4 and T3) are considered, respectively, the main factors responsible for Sertoli cells proliferation and differentiation/maturation (2). However, these factors might also direct or indirectly influence the proliferation and the function of the adult-type Leydig cells and macrophages, mainly because there are functional interations between these two cell types (3) and between Leydig cells and Sertoli cells. Therefore, the objectives of the present study were to investigate the effects of the neonatal administration of GnRH agonist (leuprolide, Tap Pharmaceuticals), antagonist (antide, Bachem), and PTU (propylthiouracil, Sigma), a drug that induces transient hypothyroidism, on Leydig cells and macrophages numbers and size in Wistar rats.

Materials and Methods

Fourty-six newborn Wistar rats were utilized (Control, n=8; PTU, n=8; Leuprolide, n=8; PTU+Leuprolide, n=7; Antide, n=7; PTU+Antide, n=8). These animals were treated during the neonatal period and sacrificed at 100 days of age. Additionally, fourty-four newborn rats (Control, n=24; Leuprolide, n=20) were treated with Leuprolide and sacrificed at 0, 1, 2, 3, 5, and 10 days of age (n=4 for each age investigated) in order to study the fetal Leydig cells size and number per testis. All animals utilized had their testis fixed in buffered glutaraldehyde. These testes samples were routinely prepared for histological and sterelogical analyses.

Results and Discussion

The results found for younger rats showed that leuprolide treatment significantly increased ($p<0.05$) fetal Leydig cell individual size 24h after injection. However, compared to the control, at days 5 and 10 fetal Leydig cell size, as well as Leydig cells number per testis, were reduced ($p<0.05$) in treated rats. These findings point out to the initial GnRH agonist effect of leuprolide that shortly after injection becomes a very potent antagonist. Concerning the adult rats, all prepubertal treatments utilized were able to affect ($p<0.05$) several adult-type Leydig cells parameters evaluated. Among these parameters it could be mentioned the Leydig cells volume density (%) in the testis parenchyma, the proportion between nucleus and cytoplasm, individual Leydig cell size, and the total number of Leydig cells per testis. Particularly, the PTU+Leuprolide treatment resulted in significantly lower ($p<0.05$) Leydig cells volume density, nuclear volume, cytoplasmic volume, and Leydig cell individual size. In a less evident fashion, all treatments also affected the macrophages. In conclusion, it can be inferred that prepubertal treatment with GnRH agonists/antagonists and transient neonatal hypothyroidism are able to induce morphofunctional changes in the adult population of Leydig cells and macrophages in Wistar rats.

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Effects of human recombinant leptin on the postnatal testis development in Sprague Dawley rats

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Introduction

Leptin is the adipose-derived peptide that has been shown to be important for the regulation of food intake, metabolism and reproductive function in both males and females. Active leptin receptor (Ob-Rb) is localized in the arcuate and ventromedial nuclei of the hypothalamus, and also in the goanadotropes, Leydig and male germ cells. However, little is known regarding the role of leptin in the male reproductive tract. Recent sudies developed by our research group have shown that the hyperleptinemia caused by the neonatal administration of monosodium L-glutamate (MSG) causes several alterations in the hyptothalamus-pituitary-testis (H-P-T) axis. However, because MSG damages the arcuate nucleus, thus altering the H-P-T axis, it was difficult to infer the direct effect of leptin in the testis. Thus, the aims of this investigation were to study the effects of postnatal human recombinant leptin (HRL) on prepubertal rat testis development.

Materials and Methods

Seventy-five newborn Sprague-Dawley rats were utilized. From day 5 to day 30 of age, approximately fourty animals received, twice a day (07:00 and 19:00 h), 30µg of HRL (Lilly) per 100g/BW. These rats were sacrificed at days 10, 20, and 30 after birth and had their testes routinely prepared for histological and stereological analyses. Also, during sacrifice blood samples were taken to investigate plasma levels of HRL, FSH, T, and 3ß-diol.

Results and Discussion

From sixteen to twenty-five days after the beginning of the HRL treatment, the body weight was always significantly lower ($P<0.05$) in treated rats, demonstrating the effect of the treatment. In fact, HRL levels were very high in treated rats. However, the gonadosomatic index was similar in both groups investigated and only at 30 days of age the testis weight was lower ($P<0.05$) in treated rats. FSH plasma levels were higher ($P<0.05$) in control group at 10 and 30 days of age, and lower ($P>0.05$) in the treated rats at 20 days of age. The same trend was observed for 3ß-diol, whereas the T levels were similar in both groups at the three ages investigated. Similar to FSH levels, the tubular diameter was higher ($P<0.05$) in control rats at 20d, and 30d. No difference ($P>0.05$) was observed for the total number of Sertoli and Leydig cells at 20d and 30d of age. Whereas the Leydig cell nuclear volume was significantly higher ($P<0.05$) at 20d in treated rats. The histological evaluation showed that the spermatogenic process was slightly more advanced in control rats at 30d, while no clear interpretation in this regard could be drawn at 20d. Besides adding to the knowledge that leptin might play an important role in the H-P-T axis, and a direct effect on the testis function, the results found in the present study suggest that leptin might present different periods of action during the postnatal testis development in rats.

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New evidences for extended Sertoli cell proliferation in the transition area between seminiferous tubules and the rete testis, in wistar rats

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Introduction

Postnatal Sertoli cell (SC) proliferation in rats occurs up to two-three weeks after birth and is regulated mainly by FSH (proliferation) and triiodothyronine (differentiation / maturation). As it occurs in all mammals investigated, the period of cessation of SC proliferation is coincident with SC barrier and tubular lumen formation, tubular fluid secretion and flow, and primary spermatocytes proliferation. However, in rats and several other mammalian species already investigated, the testis weight, the total length of seminiferous tubules, and sperm production increase for a relatively long period after the animals have reached sexual maturity, without further increase in tubular diameter. These findings suggest that SC might still proliferate after the period they are expected to stop dividing. In fact, light microscopy studies in our laboratory showed mitotic SC in the transition region (TR) located between seminiferous tubules and the *rete testis*. Also, experimental studies with hypothyroidic rats showed that these SC might be regulated by thyroid hormones. Thus, in the present work we are further evaluating the maturation status of SC present in the TR, using several different approaches.

Material and Methods

Approximately twenty Wistar rats, from 25 to 36 days of age were utilized. Testis tissue samples from these animals were adequately fixed and routinely prepared for morphological studies utilizing transmission electron microscopy (TEM) and light microscopic (LM). Additionally, imunocytochemistry (IC) investigation was performed in order to detect specific markers for SC (GATA-4), cell-cycle inhibitors (p27 and p21), and proliferation (Ki67 and PCNA). Besides that, tritiated thymidine was also utilized to detect SC cells that were synthesizing DNA at the moment of thymidine injection.

Results and Discussion

The IC for GATA-4 confirmed that the somatic cells located in TR were SC (Fig. 1A). Whereas TEM studies showed that these cells presented characteristics of immature SC, such as absence of conspicous nucleolus and tight junctions. SC mitotic figures were observed by LM investigations (Fig. 1C-D). Thymidine-labeled SC were found in this region (Fig. 1E), and other proliferation markers (Ki67 and PCNA) were also co-localized in GATA-4 positive cells. Compared to the SC in other seminiferous tubules areas, in TR staining for p27 (Fig. 1B) and p21 was weak, indicating less differentiated. Thus, the SC proliferation period extends beyond the expected 21 days post birth. Taken together, these results raise several important questions. Could these imature SC be a site for antigen leakage into rete testis fluid? Do new germ cell clonal units fill the new stem cell niches? Is this extended proliferation a potential site for chemical toxicity, leadind to single tubule atrophy? Other possible mechanisms involved in the regulation of extended SC proliferation in TR are under investigation.

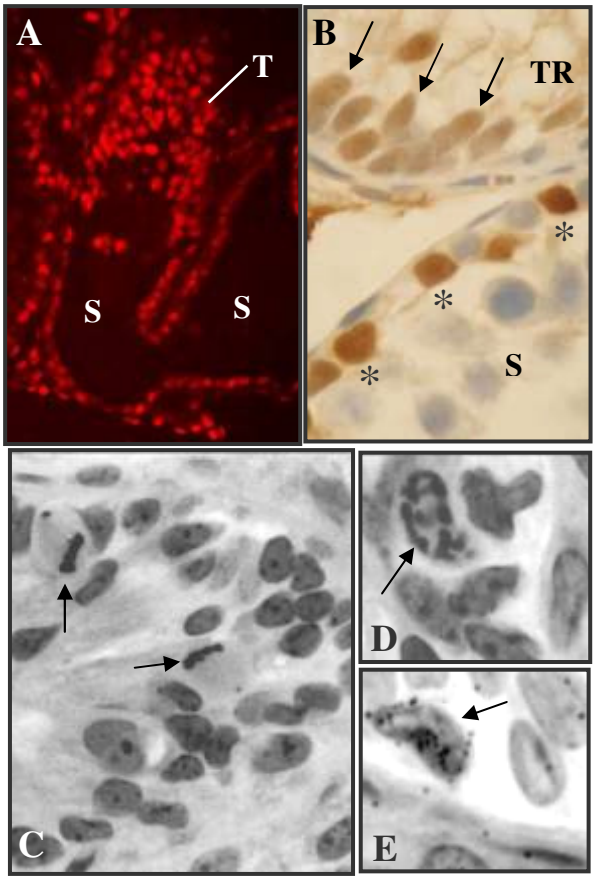

Figure 1. Transitional region (TR) at the seminiferous tubule/rete testis junction in rats 25 to 36 days of age. (A) SC located in TR seminiferous tubules (ST) stain positively for GATA-4, a specific marker for this cell. (B) Compared to the SC (*) present in other areas of ST, SC in TR (arrows) have weaker staining for P27. (C-E) Confirming the immature status of these SC in TR, mitotic figures and thymidine labeling (arrows) were observed.

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Sertoli cells proliferation in the transition region of seminiferous tubules in pigs is extended beyond puberty

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Introduction

In all mammals investigated, the period of cessation of Sertoli cells (SC) proliferation is coincident with SC barrier and tubular lumen formation, tubular fluid secretion and flow, and primary spermatocytes proliferation. Different from mice and rats, in which SC mitotic activity ends around two to three weeks after birth, SC proliferation in pigs occurs up to 4 months (mo) of age, presenting two evident postnatal mitotic peaks (1). However, in pigs and several other mammalian species already investigated, the testis weight, the total length of seminiferous tubules, and sperm production increase for a relatively long period after the animals have reached full sexual maturity, without further increase in tubular diameter. These findings suggest that Sertoli cells might still proliferate after the period they are expected to stop dividing. In fact, ongoing studies in our laboratory are showing that immature Sertoli cells are present in the transitional region (TR) located between seminiferous tubules and the *rete testis* in rats, and that these cells show mitotic activity for at least up to 36d after birth. From these results, we are now investigating SC proliferation activity in the TR in pre- and postpubertal pigs.

Materials and Methods

Eight prepubertal (1 mo, n=4; 3 mo, n=4) and five postpubertal (6 mo, n=5) crossbred pigs, from the Experimental Farm of the Veterinary School at the Federal University of Minas Gerais, were utilized. These animals were orchiectomized and testes tissue samples were obtained from the testis parenchyma near to the *rete testis* and the TR. These samples were fixed by immersion in 5% buffered glutaraldheyde, embedded in glycol methacrylate, and routinely prepared for histological analysis.

Results and Discussion

As expected, proliferating immature SC were observed in the TR of prepubertal pigs evaluated at 1 mo and 3 mo of age (Fig. 1A-B). These findings are in agreement with the literature, and are related to the first and second postnatal phases of SC proliferation that occur in pigs (1). We also found several proliferating SC in the TR of postpubertal pigs at 6 mo of age (Fig. 1C). Once puberty in pigs usually takes place at approximately 4 mo of age, this result support the hypothesis that the increases observed in testis weight and sperm production might probably be due the extended SC mitotic activity.

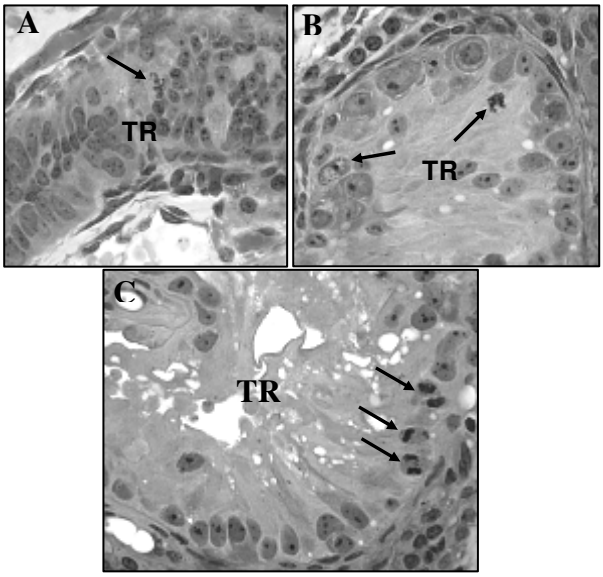

Fig. 1. Transitional region (TR) between seminiferous tubules and *rete testis* in prepubertal (A, B) and postpubertal (C) pigs. Note that Sertoli cells mitotic figures (arrows) can be observed in 1 mo (A), 3 mo (B), and 6 mo old pigs (C, arrows).

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High resolution light microscopy identification of different spermatogonial types, during the testis development in mice

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Introduction

Using high-resolution light microscopy, a recent study described different spermatogonial types in adult mice. These cells were morphologically characterized as undifferentiated type A spermatogonia (A_{und}), differentiated type A spermatogonia (A_1, A_2, A_3, A_4), intermediate type and type B spermatogonia. Despite the high-resolution method utilized, the stem spermatogonia, known as A_{single} (A_s), were not precisely distinguished among them. One possibility to identify the stem spermatogonia could be moving backward and studying the spermatogonial process since the postnatal period. In this way, the present ontogenetic approach could provide better conditions to investigate and characterize the evolution of all different spermatogonial types, including the stem spermatogonia.

Materials and Methods

Testes from mice (C57BL6, n=3) at 1, 3, 5, 7, 9, 11, 13, 15, 17, 20, 37 and 70 days *post partum (pp)* were fixed with 5% glutaraldehyde in 0.05M cacodylate buffer, embedded in araldite resin, sectioned at 1µm thickness and stained with toluidine blue-borate for high resolution light microscopy evaluations.

Results and Discussion

From birth to 3 days *pp*, gonocytes and eventual A_{und} spermatogonia were present in seminiferous cords (Table1). On 5 and 7 days, spermatogonia A_2 and A_3 were already observed in these cords, while on day 9 all the spermatogonial types, including type B, were seen. In such a way, on day 11, the spermatogonial phase of the spermatogenesis was similar to that of an adult animal. After formation of the tubular lumen, at 13 days after birth, primary spermatocytes were seen for the first time in the seminiferous tubules. At 37 days *pp*, spermatogenic process was completely developed, allowing the identification of all stages of the cycle of the seminiferous epithelium in mice by the acrosomic system. The present study using an accurate histological technique allowed the precise identification of almost all germ cell types, including the spermatogonial ones, during their ontogenetic development. Although fine details of the youngest spermatogonial types have been described in the present study, the stem spermatogonia were not yet precisely identified.

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Table 1 – Chronological occurrence of all germ cell types in the mice testes (days *post partum*)

	1	3	5	7	9	11	13	15	17	20	37	70
Gonocyte	+++	+	----	----	----	----	----	----	----	----	----	----
Undifferentiated Spermatogonia	+	+++	+++	+++	+++	++	++	++	++	++	++	++
Vacuolated Spermatogonia	----	++	+++	+++	+++	++	++	++	++	++	++	++
Spermatogonia A1	----	----	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
A2	----	----	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
A3	----	----	++	++	+++	+++	+++	+++	+++	+++	+++	+++
A4	----	----	----	+	+++	+++	+++	+++	+++	+++	+++	+++
In	----	----	----	+	+++	+++	+++	+++	+++	+++	+++	+++
B	----	----	----	+	+++	+++	+++	+++	+++	+++	+++	+++
Preleptotene	----	----	----	----	++	++	++	+++	+++	+++	+++	+++
Leptotene	----	----	----	----	+	++	+++	+++	+++	+++	+++	+++
Zygotene	----	----	----	----	----	+	+++	+++	+++	+++	+++	+++
Early Pachytene	----	----	----	----	----	----	+++	+++	+++	+++	+++	+++
Middle Pachytene	----	----	----	----	----	----	++	+++	+++	+++	+++	+++
Late Pachytene	----	----	----	----	----	----	+	++	+++	+++	+++	+++
Diplotene	----	----	----	----	----	----	----	----	----	++	+++	+++
Meiosis	----	----	----	----	----	----	----	----	----	+	+++	+++
Round Spermatid	----	----	----	----	----	----	----	----	----	----	+++	+++
Elongated Spermatid	----	----	----	----	----	----	----	----	----	----	+++	+++

Number of germ cells observed: ---- absent; + few; ++ moderate; +++ frequent.

Spermatogonial kinetics and niches in donkeys (*Equus asinus*)

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Introduction

Kinetics of spermatogonia as well as their location in niches have been described in rodents (1,2), but not in large animals or in any other species of economical interest. In this regard, and envisioning the possibility of spermatogonial transplantation from donkeys (*Equus asinus*) to mules (*Equus mulus mulus*), many parameters which contribute for better understanding of the spermatogonial biology were investigated in donkeys.

Material and Methods

Testes from five adult donkeys were routinely processed for high resolution light microscopy. Tissue samples were perfused-fixed in 4% glutaraldehyde and embedded in araldite resin. 1µm-thick sections were obtained and stained with toluidine blue-borate.

Results and Discussion

Morphological and morphometric analysis of the testis allowed the characterization of two groups of type A spermatogonia: undifferentiated (A_s, A_{pr}, A_{al}) and differentiated (A_1, A_2 and A_3) type and also the type B spermatogonia (B_1 and B_2). Undifferentiated type A spermatogonia were present in very low numbers along all the twelve stages of the seminiferous epithelium cycle characterized for this species, while differentiated spermatogonia were seen only in specific stages of the cycle. The number of differentiated spermatogonia gradually increased as the cycle progressed, despite the apparent rigid regulation of the balance mitosis/apoptosis in specific points of the spermatogenic process. Additionally, undifferentiated type A spermatogonia were found preferentially in the areas of the seminiferous tubule close to the interstitium (Fig. 1), similarly to what was already described in the literature for mice and rats (1,2). Besides adding to the knowledge about spermatogonial biology and kinetics during spermatogenesis in donkeys, our study shows that in these animals the type A undifferentiated spermatogonia are located in specific regions, called niches. Our results add to the knowledge about the spermatogenic process in large animals and encourage future spermatogonial transplantation studies in equines.

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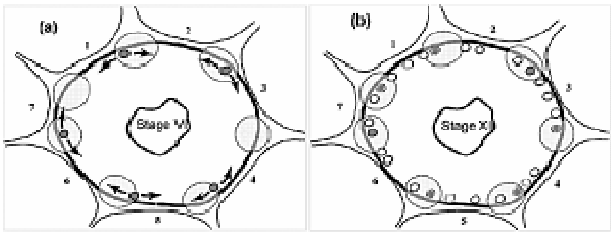
Figure 1. Cross-sectioned drawings of the seminiferous tubules illustrating the location of the niches close to the interstitial regions of the basal compartment (big circles). In (a) the undifferentiated spermatogonia (dark circles) are located in the niches and after successive mitosis, the daughter cell spread out laterally from this region (arrows). In (b) the daughter cells (light circles) are distributed randomly in the basal compartment.



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Effects of transient neonatal hypothyroidism on spermatogonial germ cell size and numbers per testis in adult C57BL/6 mice

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Introduction

The period of Sertoli cell mitotic activity is extended by approximately two weeks in laboratory rodents made transiently hypothyroidic by PTU treatment during the neonatal period, leading to a significant increase in testis size, Sertoli cells number per testis, and sperm production in adult animals. In contrast, the hyperthyroidism induced by T3 accelerates Sertoli cell differentiation/maturation shortening the period of proliferation of this cells resulting in significantly lower number of Sertoli cells per testis and sperm production. According to the literature, the higher Sertoli cell population that resulted from the hypothyroidic condition was followed by increased number of germ cells per testis in the adults (1). However, in that study the number of the different spermatogonial types were not investigated. Therefore, the main objective of the present study was to evaluate the effects of neonatal hypothyroidism in the number and volume of differentiated [type A1-4, intermediate (In), and type B spermatogonia] and undifferentiated (As, Apr, and Aal) spermatogonia, in C57BL/6 adult mice.

Materials and Methods

Eight C57BCL/6 mice (control, n=4; PTU-treated, n=4) were utilized. The hypothyroidism was induced by the administration of 0.1% (p/v) of PTU in the mother´s drinking water during the lactating period, when the pups were 1 to 20 days of age. The mice were sacrificed at 70 days of age and had their testes perfused-fixed with 5% buffered glutaraldehyde, embeded in plastic (Araldite), and routinely prepared for histological and stereological investigations

Results and Discussion

As expected, significant increase (~45%; $p<0.05$) was observed in the number of Sertoli cells per testis (Fig. 1) in PTU-treated mice. In these animals, the volume of spermatogonial germ cells was always lower (Fig. 2), being the values found significantly reduced ($p<0.05$) for type A2, A3, and type B spermatogonia. These findings suggest an adjustment for higher cell density that is expected to to ocuurs in PTU-treated animals. In both groups investigated, type A2 spermatogonia presented the biggest cell individual volume, and a clear trend toward diminution was observed until type B spermatogonia. Except for type B, the total number of spermatogonia per testis was similar ($p>0.05$) in both groups investigated (Fig. 3), suggesting that probably due to the higher number of Sertoli cells per testis, the number of apoptosis in germ cells was reduced in treated mice. In order to better understand the effects of PTU treatment on germ cells, we are currently investigating the spermatogonial germ cells kinetics in prepubertal and fully sexually mice treated postnatally with PTU.

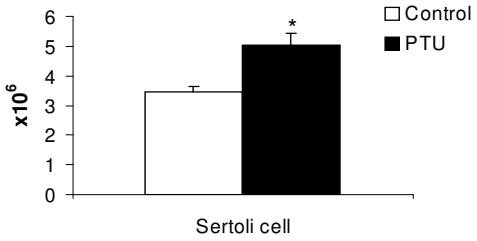

Fig. 1. Total number of Sertoli cells per testis in C57BL/6 adult mice (* $p<0.05$).

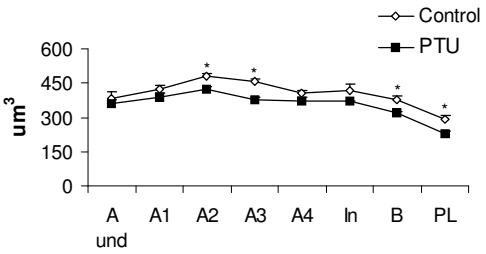

Fig. 2. Individual volume of undifferentiated (A und) and differentiated (A1-4, In, B) spermatogonia in C57BL/6 adult mice (* $p<0.05$).

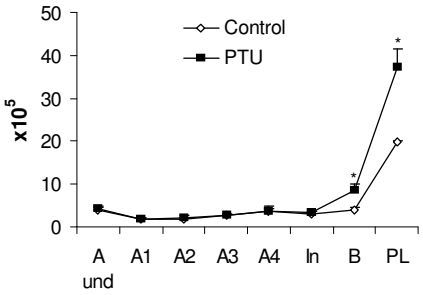

Fig. 3 – Total number per testis of undifferentiated (A und) and differentiated (A1-4, In, and B) spermatogonia in C57BL/6 adult mice (* $p<0.05$).

Reference

(1) Hess.R. A., Cooke, P. S., Bunick D., Kirby J. D (1993). Endocrinology; 132: 2607-2613.

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Spermatogonial kinetics and the Sertoli cell efficiency in zebrafish

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Introduction

The zebrafish (Danio rerio) is a small freshwater teleost fish originating from India. It has become a very important vertebrate model for biomedical and experimental research, mainly due to its frequently mentioned advantages such as small size, easy handling, external fertilization, and high numbers of progeny and easy traceable development of transparent embryos. These advantages apply to reproductive research, although very few data is available in the literature regarding to testis structure and spermatogenesis. For this reason, this work aims to evaluate testis function, particularly spermatogenesis, with emphasis on the spermatogonial kinetics and the Sertoli cell efficiency in this important teleost species.

Materials and Methods

Testes from 10 sexually mature zebrafish (Tabs) were fixed by immersion in 4% buffered glutaraldehyde, embedded in glycol methacrylate and the obtained testis sections were stained with 1% toluidine blue for histological and quantitative analyses. The stereological studies were performed in serially sectioned (at 2 or 3µm thickness) fragments in order to obtain the numbers of Sertoli and germ cells per cyst and germ cells nuclear volume. The Sertoli cell efficiency was estimated from the ratio of germ cells to Sertoli cells, per each cyst type. All data are presented as mean ± SEM.

Results and Discussion

The number of Sertoli cells per cyst increased gradually from primary spermatogonia to pre-leptotene spermatocyte cysts, showing an evident trend toward stabilization thereafter (Fig. 1). The number of germ cells per cyst increased dramatically (1 to ~1360 cells) from primary spermatogonia to early spermatid, decreasing almost 10% until late spermatids (Fig. 1), whereas the opposite trend occurred for cell nuclear diameter (~9µm to 2µm) and cell nuclear volume (~340µm^3 to ~4µm^3) (Fig.2). From the numbers of primary spermatogonia and preleptotene spermatocytes per cyst, at least 9 spermatogonial generations were found in the present work for zebrafish.

Regarding the Sertoli cell efficiency, approximately 100 spermatids were found for this very important somatic cell. Considering the the oretical number of spermatids to be produced from each primary spermatogonia, the germ cell loss during spermatogenesis was around 35%. Germ cell apoptosis were rarely seen and occurred mainly at the end of meiotic and spermiogenic phases of spermatogenesis. Sertoli cells mitotic figures were not observed.

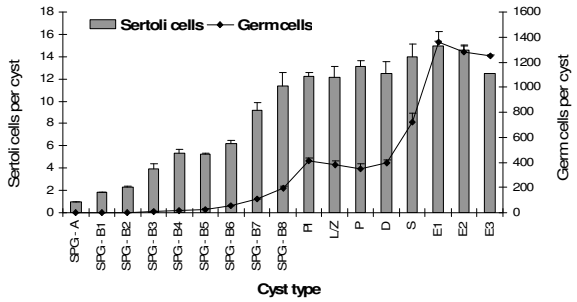

Fig. 1. Number of Sertoli cells and germ cells per cyst.

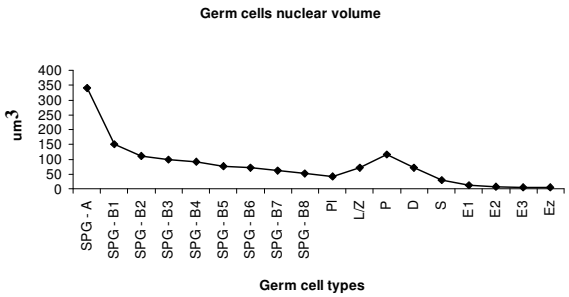

Fig. 2. Germ cells nuclear volume.

Conclusions

- At least nine spermatogonial generations are present in the zebrafish strain (Tabs) evaluated in the present study;
- The Sertoli cell efficiency in this species is very high and similar to tilapias;
- Although not prominent, germ cell loss ocurred during late meiosis and spermiogenesis.

Cell junctions in the germinal epithelium may play an important role in spermatogenesis of the Catfish P. fasciatum (Pisces, Siluriformes).

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Introduction

Catfishes are an extremely large group of diverse fishes generally regarded as a single order, the Siluriformes. *Pseudoplatystoma fasciatum* is an Amazonian freshwater catfish with commercial relevance, belonging to the Pimelodidae family. Diverse aspects of its reproductive biology have been studied in order to improve their reproductive performance in captivity (1, 2). In this perspective, an important aspect to be analysed is the production of spermatozoa by these fishes. The aim of this work was to study the topographical and functional interactions between the diverse cell types of the *P. fasciatum* testis to evaluate if the cell junctions have a key role in spermatogenesis in fish as in mammals. To evaluate this hypothesis, we studied cell-cell associations in *P. fasciatum* male germinal epithelium in mid maturation and regression phase. An ultrastructural analysis of the germinal epithelium in both phases was carried out, as well as light and electron immunohistochemistry with antibodies to proteins related to adhesive and gap junctions.

Materials and Methods

Pseudoplatystoma fasciatum (Linnaeus 1766) males from Instituto de Pesca, APTA- Núcleo de Aquicultura de Pariquera-Açu, Sao Paulo, Brazil, were obtained (5 animals every 2 months from March 1999 to January 2001) for the characterization of the reproductive cycle. For the present study, only adult males in mid maturation (n=5) and in regression stages (n=5) were selected for transmission electronic microscopy (TEM), and mid maturation stage adult males (n=5) for immunoblotting analysis and for immunohistochemical and immunogold labelling studies. The fishes were sacrificed by an overdose exposure to 0.02% benzocaine in ethanol.

We performed a immunohistochemistry light microscopy as well as an immunogold labelling electron microscopy study with antibodies to adhesive and gap junctions proteins, as: against desmoglein and cytokeratins (associated to desmosomes), against e-cadherin and α-catenin (associated with adherens junctions), and against connexin 32 (associated with gap junctions).

Results and Discussion

We identified adhesive junctions and gap junctions between Sertoli cells, between Sertoli and germ cells and between germ cells in the testis of *P. fasciatum*.

Based on our morphological studies we speculate that Sertoli-germ and germ-germ cell adhesive junctions are important for maintaining the three-dimensional structure of the germinal cysts and an organized arrangement of the germ cells inside the cysts. Connexin 32 was identified in the germ cells and in the cysts walls. Our observations also suggest that Sertoli-germ and germ-germ cells gap junctions may be involved in the mechanism of synchronous development of germ cells.

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Acknowledgments

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Interactions among Sertoli, Leydig, and peritubular myoid cells in six different phases of spermatogenesis in adult C57 mice

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Introduction

Spermatogenesis is a cyclic, complex and highly organized process in which million of spermatozoa are formed daily per gram of testis, from each spermatogonia stem cell. Although fairly well investigated in laboratory rodents (rats and mice), the dynamics and the regulation of this process is still poorly understood. In this regard, we investigated the correlations of six different phases of spermatogenesis with the disposition (parallel or perpendicular in relation to the basal membrane) and size of Sertoli cells nucleus and nucleolus, as well as the size of Leydig cells nucleus adjacent to the tunica propria, and the tunica propria thickness (including peritubular myoid cells).

Materials and Methods

Eight adult C57 mice had their testis perfused-fixed with 4% buffered glutaraldehyde. Testes fragments were embedded in plastic (glycol methacrilate), routinely prepared for light microscopy and histomorphometrically evaluated. Six representative phases of spermatogenesis were evaluated: 1) pre and 2) post-meiotic; 3) presence of acrosomic granule and 4) with acrosome formed; and 5) pre and 6) post-spermiation phases.

Results and Discussion

As expected, a significant and continuous reduction ($p<0.01$) was observed for germ cells nuclear volume, from type A1 spermatogonia to pre-leptotene spermatocytes, with further increase up to pachytene/diplotene spermatocytes, and from initial to final round spermatids. The volume of Leydig cells nucleus adjacent to the seminiferous tubule´s tunica propria, at the phase close to spermiation, was significantly bigger ($p<0.01$) than those evaluated in the five other phases investigated. These findings probably reflect the crucial importance of androgens for this specific step of spermiogenesis. In this same phase of spermatogenesis, it was observed that the size of Sertoli cells nucleus and nucleolus were also significantly bigger ($p<0.01$). The percentage of the parallel disposition of the Sertoli cells nucleus was predominant in all six phases investigated, with the lowest value observed in the phase close to spermiation.

Conclusions

Similar to what was observed in rats, the results found for mice suggest the existence of paracrine interactions among Sertoli cells, peritubular myoid cells, and Leydig cells close to seminiferous tubule´s tunica propria and that the phase close to spermiation is a critical moment for the spermatogenic process. Confirming the literature, the results found for the disposition of Sertoli cells nucleus show that important changes of the cytoarchitecture of these cells occur at this specific phase of spermatogenesis.

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Sertoli and Leydig cells in adult wistar rats show stage-dependent changes during spermatogenesis

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Introduction

Spermatogenesis is a cyclic, complex and highly organized process in which millions of spermatozoa are formed daily, from spermatogonial stem cells. Although well investigated in laboratory rodents, the dynamics and the regulation of this process, as well as the interaction between Sertoli and Leydig cells, are still poorly understood. In this regard, our main objective in the present study was to investigate the Sertoli cells and the Leydig cells nucleus volume, the disposition of the Sertoli cells nucleus in relation to the basal membrane and the correlation amongst these parameters in six representatives phases of spermatogenesis.

Materials and Methods

Eight adult Wistar rats had their testis perfused-fixed with 4% buffered glutaraldehyde. Testes fragments were embedded in plastic (glycol methacrilate), routinely prepared for light microscopy and histomorphometrically evaluated. Six representative phases of spermatogenesis were evaluated: 1) pre and 2) post-meiotic; 3) presence of acrosomic granule; 4) spermatids with acrosome formed; and 5) pre and 6) post-spermiation phases.

Results and Discussion

As expected, a continuous reduction ($p<0.01$) was observed in germ cells nucleus volume, from differentiated type A1 spermatogonia to pre-leptotene primary spermatocytes, with posterior increase from pre-leptotene to pachytene/diplotene spermatocytes, as well as from initial to final round spermatids. The Leydig cell nuclei adjacent to the seminiferous tubules, at the phase close to spermiation, were significantly bigger ($p<0.01$) than those in the others phases investigated, probably reflecting the important function of these cells in androgens production, that are important for spermiation. In this same phase it was observed that the Sertoli cells nucleus have increased ($p<0.05$) in size when compared with the nucleus volume of other phases investigated. The parallel disposition (in relation to the basal membrane) of the Sertoli cells nucleus was predominant in all phases investigated, and the lowest value was observed in the phase close to spermiation.

Conclusions

Similar to what was found for mice, the results observed strongly suggest the existence of a paracrine interaction between Sertoli cells and Leydig cells in contact with the tunica propria and that the phase close to spermiation is a critic moment for the spermatogenic process. Confirming the literature, the results found for the disposition of Sertoli cells nucleus show that important modifications in the cytoarchitecture of these cells occur in this specific phase of spermatogenesis.

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Testis structure and seminiferous epithelium cycle length in sexually mature mongrel dogs

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Introduction

The characterization of the different stages of the cycle of the seminiferous epithelium is essential for better understanding reproductive biology in mammals. The spermatogenic cycle length is a biological species-specific constant which is under the control of the germ cell genotype (1). Approximately 4.5 spermatogenic cycles are necessary in mammals for the completion of spermatogenesis, from type A stem spermatogonia up to the releasing of spermatozoa in the tubular lumen. The data published in the literature regarding the testis structure and function in dogs are scarce. In this regard, we are currently developing a very comprehensive comparative study investigating several important parameters related to spermatogenesis and the fine testis structure in eight representative (small, medium, standard and large) dog breeds. In the present work we are presenting preliminary data already obtained for the frequencies of the different stages of the cycle of the seminiferous epithelium, characterized according to the acrosomic system and the duration of spermatogenesis in mongrel dogs.

Materials and Methods

Six sexually mature mongrel dogs were utilized. Intratesticular injections of tritiated thymidine were performed in order to determine the duration of spermatogenesis, and the testes were orchiectomized at different time periods (1 hour and 27 days) after injection. The testes were fixed by immersion in 4% buffered glutaraldehyde. Testis fragments were embedded in glycol methacrylate, stained with toluidine blue-borate or PAS, and routinely prepared for histological and morphometrical analysis.

Results and Discussion

The percentage of the seminiferous tubules and Leydig cells presents in the testes of mongrel dogs were 89±2 and 4±0.4, respectively. The mean tubular diameter was 236±4µm. Based on the development of the acrosome, 8 stages were characterized for this dog "breed". The frequencies of these stages are shown in Fig. 1. As it can be noticed, stages II and IV present the highest (~25%) and lowest (~5%) frequency. The preliminary data obtained with tritiated thymidine (Fig. 2) showed that each spermatogenic cycle lasts approximately 13.0 days in mongrel dogs. Thus, approximately two months would be necessary for the entire spermatogenic process to be complete in these dogs.

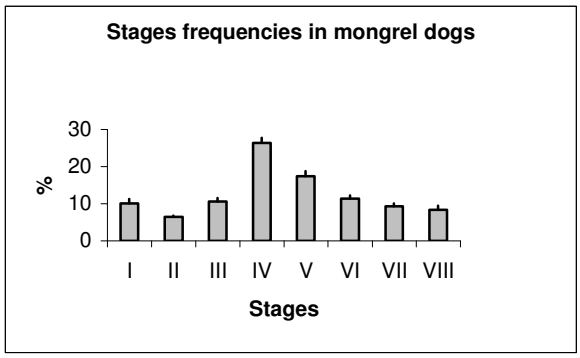

Fig. 1: Frequency of the to stages of cycle characterized according to the development of the acrosome.

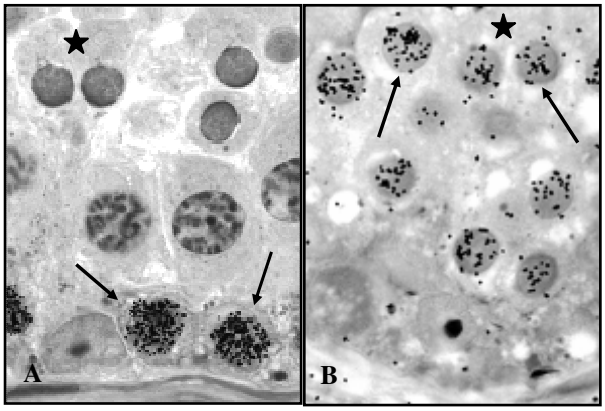

Fig. 2: Seminiferous tubules cross-sections showing pre-leptotene (A) and round spermatid (B) labeled (arrows) 1 hour and 27 days, respectively, after thymidine injection. The star is indicating round spermatid at stage V of the cycle.

Reference

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Financial support: Fapemig, CNPq
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Duration of spermatogenesis and Sertoli cell and spermatogenic efficiencies in three different wild mammalian species with potential economic interest

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Introduction

The knowledge of reproductive biology and physiology, mainly those aspects related to spermatogenesis, is critical for preventing species extinctions, improving species management, and utilizing males in natural and artificial reproductive programs. The agouti (Agouti paca), the Dasyprocta sp, and the collared peccary (Tayassu tajacu) are important mammalian species from the Brazilian fauna that present high potential to be raised and exploited commercially. However, the literature available related to the reproductive biology and testicular function for these three species is still scarce. Therefore, the objectives of the this study were to obtain data that could be utilized in reproductive and animal improvement programs. Besides that, this study will provide important information that can be utilized for comparative reproductive biology with others mammalian species already investigated.

Materials and Methods

Six agouti, eight Dasyprocta sp, and twelve adult peccaries were utilized. These animals were obtained from the Federal University of Pará and EMBRAPA/PA. Tritiated thymidine injections and investigation of the frequency of the different stages of seminiferous epithelium cycle, characterized according to the tubular morphology system, were performed to establish the duration of the spermatogenesis. The testes fragments were fixed in 4% buffered glutaraldehyde, embedded in glycol methacrylate, and routinely processed for histomorphometrical analyses.

Results and Discussion

The following comparative results were found for the agouti, Dasyprocta sp, and peccaries, respectively. A) Volume density (%) of seminiferous tubules in the testes parenchyma: 93±1; 91±1; and 77±2. B) The duration of one spermatogenic cycle and the total duration of spermatogenesis (based on 4.5 cycles): 10.2±0.4 and 46±2; 8.7±0.2 and 39.3±0.03; and 12.2±0.2; and 54.7±0.7. C) The number of spermatids per Sertoli cell (Sertoli cell efficiency): 11:1±1; 9:1±1; and 11:1±1. D) Daily sperm production per gram of testis (spermatogenic efficiency): 44±2; 56±3; and 24±2 millions.

Conclusions

The values obtained for the duration of each spermatogenic cycle in the three species investigated in the present study were in the ranged (~9 to ~12 days) found for most mammalian investigated. Mainly due to the high volume density (%) of seminiferous tubules, good Sertoli cell efficiency, and not lenghty duration of spermatogenesis, the spermatogenic efficiency in Agouti paca and Dasyprocta sp is higher than the vast majority of mammalian species already investigated.

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Stereological investigation of the testis and spermatogenic cycle length in two wild rodent species (*Trinomys moojeni;* Rodentia, Echimyidae **and** *Oligoryzomys nigripes;* Rodentia, Muridae) **from the Atlantic forest**

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Introduction

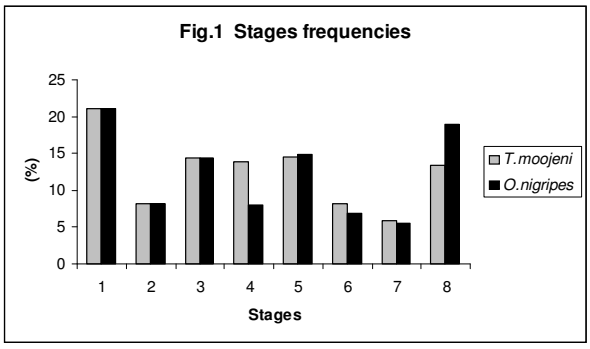
The Atlantic Forest is considered one of the most diverse and threatened biome in the world. The knowledge of reproductive biology and physiology is critical for conservation and species management, allowing also to prevent species extinction, and to utilize males in natural and artificial reproductive programs. Spermatogenenic cycle length is a biological species-specific constant which is controlled by the germ cell genotype. Approximately 4.5 spermatogenic cycles are necessary in mammals for the completion of spermatogenesis, from type A stem spermatogonia up to the releasing of spermatozoa in the tubular lumen. The main objectives of the present study were to investigate the testis structure and to estimate the duration of spermatogenesis in two wild rodent species (Trinomys moojeni and Oligoryzomys nigripes) largely distributed in the Natural Reserve of Caraça (a fragment of the Atlantic Forest) located in the State of Minas Gerais, Brazil.

Materials and Methods

Eleven sexually mature T. moojeni and twelve O. nigripes were utilized. Intraperitoneal injections of tritiated thymidine were performed at different time periods in order to determine the duration of spermatogenesis. The different stages of the cycle of seminiferous epithelium were characterized according to the tubular morphology system. The testes were perfused-fixed in 4% buffered glutaraldehyde, embedded in plastic (glycol methacrylate) and routinely processed for histological and sterological analyses of the testis.

Results and Discussion

The results found up to date showed that the volume density (%) of seminiferous tubules and Leydig cells were, respectively, 97±0.3 and 0.3±0.04 in T. moojeni and 96±0.3 and 1.4±0.1 in O. nigripes. Except for stages 4 and 8, the other stage frequencies were very similar in both species investigated (Fig. 1). The duration of one spermatogenic cycle and the total duration of spermatogenesis were, respectively, ~8.9 and ~40 days for T. moojeni and ~8.3 and ~37 days for O. nigripes. These results showed that the volume density of seminiferous tubules found for both T. moojeni and O. nigripes are among the highest observed for the mammalian species already investigated. The opposite occurred for Leydig cells volume density. The duration of spermatogenesis, particularly for O. nigripes, was one of the shortest found for mammals investigated up to date.



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Cellular proliferation in fish male germinal epithelium

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Introduction

In fish, the male germinal epithelium is composed of cysts that are formed when a single clone of primary spermatogonia is enclosed by Sertoli cells. According to the "new concept" proposed by Grier and Lo Nostro, the alternation of male germinal epithelium between continuous and discontinuous types and the stages of germ cells can be used to describe reproductive cycles in fishes. It has been accepted that the germinal epithelium shifts between mitosis-dominated and meiosis-dominated cell divisions. However, very little information is available regarding the origin of successive generations of somatic cells (Sertoli and Leydig cells). We propose to identify, through PCNA (proliferating cell nuclear antigen), the sites of cellular proliferation during the changes of the germinal epithelium (continuous and discontinuous) in two Neotropical teleosts, Serrasalmus spilopleura (Characiformes) and Pimelodus maculatus (Siluriformes).

Materials and Methods

Sexually mature males were collected in the Piracicaba river (Brazil) for two years. The animals were anaesthetized, sacrificed, and their testes were quickly removed, weighed, and immersed in fixative. For PCNA, testis fragments were fixed in Bouin, embedded in paraplast, sectioned (5µm), and submitted to immunohistochemistry according to the manufacturer's protocol (Novocastra, PC 10). For each reproductive class of P. maculatus, PCNA-positive cells were quantified per unit area. Some testes were also submitted to claudin-11 (Zymed) immunohistochemistry.

Results and Discussion

In S. spilopleura (continuous spermatogenesis), spermatogonia divide throughout the year. However, when spermatogonia mitosis diminishes, the germinal epithelium becomes discontinuous, having only Sertoli cells and few scattered cysts of non-proliferating spermatogonia. Sertoli cell mitosis occurs mainly in cysts of non-proliferating spermatogonia (Fig. 1). It is suggested that Sertoli cells should proliferate before spermatogonia in order to guarantee an appropriate number to support cyst development during spermatogenesis. Proliferation of interstitial cells was not seen in S. spilopleura. The mitotic activity throughout the reproductive cycle of P. maculatus (seasonal breeding) is shown in the graph bellow:

Sertoli cells proliferate mainly during the regressed class, when only spermatogonia and Sertoli cells are present. Sertoli cell mitotic activity ceases during the meiosis-dominated period (see Graph - maturation, final maturation and regression) when tight junctions are established in cysts (Figs. 2,3). Our results suggest that Sertoli cells are not terminally differentiated, exhibiting features common to both undifferentiated (proliferation) and differentiated (tight junctions) cells. It is proposed that Sertoli cells are in a transitional state and are capable of reentering the cell cycle.

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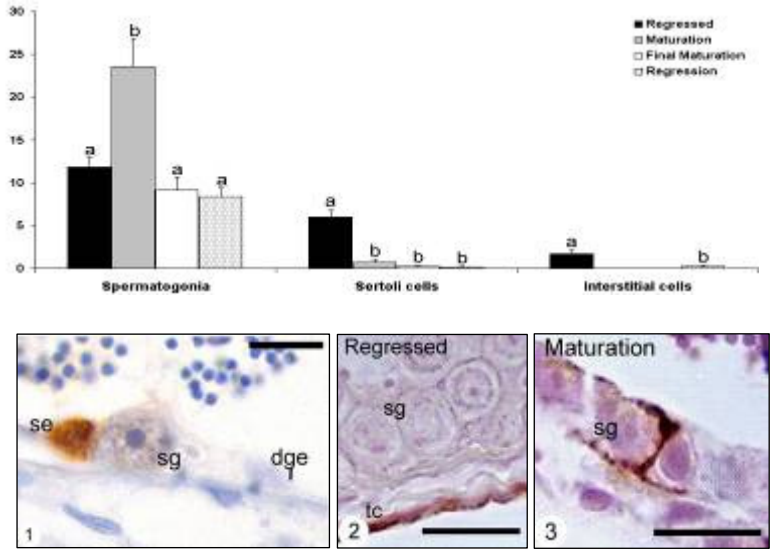

Figures. 1 PCNA in S. spilopleura. Discontinuous germinal epithelium (dge), spermatogonium (sg), Sertoli cell (se). 2-3 Claudin-11 in P. maculatus. Spermatogonia (sg), testicular capsule (tc). Bar = 10µm.

The germinal epithelium during the reproductive cycle in *Gymnotus* sp. (Teleostei, Ostariophysi, Gymnotiformes)

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Introduction

In fish, spermatogenesis occurs in cysts by the synchronic development of germ cells, which are enclosed by Sertoli cells cytoplasmic processes [1]. The structure of cysts, and in consequence that of the germinal epithelium, undergoes several changes during the reproductive cycle. The source of these alterations is the germinal epithelium alternation between continuous and discontinuous, and the developmental stage of germ cells [2]. Some histological aspects of Gymnotus sp testis are reported in the literature [3]. However, further details about the changes that take place in this organ during the reproductive cycle are unavailable. Based on these grounds, Gymnotus sp. germinal epithelium and testicular morphology during the reproductive cycle were characterized.

Materials and Methods

Gymnotus sp. sexually mature males were collected in Rio Bonito, municipal district of Botucatu. Testicular fragments were fixed in a solution of 2% glutaraldehyde and 4% paraformaldehyde in Sorensen's phosphate buffer 0.1 M, pH 7.2, for 24 hours, and processed according to the usual methods for historesin embedding (Technovit 7100).

Results and Discussion

Gymnotus sp. testis is externally covered by an albugineous tunic, and internally filled with anastomosed tubules, formed by the germinal epithelium. This type of germinal compartment organization also occurs in other basal Teleostei [2]. There are two distinct testicular regions; the cortical region, which is more peripheral, and the medular region, which is more central. In the testicular cortex the number of cysts is smaller and the lumen is more dilated than in the medular region. At the beginning of the reproductive cycle, the germinal compartment consists of just spermatogonial cords associated with Sertoli cells. After several mitotic divisions, the spermatogonia differentiate into spermatocytes, which enter meiosis. At the beginning of spermatogenesis, the germinal epithelium is continuous, and predominantly formed by spermatogonial cysts and spermatocytes. As maturation advances, and spermatozoa are released in the testicular lúmen, the germinal epithelium becomes discontinuous. Progressively, the discontinuity of the epithelium increases through the testis and only Sertoli cells and a few sparse spermatogonia or cysts are found along the tubule. By the end of the reproductive period, residual spermatozoa are found in the tubular lumen and are phagocyted by macrophages and Sertoli cells. Even when a new cycle begins, a few residual spermatozoa remain, and that can lead some investigators to misinterpretation [3], and believe that spermatozoa production occurs throughout the reproductive cycle of the species with just periods when production is high and others when it is low. The anastomosed tubules directly end into a dorsal testicular duct, which becomes very elongate as it leaves the testis (about seven times the length of the testis) [3]. The testicular structure and the changes underwent by the germinal epithelium during Gymnotiformes reproductive cycle provide new information about the about reproductive biology in Teleostei, which may be useful in future phylogenetic analyses.

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Comparative morphology of the male reproductive system in pimelodidae, pseudopimelodidae and heptapteridae

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Introduction

In Siluriforms, the male reproductive system is extremely diversified, exhibiting since saculiform-paired organs to fringed testis. In some families, the testes can be constituted only by spermatogenic fringes, or have secretory fringes in the medial/caudal region, or even accessory structures (honeycomb-like structure or ampulla) that have no spermatogenic activity but can store sperm. The knowledge of the anatomical differences between the male reproductive systems of Siluriforms may contribute to understand the gonadal evolution and to help in phylogenetic analyses in these species. For this reason, we propose to evaluate comparatively the morphology of the male reproductive system in the "old" Pimelodidae, that are recently grouped in 3 families; Pimelodidae, Pseudopimelodidae and Heptapteridae.

Materials and Methods

Sexually mature males of Pimelodidae (Pimelodus maculatus), Heptapteridae (Pimelodella gracillis, Imparfinis sp., Cetopsorhamdia sp.) and Pseudopimelodidae (Microglanis sp.) were monthly collected in rivers and streams of the municipal district of Botucatu-SP (Brazil) for two years. The animals were anaesthetized, sacrificed and their testes were quickly removed, weighed and immersed in 2% glutaraldehyde and 4% paraformaldehyde in Sorensen phosphate buffer (0.1M, pH 7,2) for at least 24 hours. The material was dehydrated and embedded in resin Technovit 7100 (Jung HistoResin). Sections of 3µm were stained with Schiff's periodic acid (PAS)+Hematoxylin+Metanil yellow.

Results and Discussion

In the studied species, the testis is composed of a variable number of fringes more or less elongated and with different formats. The fringes are connected to the main testicular ducts (right and left) that are joined together at the posterior region of the testis, forming a common spermatic duct, which extends to the urogenital papillae. Among the species, the comparative histology reveals a common testicular structure: the cranial fringes are spermatogenic (Fig. 1), showing a tubular anastomosing germinal epithelium; and the caudal fringes are strictly secretory (Fig. 1) with PAS negative secretion. In P. maculatus, a transitional region between spermatogenic and secretory fringes can also be documented (Fig. 1). Anatomically, the testicular fringes of related species present differences in color, number and format (Fig. 1). Except in Heptapteridae, the secretory fringes in Pimelodidae and Pseudopimelodidae are easily distinguished by the anatomy. These secretory fringes are more elongated, rounded and with white or translucent coloration (Fig. 1).

This work describes the morphology of the male reproductive system in the "old" Pimelodidae (Pimelodidae, Heptapteridae and Pseudopimelodidae) available informations that added to traditional characters (osteology, biometry and molecular biology) can be useful to understand the phylogeny of the group.

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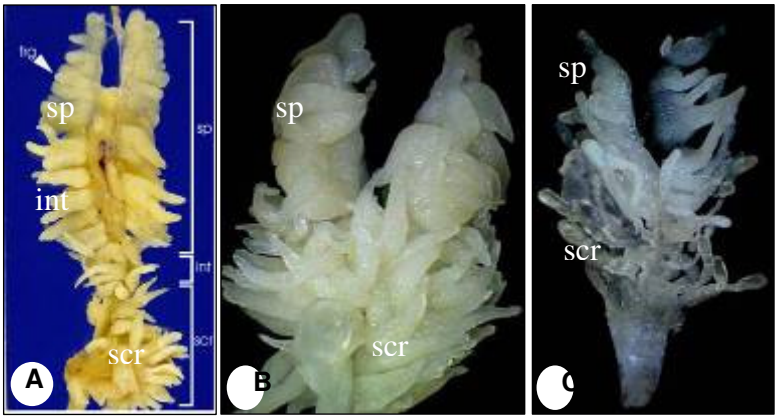

Fig. 1. Male reproductive system in *Pimelodus maculatus*/**Pimelodidae** (**A**), *Pimelodella gracillis*/**Heptapteridae** (**B**) and *Microglanis sp.*/**Pseudopimelodidae** (**C**). Spermatogenic fringes (**sp**); intermediate fringes (**int**) and secretory fringes (**scr**).

Testis morphology of the catfish *Pimelodella vittata* (Lütken, 1874) (Siluriformes: Heptapteridae)

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Introduction

The morphology of the male reproductive system of Siluriformes has been shown to be variable between the species. In some families the testes show spermatogenic lineage cells throughout their extension, others present caudal region with seminal vesicles or accessory structures that do not show spermatogenic activity, however, they can store spermatozoa (1). Moreover, the caudal region of some Siluriformes's testes is known to present secreting activity without storing spermatozoa (2). The catfish Pimelodella vittata is known popularly as "mandizinho" and occurs in headwaters streams of the upper São Francisco river basin. There are few studies focusing on reproduction of this specie. Knowledge about anatomical, biometrical and histological aspects of the testes are important in order to provide basic information for the understanding of P. vittata reproductive biology.

Materials and Methods

Fish captures were carried out trimonthly in the Paciência brook (S 19º55'59.8'' W 44º32'02.4''), São Francisco river basin. One hundred and four males of P. vittata were collected using gill nets from November/2005 to July/2006. Of these specimens, eighty were fixed in formol 10% and later, had their standard length (mm) measured. Testes were removed and maintained in ethanol 70%. Each fixed testis was divided into a cranial and caudal region. The fringes of twenty testes were counted. Testes of the other twenty four specimens were fixed in liquid of Bouin for 8-12h and kept in alcohol 70%. Later, the material was submitted to routine histological techniques. To detect carbohydrates and proteins in the caudal region of the testis, classical histochemical techniques were used.

Results and Discussion

Testes of P. vittata (665±89 mm SL) are paired and fringed organs. They anatomically related dorsally with the gaseous bladder and the kidneys and ventrally with the intestine. Each testis contained of 78 to 93 fringes in all extension. Both testes had spermatic ducts which joined together caudally forming a common spermatic duct that extended to the urogenital papillae. Testes presented two distinct regions: cranial spermatogenic and caudal secreting. In spermatogenic region, spermatogenic lineage cells occurred in cysts at the same developmental stage in all seminiferous tubule extent, which anastomosed to conduct the spermatozoa until the spermatic ducts, characteristic of an anastomosing tubular testis organization (3). The caudal region presented only tubules that exhibited secreting prismatic cells in the wall and secretion in the lumen. Histochemical analyses revealed that the secretion inside the tubules on caudal fringes contained neutral glycoproteins, acid glycoconjugates, acid carboxilates and siaomucines that may have a similar function to those of the seminal vesicles of other teleosts (4).

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Effects of different temperatures on the mitotic activity of somatic testicular cells in adult tilapias (*Oreochromis niloticus*)

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Introduction

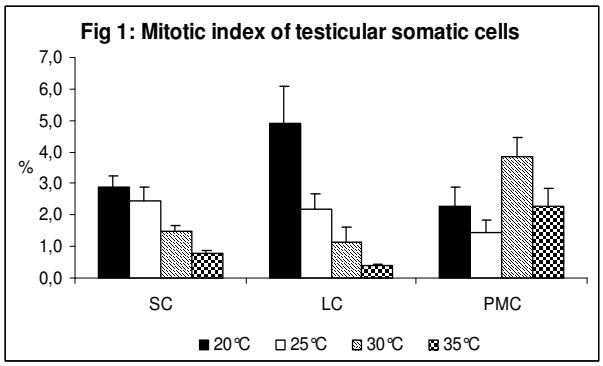
Somatic cells of the male are key to the normal functioning of the male reproduction system. For example, Leydig cells (LC) produce testosterone, and receptors for testosterone reside on LC, peritubular myoid (PMC) and Sertoli cells (SC). In intact adult testis, SC are important in the physical support and transduction of hormonal signals to the germ cells and thus essential for the successful completion of spermatogenesis. Studies developed recently in our laboratory showed that different temperatures alter the duration of spermatogenesis, that is faster in tilapias kept at higher temperatures (30-35 °C) in comparison with tilapias kept at 25 °C, which is considered the normal temperature for the reproduction of this species. Because there is no data available in the literature regarding the effect of temperature on the mitotic activity of testicular somatic cells, the main objective of the present work was to investigate the influence of this important parameter on the proliferation rate of LC, PMC and SC in Nile tilapias.

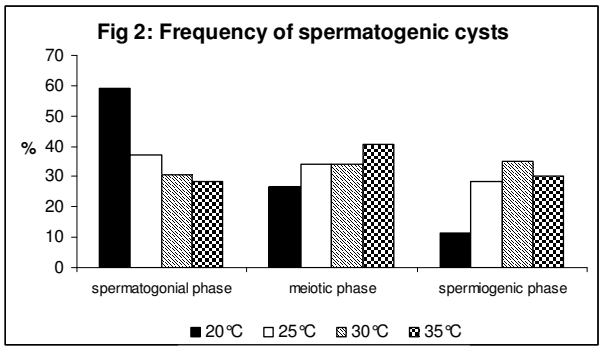
Materials and Methods

Twenty-eight adult tilapias (Oreochromis niloticus) kept at the experimental temperatures of 20°C, 25°C, 30°C, 35°C received one single intracelomic thimidine injection (~1 µCi per gram of body weight), as a marker of cells that were synthesizing DNA at the time of thymidine injection. The fish were sacrificed approximately two hours after injection and testis fragments were fixed in 4% buffered glutaraldehyde, embedded in glycol methacrylate, and routinely prepared for histomorphometrical and autoradiographical analyses. Approximately one thousand somatic cells (LC, PMC, and SC; ~3000 cells in total) were analyzed for each animal.

Results and Discussion

The mitotic index (%) for the temperatures of 20°C, 25°C, 30°C, and 35°C are shown in Fig. 1. In general, tilapias kept at higher temperatures (30-35°C) showed lower ($p<0.05$) rate of SC and LC proliferation than fish kept at the temperatures of 20-25°C. High and positive correlation ($r=0.76$; $p<0.01$) was observed between SC and LC proliferation rate, suggesting a functional interaction between these two cell types. The mitotic index of PMC was higher ($p<0.05$) at 30°C, compared to 25°C; however, no clear trend was found for the proliferation rate of this cell type. Although we still do not have an explanation for the results found, we are currently investigating other functional aspects of spermatogenesis in tilapias. We have already found that the frequency of cysts containing spermatogonia (where more SC proliferation is expected to occur) is higher ($p<0.05$) at 20°C, compared to the other temperatures investigated (Fig. 2).





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Phenotypical characterization of Leydig cell populations in the Mongolian gerbil

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Introduction

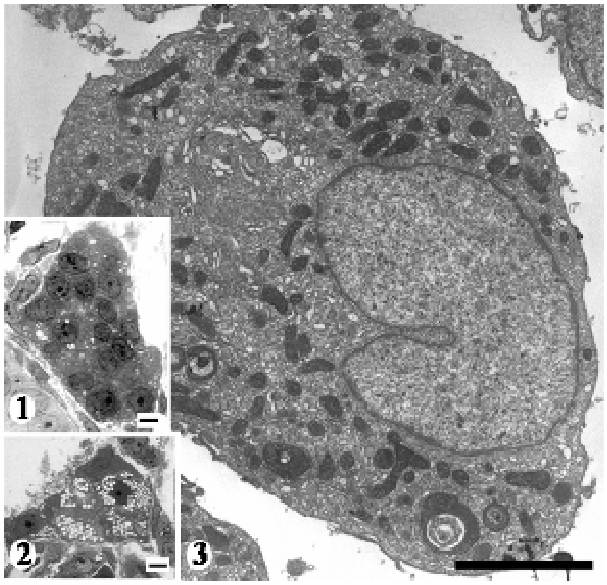
Five steps in postnatal differentiation of Leydig cells have been described in laboratory rodents [1]: mesenchymal precursor cells, progenitor cells, newly formed adult Leydig cells (NFLC), immature Leydig cells (ILC), and mature Leydig cells (MLC). Besides the morphological aspect, these progressive steps are identified according to the expression of androgen receptors and steroidogenic enzymes. Recently, several aspects of germ cell differentiation in the Mongolian gerbil have been elucidated; however, the development and structural features of Leydig cell populations are unknown. Thus, in the present study we characterized the Leydig cell types of this rodent on the basis of morphology and presence of androgen receptor.

Materials and Methods

Male gerbils (Meriones unguiculatus) were killed at the age of 1, 14, 28 and 90 days (n=4). The testes were fixed by immersion in fixative solution, and the 28- and 90- days old ones were previously perfused. The testes fixed with Bouin were embedded in historesin or paraffin and analysed both morphologically and immunocytochemically. The testis fragments fixed in 2.5% glutaraldehyde were embedded in araldite resin for analyses of thick and thin sections. Immunocytochemical detection of androgen receptor was performed using ABC/peroxidase system.

Results and Discussion

The fetal Leydig cells (FLC) of gerbil are abundant until 28 days postnatal (dpn) and rare in adult testes. They are observed in clusters surrounded by fibroblasts and exhibit nuclei with heterochromatin grumes and many large lipid droplets in cytoplasm (Fig. 1). The NFLC are particularly abundant in 14 days old animals. These are oval-shaped, contain organelles for steroids synthesis and lack lipid droplets. On the other hand, the ILC are found from 14 dpn onwards and differentiated by the polygonal shape, high electrondensity of cytoplasm which exhibit large amounts of smooth endoplasmic reticulum (SER) and tubulovesicular mitochondria (Fig. 2). The ILC population in adult gerbils showed variable levels of maturation, so that less mature ones have plenty of lipid droplets. The MLC (Fig. 3) lack lipid droplets, exhibit large amounts of SER and tubulovesicular mitochondria, usually in a concentric arrangement. These large cells are oval and, as the peritubular ones, showed intense reactivity to the androgen receptor. FLC and ILC were negative to androgen receptor. The ultrastructural and immunocytochemical data indicate that the steps of differentiation of adult Leydig lineage in the gerbil are very similar to those of rat, but with a high frequency of ILC in adult.



Figures: Thick sections of FLC from 1day old- (1) and ILC from 90 days old- gerbil (2). Ultrastructural aspect of MLC 90 days old animal (3). Bars: 1,2 =10 µm; 3 = 2µm.

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Androgen induction of testicular cyclooxygenase-2 expression

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Introduction

Prostaglandins (PGs) are derived from arachidonic acid by the action of the cyclooxygenase (COX) isoenzymes COX1 and COX2. COX2–female deficient mice are infertile. In contrast, male fertility is not affected in COX2 mutant mice from knockout experiments (1) suggesting that PGs may be not important for the functioning of the testis. This early general view is being challenged by recent observations. We have reported that whereas COX2 is not detected in normal human testes, it is expressed in testicular biopsies of men with impaired spermatogenesis and male infertility (2). Moreover, COX/PGs are upregulated in testicular cancer (3) and aging (4), affect steroid hormone production and induce testicular fibrosis (2,5). Recently, we screened testes from species ranging from mice to monkeys in order to find an animal model for further investigating testicular COX2/PGs. We only found COX2 expression in Leydig cells of the reproductively active seasonal breeder Syrian hamster (5). Thus, the active adult hamster is a readily available model to study the regulation of testicular COX2 expression and PGs production.

Materials and Methods

Testes from prepubertal to adult Syrian hamsters (*Mesocricetus auratus)* were used to investigate COX2 and androgen receptor expression by immunohistochemistry. Leydig cells were purified from reproductively active adult hamsters and incubated in the presence or absence of: 100 mIU/ml hCG, 1 μM testosterone, 10 μM bicalutamide (Casodex). After incubations, cells were used for RT-PCR, whereas media were used for determination of PGF2α concentration by immunoassay. Statistical analyses were performed using ANOVA followed by Student-Newman-Keuls test for multiple comparisons. Mean $\pm$ SEM.

Results and Discussion

Peripubertal (36d old), pubertal (45-60d old) and adult (90d old) active seasonal breeder hamsters that present high circulating levels of LH and androgens, showed expression of COX2 in Leydig cells (Fig. 1A). In contrast, prepubertal animals (12–18d old) and regressed adult hamsters exposed to a short-day photoperiod (6h light, 18h dark) with low serum concentrations of LH and androgens, did not express COX2 in testes. The influence of LH and testosterone on testicular COX2 expression and PGs synthesis was evaluated by RT-PCR and immunoassay. In Leydig cells isolated from active Syrian hamsters, hCG/LH and testosterone significantly induced COX2 expression and PGF2α production (Fig. 1B,C).

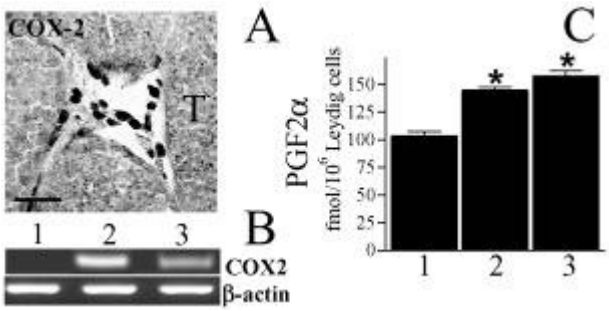

Fig. 1. COX2 expression by immunohistochemistry (A) and RT-PCR (B), and PGF2α production by immunoassay (C), in active hamster Leydig cells.
1: untreated, 2: 100mIU/ml hCG, 3: 1μM testosterone. Bar: 40μm. T: tubule. *:P<0.05. n=6.

Both, testosterone and LH stimulatory effects on COX2 expression were abolished by the pure antiandrogen bicalutamide, suggesting that LH does not exert a direct action on PGs synthesis. Moreover, androgen receptors were identified by immunohistochemistry in Sertoli cells, myoid cells as well as in Leydig cells from prepubertal to adult Syrian hamsters. Our results demonstrate that testosterone induces COX2 expression in Leydig cells. Thus, androgen environment might be crucial for the regulation of testicular PGs production at least in hamsters. Whether our results can be extended to nonseasonal reproductive species including man remains to be clarified.

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Modulation of TGF-β1 and VEGF-A expression in the testes of transgenic mice over-expressing hCG

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Introduction

Transgenic male mice (TG) over-expressing α and β subunits of hCG are infertile and show enhanced steroidogenesis. The chronic hCG hyper-stimulation leads to Leydig cell hyperplasia and hypertrophy in prepubertal mice, being reduced at adulthood (1,2). Transforming growth factor-β1 (TGF-β1) belongs to a superfamily of growth factors that regulates a variety of cellular processes, including cell cycle progression, cell differentiation, reproductive function and development. Several reports indicate that TGF-β1 is involved in vascular endothelial growth factor (VEGF) expression. The aim of this study was to analyze the expression of TGF-β1, its co-receptor endoglin and VEGF-A isoforms (VEGF-A 120, 164 and 188) and the receptors VEGF-R1 and VEGF-R2 in the testes of TG at 3 and 8 weeks of age. In order to correlate the "in vivo" studies, we analyzed "in vitro" effect of TGF-β1 on testicular VEGF-A expression.

Materials and Methods

Testes from TG and wild type FVB/n (WT) (3 and 8 weeks of age) were removed and frozen at –80ºC. Total RNA was isolated with Trizol reagent and used for RT-PCR. The expression of TGF-β1, endoglin, VEGF-A and their isoforms and receptors (VEGF-R1 and R2) were evaluated. Actin was used as house-keeping gene. Testes were also fixed in para-formaldehyde and embedded in paraffin for VEGF immunohistochemistry. Whole testes from WT (8 weeks of age) were incubated "in vitro" in 199 Medium with or without TGF-β1 (1-10 ng/ml) for 15 and 30 min.

Results and Discussion

TG showed an increase in the expression of: 1) TGF-β1 at 8 weeks of age ($p<0,05$)(Fig. 1); 2) VEGF-A (Fig 1) and its isoforms as well as VEGF-R2 in TG at both ages ($p<0,05$). Endoglin presented a non-significant increase at 3 weeks of age. "In vitro" incubation (15 or 30 min.) with 1 ng/ml of TGF-β1 induced an increase in testicular VEGF-A expression (Fig 2) whereas a decrease on this parameter is observed at 10 ng/ml of TGF-β1 ($p<0,05$). The level of expression of VEGF-R1 and R2 remained unchanged. Immunohistochemistry showed that VEGF-A is localized in Leydig cells.

In summary, high and chronic levels of hCG induced testicular TGF-β1 expression on TG at 8 weeks of age. Furthermore, TG showed an increase on testicular VEGF-A expression, its isoforms and VEGF-R2. "In vitro" studies showed that TGF-β1 exerted a dose-dependent biphasic effect on VEGF-A expression. The temporal expression of these factors might influence the growth and development of the testes.

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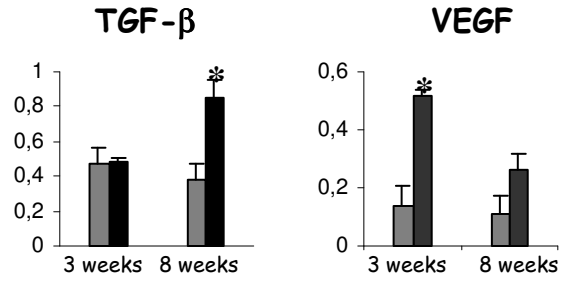

Fig. 1: Relative expression of TGF-β1 and VEGF by RT-PCR in TG ■ and WT ■ mice of 3 and 8 weeks of age. *: $p< 0,05$

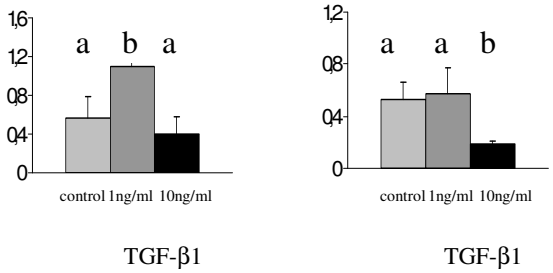

Fig. 2: "In vitro" effect of TGF-β1 (1 and 10 ng/ml) on testicular VEGF-A relative expression for 15 (Panel A) and 30 min (Panel B). Different letters indicate significant differences ($p<0,05$).

Influence of recombinant bovine somatotropin (RBST) on testicular development of beef calves

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Spermatogenesis is a complex developmental process affected by hormones and growth factors such as IGF I and II. Growth hormone (GH) also exerts effect over male gonadal fucntions, where its receptors and binding proteins are distributed ubiquitously in the reproductive system of cattle and other species.

Materials and Methods

In this trial, 54 crossbred Nelore x Simmental calves were used. Trial initial average age and weight were 7 (±15 days) month-old and 199±43 Kg, respectively. Animals were allocated to one of three treatments- 0 (n=18), 250 (n=18) and 500 (n=18) mg of rbST. Measurements were taken to determine scrotal circumference, testicular width, length and thickness. For testicle cell population and histometry, one animal of each treatment was castrated at three distinct periods at 14-day intervals, so that a total of 9 animals were castrated. For physical and morphological semen evaluation 36 animals were used ((0=12, 250=12 e 500 mg=12).

Results and Discussion

Average scrotal circumference was greater ($P<0.05$) for the 500 mg (24.35 cm) group compared to the others (23.47 and 23.02 for the 250 and 0 mg, respectively). A similar effect was observed (1) in transgenic rats which expressed hybrid genes, including human and bovine GH, where both genes led to testicle growth and increased seminal vesicle length. Epithelium height (EH) was greater ($P<0.0114$) for the 250 mg group throughout the three castration periods (46.56; 49.06 and 52.81), compared to the others (39.06, 40.31 and 48.75, and 39.37, 36.25 and 35.31 for groups 0 and 500 mg in periods 1, 2, and 3, respectively). The lower EH observed in group 500 mg may have been a function of a decrease in somatomedin receptor concentration in the germ cells, through a down regulatory mechanism set forth by the high GH dose.

It is concluded that rbST injection exerted a positive effect on scrotal circumference in a dose dependent manner, and, according to the morphometric analyses, the hormone affected germ cells, ant EH which was greater in the group receiving 250 mg of rbST. However, the 500 mg dose reversed the positive effect of the intermediate dose, suggesting an optimum point for the GH action in the testicles.

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Testicular atrial natriuretic peptide (ANP): a peptide involved in the steroidogenesis process

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Introduction

ANP is a hormone that participates actively in the maintenance of body fluid homeostasis and in blood pressure control. Besides this well established property, many works have shown the stimulatory effect of ANP on testosterone production in mouse Leydig cell culture (1, 2). The aim of this study was to verify the presence of ANP and its receptors in rat testis, as well as its effect on androgens secretion by whole testis perfused in vitro, a model that which better mimics an in vivo situation, maintaining the cellular interrelationships in the testis.

Materials and Methods

Immunohistochemistry for ANP was performed in paraffinized slices of adult rat testis through the avidin-biotin-peroxidase method, using Vectastain ABC Kit (Vector Labs.). Briefly, after blockade of the endogenous peroxidase and non-specific binding, slices were incubated overnight with antibody against ANP 1:1000. Immunostaining were visualized by DAB. Autoradiographic study for ANP receptors was done in frozen testis slices, incubated for 1 hour with ^{125}I-ANP (50 pM) alone, or with increasing amounts (10^{-10} to 10^{-6} M) of unlabeled ANP or cANF, a specific ligand to C receptor. After washes in Tris-HCl 50mM buffer, slides were dried and placed in a phosphor-sensitive cassette for 48h. Images were scanned and quantified by an imaging analyzer. Expression of mRNA for ANP and GC-A and C receptors was verified through real time PCR in immature and adult homogenate testis, following SYBR Green Master Mix protocol, using specific primers (Invitrogen Brazil). GAPDH was used as endogenous control. Perfusion of whole testis was made in a closed circuit system for 4 hours with Medium 199 (pH 7.4; 34^{o}C; 80 mmHg). LH and ANP were added to the medium and steroids profile determined by radioimmunoassay.

Results and Discussion

Immunoreactivity for ANP was identified in rat testis, confined in the interstitial compartment, mainly in the Leydig cells. Autoradiography demonstrated the presence of GC-A and C receptors in the rat testis: non-iodated ANP and cANF (10^{-6} M) displaced almost completely the total binding, indicating the presence of both ANP receptors, GC-A and C in rat testis. Relative real time PCR shown higher mRNA expression (arbitrary units) for ANP and GC-A and C receptors in adult compared to immature rat testis.

Perfusion of testis demonstrated that ANP stimulated testosterone secretion, in a dose- and time-dependent manner. Also, ANP stimulated, in the similar way, the production of testosterone precursors (progesterone and pregnenolone). When added to LH, ANP and cANF decreased testosterone production compared to LH stimulus.

Group	Testosterone ng/ml/g	Progesterone ng/ml/g	Pregnenolone ng/ml/g
Control	18.3± 4.3	17.9± 2.0	25.9±8.8
LH (100ng/ml)	150.9±21.3	237.1±37.4	218.7±48.4
ANP (0.3µg/ml)	22.3±3.8	_	_
ANP (1.0µg/ml)	53.3±9.4	111.8±17.8	80.8±11.7
LH + ANP	122.5±10.3	_	_
LH + cANF	88.3±6.1	_	_

The present data demonstrate that rat testis is a source of ANP and its receptors. Also, results points to a possible role for it as a modulator of androgens production.

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Expression of type II iodothyronine deiodinase (D2) in the reproductive system of rats.

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Introduction

The testis has been classically described as a thyroid hormone (TH) unresponsive tissue. However, in the last years, various studies have demonstrated that the TH plays an important role in the testes development and functional mechanisms. Different isoforms of receptors for the TH had been detected in the testes, during its development and also in the adult life in rodents and human beings. Additionally, our group had identified the presence of the type II iodothyronine deiodinase (D2), an enzyme evolved in the intracellular conversion of the T4 in active hormone T3, within the testes of rats and adult mice. However, the localization of the D2 in the different sub-cellular types of the seminiferous epithelium cycle was not established yet, which was the objective of this study.

Material and Methods

The testes of controls and hypothyroid adult rats (treated with 0.03% metimazol per 4 weeks) had been removed and immediately treated enzymatically to isolate somatic and germ cells. To determine the activity of the D2, the testes were homogenated with sucrose (0,25M) and DDT (10mM), labelled using marked ^{125}I and cold T4 as substratum.

Results and Discussion

Our results had demonstrated that in the adult testes the D2 are present, exclusively, in the germ cells (0,23 + 0,003 fmol/min.mg.prot). Seminiferous tubule somatic cells, as Sertoli and myoid cells, as well as the interstitial cells had been negative for the presence of this enzyme. We also evaluate the accessory glands but the activity of the D2 was detected only in prostate (0,02 fmol/min.mg.prot). The induction of the hypotyroidism increased significantly the activity of the D2 in the germ cells fraction (P=0,03), and also in the prostate (P=0,007). This present study demonstrates that the expression of the D2 in the thyroid hormone may have a direct effect on the regulation of the spermatogenesis in the adult rat testis.

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Endogenous spermatogenesis depletion in C57BL6 mice and wistar rats after fractioned X-ray irradiation

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Introduction

Spermatogonial transplantation has been widely used to investigate the biology of spermatogonial stem cells. One critical step for the transplantation success is the preparation of recipient testes. Ablation of endogenous stem cells can be done with different techniques. However, local irradiation of the testis has been effective to kill endogenous stem cells in mice and rats, without affecting other self-renewal systems. In the present study, we intended to standardize the appropriate dosage of X-ray in mice and rats, at our conditions aiming to prepare these rodents as adequate recipients for germ cell transplantation assays.

Materials and Methods

Six adult C57BL6 mice and six adult Wistar rats were submitted to two local fractioned doses of irradiation with X-ray of 1.5 and 12 Gy and 1.5 and 8.5 Gy, respectively, 24 hours apart. The testes were irradiated with a Picker-Andrex X-ray tube, operating at 240 kVp constant potential, at 4 mA (a measured HVL of 2,1 mm Cu). The dose rate was equal to 0.29 Gy/min. The animals were sacrificed at different time periods after irradiation and had their testis routinely prepared with the purpose to investigate the depletion rate (shorter period) and the recovery index (longer period).

Results and Discussion

The mice sacrificed 22 days after irradiation showed lower testicular weight (73%) compared to the controls (Tab. 1). Whereas the depletion of spermatogenesis (depletion index) was 99.6 (Tab. 1). In rats sacrificed 36 days after irradiation the reduction found for testicular weight and depletion index were 56%, and 99.6, respectively (Tab. 2). Full recovery of spermatogenesis was observed in 12% of the seminiferous tubules cross-sections evaluated for mice. Whereas in rats spermatogenesis did not advance beyond the spermatogonial phase, and only 4% of seminiferous tubules cross-sections presented spermatogonia (Table 2). From these results found we can conclude that, at least for mice, the X-ray irradiation utilized is adequated to utilize these animals for experimental studies, including spermatogonial stem cell transplantation.

Table 1. Effects of the fractioned X-ray irradiation (1.5 + 12Gy) in the adult C57BL6 mouse testis.

Days after irradiation	Testis weight (mg)	Depletion index (%)	Repopulation index (%)
Control	103 ± 0.7		
22	28 ± 0.2	99.6 ± 0.2	0
123	25 ± 0.2	88.0 ± 0.6	12 ± 0.5

Table 2. Effects of the fractioned X-ray irradiation (1.5 + 8.5Gy) in the adult Wistar rat testis.

Days after irradiation	Testis weight (g)	Depletion index (%)	Repopulation index (%)
Control	1.63 ± 0.1		
36	0.72 ± 0.2	99.6 ± 0.2	0
123	0.76 ± 0.1	96.0 ± 0.3	0

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Effects of diethylcarbamazine on male mice germ cells

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Introduction

Diethylcarbamazine (DEC) has been proven to be highly effective against lymphatic filariasis. However, its pharmacological mechanism of action remains uncertain. The effect of DEC on vertebrate cells has also been indicated. According to Fujimaki et al. DEC promotes the inhibition of microtubules assembly and induced the disassembly of the preformed ones *in vitro* [1, 2]. It is well known that dynamic changes in the cytoskeleton are present during the spermatogenesis, but until now there were no available data about DEC action on this issue. In the present work, we report several ultrastructural alterations of testis germ cells after treatment with a major anti-filarial drug, diethylcarbamazine.

Materials and Methods

Fourteen adult Swiss mice, 45-day-old and weighting 27-30g, were used in all experiments. One experimental group composed by seven animals received 200mg/kg body weight of DEC for 12 days per os in aqueous solution by stomach tube. The control group also composed by seven animals received only pure water. After treatment the experimental and control animals were killed and pieces of testis were quickly excised with a scalpel and fixed for electron microscopy evaluation.

Results and Discussion

After 12 days treatment with DEC, examination of thin sections of testes revealed numerous and large vacuoles within the Sertoli cytoplasm. Some spermatogonia showed morphological characteristics of apoptosis, as shrinkage of cytoplasm and increased chromosomal density. Enlargement of endoplasmic reticulum and deposits of electron-dense materials around the intracellular degenerated organelles were also observed. Untreated testes showed spermatids with mitochondria distributed surrounding the microtubular axis. On the other hand, in DEC-treated testes some spermatids presented vacuolated mitochondria, which were disorganized in relation to the microtubular axis of the flagella. Other spermatids presented numerous large vacuoles inside the cytoplasm and mitochondria no longer distributed along microtubule paths. Instead, these organelles were found scattered in the cytoplasm.
These cellular alterations could be explained by a microtubular disorganization, which is responsible for the dynamic stability of several cell functions. However, the present data do not exclude the possibility that DEC can also act directly on enzymatic hormonal pathways.

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The low molecular weight fraction from the *Bothrops jararaca* snake venom causes alterations in the spermatogenesis of mice.

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Introduction

The low molecular weight fraction obtained from *Bothrops jararaca* snake venom (LMWF) comprises a series of bioactive peptides. The main components of the LMWF are the BPPs (Bradykinin Potentiating Peptides), also known as Proline Rich Peptides (PRPs). One of its mechanisms of action is related to the Angiotensin Converting Enzyme (ACE) inhibition. At moment, two ACE isozymes are known: Testicular ACE (tACE) is expressed in germ cells exclusively during spermatogenesis and studies have shown the involvement of the tACE in the male fertility, even in the fertilization as in spermatogenesis[1]. Experimental evidences using models knockout of mice for the tACE indicate that this enzyme is directly associated with the male fertility. This hypothesis associated with the fact to exist great number of PRPs, presenting both functional and structural variability, had stimulated the characterization of the possible effect of the low molecular weight fraction (LMWF) obtained from the *B. jararaca* venom in mice spermatogenesis.

Materials and Methods

Male Swiss mice (30-35g) were treated or not with the LMWF (60µg/animal/day for 15 days). Testes were removed, histologically processed and the sections, stained with Mallory trichromic and about 50 sections from different sites for each testis were examined. Classification of seminiferous tubules and stage analysis were carried out for quantitative evaluation of spermatogenic cells of the seminiferous tubules[2]. The histological sections were observed in photomicroscope Zeiss Axioskop 2 and the images were captured by Pixera (Pixera Corporation, U.S.A.) connected to the optic microscope and to a microcomputer. All images were acquired and analyzed in Adobe Photoshop (Version 7.0). All data are presented as the mean ± SEM and the criteria for statistical significance was set at $P<0.05$ (GraphPad Prism 4.0, GraphPad Software, Incorporation).

Results and Discussion

The seminiferous tubule morphological data obtained from the animals treated with LMWF indicated the presence of atypical multinucleated cells in the lumen when compared with the control group and germ cell degenerated in the adluminal compartment. The morphometric seminiferous epithelium analyses showed that the LMWF treated animals present an increase in the spermatids number (23.50±1.82; $p>0.01$) and in the epithelium height (0.091±0.001 mm; $p>0.01$) when compared with the control group (15.70±2.05; 0.053±0,006, respectively). In contrast, we showed a diameter decrease in the seminiferous tubule (treated animals: 0.206±0.020 mm and control animals: 0.255±0.025 mm; $p>0.01$) in the germinal epithelium VII/VIII stages. No alterations in the spermatogonia, spermatocytes and the Sertoli cells numbers were detected. These data indicate such effects can occur through the PRPs interaction with the tACE, causing an alteration in the process of spermatogenesis. Therefore, possible inhibitors of this enzyme open new perspectives for the medical development of contraceptive with property in animals, including humans.

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Effects of sildenafil (Viagra, *Pfizer*) administratration on histological and morphometric parameters in mouse testis

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Introduction

Viagra® is the trade name of the sildenafil citrate, a new oral therapeutics for the erectile dysfunction, effective in maintaining the erection of the penis in response to the sexual stimulation. Reports indicate that the seminal characteristics were not modified in individuals treated with Viagra. However this doesn't eliminate the possibility of alterations in the testis morphophysiology, observed through histomorphometry of the testis. Thus, the present work has as objectives the structural and morphometric investigation of the testis of treated adult mouse with Viagra.

Materials and Methods

Sixteen adult male mice, with 90 days of age and weighing about 40g, were distributed in two groups: treated (n=8) with the sildenafil citrate (Viagra®, Pfizzer) suspended in water, and control (n=8), treated with the vehicle. The administration of Viagra (0,7mg/kg) and vehicle (water) was accomplished through gavage for five days a week, for 4 weeks. The treatment was accomplished between 7 and 8 hours of the morning. After the treatment the animals were weighed; anesthetized with association of the 0,02 ml of xilazina cloridrato (2,3g/100ml) and 0,07ml of ketamina cloridrato (50mg/ml), via subcutaneous, for each 40g of body weight; perfused-fixed by gravityfed perfusion with 4% buffered glutaraldehyde for 25-30 minutes, through the left ventricle; testes were removed, weighed and the gonadosomatic index established (GSI = weight of the testes x 100/body weight). Testis fragments were routinely processed and embedded in plastic (glycol metacrylate). Sections of 4µm thickness were obtained and stained with toluidine blue for histological and histometric analysis.

Results and Discussion

Compared with the group control (0,14 ± 0,01g; 0,12%), the medium weight of the testis and GSI of the treated animals with Viagra (0,15 ± 0,01g; 0,13%) were not altered. In the two experimental groups the histologic analysis showed the organization testicular with distinction of the compartments of seminiferous tubules and interstitial, and identification of the 8 stages of the cycle of the seminiferous epithelium, based on the tubular morphology system. The seminiferous tubules and cells of Leydig volume density (%), increased 4% and reduced 22%, respectively, in the treated animals with Viagra in relation to the control. The tubular diameter and length of the seminiferous tubules per testis were not different among the groups control (241µm; 2,7m) and Viagra (238µm; 2,9m). The relative frequency of the eight stages of the cycle of the seminiferous epithelium, obtained for 800 seminiferous tubule cross-sections per group, control and Viagra respectively, they were of: 1, 12,4% and 8,2%; 2, 7,2% and 5,9%; 3, 7,3% and 7,2%; 4, 11,9% and 23,3%; 5, 13,9% and 11,0%; 6, 15,2% and 13,6%; 7, 14,6% and 11,5%; 8, 17,5% and 19,3%. The stage 4 in the animals submitted to the treatment with Viagra presented an increase of 100% in relative frequency, in relation to the control. The relative frequency of the stage portrays the duration of each stage; thus we can suggest that Viagra promoted an increase in the duration of the stage 4, which is characterized by the meiotic divisions. Investigations indicate the need of the cytoplasmic Ca(2+) during the meiotic phase of the spermatogenic process (1, 2). Reports point out sildenafil citrate as promoter of reduction of the intracell calcium (3), suggesting as probable a cause of the significant increase in the seminiferous tubule in the stage 4.

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Ultrastructural characterization of leydig cells after treatment with diethylcarbamazine

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Introduction

Diethylcarbamazine (DEC), the most widely used drug, has been proven to be highly effective, but its pharmacological mechanism of action remains uncertain. The effect of DEC on vertebrate cells has also been indicated. It has a number of direct biochemical effects on a wide range of different enzyme systems, including glycolysis, folate metabolism and acetylcholinesterase [1]. There are no available data about DEC action on testis cells. In the present study, we report several morphological alterations of testis Leydig cells after treatment with a major anti-filarial drug, diethylcarbamazine.

Materials and Methods

Fourteen adult Swiss mice, 45-day-old and weighting 27-30g, were used in all experiments. One experimental group composed by seven animals received 200 mg/kg body weight of DEC for 12 days per os in aqueous solution by stomach tube. The control group also composed by seven animals received only pure water. After treatment the experimental and control animals were killed and pieces of testis were quickly excised with a scalpel and fixed for electron microscopy evaluation.

Results and Discussion

Leydig cells in control group showed normal morphologically characteristic reticulum. DEC-treated Leydig cells showed numerous lipid droplets scattered throughout the cytoplasm and some multivesicular bodies when compared to control cells. Several giant whorl-like smooth endoplasmic reticulum profiles were observed, some of them encircling large lipids droplets. Leydig cells in steatosis process showed picnotic nucleus with condensed perinuclear chromatin and cytoplasm filled with lipid droplets. Also these cells presented degenerated mitochondria around clustered lipid droplets. All these changes indicate an inhibition of the steroidogenesis and no longer drain off free cholesterol, thus allowing the free cholesterol to be re-esterified and stored in lipid droplets. Other studies showed similar alterations of Leydig cells, which were related to a declined testosterone secretion after drug treatment [2, 3]. Data from the present study showed that DEC exerts a significant effect on the spermatogenesis. Decreased steroidogenic activity has been suggested as the primary cause of spermatic production failure.

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Leydig cell modifications in wistar rats treated with cyclosporine A and Heteropterys aphrodisiaca

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Introduction

Cyclosporine A (CSA) has powerful immunosuppressive properties and is widely used in organ transplant therapy, improved graft survival rates, and in the treatment of some auto-immune diseases. However, it has a number of undesirable side-effects, such as the decrease in serum and testicular testosterone levels, as well as damage to testicular morphology. The plant, Heteropterys aphrodisiaca, known as "nó-de-cachorro", has been attributed stimulant and aphrodisiac properties. Data from the literature suggest that the root extract can increase the volume of Leydig cells (LC) in rat testis (1). Thus, the present work was undertaken to study the association of the drug and the medicinal herb in adult Wistar rats, evaluating LC morphometry and ultrastructure.

Materials and Methods

Thirty animals were used, divided into five groups: I- control (sham); II– use of CSA; III–concomitant use of CSA and H. aphrodisiaca *infusion; IV- sequential application of H. aphrodisiaca infusion and CSA and V- only H. aphrodisiaca infusion. CSA was administered at a dose of 15 mg/kg/day and* H. aphrodisiaca *at a dose of 0.5 ml of the infusion prepared with 25 g of roots/100 ml boiling water. The treatments were administered daily by oral gavage, during 56 days. Rats were anesthetized and perfusion-fixed. The testes were removed and weighed, then processed for light and electron microscopy using standard techniques. Morphometry was performed in sections of 4 μm thickness stained with toluidine blue in order to quantify individual volume of LC, nuclear and cytoplasmatic volume and number of LC per gram of testis and total number of LC per testis.*

Results and Discussion

Volume density of LC did not alter in the treated groups. The administration of CSA resulted in decreased nuclear diameter and volume in LC. On the other hand, the *H. aphrodisiaca* infusion resulted in an increase of the proportion between LC nuclei and cytoplasm, and of its nuclear diameter and volume. The LC volume was greater in the groups that received the infusion, except for group III in which the number of LC increased. Ultrastructural analysis showed a greater number of mitochondria, smooth endoplasmic reticulum (SER) and lipid droplets in these three groups. According to Castro et al. (2002), the nuclear volume of the LC correlated highly with the testicular and plasma testosterone levels. Moreover, the amount of membranous structures present in either the mitochondria and/or SER of the LC might be strongly correlated to testosterone secretion (3). Therefore, since LC produce testicular androgens, the *H. aphrodisiaca* infusion appears to help restore the levels of testosterone in animals that received it before and concomitantly with CSA.

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Ginkgo biloba protects Leydig cells from toxic effects of cadmium

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Introduction

Cadmium (Cd) is an environmental and industrial pollutant that affects mutiple organs, including the testes (1, 2). Cd administration in male rats results in severe impairment of testicular function including inibition of testicular steroidogenesis (3). The present study was performed to elucidate if the *Ginkgo biloba* extract (GbE) could protect Leydig cell (LC) from toxic effects of Cd.

Materials and Methods

Eight-week old male Wistar rats were intraperitoneally administered a single dose of 3 μmoL/Kg body weight (BW) of $CdCl_2$ and/or GbE daily at a dose of 100 mg/kg BW. The groups (G) received respectively: G1, water; G2, GbE; G3, Cd; G4 received $CdCl_2$ and GbE on the first day and only GbE on the following days; G5 received pretreatment with GbE for 30 days and $CdCl_2$ on the thirty-first day. Testicular tissue was processed histologically for glycol methacrylate using standard techniques after whole body perfusion with glutaraldehyde 2,5%. Histometry was performed in sections of 4 μm thickness stained with toluidine blue in order to quantify individual volume of LC, nuclear and cytoplasmatic volume and number of LC per gram of testis and total number of LC per testis. Ultrastructural alterations of were observed with transmission electronic microscopy using standard techniques.

Results and Discussion

The main function of LC is the production of steroid hormones, mainly testosterone (4). Cd can directly inhibit LC testosterone secretion (4), but in this work we believe that the dose of cadmium was not sufficient to cause much damage, since we did not find significant alterations in the weight of the accessory sexual organs (data not shown), which are primary indicators of testosterone level reduction. Animals that received cadmium had a significant reduction in nuclear diameter, nuclear and cytoplasmic volume and consequently, total volume of LC. However, animals that received GbE as a pre-treatment or GbE after a cadmium dose did not present reduction in these parameters. Thus, GbE maintained LC volume. These results, together with ultrastructural observations, reveal that the GbE is capable of diminishing the damage to LC usually caused by Cd, such as diminished smooth endoplasmatic reticulum and irregular nuclear boundries. The number of LCs per testis and per gram of testis did not vary significantly between the groups studied. The number of LC per testis varies from 115 to 189 million. Keeney and Ewing (5) claimed that the lutienizing hormone (LH) is the only pituitary hormone required to control LC volume and it is not required to maintain the LC number in adult rat testis We can conclude that the dose of Cd administrated was not high enough to alter testosterone production. It might modify the LH level, affecting significantly the volume of LC of animals that received cadmium and GBE, suggesting that it can protect Leydig cells from the toxic effects of Cd.

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Histamine concentrations in testis and epididymis in rams with criptorchidism and undergone to scrotal insulation

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Introduction

There is virtually little information on the physiological role of mast cells and histamine in the testis and epididymis. The mast cells and the histamine seem to be the components of the general adaptation syndrome in testicular tissue of stressed rats. The excessive and/or prolonged activation and degranulation mast cells may turn out to be harmful for testicular interstitium (1). In the present study, we compare the total concentrations of histamine in the testis and epididymis in bilaterally cryptorchid rams and an experimental group submitted to scrotal insulation.

Materials and Methods

We analyzed 28 rams of the Santa Inês breed: 16 bilaterally cryptorchid (C - age: 20.8 ± 10.5 months), 6 normal ones undergone to scrotal insulation (I - age: 10.0 ± 0.8 months) and 6 normal (IC - control group - age: 10.2 ± 1.4 months). The scrotal insulation had duration of 96 hours, reaching a rise of temperature of 1.75ºC in the scrotal surface in group I, causing changes in testicular consistence, decreasing seminal motility and increasing minor defects in spermatozoa . The animals were killed and had their testis and epididymis cauda removed for analysis. A fragment of the median region of the testis and other of the tail of epididymis were obtained for determination of the total concentration of histamine by the fluorometric method (2), with the extractions and measures made by automated method (3). Statistical was performed by using ANOVA, being considered significant the differences with $p<0.05$.

Results and Discussion

Histamine concentrations were higher in epididymis than in testis in all the groups studied. The total concentration of histamine in groups IC and I did not have significant difference. However, there was a higher concentration of histamine in the testis and in the epididymis of the animals of group C, when compared with the ones of IC and I.

Table: Mean (x) and standard deviation (sd) of the total concentrations of histamine (ngH/g) in the testis and epididymis in rams

Group	Testis x ± sd	Epididymis x ± sd
IC	182 ± 30	652 ± 242
I	177 ± 21	554 ± 206
C	333 ± 138*	3,516 ± 1,285*

* significantly different for C x IC and C x I ($p<0,0001$)

The scrotal insulation had not changed significantly the concentration of histamine in testis and epididymis in the studied animals (IC x I). Nevertheless, we observed a difference in the concentrations of histamine in the testis and in epididymis in the cryptorchidism condition (C) compared with the acute dysfunction of thermoregulation to the testis and epididymis by undergoing scrotal insulation (IC and I). More works should be done to elucidate the role of histamine and mast cells in the thermoregulation mechanisms dysfunction in testis and epididymis and in the criptorchidism condition**.**

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Could neonatal androgenic supplementation increase the reproductive performance of male rats?

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Introduction

Physiological levels of testosterone in neonatal life are necessary to assure that male rats show at the adult life a sexual behavior suitable with the genotypic gender. Thus, testosterone propionate (TP) was administered to them at birth to evaluate the sexual, motivation and behavior at adult life.

Materials and Methods

At birth, Wistar male rats received TP, 0.1 □g, s.c., (SP) or vehicle (CT)(1). In adult life, these animals (sexually inexperients) were submitted to sexual motivation test. The sexual motivation test apparatus consists in a circular arena with 2 cages in opposite sides, with 2 stimulus animals; one with a sexually active male (♂) and another with a receptive female in estrous (♀). The number of visits, duration of each visit to each animal stimulus and the total time spent in them during 20 min were evaluated and recorded. For the sexual behavior animals were placed in a cage with a receptive female in estrous. The latency for the 1st intromission, 1st ejaculation and the number of both were measured in 30min(2). In order to turn these animals really sexually experients, they were located in a cage with two females and were observed until they couple the females. The sexual, motivation and behavior were evaluated again. The Mann-Whitney test (median (Q_1–Q_3)) or Student´s *"t"* test (average±SEM) were performed (p<0.05).

Results and Discussion

All data are show in table 1. Inexperients SP remained more time in each visit to the ♀ and visited less the ♂ than CT. Despite the fact that SP visited less the ♀, they didn't remain less time in this zone. When experients, SP showed more preference to ♀ and spent less time in the ♂ zone. Despite a slightly improvement, the alterations weren't significant for the latencies for the 1st intromission, number of intromissions, neither1st ejaculation nor in number of ejaculations between groups of sexually inexperients and experients.

Table 1. Effects of neonatal androgenic supplementation in sexual motivation and behavior.

	Inexperients CT (n=10)	Inexperients SP (n=10)	Experients CT (n=7)	Experients SP (n=5)
Time spent /visit ♀ (s)	31.5±2.78	48.25 ±6.84**	70.19 ±15.92	102.62 ±20.91
Visits at ♂ Zone	16.2±1.47	10.6 ±1.08**	12.29±1.77	9.0±1.16
Visits at ♀ Zone	20.7±1.92	15.7±1.0 *	16.43±2.66	11.4±1.99
Time in ♀ Zone(s)	663.6 ±52.86	715.3 ±72.55	833.0 ±39.65	936.4 ±23.29
♀ %time Spent ♀	76.39 % (67.0-79.4)	84.77% (63.6-91.8)	84.20% (71.0-87.9)	89.02% * (88.5-89.2)
Time in ♂ Zone(s)	227.3 ±26.89	189.8±46.7	207.0 ±28.99	119.6 ±5.15*
Latecy 1st Intromis.(s)	259.5 ±96.72	113.5 ±56.76	24.14 ±13.06	13.8±3.09
Number Intromis.	26.01±7.09	39.02±5.08	26.1±7.9	39.2±5.08
Latecy 1st Ejaculat. (s)	820.4 ±146.08	809.0 ±139.7	573.71 ±195.29	305.4 ±61.74
Number of Ejaculations	1.1±0.53	1.5±0.42	2.86±0.34	3.4±0.24

*p<0.05, **p<0.01, ***p<0.001

These data show that increased TP level at birth didn´t damage the sexual behavior parameters, on the contrary can promote improvement. In conclusion, the neonatal androgenic supplementation can change the configuration of the hypothalamus-pituitary-gonad axis resulting in enhance in the sexual motivation and behavior of these animals.

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Sterilization of dogs with intratesticular injection of a zinc base solution - histophatological evaluation

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Introduction

Spermatogenesis is an elaborate process of germ cell proliferation and differentiation that leads to the production and release of spermatozoa from the testis (1). A zinc-based solution, pH neutralized in BioRelease Technologies physiological vehicle, was developed (B.E.T.Labs, Lexigton, Kentucky, USA) as a chemical sterilant for intratesticular injection in male dogs. The vehicle is non-irritating and aids in sequestering the zinc moiety within the testicular tissue. The aim of this study was to evaluate, by light microscopy analysis, the efficacy of a single intratesticular injection of zinc-based solution in causing sterility of male dogs.

Materials and Methods

Fifteen mongrel dogs were assigned to 3 groups (5dogs/group). Group one, the control placebo, was injected with saline solution. Group 2, which consisted of animals ranging from puberty to1 year old and Group three, animals ranging from 2 to 4 year old, were injected with zinc-based solution, in six different doses (0.2 to 1.0mL). The dosage was based on testicular width (10 to 27mm), determined by a caliper. All dogs were submitted to orchiectomy on day 150 post injection. Testis and epididymis were removed, fixed in glutaraldehyde, embedded in methacrylate, sectioned and stained with toluidine blue for histophatological examination (2).

Results and Discussion

There was no change in cellular structure of the testis of dogs injected with saline. The mean testis diameter of the treated animals decreased 7 to 10% when compared to the controls. Histological examination of the treated groups revealed degeneration and decreased number of germ cells, formation of multinucleated giant cells, vacuolation of gem cells and loss of elongated spermatid in atrophic seminiferous tubules. The Leydig cells showed different degree of lipidic degeneration and necrosis. The majority of the seminiferous tubules in all dogs were lined only by Sertoli cells, which were vacuolated. In response to the intra-testicular zinc injection, the dogs own immune system changed permanently the seminiferous epithelium of the testicle resulting in sterility. The epididymal canal showed normal epithelial structure and also its other structures, however no spermatozoa were found in the lumen. In conclusion, intratesticular injection of zinc-based solution showed to be effective in impair spermatogenesis. The degree of histological changes assures irreversibility, which indicates the procedure as a new method of male dog sterilization of any age.

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Expression of α_{1A} adrenoceptor in rhesus monkey male reproductive tract

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Introduction

Several α_{1A} adrenoceptor (α_{1A}-AR) splice variants have been identified in humans (1, 2). Although many of them codify non-functional truncated products, four variants ($\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}3a}$ and $\alpha_{1a\text{-}4}$) are functional, differing from the original α_{1A}-AR ($\alpha_{1a\text{-}1}$) in length and sequence of the C-terminal region. In our laboratory, the expression of α_1-ARs has been studied in different male reproductive tissues, using rat as an experimental model (4, 5, 6). Since there is no molecular characterization of α_1-ARs in non-human primates, molecular and immunohistochemical studies were conducted in order to: 1) clone and characterize the nucleotide sequences of rhesus monkey (*Macaca mulatta*) α_{1A}-AR splice variants; 2) compare the mRNA expression of α_{1A}-AR variants in different human and rhesus male reproductive tissues; 3) confirm the expression and distribution of α_{1A}-AR, at protein level, in different rhesus male reproductive tissues.

Materials and Methods

Total RNAs and paraffin-embedded tissues from adult rhesus (10-12-year old) and human (56-83-year old) were kindly obtained from Dr. F.S. French (UNC-Chapel Hill). Using rhesus seminal vesicle mRNA as template, 5'-RACE, RT-PCR and PCR screening of a cDNA library were performed in order to clone rhesus α_{1A}-AR transcripts, using primers designed against human $\alpha_{1a\text{-}1}$-, $\alpha_{1a\text{-}2}$- and $\alpha_{1a\text{-}3}$-AR sequences (2). The obtained DNA products were confirmed by direct nucleotide sequencing. Sequences were submitted to *Genbank* (NCBI) to scan gene similarities. To analyze the distribution of α_{1A}–AR variants in human and rhesus tissues, RT-PCR was performed using total RNA (5 μg) from rhesus and human testis, seminal vesicle, epididymis and prostate. To confirm the expression of $\alpha_{1A\text{-}1}$-AR at protein level, paraffin sections (6 μm) of efferent ductules, epididymis and seminal vesicles from rhesus were used in immunohistochemical studies using an antibody against $\alpha_{1A\text{-}1}$-AR C-terminal region (*Santa Cruz Biotechnology*).

Results and Discussion

The results allowed the cloning and characterization of rhesus α_{1A}-AR splice variants. Qualitative molecular studies indicated the expression of transcripts present in both human and rhesus ($\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$ and $\alpha_{1a\text{-}2c}$) and exclusively expressed in humans ($\alpha_{1a\text{-}3a}$, $\alpha_{1a\text{-}3b}$ and $\alpha_{1a\text{-}3c}$) or rhesus ($\alpha_{1a\text{-}3}$v1, $\alpha_{1a\text{-}3}$v2, $\alpha_{1a\text{-}3}$v3 and $\alpha_{1a\text{-}3}$v4). Rhesus $\alpha_{1a\text{-}3}$-related variants were originated by the use of alternative splice sites located at 5' from the human splice site, suggesting the existence of differential splicing mechanisms between these two species. Immunohistochemical studies confirmed the expression of $\alpha_{1A\text{-}1}$-AR at protein level in the rhesus male reproductive tract. In all tissues analysed, a specific immunostaining in smooth muscle cells confirmed the involvement of these AR in contractile response. A positive staining was also observed throughout the epithelial cells, suggesting additional physiological roles for these ARs. The characterization of α_{1A}–AR variants in different species is a major step to understand their physiological role. These results will enable the understanding of how sympathetic system is involved in the control and maintenance of male reproductive functions, and its role in male (in) fertility.

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Estrogen-like effect of 3beta-diol on the expression of estrogen receptor ERα in the rat efferent ductules

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Introduction

3β-diol (5α-androstane-3β-17β-diol) is a metabolite of dihydrotestosterone (DHT), which was once considered biologically inactive. However, several biological functions have now been attributed to 3β-diol. Although 3β-diol is an androgenic steroid, the actions of this hormone are mediated by estrogen receptors (ER), rather than androgen receptors (1). ERs are widely expressed in the male genital tract, especially in the efferent ductules (ED) (2). To test the hypothesis that this ER ligand may be active in the ED, we performed surgical castration followed by 3β-diol replacement aiming to study the possible effects on the ERα expression in the ED.

Materials and Methods

Bilateral castration was performed in adult male Wistar rats followed by hormonal replacement with 3β-diol (3mg), estradiol (400μg), DHT (5mg) or corn oil (control). The ERα expression in the ED was investigated by western blotting and immunohistochemistry. Testosterone and estradiol plasma levels were investigated by radioimmunassay**.**

Results and Discussion

Surgical castration resulted in a 98% reduction in the plasma testosterone concentration. Only the DHT replacement was able to recover the testosterone level. None of the treatments modified the plasma estradiol level, except the estradiol replacement, that increased the hormone level. ERα was expressed in the nuclei of the ciliated and nonciliated cells of the efferent ductule epithelium. The ERα expression was not affected by castration, corn oil or DHT injection (Fig.1). However, compared to control, the ERα expression was greatly reduced after estradiol and 3β-diol replacement (Fig.1). The estrogen-like effects of 3β-diol on the ERα expression indicate that estradiol may not be the only estrogenic hormone to play a role in the male reproductive system.

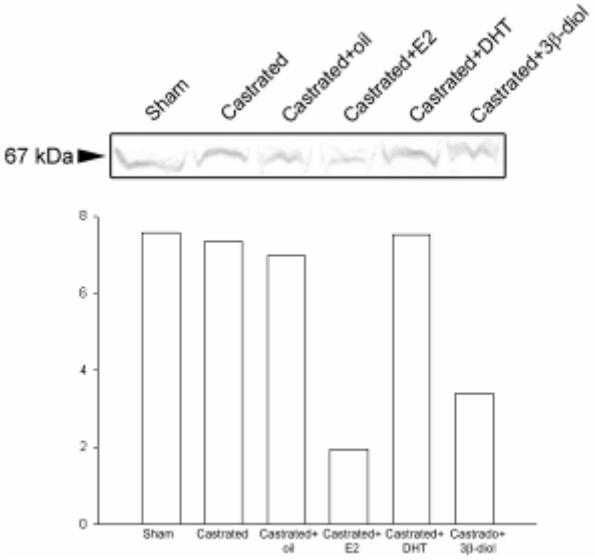

Figure 1: Western blotting analysis of ERα expression in rat efferent ductules.

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Exposure to the herbicide atrazine: effects on the morphophysiology and expression of androgen and estrogen receptor ERα in the rat efferent ductules

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Introduction

The importance of estrogens in males has been largely investigated, in face of the growing evidences regarding the relationship between xenoestrogen exposure and the increase in reproductive disorders. Atrazine (ATZ) is an herbicide widely used around the world, which has been considered a potent xenoestrogen. Potential risks on health include adverse effects in male reproduction system, such as reduction in testosterone and increase in estrogen levels, explained by its capacity to increase the levels and activity of the aromatase (1). Considering that the efferent ductules (ED) are among the main targets for estrogen action in the male tract, the present study aims to investigate the potential effects of ATZ in the morphophysiology of this segment, including changes on androgen (AR) and estrogen (ERα) receptor expression.

Materials and Methods

Adult Wistar rats were treated by gavage with ATZ 50mg/Kg for 15 days, and 200mg/Kg for 15 and 40 days, or corn oil as control. Alterations in the ED were investigated by histological and morphometrical analysis. Plasma testosterone and estradiol levels were measured by radioimmunoassay. Alteration in the AR and ERα expression was investigated by western blot and immunohistochemistry.

Results and Discussion

There was a transient increase in the testis and epididymal weights at the dosage 200mg/Kg/15d, followed by a decrease in these organ weights after exposure for 40d. At the dosage of 200mg/Kg/15d there were remarkable changes in the ED structure (Fig.1), including luminal dilation and reduction in the epithelial height and amount of lysosomes in the epithelial cells. None of the parameters analyzed were altered after treatment with ATZ 50 mg/Kg/15d. ATZ exposure at all dosages analyzed resulted in a remarkable decrease in plasma testosterone concentration, coinciding with a significant decrease in AR expression in the ED (Fig.2A). Plasma estradiol levels were increased only in animals treated with ATZ 200mg/Kg/15d. This hormonal change was in agreement with an increase in ERα expression in the ED of animals at the same group (Fig.2B). The present study extends the information about the effects of ATZ as an endocrine disruptor, once the testosterone and estradiol plasma levels are affected, resulting in histopathological changes in the male reproductive system.

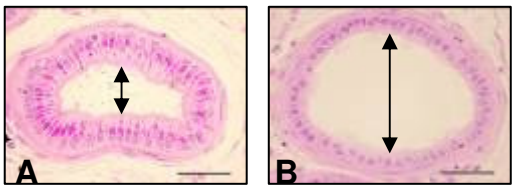

Figure 1: Structure of the efferent ductules in **(A)** control and **(B)** and ATZ 200mg/Kg/15d treated rats

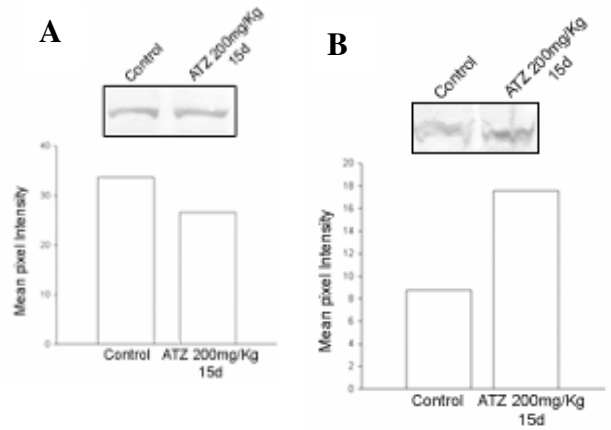

Figure 2: Western blotting analysis. **(A)** AR and **(B)** ERα expression in efferent ductules of control and ATZ-treated rats.

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Expression and immunolocalization of Toll-like receptor 4 in the rat epididymis

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Introduction

The innate immune system governs the interconnecting pathways of microbial recognition, inflammation, microbial clearance, and cell death. A family of receptors, known as the Toll-like receptors (TLRs), is crucial in early host defense against invading pathogens. Upon TLR stimulation, nuclear factor-kappaB (NF-□B) activation regulates the transcription of genes involved in the inflammatory response, injury and bacterial/viral infection. Toll-like receptor (TLR)-4 is a transmembrane receptor for lipopolysaccharide (LPS), a highly pro-inflammatory component of the outer membrane of Gram-negative bacteria (1). Recently, our laboratory has indicated that rat epididymis (caput and cauda regions) responds to an *in vivo* and *in vitro* treatment with LPS from *E. coli* with changes in the activation of NF-□B (2). Our aim in the presence study is to characterize TLR-4-positive cellular elements and the regulation of transcripts (TLR-4, CD-14 and IL-1β) during epididymal response to LPS.

Materials and Methods

Wistar rats (90-day old) were injected with sterile saline (control) or LPS from *E. coli* (1 mg/kg, i.v.) and sacrificed 2 h after treatment. The epididymis was removed, divided into caput and cauda regions. The tissues were frozen, the mRNA extracted and then used in RT-PCR with specific primers for amplification of TLR-4, CD-14 and IL-1β mRNA from rat. Tissues were also embedded in tissue freezing medium and cryostat cut (8 μm) for immunohistochemical studies, using antibodies specific to TLR-4 or ED2-like antigen (for detection of macrophages).

Results and Discussion

RT-PCR studies indicated the expression of TLR-4 and CD-14 transcripts in caput and cauda epididymis. RT-PCR studies also revealed an increase in the expression of CD-14 and IL-1□but not TLR-4 transcripts, in tissues from LPS-treated animals, confirming the response of caput and cauda epididymis to *in vivo* LPS challenge. Immunohistochemical studies located TLR-4 staining in the perinuclear and supranuclear regions of some, but not all, epithelial cells present in tubules from caput epididymis. In the cauda region, however, TLR-4 specific staining was detected only in the perinuclear compartment of epithelial cells. A higher number of these positive epithelial cells was observed in caput when compared to cauda epididymis. In both tissues analyzed, groups of interstitial cells were also TLR-4-positive. Immunofluorescent co-localization studies revealed that some of the TLR-4-positive interstitial cells were also immunostained by the ED2-like antibody, indicating staining of macrophages. No differences were observed in the expression and cellular localization of TLR-4 when caput and cauda epididymis from LPS-treated rats and saline were compared. Also the number of interstitial macrophages was not influenced by LPS treatment. All immunostainings were significantly decreased when assays were performed with antibody pre-absorbed with specific blocking peptide, confirming immunostaining specificity. The present study confirms that the epididymis presents the cellular elements involved in the recognition and in the activation of the intracellular signaling cascade induced by LPS. Further studies will be necessary to evaluate the impact of LPS treatment in the local mechanism of innate immunity in the rat epididymis.

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Aquaporins 1, 2, 7, 8, and 9 location in the dog epididymal and vas deferens epithelium

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Introduction

Recently, in rat, it was proposed the role of aquaporins (AQPs), a set of water transport proteins along plasmatic membrane, in respect to spermatozoon capacitation which occurred during its transit in the reproductive system excurrent duct lumina, such as, efferent ducts, epididymis and vas deferens. However, little is know about their role in the dog reproductive system. Thus, in this work we investigated the AQPs 1, 2, 7, 8 and 9 distribution in the epididymis and vas deferens in dog.

Material and Methods

Epididymis and vas deferens was collected from 8 adult mongrel dogs; fixed in 10% formaldehyde and processed for routine paraplast embedding. Sections were submitted to immunohistochemistry for AQP 1, 2, 7, 8 and 9 using specific primary Goat polyclonal antibodies from Chemicom, at 1:100 dilution.

Results and Discussion

AQP1 was absent from the proximal vas deferens and epididymal epithelial cell but was expressed in adjacent endothelial cells in the dog. This result was already expected, since in rats, AQP1 is expressed exclusively in the efferent ducts and in the ampulla's vas deferens (1,2). AQP2 was not detected in the adult dog epididymis or vas deferens, in agreement with previous data from literature. In the adult rat, AQP2 presents a progressive expression along vas deferens, being not detected in the proximal portion but present in the middle and distal portions (3). AQP7 had a strong expression on the proximal segments of epididymis, mainly in the apical cytoplasm and in the lateral plasmatic membranes of the initial segment. Furthermore, a strong reaction was noted in the apical brush border of epithelium as a whole and in the narrow P cells of the caput epididymis. Previously, AQP7 had been described only in the epididymis spermatozoa (4). AQP7 is absent from vas deferens. AQP8 was not detected by immunohistochemistry in our study, this result is in agreement with previous studies showing the absence of AQP8 protein in the rat epididymis (5). AQP9 is expressed in all regions of the epididymis of the dog and is clearly the predominant aquaporin in this tissue; our findings are in agreement with data in the literature (3). AQP9 staining is concentrated at the apical pole of epithelial cells and showed more evident expression on the distal segments of the dog epididymis. In vas deferens, the AQP9 was abundantly expressed on apical stereocilia of principal cells, as was previously described in the literature (6). In summary, the aquaporins 7 and 9 are the principal channels of water present in epididymis and vas deferens of the dog. In this sense they play a role in test fluid absorption and spermatozoon capacitation.

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Serotoninergic system in the rat caput epididymis during sexual maturation and variations associated with adult mating status

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Introduction

The role of the epididymis in sperm maturation is determinant in the control of male fertility. The caput epididymis of some mammals contains large quantities of serotonin (5-HT) whose origin, targets and physiological variations have been poorly studied. In this study, morphological and biochemical techniques were combined to begin describing some of the elements of serotoninergic system in the rat caput epididymis, and its relationship with both naive and breeder rats.

Materials and Methods

Epididymides of Wistar male rats of 40, 60, 90 and 120 days of age with no mating experience were used to determine the concentration of 5-HT, 5-HIAA and activity of tryptophan hydroxylase (TPH) through detection of 5-hydroxytryptophan, in the caput epididymis by using HPLC. Also, 120 days old rats were used to 1) describe the histological localization of TPH, 5-HT transporter and some receptors for 5-HT; 2) detection of TPH through western blot analyses, and 3) to assess the effects of *p*CPA, an inhibitor of TPH, over the enzymatic activity of this protein and the concentration of 5-HT. In another set of studies, we compared the concentration of this amine and the activity of TPH in the caput of males with no mating experience and colony breeders at the age of 170 days with the aid of HPLC.

Results and Discussion

Through HPLC studies, we find a progressive increase in TPH activity and 5-HT concentration with age. Immunohistochemical studies identify cells positive to 5-HT, to different 5-HT receptor subtypes (5HT1A, 5HT1B, 5HT2A and 5HT3; the flagella of spermatozoa showed immunoreactivity for 5HT2A and 5HT3 receptors), 5-HT transporter and TPH in the caput epididymis. Western blot analyses reiterate the presence of TPH in caput homogenates (we find two isoforms: 48kDa and 51kDa). pCPA treatment decreased both the activity of TPH and 5-HT concentration in adult rats. The activity of both TPH and 5-HT concentration tends to increase in breeder rats, supporting the concept that sexual activity and 5-HT concentration in the epididymis are somehow linked. All these data suggest the fact that the caput epididymis has the ability to produce 5-HT locally. Clearly, more research is needed to evaluate this fact.

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Binding patterns of cauda epididymal and accessory sex gland fluid proteins to bovine sperm

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Introduction

Comprehensive analysis of cauda epididymal (CEF) and accessory sex gland fluid (AGF) identified various groups of proteins (1,2). Proteins from CEF are the only ones leaving the epididymis with sperm and mixing with the AGF when ejaculation occurs. Prostaglandin D-synthase (PGDS), transferrin and albumin are major components of CEF and the first is associated with fertility (1,3). Bovine seminal plasma (BSP) proteins represent the most abundant component of AGF (2) and, along with osteopontin (OPN) and nucleobindin, are also empirically related to aspects of bull fertility (4,5). In this regard, understanding of how key CEF and AGF components interact with sperm is an essential step to unfold their roles and how they influence fertility. Thus, we examined in detail the binding patterns of certain CEF and AGF proteins to sperm.

Materials and Methods

Epididymal sperm was obtained by back-flushing the cauda of the epididymis followed by two washes in PBS (700 *g*, 10 min.). Semen collected by artificial vagina from 4 bulls was washed identically to obtain ejaculated sperm. Aliquots of 5 x 10^6 epididymal and ejaculated sperm were processed for immunocytochemistry using antibodies against PGDS, albumin, transferrin, BSPA1/A2, BSPA3, BSP 30kDa, OPN and nucleobindin (Nuc). The second incubation consisted of a FITC-conjugated antibody. Images were examined with a laser scanning confocal microscope and both FluoView (Olympus Inc.) and AutoBlur (Media Cybernetics Inc.) softwares.

Results and Discussion

From all proteins probed, only PGDS was found on epididymal sperm, restricted to the acrosome. After ejaculation, PGDS retained the same pattern, although with more intense fluorescence. Binding of BSA and transferrin to either epididymal or ejaculated sperm was not visualized. BSPA1/A2 was only detected on ejaculated sperm (midpiece, equatorial and post-equatorial regions and acrosome). Binding on the midpiece was significantly more intense than on the other regions. A similar pattern was observed for BSP 30kDa and BSPA3. However, BSPA3 binding to the equatorial segment was less intense, as compared to the other two. Interaction of OPN with ejaculated sperm was detected on the equatorial segment and acrosome. If sperm binds to the oolema through the equatorial region (6), it is plausible that OPN contributes with that event. A weak signal of nucleobindin was present on the midpiece and equatorial region of epididymal sperm. Nuc, an extracellular matrix protein with ability to bind calcium, had been recently detected in the male fluids for the first time by our laboratory, but the biological significance of its interaction with sperm is still unknown. In conclusion, using tools of immunocytochemistry and confocal microscopy, we show the existence of unique binding patterns of certain epididymal and accessory sex gland proteins to sperm. Such patterns may be linked to functional attributes of those proteins and how they affect the sperm while in the female reproductive tract.

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Impact of adrenalectomy on the rat epididymis: effects on distribution of glucocorticoid receptors and on spermatic parameters

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Introduction

Glucocorticoids (GC) are stress-induced steroid hormones, synthesized by adrenal gland. They regulate several functions in vertebrates such as immune responses, metabolism and reproduction. The actions of GC are mediated by glucocorticoid receptor (GR), which is a ligand-activated transcription factor. Clinically, GC represent one of the most prescribed drugs world wide. Curiously, little is known about GC modulation in the epididymis, an androgen-dependent male reproductive accessory organ which plays a vital role in the maturation, transport and storage of the sperm. Here we present a systematic study to immunolocalize and to evaluate the impact of adrenalectomy (ADX) on the expression and cellular distribution of GR (as well as androgen receptor, AR). The effect of ADX and dexamethasone (Dex) on testicular and epididymal sperm count was also evaluated.

Materials and Methods

Wistar rats (90 days) were sham-operated (S) or bilateral adrenalectomized (ADX) for 1, 2, 7 and 15 days or ADX and treated with Dex (5 μg/kg ip) for 7 days. Plasma corticosterone (C) and testosterone (T) concentrations were monitored by RIA. Caput and cauda epididymis were used in Western blot (total protein extracts) and immunohistochemistry (cryosections) with GR and AR antibodies (negative controls with specific blocking peptides). Homogenization-resistant testicular spermatids and sperm in the caput/corpus and cauda epididymis from S and ADX rats were submitted to sperm counting. Results were analyzed by ANOVA followed by Newman-Keuls test ($p<0.05$).

Results and Discussion

A significant reduction on plasma C, but not T levels, was observed with progression of ADX. Western blot studies indicated the expected MW for GR (~85kDa) and AR (~120 kDa) in control caput and cauda epididymis. Densitometric analysis revealed a significant increase in GR, but not AR protein levels, with ADX 7 and 15 days in caput when compared to S group. Both AR and GR levels increased with ADX 7 and 15 days in the cauda. Specific GR and AR immunostaining in control caput and cauda epididymis was detected in different cell compartments of epithelial, smooth muscle and interstitial cells (nuclear, perinuclear and cytoplasmic localization). Significant changes in the dynamic of nuclear and cytoplasmic GR and AR immunostaining were observed with ADX progression as a consequence of the reduction in plasma C levels. These effects were partially reversed by Dex treatment. A significant reduction of the number of testicular homogenization-resistant spermatids and the daily sperm production was observed with prolongation of ADX. The sperm transit time in caput/corpus was increased on ADX 7 days, while the sperm number in cauda was reduced on the same group. These effects were reverted to control levels with Dex treatment, confirming the participation of GC on such alterations. In summary, our results show for the first time the distribution of GR and the role of GC on the modulation of GR and AR in the epididymis. The progression of ADX had effects on sperm parameters in both testis and epididymis, reversed by treatment with a synthetic GC, suggesting a key role for GC on sperm production and in the regulation of epididymal function.

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The impact of altered epididymal sperm transit time on sperm parameters and fertility of rats

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Introduction

The epididymis is an organ of the male reproductive system where sperm undergoes the maturation process, acquiring motility and fertility capacity. The epididymal sperm transit time (number of days necessary for the sperm to be transported through the organ) seems to have an important role in sperm maturation, and it seems that an alteration of the duration of this transit can harm the process (1). Data from the literature show that the exposure of male rats to estrogenic substances, such as diethylstilbestrol (DES), decreases plasma testosterone levels and provokes an acceleration of sperm transit in the epididymis, damaging the fertility of the animals (2). The aim of present work was to evaluate the influence of altered sperm transit time through the epididymis on sperm parameters and fertility of rats, as well as the role of testosterone in the process.

Material and Methods

Two experimental models were used: DES was administered to the rats to accelerate sperm transit time, and guanethidine, to delay it, through a selective chemical sympathectomy of the male internal organs. Sprague-Dawley adult male rats were divided into four experimental groups: 1) treated with sc injections of DES, for 12 days, 10µg/rat/day, dissolved in corn oil; 2) treated with guanethidine sulfate via ip injections, for 12 days, at the dose of 6.25mg/kg/day, dissolved in saline solution; 3) same treatment as group 1, plus androgen supplementation, using testosterone-filled subcutaneous implants; 4) control animals received the vehicles. After treatment the animals were killed and the following parameters were compared: corporal and organs weights, number of germ cells in testis and epididymis, epididymal sperm transit time, sperm motility and morphology, and fertility after *in utero* artificial insemination (AI), using sperm collected from the proximal cauda epididymidis. Results are expressed as mean ± SEM; $p<0.01$ (**).

Results and Discussion

Guanethidine treatment delayed the sperm transit time (days) through the epididymal cauda (control = 5,73±0,25, guanethidine = 12,07±0,39**), provoking an increase in the sperm reserves in this region. On the other hand, exposure to DES accelerated the transit in the epididymis, decreasing the sperm density in both epididymal regions, caput-corpus (control = 3,01±0,13, DES = 1,81±0,10**) and cauda (control = 5,73±0,25, DES = 2,14±0,18**) and diminishing sperm motility. In both cases sperm production was not altered. Testosterone supplementation was able to reverse the acceleration of transit time and sperm motility in DES treated rats. Rats exposed to DES presented a trend toward lower fertility after AI. Thus, it was concluded that the acceleration of sperm transit time seemed to harm the normal sperm maturation in the rat, decreasing sperm quality and fertility capacity, in an androgen-dependent way.

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Expression of estrogen receptors ERα and ERβ in the epididymal region of roosters (*Gallus domesticus*) is differentially affected by epididymal lithiasis

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Introduction

The epididymal lithiasis is characterized by the formation of stones rich in calcium in the epididymal region of roosters, resulting in long-term effects on fertility (1,2). The intraluminal stones affect mainly the efferent ductules, leading to drastic morphological alterations in these segments. As a secondary effect, the testes also present morphological alterations in affected animals (2). The efferent ductules reabsorb about 90% of the fluid coming from the testis, an important function to guarantee male fertility. It is well known that fluid reabsorption depends on estrogen and estrogen receptors (ERα and ERβ), which can be widely found in the efferent ductules. This study aims to investigate alterations in the expression of ERα and ERβ in roosters affected by the epididymal lithiasis in order to clarify the physiopathology of the disease.

Materials and Methods

Adult roosters obtained from commercial sources were used in this study. The animals were considered as *affected* or *non-affected* due to the presence or absence of stones and morphological alterations in the epididymal region, respectively. Fragments of the epididymal region were frozen in liquid nitrogen or fixed in neutral buffer formalin for the investigation of ERα and ERβ expression by Western Blotting or immunohistochemistry, respectively.

Results and Discussion

The epididymal region of the rooster is formed by the rete testis, proximal and distal efferent ductules, connecting ducts and epididymal duct. Western Blotting analysis showed that ERβ was overexpressed (26%) in the epididymal region of affected roosters when compared to non-affected animals (Fig.1). On the other hand, ERα expression was not altered in affected animals. All the segments of the epididymal region showed positivity for ERβ immunostaining (Fig.2). When compared to the non-affected animals, the immunoreactivity for ERβ was more intense in all segments of the epididymal region, especially in the distal efferent ductules and epididymal duct. The results suggest that epididymal lithiasis is related to an unbalance of ERβ, but not ERα expression. Higher ERβ expression can be involved in the formation of intraluminal calcium stones, as already described in other tissues.

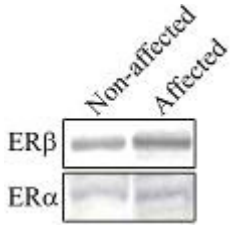

Figure 1: Western Blotting analysis of ERβ and ERα expression in the rooster epididymal region.

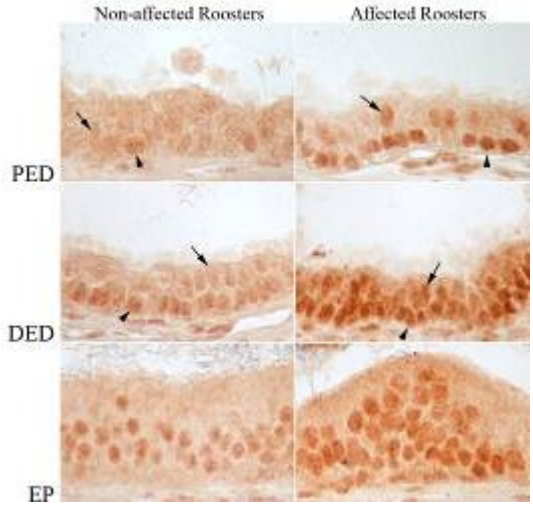

Figure 2: ERβ expression in the epididymal region of non-affected and affected roosters. PED=proximal efferent ductules; DED=distal efferent ductules; EP=epididymal duct; arrowheads=non-ciliated cells; arrows=ciliated cells.

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Vitamin D3 receptor (VDR) is overexpressed in the epididymal region of roosters (*Gallus domesticus*) affected by epididymal lithiasis

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Introduction

The epididymal lithiasis is an illness of unknown etiology that affects about 90% of domestic roosters (*Gallus domesticus*) in Minas Gerais state, Brazil (1). This pathology is characterized by the formation of stones rich in calcium in the epididymal region, concomitant with injuries in the epithelium of the efferent ductules and precocious infertility (2). The segment more affected by epididymal lithiasis is the efferent ductules, which, in birds, are responsible for the reabsorption of significant amount of calcium, in a process that follows the reabsorption of sodium and luminal fluid. It is possible that the formation of the intraluminal stones may be due to a disruption of calcium homeostasis, a process dependent on vitamin D3 and its receptors VDR. The VDR are widely expressed in the efferent ductules. As a first step in unraveling the molecular mechanisms involved in the formation of the stones rich in calcium in the efferent ductules, this study investigated possible alterations in the expression of VDR in roosters affected by the epididymal lithiasis.

Materials and Methods

The roosters used in this experiment were considered as *affected* or *non-affected* due to the presence or absence of stones in the epididymal region, respectively. Fragments of the epididymal region from animals of both groups were fixed in neutral buffer formalin, embedded in paraffin and processed for the immunohistochemical detection of VDR. Western Blot analyses were performed in frozen fragments of the epididymal region to confirm the results. Fragments of duodenum were used as positive control.

Results and Discussion

The immunoreactivity to VDR was noted as a nuclear staining seen in duodenum as well as the epithelium and some cells of the connective tissue in all the segments composing the epididymal region. Both proximal and distal efferent ductules of the affected roosters exhibited more intense staining for VDR when compared to non-affected animals, while the expression of this receptor in the connecting and epididymal ducts were similar to non-affected animals. Western Blot assay showed that VDR expression increased about 42% in the epididymal region of affected when compared to non-affected roosters, confirming the results obtained with immunohistochemistry. These results suggest that epididymal lithiasis is related to an alteration of VDR expression which could lead to a disruption in calcium transport in the efferent ductules culminating in the formation of stones rich in calcium in the epididymal region.

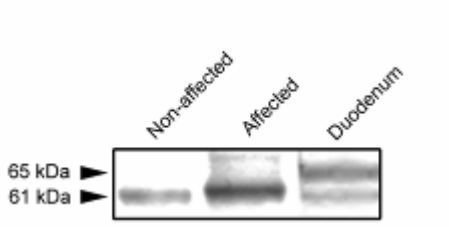

Figure 1: Western Blot analysis of VDR expression in the epididymal region of non-affected and affected roosters. Duodenum is the positive control.

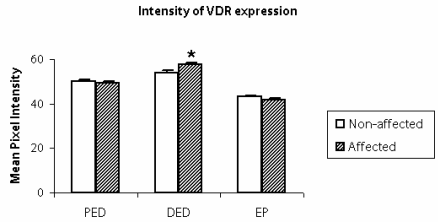

Figure 2: Quantification of the immunoexpression of VDR in different segments of the epididymal region. PED – Proximal efferent ductule; DED – Distal efferent ductule; EP – Epididymal duct.

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3beta-diol modulates the expression of estrogen receptor beta in the rat ventral prostate

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Introduction

The prostate gland is a sex hormone target organ. Androgens and estrogens are involved in the maintenance of structural and functional integrity of the gland. Dihydrotestosterone (DHT), acting through androgen receptors, is the main androgen regulating the prostate. However, DHT may also be metabolized in 3β-diol, which acts through estrogen receptors, especially ERβ (1). The ERβ is highly expressed in the normal prostate gland, but in both benign prostatic hyperplasia and prostate cancer its expression is decreased or even absent (2), indicating that a relationship between the ERβ expression and these pathologies may exist. However, the mechanism of regulation of ERβ expression in the prostate is still not defined. Therefore, considering that its ligand 3β-diol is present in high concentrations in the ventral prostate, we hypothesized that this hormone could be involved in local functions, such as modulation of its receptor, ERβ. To investigate this hypothesis is the aim of the present study.

Materials and Methods

Bilateral surgical castration was performed in adult male Wistar rats. Following castration, the animals were injected with estradiol (400μg), DHT (5mg), 3β-diol (3mg) or corn oil, as control. Ventral prostates were frozen in liquid nitrogen or fixed in neutral buffer formalin for the investigation of ERβ expression by Western blotting or immunohistochemistry, respectively.

Results and Discussion

In the prostate, ERβ expression was detected in the nuclei of epithelial cells and in some stromal cells. Both immunohistochemical (Fig.1) and Western blotting (Fig.2) analyses showed that the expression of ERβ was drastically decreased in the prostate epithelium of the castrated rats when compared to control animals. Hormonal replacement with DHT recovered the ERβ expression to levels similar to control prostates, while this receptor was overexpressed in the prostate epithelium after 3β-diol injection. On the other hand, replacement with estradiol resulted only in a slight increase in ERβ expression when compared to castrated animals. Taken together, these results suggest that estrogenic effects on prostate may be mediated by 3β-diol via ERβ, rather than by estradiol.

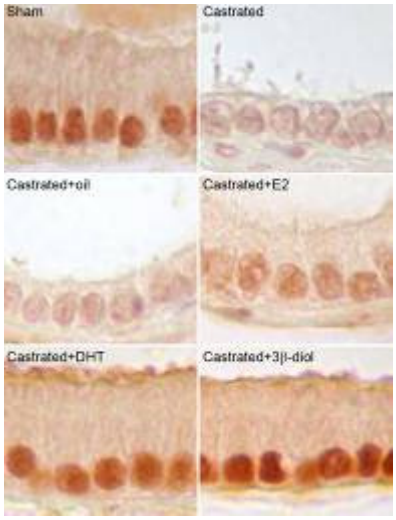

Figure 1: ERβ expression in the prostate. E2=estradiol, DHT= dihydrotestosterone.

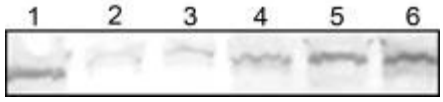

Figure 2: Western blotting analysis of ERβ expression in the rat prostate. lane 1= sham; lane 2= castrated; lane 3= castrated + oil; lane 4= castrated + estradiol; lane 5= castrated + DHT; lane 6= castrated+3β-diol.

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Immunohistochemical characterization of prostatic epithelium of the guinea pig (*Cavia porcellus)*

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Introduction

Adult prostatic epithelium consists of basal, luminal and neuroendocrine (NE) cells, which are presumed to differentiate from common progenitor/stem cells. In the basal compartment, a layer of cells is situated between the basement membrane and the overlying secretory cells. The human secretory compartment consists of a luminal layer which expresses the androgen receptor, cytokeratin (CK) 8 and 18 and prostatic specific antigen. The basal cells can be distinguished from secretory cells, because they express p63, CK5 and 14. Prostatic NE cells expressing chromogranin A, serotonin and others markers. (1) Hudson *et al* (2001) have demonstrated sub-populations of epithelial cells in the basal layer expressing CK15, 17 and 19 in various combinations. The present study describes phenotypic identification of prostatic cells of guinea pig.

Material and Methods

Seven male guinea pigs of 120 postnatal days were used. The animals had been submitted to the perfusion with formaldehyde l0% and Bouin solution. Sections of the prostate were stained immunohistochemically using the avidin-biotin-peroxidase complex method. Sections were incubated with the primary antibodies (serotonin, CK5, 18 and 19) overnight at 4°C After, the slides were rinsed with PBS and incubated with biotinylated anti-mouse IgG, 1:160, Vector Laboratories®, Burlingame, USA) for 1 hour and rinsed with PBS and incubated with ABComplex for 45 minutes. The slides were rinsed with PBS and then the site of antigen-antibody reaction was revealed by diaminobenzidine (DAB).

Results and Discussion

The epithelium possesses basal and secretory epithelial compartments and rare NE cells. The secretory cells express mainly CK18 and 19. In the basal compartment, the majority of basal cells expressed CK5, being negative for CK18. A considerable number of basal cells co-expressed CK19. A small population of NE cells presented positive reaction for serotonin and CK19. (2) Signoretti *et al* (2000) related that the CK5, 14 and p63 are always expressed in basal cell and can be considered to be basal cell specific in adult prostate. CK 19 is a protein mainly expressed in adult prostatic basal but is also expressed in a subset of secretory cells. Hudson *et al* (2001) considered that basal cells co-expressing secretory and basal markers were basal cells in transition differentiating into secretory cells. (3) Wang *et al* (2001) considered in the prostatic epithelium: (1) Basal cells expressing only basal markers; (2) basal cells expressing only basal markers and CK19; (3) intermediate cells expressing CK19; (4) secretory cells expressing luminal markers (CK8 and 18) and (5) NE cells expressing specific markers. The prostatic cells population of guinea pig presented results similar to the human, rat and mouse prostate. The guinea pig can be considered a useful model for experimental studies of prostatic epithelium, because expresses all CKs found in the human epithelium prostatic.

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Morphological and histological study of the gerbil prostate

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Introduction

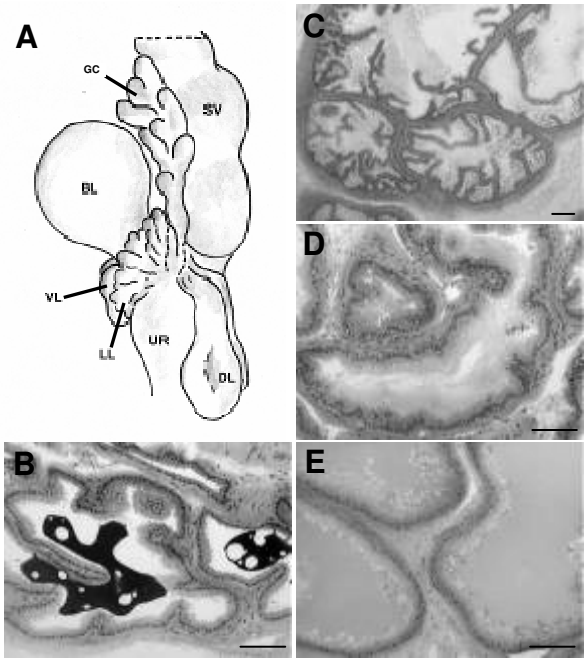
The prostate gland in rodents is composed of several distinct lobes which differ with regard to their histology, secretion, hormonal sensibility and incidence of diseases. In most of rodent models, including the Mongolian gerbil, special attention has been given to the histophysiology of ventral lobe (VL) and little is known about the other prostate components. This information is essential to improve the knowledge on reproduction of the gerbil and to establish comparisons with other rodents. Hence, the investigation reported herein is an analysis of the macro and microanatomy of prostatic complex of Mongolian gerbil.

Materials and Methods

The anatomy of the prostatic complex of 10 adult gerbils (*Meriones unguiculatus*) was examined in fresh and previously fixed specimens. Ductal networks were evaluated for each lobe after incubation with 1% collagenase, followed by microdissection. For serial histological sections, the whole prostatic complex or isolated lobes were fixed in Karnovsky and embedded in historesin. Ultrastructural features were examined at Transmission and Scanning Electron microscope using conventional procedures.

Results and Discussion

The topographical relationship of gerbil prostatic lobes with the pelvic urethra is shown in Figure A. The anterior prostate lobes (GC) are placed in ventral curvature of seminal vesicle and contain 7-10 secretory ducts which converge to a main principal duct. As regards the remaining pairs of lobes, the dorsal one (DL) is the most prominent and consists of 2-5 primary ducts emerging from the dorsal side of urethra, under the ducts of seminal vesicle (SV). Its tubuloacinar unities are large, little ramified and the low columnar cells produce a basophilic secretion. In this lobe the fibromuscular stroma is abundant and dense. The VL in adult gerbil contains 2-4 main ducts and the ramified duct shows marked regional differences along the proximal/distal axis. The stromal component of this lobe is very loose. The lateral lobe (LL) exhibits 4-7 main ducts which emerge from dorsal side of urethra and show short secretory branches. This lobe is histologically very similar to the VL, except for the presence of more epithelial folds, acidophilic secretion, high frequency of smooth muscle cells and epithelial neuroendocrine cells. The analysis of glandular architecture of gerbil prostatic complex indicate that, although all lobes are compound tubuloacinar glands, the branching pattern and histological features are lobe specific. Differently from mouse and rat prostate, the DL in the gerbil is more conspicuous and it is not associated with the LL. In addition, the later is positioned between the VL and DL. The anatomical and histological peculiarities described in this paper probably reflect a specific need concerning the prostatic secretion in gerbil, in comparison with other laboratory rodents.



Legend: Lateral view of prostatic complex in the adult gerbil **(A)** and histological aspect **(B-E)** of each lobe. **(B)** Dorsal Lobe, **(C)** Coagulating Gland or Anterior Lobe, **(D)** Lateral Lobe, **(E)** Ventral Lobe. SV- seminal vesicle; UR – pelvic urethra. Bars= 100μm

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The use of Alpha-methylacyl coenzyme a racemase immunohistochemistry in the diagnosis of mongolian gerbil prostate cancer

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Introduction

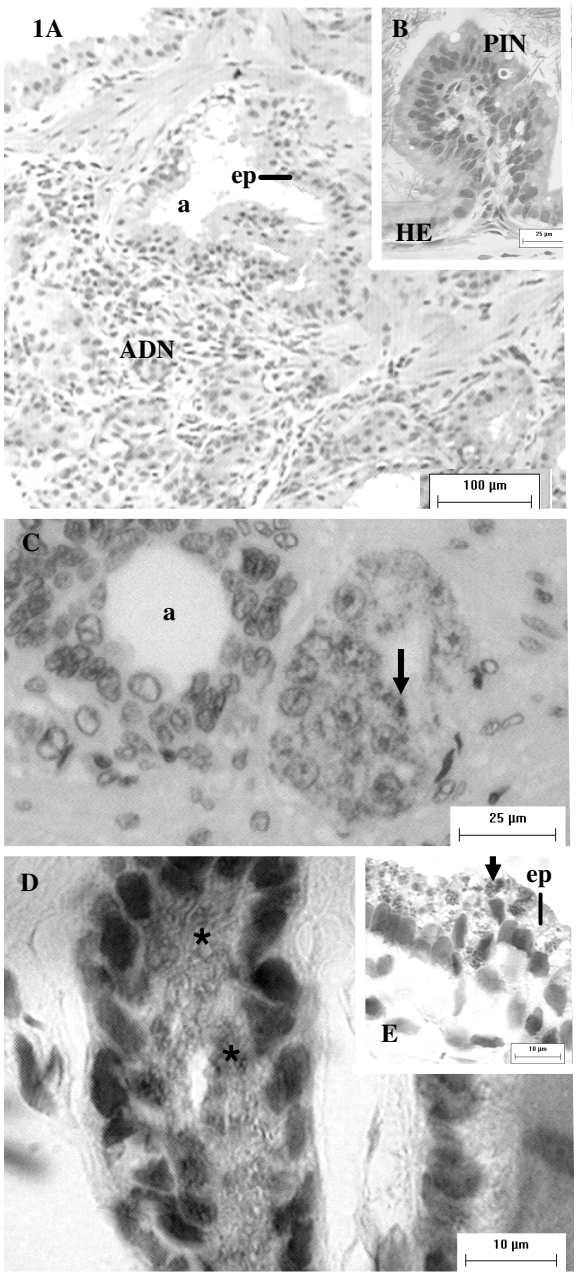
Alpha-methylacyl coA racemase (AMACR/p504S) is an enzyme involved in β-oxidation of branched-chain fatty acids and bile acid intermediates. Recent studies have identified this enzyme as a new diagnostic marker for prostate cancer (1). Until the present time, no mention about the possible application of this enzyme in prostatic tumor diagnosis of rodent models has been verified in the scientific literature. Therefore, in the present study, we evaluated the AMACR/p504s expression in spontaneous prostatic lesions of old gerbil and its potential as a new molecular marker.

Materials and Methods

Prostatic fragments of old gerbil (average age of 18 months) were fixed for 24h in 4% paraformaldehyde phosphate-buffered and embedded in Histosec for immunocytochemical analysis. In the last method, human prostatic fragments were used as positive control of the reaction (Fig. 1C, arrow). Initially the prostatic samples were diagnosed by H/E and the prostatic fragments containing proliferative lesions were submitted to AMACR/p504s immunostaining (1:100, monoclonal rabbit anti-human p504s, Dako, USA).

Results and Discussion

The most frequent spontaneous alterations observed in this rodent model were from epithelial origin, such as prostatic intraepithelial neoplasias (PIN), microinvasive carcinomas and adenocarcinomas (ADN) (Fig. 1A-1B. a, acinus; ep, epithelium). The data showed although in moderate intensity, there is racemase expression in the epithelial cell cytoplasm of prolifetive aggregates (Fig. 1D-1E; asterisks and arrows). In this preliminary analysis, it was not already possible to differentiate, by immunostaining intensity, the expression degree of this enzyme among the prostatic lesion types. However, as in humans, AMACR/p504s seems to be essential for the proliferation of anomalous epithelial cells in the evolution of gerbil prostatic carcinogenesis.



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Steroid hormone metabolism in the prostate of finasteride plus letrozol treated gerbils: a possible explanation for the differential action of testosterone and estradiol

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Introduction

In the prostate, androgens play a central role in its biology and estrogens also affect growth and differentiation of the gland. Prostate tissue contains also a variety of steroid-metabolizing enzymes required for the local formation of active androgens and estrogens, as the 5-alpha reductase (*5α-r*) and aromatase (*aro*), respectively. In this gland, testosterone is converted to the more potent androgen dihydrotestosterone by the *5α-r* and the *aro* provides an alternative pathway for the metabolism of testosterone to 17β-estradiol. Since local steroid metabolism controls the bioavailability of active steroid hormones in the prostate, the aim of this study, therefore, was to elucidate the differential roles and actions of these steroids hormones and to investigate what the absence of these enzymes provoke to the regulation of the growth and function of the prostate during the post-natal development.

Materials and Methods

Male gerbils (*Meriones unguiculatus*), young, adult and old, aging 7, 20 and 78 weeks respectively, were treated orally, once a day, for 30 days, with a combination of finasteride (0,5; 1; 1,5mg/animal, respectively) and letrozol (0,025; 0,5; 0,075mg/animal, respectively) diluted in 0,1ml of cotton oil. Finasteride causes the inhibition of *5α-r,* whereas Letrozol blockades the *aro*. Besides, in each age, the drugs had being also administrated separately. All animals were sacrificed 24 hours after the last day of treatments, the entire prostatic complex was removed, weighed and only the ventral prostatic region was fixed with Karnovisky solution. The samples were then processed for light microscopy and embedded in Historesin Leica. Sections of 5µm were dewaxed and subjected to Hematoxylin-eosin staining for evaluation.

Results and Discussion

In comparison with the control group and the administration of the drugs separately, the data obtained after the enzymatic dual inhibition demonstrated a marked remodeling of epithelial and stromal compartments. The ventral prostate of the adult and old gerbils decreased, whereas it increased in the youngs. Serum testosterone concentration increased in all ages and the estrogenic levels decreased in the adult animals and increased in the others. Irrespective of the age, the administration of finasteride decreased the weigh of the ventral prostate and increased the seric levels of testosterone and estradiol. The administration of Letrozol provoked an increase in weight of young and old ventral prostates and a slight decrease of it in the adult animals. The serum concentrations of testosterone were higher in young and old animals and lower in the adult. Surprisingly, the estradiol levels increased in animals of all ages after the administration of letrozol. Based on these results, it is possible to comprehend that testosterone and estradiol have synergic effects. It is evident that the balance between androgens and estrogens action, rather than the absolute levels of either hormone, may be of fundamental importance in determining normal or abnormal development and maintenance of the prostate. The blockade of these steroid-metabolizing enzymes provided an important novel tool to study the relationship between sex steroids and reorganization in normal physiology and diseases of this gland.

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Ultrastructural study of pirapitinga *Brycon nattereri* spermatozoa

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Introduction

The pirapitinga (*Brycon nattereri*, Günther 1864) is one of the Brazilian endangered fish species. The spermatozoa of many fish species, particularly teleost, have been extensively examined by electron microscopy. However, there is no ultrastructural study of this Characidae sperm published so far. The aim of this work was to investigate the spermatozoon of pirapitinga at electron microscopic level.

Material and Methods

Fish were originated from Hydroelectric Company of Minas Gerais (CEMIG). Three fish (BW=400 g) were injected with carp pituitary extract to induce spermiation. Semen was pooled, fixed in Karnovisky solution, transported to the Electron Microscopy and Ultrastructural Analysis Laboratory at UFLA, post-fixed in aqueous solution of OsO_4 and dehydrated through an acetone series. For scanning electron microscopic (SEM) analysis, samples were dehydrated at the critical point dryer, coated with gold under vacuum and examined in LEO EVO 40 XVP ESC. For transmission electron microscopic (TEM) analysis, samples were embedded in resin, cut as ultrathin sections (ultramicrotome), double-stained in uranyl acetate, lead citrate and examined in Zeiss EM 109 TEM operated at 80 kV. Spermatozoa (n=50) dimensions (head width, head length, midpiece length, flagellum length and total length) were measured using the image analysis program.

Results and Discussion

The *Brycon nattereri* spermatozoon has a total length of 35.056 ± 2.278 µm (Fig. 1). Sperm head is small and ovoid, nucleus is U-shaped with highly condensed chromatin forming coarse clots, centriolar complex is in nuclear fossa, acrosomal vesicle is absent, length of 2.002 ± 0.149 µm and width of 1.218 ± 0.091 µm (Fig.2). Sperm midpiece is 2.152 ± 0.377 µm long, is surrounded by a small mitochondrial necklace (Fig. 3) and the cytoplasmic channel is completely encircled by concentric membrane rings (Fig. 4). Only one long flagellum is present with the typical axoneme pattern of 9+2 characteristic of primitive species (1) and it is 30.901 ± 2.320 µm long (Fig. 4).

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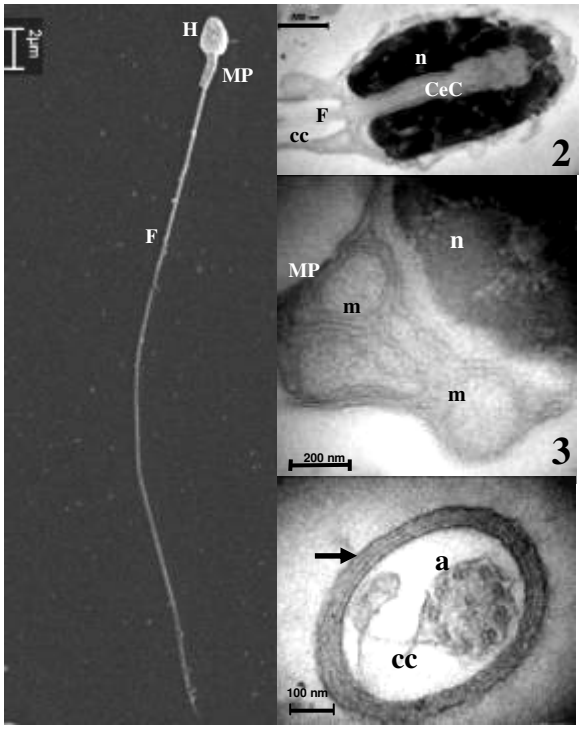

Figures (1) Scanning electron micrograph of *B. nattereri* spermatozoa: head (H), midpiece (MP) and flagellum (F); (2-4) Transmission electron micrographs: cross-sections showing nucleus (n), centriolar complex (CeC), mitochondria (m), cytoplasmatic channel (cc), axoneme (a), arrow = concentric membrane rings.

The sperm serine protease BSp66 IS included in membrane rafts

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Introduction

We have partially purified and characterized a serine proteinase named BSp66, that is located on the surface of the acrosomal region of various mammalian spermatozoa [1, 2]. As other sperm serine proteases, BSp66 has been shown to have an important role in early steps of fertilization in bovine and hamster [3, 4]. BSp66 possessed an abnormal electrophoretic migration when isolated from capacitated and cryopreserved sperm [3, 5]. Several attempts to isolate this protease in a soluble form have failed, and we also failed in obtaining its amino acid sequence by both Edman and MS methods. The evidence obtained prompt us to hypothesize that BSp66 is included in membrane insoluble microdomains called rafts, based on the reported data for other proteins with similar behavior. In this work we investigated if BSp66 is also included in microdomains in bovine and mouse sperm.

Material and Methods

Bovine cryopreserved sperm were obtained from INTA-Balcarce. Mouse sperm were obtained from *cauda* epididymis [2]. After extensive washing, sperm membrane rafts were prepared as reported [6]. Proteins from each fraction were electrophoresed in 12% SDS-PAGE under non-reducing conditions. Fractions containing rafts were monitored by WB with an antibody to caveolin 1. The presence of BSp66 was evaluated by WB using polyclonal anti-BSp66 [1].

Results and Discussion

Membrane rafts are formed by polymerization of caveolin related integral membrane proteins which tightly bind cholesterol and are implicated in signaling events related to sperm capacitation [6]. In this work, sperm membrane rafts were separated from soluble proteins by sucrose gradient in both bovine and mouse sperm. Caveolin signal was distributed from fraction 4 to 6 in mouse sperm and fraction 6-8 in bull sperm, indicating the presence of the light buoyant density of raft domains. When anti-BSp66 was used, a 66 kDa immunoreactive protein was detected in the same fractions were caveolin was present in bull sperm, providing evidence that BSp66 is included in rafts. However in mouse, a 66 kDa signal was detected in fraction 6 and higher molecular mass immunoreactive proteins were distributed from fraction 8 to 10 (bottom). This result suggests that one isoform of BSp66 is included in mouse sperm rafts, while protein covalent aggregates exist in the soluble fraction. Because of the methodology used, the possibility that BSp66 is localized to the inner or outer acrosomal membranes cannot be eliminated [6]. The discrepancies between species might be due to different sperm architectures or to changes in membrane composition accomplished to capacitation or cryocapacitation [6], since bovine sperm were frozen-thawed while mouse sperm were fresh.

The identification of proteins included in mouse sperm rafts [6], revealed four serine proteases after a bioinformatics search of an EST database: TESP1, TEX101, TESP2, 1700036D21RIK. This information suggests that BSp66 may be one of these proteins.

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Immunolocalization of SP22, a novel fertility biomarker in ram sperm

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Introduction

Recent studies have shown that the SP22 sperm membrane protein (28kDa) is highly correlated with fertility (1,2,3). The identification of this protein or its homologous in different species suggests its high conservation and its importance for the masculine reproductive capacity (2). The investigation of the sperm biomarker SP22 in domestic animals, especially ovines, has great importance since can contribute to select males to artificial insemination, increasing the reproductive efficiency or to improve strategies of semen criopreservation, since some sperm proteins indicate semen freezability. Thus, the aim of this study was to identify and to immunolocalize the expression of SP22 on ram sperm.

Materials and Methods

Semen was collected from a mature ram by electroejaculation. An aliquot of fresh semen was added to a conic tube and 1 mL of Sperm Isolation Buffer was put gently on top. After 10 minutes, the sperm that swum out into the overlay buffer was removed and fixed (4% paraformaldehyde in Sorenson phosphate buffer) for 5 minutes. After an initial wash in DPBS (1,000 rpm, 5min, 4ºC) the pellet was resuspended and sperm were incubated 1 hour at room temperature in affinity-purified anti-rSP22 Ig (1:200). After another wash the sperm were incubated with FITC conjugated rabbit anti-sheep Ig (1:50) for 1 hour at room temperature. After last centrifugation, a small aliquot of sperm pellet was put on a slide and coverslipped with Vector's fade retardant mounting medium. Images was analyzed and captured by fluorescent microscope (Olympus BX6 1).

Results and Discussion

Sperm protein SP22 was identified on ram spermatozoa. The immunolocalization was specific to the equatorial region of the gamete head. This localization is similar in several species like rabbits, bulls and humans (2), suggesting the involvement of the protein on spermatozoa-oocyte interaction during fertilization process.

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Protein standard of dairy gyr bull seminal plasma

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Introduction

Several studies show that seminal plasma proteins are essencial for the biological processes of fertilization (1). The discovery of more acurate methods to identify fertility associated atributes helps the selection of bulls with high fertility potential (2). The objective of this study was to evaluate the variability and the profile of the seminal plasma proteins of Dairy Gyr (D-Gyr) bulls.

Materials and Methods

Semen from 15 andrologicaly approved bulls was collected and seminal plasma was isolated by centrifugation. Total protein concentration was determined according to (3). Molecular exclusion (Superose 12) chromatography, using Fast Performance Liquid Chromatography (FPLC), was calibrated with proteins of known molecular weight. The samples were loaded in this system for the verification of different fractions or proteins peaks present in the material.

Results and Discussion

Seminal plasma total protein concentration ranged from 4.1 to 167.9 mg/ml, with an average of 47.1 ± 48.5 mg/ml, showing great variability among bulls. The chromatographic profile of seminal plasma proteins presented eight different peaks, ranging from 640 to less than 2KDa (Figure 1).

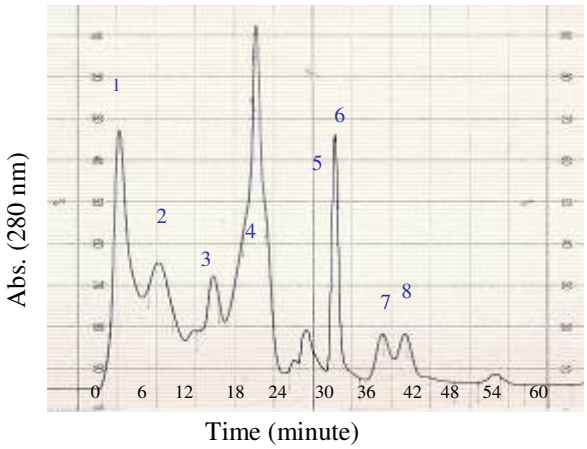

Figure 1. Chromatograms peaks of D-Gyr seminal plasma protein profile by Supherose 12 column.(▪ peaks)

These peaks were present in more than 75% of the bulls. However, despite the similarity of these profiles, it was possible to observe a high variability and large standard deviations values for the areas of the respective peaks among bulls. One can conclude that, despite the evidence of a pattern amongst seminal plasma protein fractions, there is a large variability in the total protein concentration and also in specific fractions among D-Gyr bulls.

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Identification of a Trypsin-like enzyme in canine seminal plasma

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Introduction

Seminal fluid is the liquid component of sperm, providing a safe surrounding for spermatozoa. Moreover, seminal plasma has the feature common to many other body fluid, that it is characterized by a high dynamic range of proteins.

Analysis of enzyme activities and concentrations of elements can estimate integrity and function of sperm cell membranes. However, not many studies have been conducted to characterize the canine seminal plasma.

The studies of proteolytic enzymes, at first detected by their fibrinolytic and fibrinogenolytic properties, have long been known to exist in seminal plasma (Mann and Lutwak-Mann 1981). Indeed, some early reports have described the presence in canine prostatic fluids of numerous proteolytic activities (Bhoola et al, 1962).

Furthermore, the study of the constituents of canine seminal plasma, specially the proteins, appears as a way of understanding the mechanism of fertility as well as of biomarker discovery (Moura , 2005; Pilch and Mann, 2006).

Material and Methods

Fresh semen was collected from 6 dogs and all samples were immediately cooled the $+5^0$C. The seminal plasma was brought to 36% saturation with solid ammonium sulfate; decantation of spermatozoa occured and the supernatant was dialyzed and the lyophilized. The proteins were analysed by SDS-PAGE electrophoresis and the protein concentration and the amidasic activity were evaluated. The sample was chromatographed in a Supherose 12 (FPLC system); The fractions were analysed by SDS-PAGE electrophoresis and the amidasic activities were determined.

Results and Discussion

The presence of a Serine proteinase in the canine seminal plasma was established by an enzymatic assay based on the it's amidasic activity (using BApNA as substrate). The molecular mass of the protein was assessed using a SDS-PAGE gradient (5% to 20% acrylamide). It was found to be in the low molecular weight range (around 25 KDa). These results showed the presence of a serine proteinase in canine seminal plasma.

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Identification of serine proteinase inhibitor from seminal plasma of stallion semen

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Introduction

Study of the seminal plasma proteinase inhibitors can help uncover various changes in protein functions and protein-protein interactions, which can modulate sperm function, such as hyperactivation and acrosome reaction (Strzezek et al. 2005).

Proteinase inhibitors are present in various glands, tissues, and secretions of the male reproductive tract. Some of these inhibitors bind to the acrosomal region of the sperm, and their release during *in vitro* or *in utero* incubation suggests that they may play a role in fertilization process (Lai, et al, 1991).

Trypsin inhibitor protein which inactives a membrane-bond trypsin-like proteinase of epididymal spermatozoa has been reported in boar (Jonáková et al, 1988), mouse (Lai, et al,1991) and guinea pig (Winnica et al, 2000).

The aim of this work was to isolate and identify equine seminal plasma proteinase inhibitors and evaluate its inhibition potential.

Material and Methods

Fresh semen was collected from 6 stallions with artificial vagina model "Hannover". All samples were immediately cooled to $+5^0$C and centrifuged (600g/30min at $+4^0$C) in order to separate seminal plasma from spermatozoa. The supernatant (seminal plasma) was brought to 36% saturation with solid ammonium sulfate and thus decantation of spermatozoa will occur and the supernatant was dialyzed and then lyophilized. The proteins were chromatographed in a Superose 12 (FPLC system) column followed by C_{18} HPLC reverse fase. Seminal plasma fractions were also evaluated for inhibition of amidasic activity. The samples were analysed by mass spectrometry MS/ES.

Results and Discussion

Sample fractions of seminal plasma obtained by cromatography were analysed by mass spectrometry MS/ES and a molecular mass protein of 6.3 to 7 KDa was observed. The molecular mass of equine serine proteinase inhibitor is similar to that observed in mouse (Lai, et al, 1991), boar (Jonáková et al, 1988) and guinea pig (Winnica et al, 2000) seminal plasma. As seminal plasma fractions were evaluated for amidasic activity with BApNA substrate in the presence of bovine Trypsin, an inhibition of 97% in the active fraction was observed.

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Correlation between dna integrity and high levels of proximal cytoplasmatic droplets in bovine spermatozoa

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The objectives of this research were to evaluate the correlation between the integrity of chromatin of bovine spermatozoa and the presence of high levels of proximal cytoplasmic droplets (PCD).

Materials and Methods

Five *Bos indicus* bulls were selected and maintained on native pasture with supplementation at artificial insemination centers. Animals were collected twice a week, completing 3 samples (n=15). The design criteria for the control group was: $\geq 20x10^6$ spermatozoa/mL, ≥ 40% of motility and percentage of total defects ≤ 15%. The pathology group consisted of 5 *Bos indicus* bulls (samples=15) with at least 15% of PCD. After thawing the samples at 38°C for 30 seconds the following tests were carried out: motility, vigor, concentration, sperm morphology and integrity of the chromatin with the Acridine Orange (AO) stain. The samples were incubated for 3 hours at 37°C for the test of slow term resistance (TTL), motility and vigor, the injured percentage of DNA and the complete percentage of intact acrosomes (PIA) were evaluated. For AO staining, the spermatozoa were separate from plasma by centrifugation with saline solution 0.9% at 37°C, two smears of each sample were dried and fixed "overnight" in Carnoy's solution. The slides were dived for 5 minutes in a solution containing 0.2% of the AO in a buffered solution of citrate and phosphate, washed in distilled water and covered with cover slides. A total of 200 cells were counted differently through a fluorescent microscope using filter with 490 excitements and 640 to 530 nm emissions. The cells were classified in accordance to the emitted fluorescence: sperm cells with intact DNA stained green and the ones with injured DNA red. For statistical analysis the data were submitted to analysis of variance, means were compared by tukey test and a correlation test (significance level of 5%).

Results and Discussion

The results demonstrated a significant difference between the control and pathology group after thawing (45.67±1.75 and 33.33±6.10, respectively) as after the TTL (35.33±2.015 and 16.25±3.85, respectively). A significant difference between vigor of the control group (4.47±0.03 and after TTL 3.8±0.20) and pathology (3.42±0.51 and after-TTL 1.96±0-44) also were shown. The PIA of the control group and pathology group varied significantly (55.27±1.68 and 31.5±4.98, respectively). The total percentage of major defects (control 4.7±0.70 and pathology 35.58±4.79), of minor defects (5.40±0.60 and pathology 13.04±1.91) and of total defects (control 10.10±0.92 and pathology 48.63±5.58) also differed significantly. The motility, the vigor, and PIA presented negative correlation with the percentage of CPD. The control and pathology group did not show significant difference considering the percentage of the injured DNA, however a positive correlation was verified at two moments (before 0.4106, P=0.0094 and after TTL 0.6055, P<0,0001). The results indicate that the presence of high levels of PCD contributes negatively the semen quality and the higher the percentage of this defect, the greater the percentage of spermatozoa with injured DNA will be.

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Rapid technique for simultaneous evaluation of the plasma, acrosomal and mitochondrial membranes of ram spermatozoa

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Introduction

The integrity and function of all sperm cell membranes are crucial to sustain the ability to fertilize the oocyte. Fluorescent probes can be used for assessment of plasma, acrosomal and mitochondrial membranes. Some fluorescent probes, specific for each cellular membrane, have been employed, such as: propidium iodide (PI), a specific stain that binds to DNA and does not cross the intact plasma membrane; fluorescein isothiocyanate-conjugated *Pisum sativum* agglutinin (FITC-PSA) that marks damage in acrosomal membranes; and 5,5',6,6'-tetrachloro-1,1,3,3'-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) with specificity for detection of mitochondrial function, which dyes mitochondria with high membrane potential in red, and low membrane potential in green. This experiment was designed to validate a technique for simultaneous evaluation of the plasma, acrosomal and mitochondrial membranes in ovine spermatozoa, using the fluorescent probes: PI, FITC-PSA and JC-1.

Materials and Methods

For experimental purposes, four ejaculates were collected weekly from each of three rams (n=12). Only ejaculates showing motility > 80% and abnormal morphology < 10% were diluted in TALP medium ($25x10^6$ spermatozoa/mL) and split into 2 aliquots, 1 aliquot was flash frozen in liquid nitrogen and thawed in three continuous cycles, to induce damage in cellular membranes and to perturb mitochondrial function. Three treatments were prepared with the following fixed ratios of fresh semen: flash frozen semen: 100:0 (T100), 50:50 (T50), and 0:100 (T0). The three samples (T100, T50 and T0) were submitted to a stain technique modified from Celeghini et al. (2). A 150-µL aliquot from diluted semen was mixed with 3 µL of PI (3 mM, Sigma, 28,707-5), 3 µL of JC-1 (153 µM, Molecular Probes, M-7514) and 50 µL of FITC-PSA (100 µg/mL, Sigma, L-0770); incubated at 38.5°C/8 min, in the dark. A 5-µL sample was put on a slide, coverslipped and evaluated immediately by epifluorescence microscopy. Two hundred sperm cells were assessed per slide. The effects of treatments (T100, T50 and T0) were tested by analysis of variance, and their means across all samples were compared by Fisher's LSD test. After detection of a significant difference ($P<0.0001$) between treatments, the data obtained for plasma membrane integrity, intact acrosome, and mitochondrial function (dependent variables) for each treatment (independent variable) were submitted to a linear regression using StatView software (SAS Institute, 2.ed., Cary, NC, USA, 1998).

Results and Discussion

The association of the fluorescent probes resulted in eight-cell classes (1), according to plasma membrane integrity, intact acrosome and mitochondrial function. To plasma membrane integrity, detected by PI probe was obtained the equation: $Y=1.09+0.86X$ ($R2=0.98$, $P<0.0001$). The intact acrosome, verified by FITC-PSA probe produced the equation: $Y=2.76+0.92X$ ($R2=0.98$, $P<0.0001$). Ended the mitochondrial function, marked by JC-1, was estimated by equation: $Y=1.90+0.90X$ ($R2=0.98$, $P<0.0001$).

We can conclude that this is an efficient and practical technique for simultaneous evaluation of the plasma, acrosomal and mitochondrial membranes in ovine spermatozoa.

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Validation of a radioimmunoassay for growth hormone quantification in bull seminal plasma

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Introduction

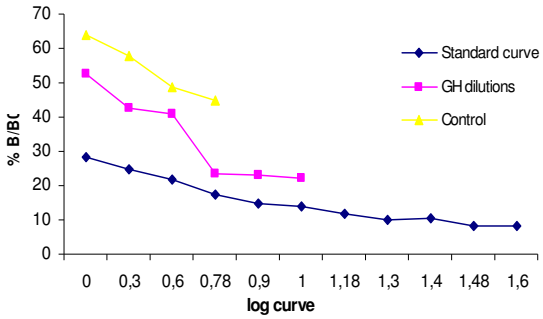
The pulsatile nature of growth hormone (GH) secretion makes difficult an accurate measurement of circulating levels unless from sequential blood sampling. Davis et al. (1984) observed that bovine seminal plasma contains higher levels of GH compared to blood plasma and suggested that it was provided by an active transport from the blood, since the simply diffusion would result in the same ratio between the concentration in both fluids. The objective of this study was to validate a Radioimmunoassay (RIA) to measure GH levels in seminal plasma of purebred Simmental bulls.

Materials and Methods

A parallelism test was performed between standard curve, a control dilution (pool of samples) and a curve made by addition of known amounts of GH in plasma. This test was made by a double antibody method. Five µg of GH was radioiodinated by the Tetracloro method (Iodogen®) as previously described. The labeled GH was purified by applying the reaction mixture to a Sephadex G-25H column (Pharmacia®) equilibrated with the assay buffer for GH RIA (0,01M phosphate buffer containing 1% of bovine serum albumin). Standard curve and plasma samples (100µl) were incubated with 200µl assay buffer and 100 µl of specific antibody (from NDDK, NHPP-National Hormone an Pituitary Program, final dilution: 1:130.000) for 48 hours at 4°C. The nonspecific binding tubes contained only assay buffer and normal monkey serum (1:300, 100µl). After this first incubation, 100 µl of ^{125}I-GH (approximately 25.000 cpm/100 µl) was added and the incubation continued for more 24 hours at 4°C. After the second incubation, 100 µl of precipitant antibody (goat anti monkey gamaglobulin, 1:30) and 500 µl of polyethylene glycol 6% was added to all tubes, except to TC ones and incubate for 3 hours at room temperature. Centrifugation was performed at 30.000 rpm for 30 minutes and 4°C. Supernatant was decanted and the pellet was counted for 60 seconds.

Results and Discussion

The maximum binding of the assay was 23.43%, nonspecific binding 2.77%. The sensitivity of the assay was 0.5 ng/ml of GH and the precision was 1,93%. The serial addition of known amounts of GH and the dilution of control were parallel to the standard curve.



We concluded that seminal plasma GH concentrations were precisely measured by this RIA.

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Characterization of rooster seminal plasma proteins by SDS-PAGE

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Introduction

Seminal plasma contains many types of components, among these, proteins, that are major organic constituent of this fluid with relevant physiologic functions on semen. The composition, conformation and molecular weight of these proteins are specific for different species. Some seminal plasma proteins have influence on sperm motility, viability and fertilization. One-dimensional poliacrilamide gel electrophoresis (SDS-PAGE) is a valuable tool for the separation and characterization of proteins from complex biological samples. Many proteins from seminal plasma of various species have been described and characterized by SDS-PAGE or by two-dimensional poliacrilamide gel electrophoresis (2D-PAGE). However, the protein profile of rooster seminal plasma has not yet been reported. The objective of this study was to determine the protein profile of rooster seminal plasma by SDS-PAGE.

Materials and Methods

Semen samples from 15 mature healthy roosters were collected by abdominal massage and centrifuged at 4000 x g during 10 min at 4°C. Supernatant (seminal plasma) was used in the analysis. Seminal plasma was re-centrifuged at 14000 x g during 10 min at 4°C. SDS-PAGE was carried out in a TRIS buffer system. Electrophoresis was performed in a BIO-RAD Mini-Protean 3 Cell® system using 15% bis-acrylamide gels. Samples were concentrated at 60 V for 20 min, and the separation was performed at 120 V for 70-80 min. The BenchMark Protein Ladder™ (Invitrogen®, Carlsbad, USA) was used as the standard of molecular weight. Gels were stained with Coomassie Brilliant Blue and scanned and analyzed using the TotalLab TL 100 analysis software (Nonlinear Dynamics, UK).

Results and Discussion

The protein profile of seminal plasma detected the presence of 18 bands ranging from 214.6 to 7.7 kDa. The bands with 52.9 ± 0.65 and 25.9 ± 0.5 kDa were present in all samples. Proteins with 115.5 ± 0.2 and 62.4 ± 0.3 kDa were detected in 12.5% of the analyzed samples. Six protein bands with 214.6 ± 12.1, 101.3 ± 3.7, 57.3 ± 0.8, 45.0 ± 0.82, 35.9 ± 0.5 and 11.3 ±0.1 kDa were detected in 87.5% of the samples. The band with 57.3 ± 0.8 kDa presented concentration (35% of total sample protein content) higher than that observed for other bands. No male presented all 18 protein bands. The characterization of seminal plasma proteins is critical to identify properties and functions involved in semen physiology. In one study (1), a rooster seminal plasma protein that inhibits sperm motility and has antibacterial property was identified and isolated. The knowledge about the seminal plasma protein profile obtained in the present study may help to elucidate the function and properties of these proteins.

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Seminal characteristics of curimba *Prochilodus lineatus* (Valenciennes, 1836) induced with carp pituitary extract and equine chorionic gonatotropin

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Introduction

The curimba (*Prochilodus lineatus*) presents high fertility, fast growth, high rusticity and serve as food source for predatory fishes. Carp pituitary extract (CPE) is the more used method for the induction of reproduction of curimba and several other fish species (1). The mammals gonadotropin, as the equine chorionic gonadotropin (eCG), being easily gotten in the commerce, and of low cost, are presented as alternative substances for induction of fish reproduction. The objective of this work was to evaluate the effect of the CPE and eCG (Novormon 5000®), combined or not, on the seminal characteristics of curimba.

Materials and Methods

The experiment was conduced at the Environmental Station of Itutinga of the Energetic Company of Minas Gerais (EAI-CEMIG). Thirty-three males of *Prochilodus lineatus* were submitted to four different hormonal treatments with two doses in 12- hour interval of (I: 0,5 and 5,0 mg/kg CPE; II: 0,5 mg/kg CPE and 5,0 mg/kg CPE + 500 IU/kg eCG; III: 0,5 mg/kg CPE and 500 IU/kg eCG; IV: 500 and 500 IU/kg eCG). The semen was collected in graduated tube, through the compression of the abdominal region of the animal. Semen volume (ml), sperm motility (%) and motility duration (seconds), spermatozoa concentration (sptz/ml) and sperm morphology (%) were analyzed. For the variables semen volume, sperm motility, motility duration and spermatozoa concentration, analyses of variances was applied, being the averages contrasted for the Tukey test ($P<0.05$). For the sperm morphology was adopted chi-square test.

Results and Discussion

No significant statistical differences ($P>0.05$) between hormonal treatments related to semen volume, sperm motility, motility duration and spermatozoa concentration were found. Average volume of the semen produced for the males in this work was 2.34 ml. Average values of spermatozoa concentration, sperm motility and motility duration for all treatments, had been higher than those found by other authors (2). For spermatozoa morphology, we found significant statistic differences ($P<0.05$) among reatments. Animals that received treatment IV presented 97.2% of normal spermatozoa. The results indicate that eCG can substitute the CPE without modifying the seminal characteristics of *Prochilodus lineatus*.

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Scrotal circumference and semen characteristics of nelore bulls at 12 and 18 months of age

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Introduction

Scrotal circumference (SC) related to parameters to measure fertility, such as morphologic and physical characteristics of the semen has become a basic point in the process of selection of sires and its offspring (1). The objective of this study was to search for standard patterns for testicular biometry along with seminal parameters in young bulls considered of high pedigree, from 12 to 18 months of age, as an aid for selection criteria.

Materials and Methods

Twenty four Nelore young bulls (*Bos taurus indicus*) from 12 to 18 months of age were used to form groups based in the scrotal circumference, resulting in 3 groups of 8 animals each: Group 1 (SC>22,8cm); Group 2 (SC between 21,6 and 22,6cm) and Group 3 (SC between 20,1 and 21,2cm). Measures of the SC and physical characteristics of the semen were evaluated.

Table 1 – Scrotal circumference (SC), motility (Mot) and seminal concentration (Conc) of Nelore young bulls of 12 and 18 months of age.

Groups	**SC and seminal values**					
	12 months			**18 months**		
	SC (cm)	Mot (%)	Conc (x10^6)	SC (cm)	Mot (%)	Conc (x10^6)
1 SC > 22.8cm	30.06 ± 1.87	33.75 ± 20.65	18.46 ± 8.61	34.18 ± 1.55	57.5 ± 24.34	98.22 ± 48.07
2 SC between 21.6 and 22.6cm	25.37 ± 3.28	5.62 ± 11.16	4.48 ± 4.89	31.5 ± 2.77	48.75 ± 27.99	75.18 ± 52.29
3 SC between 20.1 and 21.2cm	24.75 ± 2.25	1.87 ± 5.30	1.38 ± 3.92	32.81 ± 2.67	58.75 ± 22.95	65.72 ± 38.62

Results and Discussion

Nelore young bulls are classified as precocious when they present spermatozoa (sptz) in its seminal plasma at 12 months of age, with low motility (5 to 10%), concentration beteween 25 to 50x10^6 sptz/ml and SC between 24 and 26 cm; super-precocious are those that, in this same age, present progressive sptz motility between 20 and 30% and concentration of 50x10^6 sptz/ml, besides SC equal or superior the 26 cm (2). The animals of Group 1 had been classified as super-precocious at 12 months of age, showing SC greater than 30 cm, mobile sptz in the first ejaculate and motility of 33.75%, however the sperm concentration was low with 25x10^6 sptz/ml. The animals of groups 2 and 3 at 12 months of age had the SC greater than 24 cm, however some of them had low sperm motility and concentration or absence of sptz in the seminal plasma. At 18 months of age the animals of the 3 groups showed complete sexual maturity indicated by the SC above 31 cm, motility greater than 50% and concentration above 50x10^6 sptz/ml (Table 1).

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The effect of season on seminal characteristics in dogs

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Introduction

Photoperiod and environmental temperature are two important factors that may influence the reproductive cycle of different species. Usually at lower latitudes there is little seasonal variation in environmental temperature and marked variation in rainfall [1]. The objective of this study was to investigate any seasonal influence in seminal characteristics in different breeds of dogs living in identical conditions in a tropical zone, at 23º 35' S latitude and 48º 59' W longitude. In this region, the variation in day length between winter and summer solstice is approximately two hours and thirty minutes.

Materials and Methods

Eight adult dogs of 3 breeds (2 Blood Hound, 2 Golden Retriever, 4 Springer Spaniel) aged between 1.2 and 6 years were used in the study. The dogs were active breeding animals that were housed in the same kennel environment throughout the 14-month period of the study (July 2002 to August 2003). Commercial dog food and water were offered *ad libitum*. Semen samples were collected by digital manipulation each 15 days. Immediately after the collection, spermatic progressive motility, progressive velocity, pH and abnormalities of the acrosomes, midpieces, and sperm tails, and head defetcs were evaluated. The year was arbitrarily divided into 4 seasons giving 5 research periods: winter 2002 (I - July to September 2002), spring 2002 (II - October to December 2002), summer 2003 (III - January to March 2003), autumn 2003 (IV - April to June 2003) and winter 2003 (V - July and August 2003). Environmental temperature and pluvial index data were obtained from the Information Center for Agronomy and Meteorology (www.iac.org.sp).

Results and Discussion

Throughout the research period, temperature ranged from 10.2ºC to 32.8ºC and the pluvial index from 33 mm to 476 mm. The highest temperatures and greatest rainfall were observed during the summer period. Median values of semen characteristics were described in Table 1.

Table 1: Seminal characteristics in 8 stud dogs, during 5 season. Median of pH, sperm motility (%), sperm velocity (score 0 to 5), total concentration ($x10^6$), normal sperm (%), major (%) and minor defects (%).

Characteristic	I	II	III	IV	V
pH	6.4	6.2	6.3	6.1	6.2
Motility	84.0	84.2	89.6	88.0	88.8
Velocity	4.2	4.1	4.4	4.5	4.6
Concentration	346.5	369.5	403.9	461.2	466.0
Normal	61.5	61.9	65.5	73.8	73.5
Major Def	24.9	27.7	22.4	17.2	16.4
Minor Def	12.0	10.5	12.7	9.0	10.3

Semen characteristics were normal during the study. Significative increase was observed in progressive spermatic motility during Summer 2003 versus Spring 2002 (84,2% versus 89,6%, p=0,004). High temperature and high pluvial index result in significantly increased humidity, which may be the major stressor. In conclusion, in tropical zones, the seasonality has only a minimal influence on canine testicular function.

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Climatic seasonality influence on physical and morphological aspects of goat semen.

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Introduction

The reproductive performance of a goat herd depends directly on the genetic potential of the animals, on the environment and on the conditions of management. The interrelation of these factors produces a morphophysiological adaptation of the animals, i.e., it contributes to an increase in their reproductive efficiency. This research work had as its objective to evaluate the influence of the climatic seasonality on the physical and morphologic aspects of goat semen.

Materials and Methods

Eleven crossbred goat males were studied, with age varying between 10 months and three years, raised in different regions of the State of the Piauí. The semen collects were taken weekly in the dry period (October-December/05), and in the rainy period (February-Abril/06). The following aspects of the semen were analyzed: volume, total and individual motility, vigor, concentration and spermatic morphology

Results and Discussion

For the parameters total motilities, vigor and individual motilities evidence was found that higher quality was demonstrated in the rainy period in comparison to the dry period, but that this difference was not observed for the morphology of the spermatozoa. No difference was observed between the dry and rainy periods for any of the types of defects under study, indicating that the season of the year did not influence the emergence of spermatic defects in any of the studied animals;

Conclusion

The rainy period presented a short improvement in the quantitative aspects of the semen. However, it did not have influence in respect to the qualitative of aspects.

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Seasonal effects on semen quality of murrah buffalo bulls

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Introduction

In this work we analyzed the seasonal effects on semen quality of Murrah Buffalo bulls. Some physical and biochemical parameters were measured in ejaculates during the different season of the year.

Material and Methods

Four Murrah Buffalo bulls with initial age varying from 13 to 18 months were used in this study during one year (from September to August). The semen was collected once a week and the volume of the whole ejaculation was measured. The concentration of spermatozoa per cubic mm^3 of semen was determined by a direct cell count on the Neubauer chamber. An aliquot of whole semen was taken for the determination of fructose concentration (1). The remaining semen was centrifuged at 2600 rpm for 15 minute and an aliquot of seminal plasma was taken for estimation of the content of protein (1) and alkaline and acid phosphatases (1). The spermatozoa were washed three times with saline, lysed and ressuspended for the assay of glutamic-oxaloacetic and glutamic pyruvic transaminase (1).

Results and Discussion

The data on means and standard deviation of the attributes of semen in different seasons of the year are given in Table 1.

The data in Table 1 revealed that significant variation was observed in the ejaculate volume between autumn and summer. The sperm concentration was significantly affected by seasons and the lowest concentration was found in summer. The content of proteins showed no significant variation between seasons. No significant variation was recorded in those pH values between seasons. The activity of alkaline phosphatase varied significantly between seasons being greater during autumn, lower in summer and intermediate in winter and spring and the acid phosphatase level was not significantly different between seasons. In relation to the transaminases, the acitivity of the glutamic- pyruvic transaminase (GPT) was not significantly during the different seasons. The activity of glutamic- oxaloacetic transaminase (GOT) showed an apparent variation in the enzymatic activity during the different seasons but the differences could not reach a statistical significance. The data showed that the winter season does not present unfavorable conditions to the production of semen from buffaloes and suggest that the best quality of semen is during the autumn.

Table 1 –Means and standard deviation from physical and biochemical parameters of Murrah Buffalo semen during spring, summer, autumn and winter.

Parameters	Seasons			
	Spring	Summer	Autumn	Winter
Volume (ml)	3.06±0.69ab	2.81±0.87b	3.58±1,05a	3.25±1,21ab
pH	6.82±0.20a	6..83±0.20a	6.92±0.24a	6..93±0.21a
Sperm concentration (cubic mm^3)	1.26±0.15a	1.12±0.09b	1.24±0.09a	1.33±0.15a
Fructose (mg percent)	625.57±115.1b	655.79±107.14b	683.70±88.03ab	729.70±76.69a
Protein (g percent)	3.2±0.7a	2.88±0.79a	3.09±0,78a	3.13±0.73a
Alkaline phosphatases (mIu)	694.97±111.4ab	616.00±141.68b	709.56±140.26a	688.46±108.36ab
Acid phosphatase (mIu)	609.41±127.5a	627.41±128.76a	662.38±156.83a	620.78±104.9a
GOT (UF) **	127.9±28.46a	125.96±21.36a	119.9±19.02a	119.88±17.55a
GPT (UF)**	34.24±13.64a	34.59±9,5a	35.9±8.96a	35.44±8.38a

* Means on the same line followed by a dissimilar letter differ significantly (P<0.05); ** GOT- Glutamic- oxaloacetic transaminase; GPT- Glutamic- pyruvic transaminase.

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Winter-springer and summer influence upon seminal plasma proteins in bulls

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Introduction

Seminal plasma, the fluid in which mammalian spermatozoa are suspended in semen, is made up by the secretions of the male accessory reproductive organs and appears to exert important effects on sperm function (6). The protein quality of the seminal plasma may affect positively the bulls fertility (4). Peptides of 55 and 66 KDa were present in bulls with excellent spermatic conditions. On the other hand, 16 and 36 KDa peptides were observed with unfavourable spermatic conditions (2). The present work has the objective of evaluating the season year influence upon seminal plasma proteins in bulls.

Materials and Methods

Twelve, 2-year-old Limousin, bulls were semen collected throughout electroejaculation, during the months of August and September (winter – springer), from each bull, at 7 days intervals, in a total of 55 semen samples. In January (summer), nine of these bulls were re-evaluated through the andrology tests according to CBRA norms (3). Samples of seminal plasma were centrifuged (1500g/15min) and conditioned in assay test tubes and stored at –20ºC until further processing. 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. Protein was quantified according to (1) 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 with 2% of Comassie Blue R250. If background was too dark gel it was distained in 7% acetic acid.

Results and Discussion

In Bulls A, C, D, E and I the absence of high molecular weight (HMW 55 KDa, 66 KDa and 80 KDa) proteins was verified in the winter - springer and the one which it was supposed to be of low fertility (LF – 40 KDa, bull D), in the summer. The bulls F, G and J showed presence of HMW (55 KDa) in the winter – springer. In bulls H, I and J HMW (55 KDa, 66 KDa or 80 KDa) proteins were present with a satisfactory semen condition in accordance with (4). The bull I showed presence of HMW proteins (66 KDa and 80 KDa) in summer. It is suggested that different seasons of the year may influence the presence or absence of proteins in seminal plasma. There was a direct relationship between the presence of the proteins of 20KDa, 55 KDa, 66 KDa and 80 KDa and the quality of the spermatic condition.

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Integrity of sperm dna of native srd bucks agreed to scrotum conformation

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Introduction

Native bucks of tropical areas frequently present scrotum bipartition, a characteristic which is associated to best reproductive capacity (Nunes, 1983). Motility, vigor, concentration and sperm morphology are parameters usually used to evaluate male reproductive potential. In humans, Acridine Orange technique (AO) is used for spermatozoid evaluation (Tejada et al, 1984). However, in Boer and Alpine buck, this technique was not a good indicative of fertility prediction (Cavalcante, 2003). This work aimed to evaluate if the integrity of the sperm chromatin (DNA) of native SRD bucks was associated to scrotum conformation.

Materials and Methods

We studied 154 ejaculates from SRD bucks which were grouped according to scrotum conformation: GI (n=4), males with normal scrotum; G2 (n=4), <50% of scrotum bipartition and G3 (n=3), >50% of scrotum bipartition. Samples of blood were obtained by occasion of semen collections. After having been submitted to physical and morphologic classic evaluation, the semen was diluted in tris-citrate-yolk extender (1:9) and frozen in 0,5ml straws. After thawing (37°C/30s), 250µl of the semen were added to 1000µl of PBS, and the mixture submitted to two centrifugal washes, the precipitate suspended in 700µl of PBS, from which 10µl were taken for preparing semen smears. The smears were fixed in Carnoy solution, air dried and then dipped in 3ml of the staining solution during 5 minutes in (Tejada et al, 1984). Then they were washed in Mili-Q water, covered with cover glass and the excess of water removed with filter paper. The slides were examined under immune-fluorescence microscopy, using excitement filter (490nm) and barrier filter (530 nm). One hundred cells/slides were evaluated. Entire spermatozoids were stained green and the damaged stained orange. The experimental design was entirely casual and the results submitted to the ANOVA and test t (p=5%).

Results and Discussion

Among the evaluated classic sperm characteristics, mass movement, vigor and concentration differed among the groups ($p < 0.05$), with G3 showing higher values than the other groups. The percentile of damaged cells (0.55±0.26) was considered low, indicating that the AO technique was not efficient in detecting the lesions. Cavalcante (2003) also verified in Boer and Alpine bucks, in reproductive station and in no reproductive station, very low indices of damaged cells. Plasma testosterone level (overall average=10.84±1,60ng/ml) was different among the groups ($p < 0.05$), but it did not present positive correlation with libido. Under the study conditions, it is concluded that there was no influence of the scrotum conformation on the integrity of the spermatozoid DNA.

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Effects of dietary supplementation with gamma-oryzanol on seminal characteristics of stallions

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Introduction

The production of reactive oxygen species (ROS) is a normal physiological event in various cell types. There is ROS production during the steroid hormones synthesis and by spermatozoa, therefore antioxidants are necessary to protect them to the deleterious effects of lipid peroxidation. Dietary supplementation with numerous antioxidants has proven to have beneficial effects on male reproductive characteristics. Gamma-oryzanol (GO), extracted from rice bran oil, has a wide spectrum of biological activities, including antioxidant properties in *in vitro* systems (1). The aim of this study was to evaluate the effects of dietary supplementation with GO on seminal characteristics of stallions.

Materials and Methods

Six light-horse stallions were split in two groups and supplemented for 60 days. The control group, received 150 mL of soy oil, and the treatment, received 150 mL of rice oil containing 1,1% of GO (Gamahorse® [3]). Eight semen collections from each stallion were performed, two before the supplementation, and six after, with 15 days interval. Semen was collected using artificial vagina. Volume was measured by a graduated cylinder. Progressive motility was estimated visually under light microscopy 100 x magnification at 37°C on a heated stage. Concentration was calculated using a hemocytometer. Morphology was evaluated on a wet mount, with spermatozoa fixed in buffered formol saline, using differential interference contrast (DIC) microscopy 1000 x magnification. Sperm membrane integrity was evaluated using the hypoosmotic swelling test (HOS). Statistical analysis was performed using SAS program (SAS Institute, Cary, NC). Experimental design were random blocks and data were analyzed by repeated measures ANOVA. P values < 0.05 were considered statistically significant.

Results and Discussion

Results are shown in the table below. Data are presented as mean ± SEM. There were no differences (P>0.05) in the analyzed variables. There are several studies about antioxidant dietary supplementation, but just a few were found about GO. We expected that GO would decrease ROS production, optimizing the action of steroids hormones and spermatic membrane integrity, improving the seminal quality. Young (2) reported experiments with antioxidants that improve steroid hormone synthesis. Previous studies with other antioxidants supplementation had beneficial effects on seminal characteristics. Juliano (1) reported that antioxidant properties of GO are not exceptional but they could be enhanced by the association with other natural antioxidants. Probably this association or a prolonged supplementation would give better results. It is interesting to do future studies with GO, once it possesses many biological activities, which make it an useful multifunctional ingredient for pharmaceutical formulations and foods.

Variable	Control	Treatment
Volume (mL)	74.7 ± 7.2	67.2 ± 4.6
Motility (%)	70.8 ± 1.8	68.3 ± 2.7
Concentration ($x10^6$)	125.6±12.5	147.6±16.1
Minor defects (%)	5.7 ± 0.5	4.6 ± 0.4
Major defects (%)	15.8 ± 1.5	18.6 ± 3.2
HOS (%)	87.9 ± 0.7	83.6 ± 1.8

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Effect of Selenium + Vitamin E suplementation on testicular parameters of goats submitted to scrotal insulation

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Introduction

Variations on the testicular temperature by inflammatory process, environmental or artificially induced factors can interfere on the sperm production. The efficiency of sperm production is related to the number of the cells produced per day and per gram of the testicular parenchyma (1) and it has a correlation with weight and scrotal diameter (2). That is why the measurement of the testis is important to andrologic evaluation of the males. The objective of this study was to evaluate the effect of the Se + Vit E supplementation on the biometric testicular parameters on goats of no defined breed (NDB) submitted to scrotal insulation.

Materials and Methods

Twelve adult goats (NDB) were submitted to scrotal insulation (SI) for 18 days. During this period, 6 animals were supplemented with Se + vit E (Treated group) and 6 animals did not receive supplementation (Control group). On the 18th day, corresponding of the end of SI period, the scrotal circumference (SC) was evaluated. After this, 6 animals were randomly chosen: 3 from the Treated group and 3 from the Control group, which were submitted to bilateral orchiectomy. Body and testes (right and left) and epididymis (right and left) were weighted. The 6 animals of the Control group were also castrated. They received the same food management from the beginning of the experiment until 36 days after the end the insulation (moment of the castration) aiming to analyze the testicular and epididymal parameters. The data were analyzed by MINITAB RELEASE 14 computational program, applying ANOVA, at 5% of significant level.

Results and Discusion

It was not observed significant difference between body weight of the Treated and Control group animals during all the period analyzed. On the beginning of the experiment, no difference between SC of the Treated (23.00 cm) and Control group (22.80 cm) were observed. However, after 18 days of SI the SC of Treated animals (23.00 cm) had significant difference ($P<0.05$) when compared with those of the Control group (20.00 cm), which recovered 36 days after the end of the SI (22.33 cm). In relation to the right testis weight, it was observed significant difference ($P<0.05$) between the Treated (60.51g) and Control group (42.92g), which recovered the weight on the 36th day after the end of the SI, weighting 59.65g (Treated) and 54.52g (Control) with no difference between groups. The weight of the left testis had also significant difference ($P<0.05$) at the end of the SI period (Treated = 65.47g; Control = 46.32g), but had no difference ($P>0.05$) on the 36th day after the end of the SI (Treated = 55.27g; Control = 55.64 g). The weight of the left epididymis on the Treated (10.10g; 7.83g) and Control group (8.98 g; 8.49g) were the same ($P>0.05$) on the end of the SI and 36 days after, respectively. Similarly, it was not observed difference between left epididymis of the Treated (9.66 g; 7.84g) and Control group (7.93g; 8.44g) animals on the end of the SI and 36 days after, respectively. These data confirm the results of other authors who showed correlation between testis weight and scrotal circumference (2). In conclusion, the supplementation with Se + Vit E prevents alterations on the biometric testicular parameters on the NDB male goats submitted to SI.

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Effect of the Selenium + Vitamin E suplementation on the seminal parameters of goats induced to scrotal insulation

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Introduction

Oxygen is essential to the life of aerobic organisms, but excessive production of reactive oxygen species (ROS) determine oxidative stress and cellular damage. Oxidative stress on the testis has been observed on patients with cryptorchidism (1) or varicocele (2), because of the increase of testis temperature that blocks the antioxidant defense systems, reducing the concentration and the percentage of mobile sperms of human semen (3), as a result of the lipidic peroxidation of the cellular membranes. The aim of this study was to analyze the effect of the administration of Selenium and vitamin E on the seminal characteristics of the goats with no definite breed (NDB) submitted to scrotal insulation.

Materials and Methods

Twelve adult goats (NDB) were submitted to scrotal insulation (SI) for 18 days. During this period, 6 animals were supplemented with Se + vit E (Treated group) and 6 animals did not receive supplementation (Control group). Semen samples were obtained using electroejaculation method with intervals of 6 days and the samples were analyzed to assess volume, motility, vigor, concentration, acrosome and DNA integrity. On the 18^{th} day, corresponding of the end of SI period, all animals had the scrotal insulation pockets retreated and 3 animals of each group were randomly selected and submitted to orchiectomy. After this, the non-orchiectomized animals of the Treated (n=3) and Control (n=3) groups were submitted to the same management used before to each group and to semen collection (n=7) aiming to analyze the effect of the supplementation on the recovery of the seminal parameters of the males. The data were analyzed by MINITAB RELEASE 14 computational program, applying ANOVA and 5% of significant level.

Results and Discussion

After the end of SI period (18^{th} day), the semen samples of the 12 goats showed the following values of the analysed parameters: volume (0.42 and 0.41 mL), motility (12.83 and 8.83 %), vigor (1.83 and 1.50), concentration (0,17 and 0,20 x 10^9), acrosome (36.17 and 76.09 %) and DNA integrity (37.92 and 54.5%) on the treated and control animals, respectively, with no difference between groups. However, 36 days after the end of SI, even though it was not registered difference ($P>0.05$), the concentration and DNA integrity average showed higher values on the supplemented animals with Se + Vit E, pointing a quicker recuperation of these parameters. After the 60^{th} day (42^{th} day of the end of SI period) these values were the same as those observed on the day 0, on both groups, making evident that there was recovery from testicular damage caused by high scrotal temperature. These results show that the high testicular temperature caused by SI altered seminal parameters, confirming other authors (1, 2, 3). The supplementation with Se + Vit E did not minimize the negative effect of the high scrotal temperature on the seminal characteristics of the male goats.

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Precocious and super-precocious dairy gyr young bulls (*Bos taurus indicus*) raised under pasture conditions

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Introduction

The dairy Gyr (D-Gyr) breed have been responding for approximately 43% (537,000) from the total doses of semen for AI commercialized in Brazil, considering the national zebu's dairy breed production (1). This calls for a rigid selection for sexual precocity and seminal quality. The aim of this study was to screen super-precocious (SPre), precocious (Pre) and to cull the immature bulls (Immat.), at this stage, in a D-Gyr herd in Uberaba, MG.

Material and Methods

After more than 30 years, under selection pressure for milk production, at the same farm, from a group of 90 male calves born in 2004, a sample of 20 clinically normal D-Gyr bulls, were andrological (2) and zootechically (1) evaluated, based in three semen collections at an average of 21.0 (ranging from 19.6 to 22.5 months) and another evaluation at 23.8 months of age. These animals were raised under pasture conditions (Brachiaria brizantha + mineral mixture) from weaning to 21 months. (period of 14 months). Thereafter, until 24 months, they were kept in a feeding-lot, individualized, under full diet management. Bulls sexually mature in the first and second sampling were classified as SPrec. and Prec., respectively. Data was analyzed by the SAS (1998) software.

Results and Discussion

Analyzed parameters in this study are shown in Tab.1.

Table 1 –Parameters of D-Gyr bulls at two-years-old, classified by sexual maturity status

AGE	STATUS	N(%)	BW(Kg)	SC(cm)	MOT(%)	VIG.(1-5)	MM(1-5)	MSD(%)	mSD(%)
21.0	S-PREC.	4(20)	299.73[b]	30.0[a]	33,5[a]	3,5[a]	1.2[b]	13.64[b]	9.09[a]
	IMMAT.	16(80)	287.31[b]	28.3[a]	36.7[a]	3.6[a]	0.6[b]	63.15[a]	13.52[a]
23.8	PREC.	14(70)	354.11[a]	30.7[a]	53.3[a]	4.8[a]	2.8[a]	13.44[b]	9.67[a]
	IMMAT.	6(30)	360.67[a]	30.8[a]	48.3[a]	4.5[a]	1.7[b]	45.50[a]	17.17[a]

BW = body weight; SC = scrotal circumference; Mot = motility; Vig = vigor; MM = mass motility; MD = major defects; mD = minor defects. *P< 0.05

Four (20%) young bulls, with average BW of 299.73 kg, only receiving good quality pasture and mineralization reached sexual maturity at 21 months of age, being classified as top, similar to (3,4,5); however in the first case (Gyr bulls) they received full diet since the weaning time; in the second and third cases, (Nelore and Tabapuã bulls, respectively), they received only pasture, similarly to the present study. However, after the supplementation from 21 to 24 months of age, 70.0% of the other bulls also reached sexual maturity at 24 months and BW of 354.11 kg. It can be concluded that MSD was the same to the Prec and SPrec bulls, as well as the SC.

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Andrologic aspects of Tabapuã (*Bos taurus indicus)* bulls, under two year old, raised on adequated pasture conditions

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Introduction

Brazil detains the world's biggest commercial herd, eventhough with low productive and reproductive levels due to genetic and nutritional conditions of the animals. Focusing on the economical return of the activity, zebu breeds specially Tabapuã breed, even though not well studied, presents great interest due to its rusticity, fertility levels, carcass characteristics and high complementarities in crossbreeding programs, even when raised under pasture conditions.

Material and Methods

From a group of 120 young Tabapuã bulls sired by 8 different bulls, a total of 24 (20%), raised under pasture conditions, were continuously selected from weaning to an average age of 21.4 ± 0.14 months by body weight (BW)(ranging from 353.0 to 471.0 kg) and zootechnical characteristics. Thereafter they were andrologicaly evaluated according to (1), and classified by sexual maturity condition, according to Breeding Soundness Evaluation for Zebu bulls (BSE-Z) (2).

Result and Discussion

Even though no difference in BW was recorded (P>0.05) it was registered significant differences (P<0.05) for scrotal circumference (SC) and semen characteristics (motility – MOT; major sperm defects – DSM and total sperm defects – DST) because of the genetic selection pressure for production and reproduction.The animals had the ability to express these characteristics even young and raised on pasture conditions The BSE-Z values were considered high, with mean of 78.64 points, similar to (2, 3), although the animals from the immature group could not be classified by the BSE-Z system, due to the high percentage of MSD (59.85%)(Table 1), differently from the animals of the mature group, with 12.82% of MSD. Four out of the 8 sires, two sired a larger number of sons that reached early sexual maturity, and 2 sired sons with late sexual maturity. Based on the results it is recommended to discard from reproduction sons of bulls that reached sexual maturity in an age over 24 months, showing the importance of selection for BW and SC at an early age.

Table 1 - Age, weight, SC and physical and morphologic aspects of 21.4 ± 0.14 months old Tabapuã bulls, raised under pasture conditions

Age	Category	N	Weight	SC	Motility	Vigor	MSD	mSD	TSD	BSE-Z
(years)			(kg)	(cm)	(%)	(1-5)	(%)	(%)	(%)	(1 - 100 points)
21.4	Mature	17 (70.8%)	400.76	31.80(a)	56.47(a)	5.00	12.82(a)	7.00	19.82 (a)	78.64
	Immature	7 (29.2%)	404.30	30.08(b)	43.33(b)	4.66	59.85(b)	9.43	57.66 (b)	--

P<0.05 mSD = minor sperm defects

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Comparing andrological profiles of sexually mature and immature two-years-old dairy Gyr bulls (*Bos taurus indicus*)

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Introduction

Selecting bulls that are able to reach sexual maturity as precociously as two-years-old (2 y.o.) must be the goal of most Brazilian bovine breeding programs, once it enhances the genetic gain per generation, therefore the lucrativity of the production systems. The aim of this study was to compare, based on andrological and zootechnical aspects, sexually mature and immature dairy Gyr (D-Gyr) bulls at 2 y.o., as a tool for fertility selection.

Material and Methods

A standardized group of 20 clinically normal bulls from a traditional D-Gyr herd was evaluated under andrological (1) and zootechnical parameters at 2 y.o. These animals were raised under pasture conditions (*Brachiaria brizantha* + mineral mixture) from weaning to 21 months of age (a total period of 14 months). Thereafter, until 2 y.o., they were kept in a feeding-lot, individualized, under full diet management. Data was analyzed under Tukey's Studentized Range Test by (2) to compare mature sexually developed bulls to immature ones (1).

Results and Discussion

Analyzed parameters in this study are shown in Tab.1.

Table 1 – Zootechnical and andrological parameters of D-Gyr bulls, at two-years-old.

Status	N(%)	BW(kg)	SC(cm)	MOT(%)	VIG(1-5)	MM(1-5)	MSD(%)	mSD(%)
Mature	14 (70)	366,3	30.7	53.1	4.9	2.7	12.1[a]	9.2[a]
Immature	06 (30)	360,7	30.8	48.3	4.5	1.7	45.5[b]	17.2[b]

BW = body weight; SC = scrotal circumference; Mot = motility; Vig = vigor; MM = mass motility; MSD = major sperm defects; mSD = minor sperm defects. $P < (0.05)$

Even being raised mainly under pasture conditions and supplemented with full ration for only three months, 70% of the animals reached sexual maturity at 2 y.o., showed by the low percentage of MSD and mSD in the semen of the mature group comparing to the immature one. This reiterates the rusticity and high genetic expression for early sexual maturation under reasonable food management conditions when, associated to the selection for milk production, the parameters for fertility are also observed in D-Gyr breeding programs. This is in agreement to (3) and to (4) which researched the effects of food management on Tabapuã and on Gyr bulls' andrological profiles, respectively. Therefore, it was concluded that MSD and mSD are important seminal parameters to classify 2 y.o. D-Gyr bulls as reproductors and/or semen donors.

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Screening and andrological correlations of two-year-old Tabapuã (*Bos taurus indicus*) bulls, raised under pasture conditions

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Introduction

The Tapabuã breed has been increasing in number due to its importance in beef production parameters at an early age, even under pasture conditions**.** The aim of this study was to evaluate andrological and semen freezing viability in two-year-old Tapabuã bulls, raised under pasture conditions.

Material and Methods

From a total of 156 young Tabapuã bulls (*Bos taurus indicus*) receiving pasture supplementation during the first dry season after weaning, and previously selected at 12 months old by scrotal circumference (SC) and live body weight (BW), and that reached sexual maturity at 24 months of age, measured by SC > 30 cm, major defects (MD) < 15%, total defects < 30%, sperm motility > 60% and vigor (1-5) = 5 (2) and mean Breeding Soundness Evaluation for Zebu bulls (BSE-Z) of 69.91 (1-100) points, a group of 35 young bulls was taken, at random, 20 with BSE-Z > 60 points, being 45.0% of them with > 84; 25.0% ranging from 74 to 83 and 30,0% ranging from 60 to 73 points. From these 20 animals, three (17.6%) showed motility = 50% and vigor = 5; six (35.3%) showed motility = 40% and vigor = 5; other six (35.3%) showed motility = 35% and vigor = 5 and two (11.6%) showed motility = 30% and vigor = 5, to evaluate andrological correlations almong the parameters measured. The others 3 animals were discarded based on inability to reach the recommended freezing standards (2).

Results and Discussion

The observed results were similar to those registered in the Nelore breed (3), when comparing SC and its correlations with BW (0.77) and sperm motility (0.57) (Table 1). A negative and of low magnitude correlation between SC and MD (-0.09) was recorded, similar to that observed in the Nelore breed (4). The results from post freezing agreed to those registered from (1).

Table 1 – Pearson correlations among the andrological parameters and frozen semen parameters

Parameters	M1	M2	BW	BSE-Z	MSD
M2	0,68*				
BW	0,45	0,35			
BSE-Z	0,13	0,19	0,22		
MD	0,14	-0,0099	-0,06	-0,87*	
SC	0,57*	0,4	0,77*	0,23	-0,09

M1- pre-thawing motility; M2- post thawing motility; BSE-Z – Breeding Soundness Evaluation for Zebu; MsD- major sperm defects t-student *(P< 0,05)

Based on the results observed with Tabapuã bulls, it can be concluded that previous selection of animals by SC and BW at one year of age and BSE-Z at age of two years, along with tests of semen thawing, results in screening of animals with characteristics of high meat production and seminal parameters within the recommended standard.

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Pearson's correlations among andrological and zootechnical parameters in dairy gyr breed (*Bos taurus indicus*)

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Introduction

The Dairy Gyr (D-Gyr) breed represents an outstanding role in the low cost milk production, along with its rusticity, high production and docility. The aim of this study was to evaluate the Pearson's correlations among andrological and zootechnical parameters for an objective selection of young D-Gyr bulls for high fertility.

Material and Methods

A group of 22 clinically normal young D-Gyr bulls, ranging from 18.0 to 25.0 months of age, body weight (BW) ranging from 224.0 to 417.0 kg and scrotal circumference (SC) ranging from 23.0 to 36.5cm, were evaluated. A total of 88 semen samples, collected at 4 different average ages (19.6, 20.5, 22.5 and 24.0 m.a.), were taken by eletroejaculator and evaluated for physical and morphological parameters (1). Data were analyzed according to (2).

Results and Discussion

Pearson's correlations among the different andrological and zootechnical parameters of young D-Gyr bulls are shown in Tab. 1.

Table 1- Correlations among the andrological and zootechnical parameters of young D-Gyr bulls

Parameters	BW (kg)	SC (cm)	MOT (%)	VIG. (1-5)	MM (1-5)	MSD (%)	mSD (%)
AGE	0,70*	0,65*	0,42	0,47*	0,44*	- 0,39*	-0,07
BW		0,53*	0,40*	0,33*	0,43*	- 0,41*	0,02
SC			0,42*	0,40*	0,38*	- 0,47*	0,18
MOT				0,77*	0,67*	- 0,35*	-0,04
VIG					0,59*	- 0,38*	-0,14
MM						- 0,47*	-0,14
MSD							-0,11

Mot = motility; Vig = vigor; MM = mass motility; MSD = major sperm defects; mSD = minor sperm defects. *P<0.05

It was observed high correlation of age with BW and SC; medium correlation was also registered for BW and SC with all semen parameters, except mSD. The results are in agreement to (3), working with two-year-old Nelore bulls, raised under pasture conditions and (4) working with young Gyr bulls, raised on pasture and supplemented with full ration. It can be concluded that selection for larger SC and semen parameters leads to earlier sexual maturity, once it increases BW, MOT., VIG., MM. and decreases MSD. (4, 5).

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Buck individual effect on the motility degradation rate of the semen cooled at 4ºC and stored for 48 hours

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Introduction

The purpose of semen storage (cooled or frozen) is to prolong the spermatozoa fertilizing ability by temporary reduction of its metabolic activity (Salamon & Maxwell, 2000). However the cryopreservation of the spermatozoa at low temperatures must be applied carefully, because this process could provide irreversible damages on the sperm membrane (Salamon & Maxwell, 2000). Probably male individual effect is a factor that influences semen quality after cooling. This study aimed to evaluate the male individual effect on motility degradation rate of buck semen stored at 4ºC during 48 hours.

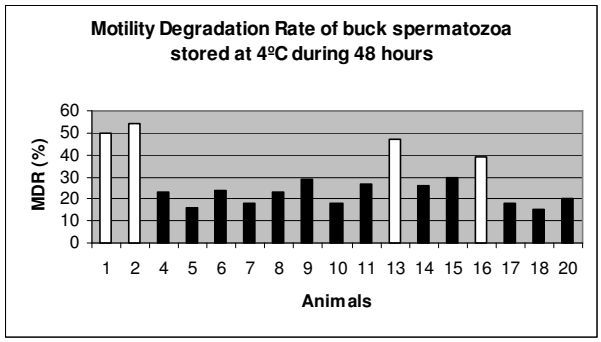
Materials and Methods

The semen of seventeen native male goats was collected biweekly by artificial vagina during the period of February to May of 2006 (8 collections per animal). The ejaculates were diluted at 32ºC with coconut water added of 2,5% of egg yolk, at final concentration of $200x10^6$ spermatozoa per mL. Afterwards, the semen was cooled during 2 hours until reaching 4ºC and stored for 48 hours. Semen samples evaluations were performed at 2, 24 and 48 hours after cooling by thermoresistance test at 38ºC. The semen parameters: forward progressive motility (FPM) and percentage of motile spermatozoa (PMS) were appraised at 5, 60 and 120 minutes of incubation at 38ºC. The motility degradation rate (MDR) was calculated based on the FPM (score from 0 to 5), using the following formula:

$$MDR = \left[\frac{FPM\ 5min - FPM\ 120\ min}{FPM\ 5\ min} \right] \times 100$$

Results and Discussion

Many factors can affect buck semen quality after cooling at 4ºC. Campos (2003) proved that time of storage influences both forward progressive motility and percentage of motile spermatozoa in vitro. The author suspected that an individual effect also influences semen quality, but the number of animals (4) in the study was insufficient to prove this fact. In the present study, the results of MDR (**showed in the graph below**) confirm the presence of an individual effect **(white colour)** on the forward progressive motility evaluated by the average values obtained during 48 hours of semen storage. The MDR measures the spermatozoa efficiency to maintain its forward progressive motility during incubation at 38ºC and a high MDR means bad semen preservation.



Maybe, interaction between phospholipase A in the seminal plasma and egg yolk in the extender led to bad semen preservation after cooling. We suggest that the male individual effect must be investigated though biochemical analysis of seminal plasma Phospholipase A levels.

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Effect of insulin on rabbit semen stored at 15^0 C

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Introduction

The importance of insulin on cell growth, differentiation and metabolism is well recognized. However, recent data have shown that, both, the sperm plasma membrane and the acrosome are cytological targets for insulin. Rabbit sperm storage is one way to enhance rabbit production in Brazil. This study was designed to investigate the effect of insulin addition to medium, on rabbit semen cooling at 15°C for 8 hours.

Materials and Methods

A ten-week trial was carried out from March to May of 2006 at the experimental rabbitry of the Animal Science Department at Lavras Federal University. Seven New Zealand White rabbit bucks (weighing 3.0 kg on average) were housed in individual cages, under a constant photoperiod of 16 h of light/day, under non-controlled environmental conditions. During the experiment, temperature and humidity were recorded. Rabbits were fed 150 g/day of a commercial diet containing 15% crude protein. Water was provided *ad libitum* with nipple drinkers. Semen from every buck was collected once a week using an artificial vagina. Each ejaculate was examined under optical microscopy and the percentage of mobile sperm was subjectively estimated. Only ejaculates that had motility greater than 60% and vigor score of at least 3 were pooled (total volume ranging from 4.0 to 4.8mL) and diluted in TRIS-sucrose-citrate (1:1). One mL of the pooled sample was added to four 10 mL glass tubes containing 0, 5, 10 or 15 µl of insulin. This procedure was replicated once for each collection, throughout a ten week period. Tubes were kept at 15°C and evaluated every two hours (0, 2, 4, 6 and 8). Sample aliquots were laid over a pre-warmed Makler® chamber at 37°C. The fixed effects of treatment and storage time on semen motility and vigor were analyzed using the Proc Mixed procedure (SAS, 1999). The significance level was taken as $p < 0.10$.

Results and Discussion

Storage time affected (P<0.0001) semen motility and vigor, such that these characteristics lowered as the time post-cooling increased. Motility and vigor were subject to a square effect of insulin (P=0.0907 and P=0.0886, respectively). Motility and vigor were higher at the insulin levels of 5 and 10µL, compared to 0 and 15µL. The role of insulin on male fertility is not completely defined, although it is expressed in human ejaculated spermatozoa. Both insulin transcripts and protein were detected (1). In conclusion, rabbit semen was better preserved in 5 and 10 µL insulin levels, at 15°C for four hours. Under these conditions minimal required seminal quality was maintained (60% motility and vigor score 3).

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Viability of equine sperm submitted to freezing using piruvate and trolox on extender

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Introduction

Sperm has capacity to produce reactive oxygen species (ROS), which is responsible for sperm alterations that are indispensables to the fertilizing process. However, the excessive production causes oxidative stress damaging its survival and fertilizing capacity (1). Antioxidant substances are used in semen samples aiming to reduce oxidative damage caused by high concentration of ROS. It is believed that vitamin E is the primary inhibitor of ROS found in quantities in cellular membranes of mammals and seminal plasma (2). Piruvate has antioxidant action (3), besides being an excellent energetic substrate. The purpose of the present study was to analyze the viability of equine sperm submitted to freeze using piruvate and Trolox (hidrosoluble analogue of vitamin E) on extender.

Materials and Methods

We used 5 ejaculates collected from 3 stallions classified as fertile based on history of pregnancy rates, using artificial vagina method. The samples were analyzed to assess sperm quality: total and progressive motility, vigor and concentration. After dilution, the samples were centrifuged and diluted in Palmer extender, according to treatments: T1 – Palmer without antioxidants; T2 – Palmer plus 2mM of piruvate and T3 – Palmer plus 120 mM of Trolox. After an hour under refrigeration (4 ^{o}C), the samples were drawn into straws (0,5 mL), placed on liquid nitrogen vapor, frozen and stored at -196 ^{o}C. After thawing the samples (37 ^{o}C, 30 s), the following sperm characteristics were evaluated: sperm motility rate, progressive motility, viability (IP + SYBR), mitochondrial potential (JC-1), acrosome (FITC-PNA) and DNA integrity (Acridine Orange). The data were analyzed (SPSS Statistical analyzes) applying ANOVA , at 5% of significant level.

Results and Discussion

After thawing, we observed total motility (12.,08%; 9.17%; 18.92%), progressive motility (7.75%; 4.67%; 12.50%), vitality (27.65%; 32.00%; 28.50%), mitocondrial potential (24.55%; 24.00%; 26.95%), acrosome (88.78%; 88.33%; 84.13%) and DNA integrity (82.83%; 78.58%; 79.62%), respectively, on groups T1, T2 and T3. It was observed significant differences ($P<0.05$) only between T2 and T3 groups in respect to total and progressive motility parameters. However, among groups (T1, T2 and T3), we observed cells with low percent of motility making unviable their use on artificial insemination in mares, thus differing from other authors (2,3) Trolox and piruvate additions, on the concentrations used, were not efficient to preserve the viability of sperm cells, and that the cryopreservation process was not efficient to preserve the viability of the semen samples. In conclusion, more research should be done to study different types and concentrations of antioxidants on the stallion semen freezing process.

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Effect of extenders minimum contamination medium (MMC) and modified egg yolk lactose extender (LGM) on canine semen viability after cooling at 4ºC for 24 hours in Equitainer®

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Introduction

Cooled transported semen has been used with success in the canine artificial insemination (AI). Seminal extenders must give energetic components for spermatozoid metabolism and also prevent the injuries during the cold shock. Some extender component like yolk egg and milk prevent the integrity and functional characteristics of the sperm plasma membrane (2). The aim of this study was to compare the canine semen viability after dilution in Minimum Contamination Medium (MMC) and Modified Egg Yolk Lactose Extender (LGM) and incubation at 4ºC for 24 hours.

Materials and Methods

Thirteen ejaculates were collected from five adults dogs by digital manipulation. Microscopic and microscopic characteristics were assessed right after collection. Semen was divided to be diluted (1:3) in MMC (1) and LGM (3) and subsequently chilled in Equiteiner®. Seminal parameters of motility, spermatic morphology, membrane integrity (hiposmotic test), spermatic viability, and spontaneous acrosome reaction (Trypan-blue-Giemsa stain) were evaluated in fresh and chilled semen after 12 and 24 hours of incubation. Statistical analize (ANOVA) was performed with the help of Software GraphPad Instat 3.05 32 bit for Wi 95/NT.

Results and Discussion

The mean of progress motility in fresh semen was 89,61(±4,77)%, the percentage of spermatic defects was 31,84 (±5,65)%, sperm plasma membrane integrity was 86,54 (±3,6)% and spermatic viability 92,23 (± 6,37). All seminal parameters were in conformity with accepted values for artificial insemination (IA), except the percentage of spermatic defects which mean was higher than 30%. LGM medium was better than MMC in maintaining semen characteristics after 12 hours, probably because the properties of egg yolk (2). The mean of progress motility in semen in semen diluted in LGM and incubated during 12 hours was 70,0(± 12,74)%, percentage of spermatic defects was 36,77 (± 8,7)%, sperm plasma membrane integrity was 77,53 (±5,7)% and spermatic viability was 90,30 (±7,01). Only spermatic defects percentage showed different values from the the accepted for IA, but it was already high in fresh semen. The main of true and false acrosome reaction didn't exceeded 2.0% in both semen extenders, which demonstrates no influence of media components and incubation period on these phenomena at 4ºC. After 24 hours of incubation none of the extenders kept semen characteristics properly, what could be explained by the quality of ejaculates used in this study, which had a mean of 31% of sperm pathologies that showed direct effects on semen viability after cooling. In conclusion, these results indicate that LGM diluent keeps canine fresh semen characteristics at 4ºC in transport container during 12 hours and both extenders used in these study did not induce the spontaneous acrosome reaction.

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The effects of two isosmotic solutions in the morfological characteristics and in the freezing of spermatozoa obtained from epididymis of dogs and cats: preliminary results

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Introduction

Germplasm conservation and assisted reproduction are necessary in mammalian species that can be extinct. The dog and the domestic cat can be used as an experimental model to the wild canine and feline. As the sperm function and viability are affected deleteriously by cryopreservation, a basic knowledge of the reproductive physiology and the development of an adequate conservation medium are indispensable for the success of the genetic material preservation (1). The aim of this study was evaluated two isosmotic solutions to recuperate the spermatozoa that comes from the vasa deferentia and the caudae epidydimis of orchiectomized dogs and cats.

Materials and Methods

Six cats and four dogs, mixed breed, were submitted to elective orchiectomy. After removal and dissection of the testicles, the distal portion of the epididymis and part of the ducts deferens were squeezed, with an anatomic clamp into a Petri dish containing Saline Solution (T1) and Ringer without Lactate (T2). Immediately after the collection, progressive motility, spermatic velocity and morphological spermatozoa were evaluated. Samples were centrifuged at 800xg/10 minutes, the supernatant was removed and the pellet was diluted in one step with Tris-ac.citric-glucose-amicacina-oep-20%egg with 7%glycerol for dogs and 3.5%glycerol for cats. Samples were kept on 0.5mL French straws with 50 x 10^6 sperm per straw, and cooled to 5ºC during 60 minutes. The straws were placed 6 cm above liquid nitrogen for 20 minutes and then emerged into it, after they were stored in cryogenic container. Semen was thawed in water bath, 70 ºC, during 8 seconds. The following parameters were evaluated on semen post-thawed: sperm motility, membrane integrity by fluorescent probes and sperm morphology.

Results and Discussion

Median values of semen characteristics obtained from epididymis were described in Table 1.

Table 1: Seminal characteristics of 4 dogs and 6 cats obtained from epididymis in isosmotic solutions (M1:Fresh) (M2: Thawed).

		Saline Solution		Ringer without Lactate	
		M1	M2	M1	M2
Dogs	Mot. (%)	69	35	73	45
	Vel. (0-5)	3.25	2.25	3.5	2,5
	Memb. Int.		48		53
	Major (%)	19.2	16.7	23.5	19
	Minor (%).	38.7	27.7	39.7	20.7
Cats	Mot. (%)	77.5	40	77.5	45
	Vel. (0-5)	3	2.6	3.6	2.6
	Memb. Int.		56		49
	Major (%)	57.2	63.8	57	59
	Minor (%).	18.5	10.3	13	9.6

There was no significant difference between the treatments (Saline Solution *versus* Ringer without Lactate) in the seminal characteristics. Although these are preliminary data, both isosmotic solutions were efficient in the epididymis spermatozoa recuperation, making possible to preserve the sperm cells.

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Seminal plasma reduce acrosome reaction in post-thawed equine spermatozoa

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Introduction

In the last few years, several investigators have reported changes in cryopreserved spermatozoa that are similar to those occurring during capacitation, and thus the term ''cryocapacitation'' was coined. It has been known that seminal plasma can suppress capacitation (1) and decapacitate previously capacitated spermatozoa (2). Cross (3) established that seminal plasma can prevent or reverse the development of acrosomal responsiveness in human spermatozoa. Seminal plasma from stallions with high post-thaw motility improves semen quality from stallions with low, when added prior the cryopreservation process (4). Therefore, this study was intended to find out whether the incubation of post-thawed semen with seminal plasma from a stallion with high post-thaw semen quality prevent or reverse acrosomal reaction in equine spermatozoa.

Materials and Methods

Three ejaculates were obtained from each of three mature stallions. Semen was packaged into 0.5 mL straws with 200 x 10^6 cells/mL in a cryopreservation media (Botu-Crio™ - Biotech-Botucatu-Ltda / ME, Botucatu, Brazil). Straws were frozen by automated technique using a programmed machine (TK3000-TK Tecnologia em Congelação Ltda., Uberaba, Brasil). Seminal plasma was obtained from one stallion with high post-thaw motility (≥50%). Semen was centrifuged at 3000 g for 20 min. The sperm-free supernatant was aspirated and stored at -196 ºC until use. Two straws of cryopreserved semen of the same collection and stallion were thawed at 37 ºC for 30 s, for each treatment: (T0) 1 mL semen + 1mL of cryopreservation media without cryoprotectants; (T50) 1 mL of semen + 0.5 mL of cryopreservation media without cryoprotectants + 0.5 mL of seminal plasma; (T100) 1 mL of semen + 1 mL of seminal plasma. All samples of semen (three treatments) were diluted at a final concentration of 100 x 10^6sperm/mL, placed in 2 mL microcentrifuge tubes and maintained in water bath (37 ºC) for 120 min. To assess number of live sperm with acrosome reaction, at time 0, 60 and 120 min, 3 µL of propidium iodide (0.5 mg/mL) and 80 µL of fluorescein isothiocyanate (FITC)- conjugated pea (Pisum sativum) agglutinin (PSA) (100 µg/mL) were added to suspension of 25x10^6 cells/mL in TALP, and incubated for ten minutes (25 ºC). The analysis of each sample was determined by flow cytometry (FACSaria, Beckton Dickeson, San Jose, USA).

Results and Discussion

Results of live sperm with acrosome reaction at different times are shown in table below. Data are presented as mean ± SEM. The acrosome status of spermatozoa was influenced by interaction between treatment and time ($p<0.05$). In time 0, the addition of seminal plasma (T50 and T100) reduced ($p<0.05$) the number of sperm showing acrosome reaction, although after one hour there was no statistical difference ($p>0.05$) between treatments. The addition of 50 % of seminal plasma (T50) reduced ($p<0.05$) the number of acrosome reacted spermatozoa after two hours of incubation, while 100 % (T100) increased the number. It's showing a harmful effect of seminal plasma in high concentration. The results of this investigation show, in fact, that the number of acrosome reacted sperm were reduced by addition of 50 % of seminal plasma to the post-thawed semen, probably through its decapacitation effect. Decapacitation factors adsorbed onto the plasma of sperm might protect some spermatozoa from premature acrosome reaction. Further investigations to compare the decapacitation capacity of seminal plasma from sires of different post-thaw semen quality is currently in progress.

	Treatment		
Time(hr)	0	50	100
0	3.52 ± 0.24^{a}	2.18 ± 0.27^{b}	2.73 ± 0.22^{b}
1	3.26 ± 0.35^{a}	2.16 ± 0.29^{a}	2.80 ± 0.31^{a}
2	$2.74 \pm 0.31^{a,b}$	2.55 ± 0.16^{b}	3.83 ± 0.46^{a}
Mean	3.17 ± 0.18^{a}	2.29 ± 0.14^{b}	3.12 ± 0.21^{a}

Means in the same row with different superscripts are significantly different ($p<0.05$).

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Association between post-thawing boar sperm membrane integrity and the presence of a 26 kDa factor in boar seminal plasma

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Introduction

An understanding of the molecular mechanisms involved in the sperm maturation process and their associations with seminal plasma components can generate knowledge about the presence of biochemical markers related to semen quality. Such biochemical markers may be used to identify biological properties related to sperm function during cryopreservation. This study aimed to identify polypeptides in the swine seminal plasma associated to membrane integrity of boar spermatozoa submitted to freezing and thawing.

Materials and Methods

Semen samples were collected from 3 males, each one collected 6 times. Semen was diluted in *Beltsville Thawing Solution* and then cooled at 24ºC during 90 min and later cooled at 15ºC for 90 min. The sperm was centrifuged and the seminal plasma was removed. The cryoprotectant used for freezing was N,N-Dimetilacetamide (DMA – C_4H_9NO). Frozen semen was stored in straws. Thawing was conducted at 37ºC during 20 s. Post-thawing plasma membrane integrity (PMI) was conduced through fluorescent probes and categorized as <55% or ≥55%. An aliquot of seminal plasma was used for the protein profile, conducted by one-dimensional poliacrilamide gel electrophoresis (SDS-PAGE). Electrophoresis was carried out in a TRIS buffer system. Electrophoresis was performed in a BIO-RAD Mini-Protean 3 Cell® system using 15% bis-acrylamide gels. Samples were concentrated at 60 V for 20 min, separation was performed at 120 V for 70-80 min. The BenchMark Protein Ladder™ (Invitrogen®, Carlsbad, USA) was used as the standard of molecular weight. Gels were stained with Coomassie Brilliant Blue and scanned and analyzed with TotalLab TL 100 analysis software (Nonlinear Dynamics, UK). Association between the presence of specific proteins and distinct categories of membrane integrity was evaluated by chi-square test.

Results and Discussion

In this study, we identified the presence 18 proteins in boar seminal plasma. However, among them, only one protein band having molecular weight of 26 kDa was associated with PMI. The presence of this protein was related to PMI lower than 55% ($P < 0.05$). The plasma membrane undergoes extensive modifications when spermatozoa are in contact with seminal plasma. So, two situations may influence this process: (i) interaction among seminal proteins and plasma membrane and (ii) mutual interactions among seminal plasma proteins. Many boar seminal plasma proteins have been identified and characterized (1). However, no association between any seminal plasma protein and post-thawing plasma membrane integrity has been described. The 26 kDa factor identified in the present study, which is associated with PMI after cryopreservation, may be an important biochemical marker associated to post-thawing semen freezability. Nevertheless, further studies still need to be conducted to elucidate that subject.

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Hyperosmotic conditions and thawing temperatures for goat semen: application in freezing protocols devoid of glycerol

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Introduction

Disaccharides like sucrose and trehalose added to semen extenders proved to be beneficial in a number of studies (1, 2). A higher content of non-permeating cryoprotectant would allow for a more controlled de- and rehydration of cells during freezing and thawing (3), but to recover motility, the cells must be returned to near isosmolality (1). The uppermost tolerance limit of goat spermatozoa to sucrose solutions was found to be 900 mOsm. Promising results were obtained when freezing goat semen in a hyperosmotic Tris-egg yolk extender added with 375mM sucrose and only 1.7% glycerol (4). Thus, two experiments were designed to evaluate the effects of 2 different disaccharides, various thawing regimes and rehydration rates.

Materials and Methods

In Experiment 1, 15 from 4 Boer bucks were split into aliquots to be allocated to one of 5 treatment groups. In Group 1 (Control), semen was diluted in a standard Tris-egg yolk-glycerol extender, chilled at 4°C for 2h, frozen at -120°C in LN2 vapor and stored in LN2. In Group 2, Tris-egg yolk extender (TY) was supplementend with 375mM sucrose and 1.7% glycerol, in Group 3, with 300mM sucrose and 3.4% glycerol and in Group 4, with 300mM sucrose and 1.7% glycerol. In Groups 2, 3 and 4 samples were first diluted in TY, chilled to 4°C for 2h before addition of sucrose and glycerol and were equilibrated for 5 min before freezing. Samples of the Group 1 were thawed at 38°C for 30s. In the other 3 groups, semen was thawed at 4 or 20°C and rediluted with TY to near isosmolality by one of 3 protocols: 5 steps at 4°C; 5 steps at 20°C or 2 steps at 20°. This was conducted as a 3x3 factorial. Eosin-nigrosin staining was used to assess post-thaw membrane integrity (MI). Motility (MOT) was assessed after 0, 2 and 6h of incubation at 38°C. The results of MI were normalized to those of the Control group and those of MOT were normalized to those of the native sample.

In Experiment 2, 9 ejaculates were collected and samples frozen as described for Groups 1 and 3 of Exp.1 were compared to: TY + 300mM sucrose and TY + 300mM trehalose, both devoid of glycerol. Samples were chilled, frozen and evaluated as described before. The Control was thawed at 38°C, and groups with added disaccharides, were thawed and rediluted in 5 steps at 20°C or in 1 step at 38°C.

Results and Discussion

Experiment 1: MI in Group 4 was superior to the Control regardless of the dilution protocol. This suggests that moderate dehydration by sucrose and a low level of permeating cryoprotectant allows for faster rehydration of the cells at a higher temperature, causing the least cell damage. The advantage of a lower sucrose content on MOT became evident after 2h and, more so, after 6h of incubation in Groups 3 and 4 when thawing at 20°C. MOT after 6h was at a maximum in Group 3 when rediluting in 5 steps. As shown by others (5), optimum conditions for MI and MOT were not identical.

Experiment 2: In all groups containing a disaccharide post thaw-MI was better than in the Control, best results being achieved with 300 mM trehalose and thawing and rediluting at 38°C in one step (320% vs 100% in the Control). After thawing MOT did not differ, but at 2h and 6h the superiority of the samples containing trehalose was evident.

In conclusion, when freezing goat semen, the use of permeating cryoprotectants, such as glycerol, may be avoided if the cells undergo a previous dehydration in a Tris-egg yolk extender containing sucrose or trehalose. The best membrane integrity and motility results were obtained with 300mM trehalose.

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Effects of fractioned addition of dimethyl formamide in the viability of cryopreserved stallion semen in different procedures of freezing

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Introduction

Protocols for equine spermatozoon cryopreservation are not standardized, and success of this technique varies substantially. The aim of this research was to evaluate the efficiency of adding 5% dimethyl formamide to modified base INRA 82 extender in a fractionated manner using three different schedule and to study the interaction of manner of cryoprotectant adding with equilibtration time and rates of freezing in the criopreservation of equine spermatozoa.

Materials and Methods

One ejaculate of six stallions was used. Five percent dimethyl formamide was added as follows: Time 1: addition of one tenth part of the dimethyl formamide per minute; Time 2: addition of one tenth part of the dimethyl formamide every two minutes; Time 3: addition of one tenth part of the dimethyl formamide every three minutes. For all three treatments extended semen was maintained at room temperature for an average period of one hour and eight minutes. Afterwards samples were cooled down to 5°C using a computerized rate of cooling of 0.25°C/min (TK – 3000 – TETAKON, Brazil). Freezing curves used were as follows: a) with no additional equilibration time at 5°C, freezing in the vapor 4 cm above of the nitrogen level for fifteen minutes; b) with 45 minutes equilibration time at 5°C in the vapor 4 cm above of the nitrogen level, c) with 45 minutes equilibration time at 5°C followed by freezing in a computer systems at a rate of -10°C/min down to -127°C. After freezing all samples were plunged in the liquid nitrogen. Thawing was made at 52°C for ten seconds, followed by immersion of samples in a water bath at 37°C for thirty seconds. Immediately post thaw total motility, progressive motility and spermatic vigor were evaluated under optic microscopy (400X). The integrity of the plasmatic membrane of the tail was evaluated through the hiposmotic swelling test and the functional and structural sperm membrane integrity were evaluate by the fluorescent dyes, carboxyfluorescein diacetate and propidium iodide, respectively. Samples were also submitted to the thermo-resistant test at 37 °C. Statistical variance analyses were compared through the Spearmann and Kruskal-Wallis test.

Results and Discussion

The percentage of progressive motility was 40.8; 38.4 and 35.8 for procedure a; 37.5; 34.2 and 36.7 for procedure b; 33.4; 37.5 and 36.7 for procedure c in time 1, 2 and 3 respectively. There was no significant difference in the evaluated parameters (P> 0,05) among the three times of addition and among the freezing rates. The results showed that there was no beneficial in the sperm cells viability evaluated in vitro after thaw increasing time of cryoprotectant addition above 10 minutes, or adding equilibration time at 5°C or adjusting rate of freezing.

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Effects of low density lypoproteins and dimethyl formamide in differents freezing rates on equine frozen semen

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Introduction

The process of equine spermatozoa cryopreservation depends on a number of inter-related factors including among others semen extenders, cooling and freezing rates. Few experiments were conducted aiming to evaluate effectiveness of alternative cryoprotectants agents (1). It has been shown that low density lypoproteins (LDL) of the egg yolk exert protective effect on frozen stallion sperm cells (2). The aims of the present study were to evaluate the protective effect of different LDL concentrations in association with dimethyl formamide (DMF), and to detect possible interaction of extender composition with cooling rates during the criopreservation process of equine spermatozoa.

Materials and Methods

Seven stallions of known fertility were used. One ejaculate per stallion, free of gel, was diluted (1:1) in a modified milk extender (INRA 82) and centrifuged. The pellets were re-diluted in the same extender containing 5% DMF and the following proportions of LDL: T1 – 5%; T2 – 10%; T3 – 20%. T4 samples were re-diluted in the conventional – INRA 82 with 5% DMF (control). Samples were packaged in 0.5 ml straws. Two different procedures for cooling and freezing were used: protocol 1) immediate freezing 4cm above of the nitrogen level for fifteen minutes, followed by samples submersion in the liquid nitrogen; protocol 2) cooling rate – 0.5°C/min. from room temperature down to 5°C and -20°C/min. from 5 to -120°C, using a programmable system (TK – 3000 – TETAKON, Brazil). Thawing was made at 52°C for 10 seconds, followed by samples immersion in a 37°C water bath for 30 seconds. Total and progressive motilities and integrity of plasma membrane of spermatozoa assessed by the hyposmotic test and fluorescent dyes were evaluated immediately post-thaw. Variance analysis was used and means were compared through the Scott-Knott test.

Results and Discussion

A significant improvement (P<0.05) on motility parameters was observed for T1 to T4 when submitted to protocol 2 of freezing. Results are shown in table 1.

Table 1. Progressive post-thaw motility of stallion sperm.

Treatments	PM Prot. 1	PM Prot. 2
T 1	42.8 ± 11.2	52.1 ± 11.3
T 2	45.0 ± 10.4	55.0 ± 10.2
T 3	42.8 ± 9.3	56.4 ± 10.3
T 4	42.8 ± 8.6	57.1 ± 8.1

PM = progressive motility (P<0.05)

Results of the hyposmotic test and fluorescent dies test were similar between treatments. The results of the present work allow concluding that there was no difference in sperm parameters evaluated after thaw between extenders and that using DMF sperm parameters post thaw were improved using a programmable slow cooling rate as compared to a rapid freezing rate.

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Thermo resistance and hypoosmotic swelling tests for evaluation of post-thawing viability of two years old tabapuã bull sperm

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Introduction

Functional tests are important for evaluation of post-thaw sperm viability. The Thermo Resistance Test (TRT) indicates the capability of sperm to resist to warm shock during thawing. The fast TRT is performed until 30 minutes and slow TRT is performed up to 240 minutes after post freezing. Hypoosmotic Swelling Test (HOST) is a powerful tool to evaluate sperm membrane functionality.

Materials and Methods

Semen samples were collected from 12 two-years-old Tabapuã bulls raised in pasture condition. The samples were frozen in liquid nitrogen (-196ºC) until evaluation. The fast and slow TRT were performed according to 1. The HOST was performed using three concentrations of the testing solution (75, 100 and 125%) according to (2).

Results and Discussion

There were no statistical differences ($p>0.05$) between the methods, indicating that the fast TRT shows similar results compared to the slow TRT (Table 1). The results are in agreement with those reported by (1). Similarly, there were no statistical differences ($P>0.05$) among the concentrations of testing solutions of hypo osmotic test (Table 2).

Table 1 – Descriptive statistics of fast and slow TRT on Tabapuã post-thawed sperm motility.

Test	Average motility (%)	Standard deviations
Slow TRT	7.58[a]	9.34
Fast TRT	5.50[a]	7.46

Means with different letters in the columns are different by Fisher's test ($p<0.05$)

These findings are according to (2) and also allow the use of this test. In conclusion, fast TRT along with HO with 75 mOsm/l can be safety added to andrological exam routines with minor losses of time and material.

Table 2. Descriptive statistics of three concentrations of HOST on Tabapuã post-thawed sperm membrane functionality.

HO mOsm/l	% of reacted sperm	Standard deviations
75%	16.67[a]	11.26
100%	18.88[a]	12.18
125%	26.08[a]	32.60

Means with different letters in the columns are different by Student's test ($p<0.05$)

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Functionability of sperm membranes in frozen-thawed semen from two-year-old tabapuã bulls, as predictors for higher fertility

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Introduction

The spermatic membrane participates in capacitating, spermatozoid pelucida zone interaction, acrossomic reaction, and in spermatozoid/oolema interaction and can suffer injuries in the freezing/defrosting semen process. In this study, hyposmotic (HOS) and thermal resistance (TTR) tests were used to evaluate the functional sperm membrane integrity. The aim of this study was to obtain information on these tests at post freezing semen samples, as well as to find correlations among conventional andrological parameters and the normal function of sperm membranes in frozen semen.

Material and Methods

The methodology for the hyposmotic test in frozen bovine semen consists in a mixture of a sample of 30 microliters of semen and 300 microliters of a hypotonic solution containing trisodium citrate and fructose at 100mOsm/L incubate at 37º C for an hour (1). After that the samples were fixed in 0.5 ml of tamponated formol-saline solution for reading in a phase-contrast microscope of x1000 (3). The slow TTR consist of defrosting the semen in water bath at 38º C for 30 seconds, being that maintained in a test tube at 38º C for a 4 hours period, followed of repeated analyses every 15 minutes. Semen samples of 17 two-year-old Tabapuã bulls were used, with good motility recovered (>30%), vigor (1-5) 5, after defrosting and screening of 35 animals taken at random from a group of 156 bulls with sexual maturity at two-years of age.

Results and Discussion

The values in Table 1 show positive, significant correlations ($P<0.05$) among values of sperm motility from TTR with values of pre freezing (M1; 0.64), post-freezing (M2; 0.60) and MT (0.52). These results are similar to those found by (3 and 4), working, respectively, with equine and goat semen.

Table 1- Pearson correlations among TTR, HOS and andrological parameters

Tests	M1	M2	BW	Conc.	BSE-Z	MD	HOS
HOS	0.64*	0.34	0.26	0.045	0.11	0.04	
TTR	0.64*	0.60*	0.63*	0.47	0.14	-0.03	0.52*

M1 = pre freezing motility; M2= post-freezing motility; Conc. =- pre freezing sperm concentration; BSE-Z= Breeding Soundness Evaluation for Zebu bulls; MD= major sperm defects *($P < 0.05$)

Data from HOS test are similar to those reported by (2), where they found 0.61, whereas in this study it was observed 0.64 between HOS and sperm progressive motility. The same authors also reported very low correlation between morphology and HOS test, like in this study. The high correlation of HOS test and sperm progressive motility can indicate good biochemistry functionality of mitochondrial membranes located in the sperm tails, place where swelling reaction to HOS test are observed indicating a perfect function of the membranes (2). In the same way it was observed high correlations of TTR with pre and pos freezing semen motility (4). Based on the results found it can be concluded that selection of semen samples for high pre and pos freezing motility allow individualize samples with better resistance 4 hours semen incubation at 38º C, post freezing and with high fecundation power even for using in IVF.

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In vitro interactions between frozen-thawed canine sperm and homologous oocytes

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Introduction

Cryopreservation induces serious damage in sperm structure. Evaluation of *in vitro* sperm oocyte interactions could be an efficient test for sperm function after frozen-thawing procedures. In vitro studies indirectly reflect damage in the complex system of proteins, glycoproteins and carbohydrates that participate in the sperm-oocyte interaction. However, there is a lack of information on how the interactions between sperm and oocytes in canine species are affected by cryopreservation, as reported for other mammalians. The aim of this study was to evaluate in vitro interactions between frozen-thawed canine sperm and homologous oocytes.

Materials and Methods

Ten ejaculates from five stud dogs (two ejaculates/dog) were collected by digital manipulation. Semen samples were evaluated; extended in Tris-egg yolk-glycerol; frozen and stored in liquid nitrogen, and thawed several weeks later. Grade-1 oocytes, containing an even dark ooplasm surrounded by a well-formed, multilayered cumulus cell mass, were collected from 44 canine ovaries obtained by ovariohysterectomy. Oocytes were washed in PBS and transferred to droplets (95µL) of FIV-TALP plus BSA. Sperm samples were thawed, evaluated, extended in Sperm-TALP and centrifuged (700g/5 min). Supernatant was discarded and the sperm pellet was diluted in Sperm-TALP. This suspension (5 µL) was added to the oocytes in each droplet. A minimum of 30 oocytes (three droplets) was used per ejaculate. After incubation (38 ºC/18 h under a humidified atmosphere containing 5% CO_2), the oocytes were removed, washed (sodium citrate 1%) and vortexed for cumulus-cell retrained. Oocytes were stained using Hoechst 33258 and evaluated at 400 x under fluorescence microscopy. The percentage of oocytes bound to sperm, oocytes with sperm penetrated into the perivitelline space or ooplasm, and the number of sperm that had bound and penetrated the oocytes were determined. Results were expressed as means and standard deviation (Statview 5.0).

Results and Discussion

The number of oocytes obtained from each ovary ranged from 0 to 222. The total number of oocytes collected was 1346 oocytes; from that number, 322 Grade-1 oocytes were selected for use in this trial. There was a high rate of interactions among canine frozen-thawed sperm and homologous oocytes (72.0 ± 19.8% oocytes with sperm bound or penetrated). We found 36.0 ± 14.8% penetrated oocytes and 57.5 ± 21.0% with sperm bound to the zona. Our results seemed lower than the 60% penetration rate for fresh canine semen, reported by Hewitt and England (1). Between one and three sperm penetrated the oocytes in this study, indicating the presence of polyspermy. We also observed from one to 11 sperm bound to the zona (1.9 ± 1.1 sperm/oocyte). These results showed that *in vitro* conditions alter sperm-oocyte interactions, since polispermy is not observed in *in vivo* studies (2). In conclusion, cryopreservation conserve the ability of canine spermatozoa in promote initial interactions with homologous oocytes under *in vitro* conditions.

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Effects of thawing temperature and straw size on sperm motility of cryopreserved curimba *Prochilodus lineatus* semen

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Introduction

Migratory Brazilian fish are known as piracema fish. During the spawning season (October–February), piracema fish species migrate up-stream to spawn. The curimba (*Prochilodus lineatus*) is an important species for aquaculture, as it serves as forage fish (food) to carnivorous species. Semen cryopreservation is a simple technique that facilitates larvae production and allows exchange of new genes between hatcheries. There are some reports concerning the efficiency of cryopreserved sperm in large straws (4 mL) for commercial purposes, but only a few in Brazilian species. Furthermore, the success of cryopreservation also relies on a standardized thawing protocol. Therefore, the aim of this study was to evaluate the effects of straw size and thawing temperature on sperm motility of cryopreserved curimba semen.

Material and Methods

This study was carried out at Hydroelectric Company of Minas Gerais (CEMIG) and Laboratory of Animal Sciences of the Federal University of Lavras, MG, Brazil. Four fish received an injection of carp pituitary extract (5 mg/kg BW) to induce spermiation. Semen was diluted 1:10 (v/v final volume) in a medium containing 10% methylglycol as cryoprotectant and glucose 5% as extender (1). Diluted semen was then aspirated into 0.5- or 4 mL straws, placed in a nitrogen vapor vessel (Cryoporter^{TM} LN_2 dry vapor shipper) at -170°C for 24 h and plunged into liquid nitrogen for storage. To evaluate the effect of two thawing temperatures, 0.5 mL straws were thawed in a 30°C or 60°C water bath for 8 or 16 s, respectively, and 4-mL straws were thawed at 60°C for 24 s. Sperm motility was evaluated immediately after thawing. NaCl 50 mM (2) was used as sperm motility activating solution.

Results and Discussion

A very high post-thaw sperm motility was observed (above 86%) in all semen samples and neither thawing temperature nor straw size affected (P>0.05) post-thaw sperm motility (Table 1). Differently, some studies suggest enzyme denaturation on sperm cells displayed in the peripheral area of larger straws caused by higher water bath temperature. It has been observed in the Colombian yamú *Brycon amazonicus* semen cryopreserved in 0.5, 1.8, 2.5 or 4.0 mL straws, a similar fertilization rate among straw sizes (3).

Table 1. Motility rate (%; n=4 males) of curimba semen cryopreserved in glucose and methyl glycol, in 0.5- or 4.0-mL straws and thawed in a water bath at 30 or 60°C.

Thawing temperature (° C)	Straw size (ml)	
	0.5	4.0
30	87 ± 8	---
60	86 ± 8	95 ± 0

P>0.05; Scott-knott.

Sperm cryopreservation using larger straws reduce the time required for semen packaging and thawing, facilitating sperm handling during the fertilization process.

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Effects of gentamicin on bacterial growth and sperm motility of refrigerated semen of piracanjuba *Brycon orbignyanus*

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Introduction

The piracanjuba *Brycon orbignyanus* (Valenciennes, 1849) is an endangered Brazilian teleost fish. Semen refrigeration is a simple technique that can preserve sperm cells for a few days, facilitating exchanges of semen between hatcheries. The storage period is believed to be limited mainly by bacterial growth that would compete with sperm cells by oxygen and nutrients. The aim of this study was to improve refrigeration conditions of piracanjuba semen by controlling bacterial growth.

Material and Methods

Fish were originated from Hydroelectric Company of Minas Gerais (CEMIG). Eight males were injected with carp pituitary extract to induce spermiation. Only samples that were free from water, urine or feces were used. Semen of each male was diluted in NaCl-tris solution containing gentamycin (1) in five concentrations (mg/mL): 0 (control), 0.01; 0.1; 0.5; and 1.0. Semen was then placed in open 10-mL glass tubes and refrigerated for 8 days. Motility rate (NaCl 50 mM as activating solution; light microscope) and colony forming unit (CFU/mL) in Petri dish containing trypticasein soya agar (TSA, Biolife, Italy) were determined every 2 days.

Results and Conclusion

Bacterial growth was successfully controlled by addition of gentamycin in semen extender in all concentration tested (Table 1). However, sperm motility was improved by gentamycin addition only from day 4 to 6. Our next step is testing an extender that provides energy to sperm cells in combination with the addition of gentamycin to provide a better environment for the refrigeration of semen.

Table 1. Sperm motility (%) and bacterial growth (CFU/mL) of piracanjuba *Brycon orbignyanus* semen diluted in NaCl-tris solution containing different concentrations of gentamycin, and refrigerated for 8 days.

Gentamycin mg/mL	Cool storage (days)				
	0	**2**	**4**	**6**	**8**
	Sperm motilidade (%)				
0	95 ± 6 $^{a\,A}$	68 ± 10 $^{a\,A}$	26 ± 19 $^{b\,B}$	11 ± 14 $^{b\,D}$	1 ± 2 $^{a\,d}$
0,01	95 ± 6 $^{a\,A}$	56 ± 7 $^{a\,B}$	36 ± 7 $^{a\,C}$	19 ± 16 $^{b\,D}$	0 ± 0 $^{a\,D}$
0,1	96 ± 4 $^{a\,A}$	67 ± 10 $^{a\,B}$	47 ± 18 $^{a\,C}$	31 ±19 $^{a\,C}$	10 ± 16 $^{a\,D}$
0,5	95 ± 6 $^{a\,A}$	69 ± 7 $^{a\,B}$	40 ± 14 $^{a\,C}$	26 ± 13 $^{a\,D}$	5 ± 7 $^{a\,D}$
1,0	95 ± 4 $^{a\,A}$	68 ±7 $^{a\,B}$	44 ± 10 $^{a\,C}$	24 ± 14 $^{a\,D}$	6 ± 12 $^{a\,D}$
	Bacterial growth (CFU/ml)				
0	1,4 x10^4	1,3 x 10^6	4,31 x 10^8	4,12 x 10^{10}	3,25 x 10^{11}
0,01	not determined	2,3 x 10	0	4,79 x 10^2	3,50 x 10^2
0,1		0	0	0	0
0,5		0	0	0	0
1,0		0	0	0	0

Mean followed by different superscript (lowercase in columns and uppercase in lines) differ (P<0.05; Scott-Knott)

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Motility and eyed-egg rates of cryopreserved semen of masculinized rainbow trout females: extenders and straw volume

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Introduction

Female rainbow trout *Oncorhynchus mykiss*, as others salmonid, grow faster than males. To produce all-female progenies, females are sex-inverted by androgen diet during larval stages. After sexual maturation, these masculinized females produce XX semen, and thus all-female progenies. The aim of this study was to test different semen extenders and two straw volumes on the cryopreservation of masculinized female rainbow trout semen.

Material and Methods

Five masculinized females were anaesthetized (benzocaine**,** 1:10000) and hand-stripped. Semen was individually collected and diluted (1 semen:3 medium) in a medium containing 10% dimethyl sulphoxide (DMSO) + 10% egg yolk + 80% extender (0,9% NaCl, NaCl 200 mM + tris (Saad) or 5,4% glucose). Diluted semen was then aspirated into straws (0.5 or 4.0 mL), placed inside nitrogen vapor vessel (CP 100, Taylor-Wharton; dry-shipper) at -170°C for 12-16 h, then plunged into liquid nitrogen. All straws were thawed in a water bath at 70°C for 3 sec (0.5-mL straws) or 15 sec (4.0-mL straws). Immediately after thawing, sperm motility was determined under light microscope, after activation with 1% $NaHCO_3$ and post-thawed semen was used for fertilization (pool of oocytes from 5 females) in a ratio of 4.5 x 10^7 sperm cells:oocyte. Eyed-egg rate was determined after 17 days of incubation (190ºC/day) in triplicate batches of eggs.

Results and Conclusion

There was no interaction (P>0.05) between extender and straw size. Highest sperm motility and eyed egg rates were observed when semen was cryopreserved in 0.5 mL straws compared to 4.0 mL straws. Semen cryopreserved in glucose or in NaCl produced higher motility rates, compared to semen cryopreserved in Saad solution. Semen cryopreserved in glucose produced higher eyed-egg rate, than semen cryopreserved in NaCl or in Saad solution (Table 1). Similar results were obtained by another study on cryopreservation of masculinized female rainbow trout semen (1).

Table 1: Motility (%) and eyed-egg rates (%) of semen cryopreserved in different extenders and in 0.5- or 4.0-mL straws, of masculinized females rainbow trout.

	Straw	Extenders			
	(mL)	NaCl	Saad	Glucose	Mean
motility	0.5	50±7	22±8	60±0	**44**[a]
	4.0	43±6	17±6	47±6	**36**[b]
mean		**47**[A]	**20**[B]	**54**[A]	
eyed-egg	0.5	33±5	16±6	46±4	**32**[a]
	4.0	25±4	13±6	30±4	**23**[b]
mean		**29**[B]	**15**[C]	**38**[A]	

Means followed by different superscripts (uppercase for lines and lowercase for columns) differ (P<0.05; Scott Knott).

Based on these results it can be concluded that semen of masculinized females can be successfully cryopreserved in a medium containing 5,4% glucose + 10% egg yolk + 10% DMSO, 0.5 mL straws, and exposed to -170°C in a dry-shipper for 12-16 h.

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Mlh1 deficiency results in sterility in male and aneuploid progeny of female zebrafish

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Introduction

Proper formation of chiasmata during meiosis I is necessary for subsequent spindle attachment and segregation of chromosomes in anaphase. The molecular mechanisms of meiosis are still largely unknown, but numerous genes are known to be involved, among which many DNA mismatch repair genes. One of them, *mlh1*, colocalizes with presumptive sites of crossing-over, although its exact action remains unclear. *Mlh1* mutant male and female mice are infertile, showing problems during meiosis and hypogonadism. Also *mlh1* mutant male zebrafish are infertile, but show increased testis weight; surprisingly, female mutants are fertile. Here, we studied the morphological and genetic basis of the absence of *mlh1* on zebrafish reproduction.

Materials and Methods

Mutant *mlh1* zebrafish were isolated by tilling after ENU-induced mutagenesis. To characterize the testicular phenotype, testes from 6 wild-type and 6 homozygous mutants were prepared for histomorphometric analysis. Mutant *mlh1* females were crossed with wild-type males and the progeny was genotyped, karyotyped, and its development was followed to adulthood.

Results and Discussion

Although male *mlh1* mutants are sterile and display arrest in spermatogenesis, they show – different from mammals – increased testis weight and GSI (~45%) due to an accumulation of primary spermatocytes (~150%) in prophase I. Metaphase I spermatocytes present abnormal meiotic figures, become apoptotic and eventually are phagocytised by Sertoli cells (Fig. 1). In contrast, females are fully fertile, but their progeny shows high rates of dysmorphology and mortality (~99%) within the first days of development (Fig. 2). This is caused by aneuploidy, resulting from meiosis I chromosomal missegregation. Surprisingly, from the ~1% progeny that develops normally, all are male, also show spermatogenesis aberrations (Fig. 3), and have a complete triploid genome, consisting of both sets of maternal and one set of paternal chromosomes. The frequency of triploid progeny (~1%) is much higher than expected for random chromosome segregation, suggesting that additional mechanisms exist to circumvent meiotic problems in oocytes.

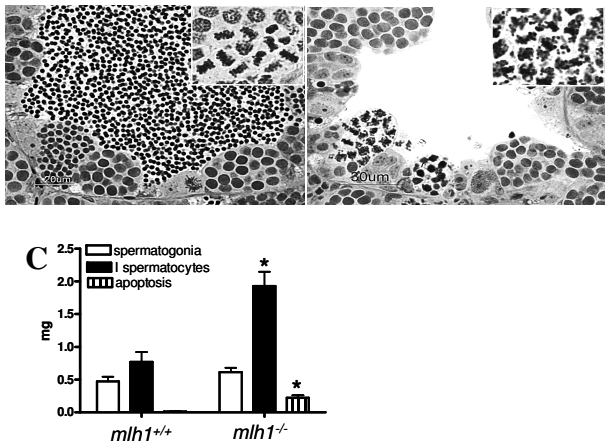

Fig. 1. **Testis histology and morphometry of *mlh1* mutant zebrafish**
A-B) Cross section of seminiferous tubule in *mlh1*$^{+/+}$ and *mlh1*$^{-/-}$ zebrafish, showing full spermatogenesis in wild-type (A) and arrested at metaphase I in mutant (B). Insets show cells in first meiotic division. C) The mutant shows a significant increase in amounts of spermatocytes and apoptotic cells, but not of spermatogonia.

Fig. 2**. Progeny from *mlh1*$^{-/-}$ females is severely aneuploid**
At 24 hpf embryos are already strongly malformed.

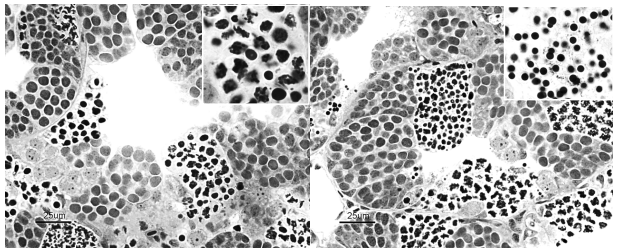

Fig. 3**. Testis histology of triploid *mlh1* zebrafish**
A-B) Cross sections in infertile (A) and fertile (B) triploid *mlh1* zebrafish.
Insets show second meiotic division (A) and spermatozoa in the efferent ductules (B).

Conclusion

We show here that the requirement for *mlh1* during male meiosis in zebrafish is similar to that in mammals. Although females are fertile, aneuploid offspring and the high incidence of triploid offspring demonstrates that important features of meiosis remain to be unravelled.

Alterations on the reproductive system of male rats exposed during gestation and lactation to Solanum lycocarpum fruits

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Introduction

Solanum lycocarpum (Solanaceae), popularly known as "fruta do lobo", is a native plant very common in the Brazilian savanna. Solamargine and solasonine are two major glycoalkaloids found in the *S. lycocarpum* fruit and their steroidal aglycone, solasodine, present chemical structure similar to sexual hormones and can penetrate the cell membrane by simple diffusion (1). If solasodine can penetrate the cell membrane, once inside the placenta, and consequently inside the fetuses, this steroidal compound might possibly disrupt this important hormonal role and may promote morfophysiological alterations that possible impair reproduction at adult age. This study was undertaken to evaluate the possible effects of *S. lycocarpum* fruit on the reproductive system of male rats exposed during gestation and beginning of lactation.

Materials and Methods

Wistar pregnant rats were divided into two groups: control and experimental (n=7/group). The experimental dams received from gestation day 06 (GD 06) to post-natal day 07 (PND 07) chow mixed with 10% of dry milled unripe *S. lycocarpum* fruits. On PND 01 the litters were organized in groups of eight pups each, four males and four females. At PND 60 and PND 90 one male per litter were euthanized. Testes and epididymis were weighed and specimens were fixed in 10% formaldehyde for histopathologic evaluation. The intact right testis and epididymis were employed for spermatic morphology study.

Results

At PND 60 and PND 90 the experimental males presented important vacuolization and reduced number of germinative cells (spermatogonias) in seminiferous tubules. A significant decrease in the cauda sperm transit time (control: 7.57±0.17; experimental: 5.97±0.37) was observed in the epididymis of the experimental males at PND 90, possibly promoting the reduced spermatic storage observed in the epididymis of these animals (control: 192.83±6.09; experimental: 142.54±5.73).

Discussion

If the steroidal alkaloid solasodine penetrate the placental and hematoencephalic barriers, possible act at the central nervous system of the fetuses during the organizational period, critical for sexual differentiation of the brain and sensitive to hormonal effects. It is well known that estrogenic compounds promotes degeneration of the testis and alterations in sperm production and transit time as consequences of perinatal exposition (2). Solasodine possible act as estrogen, due to the important vacuolization observed in the testis and to the decreased sperm transit time observed in the cauda/corpus epididymis of the experimental males at PND 90. However, previous study (3) showed that the fertility capability of these animals was not impaired. We can conclude that the alterations observed in this study were not sufficient to promote fertility impairment.

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Reproductive disorders in male rats exposed to the insecticide Fenvalerate

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Introduction

Fenvalerate is a synthetic pyrethroid insecticide widely used in agriculture to control a variety of insects. Although it is considered to be of low acute toxicity to mammals, studies have showed that pyrethroids can have estrogenic activity and can act as endocrine disruptors, causing important reproductive impairment in males. However, little is known about reproductive effects of fenvalerate; thus, the objective of the present study was to investigate the estrogenic activity and the effects of fenvalerate on the reproductive system and fertility of adult male rats.

Materials and Methods

Adult male rats received, for 30 consecutive days, by gavage, 40 mg/kg/day (2/5 of LD50) of fenvalerate (FEN) (96.8% purity) (1). The control group (CON) received only corn oil, in the same experimental conditions. At the end of the treatment the following parameters were analyzed: body weight; absolute weights of reproductive organs, liver and kidneys; plasma testosterone levels; germ cell counts in the testis and epididymis; sperm morphology; fertility tests by natural matings and artificial insemination *in utero* (AI); sexual behavior; analysis of testis and epididymis at the optical and electron microscopic levels, and evaluation of estrogenic activity of fenvalerate by the uterotrophic test. Fenvalerate residues were quantified using HPLC in reproductive and vital organs. Results, expressed as mean ± SEM, were considered statistically different at $p<0.05$ (*).

Results and Discussion

The treatment with fenvalerate de creased the absolute weights of testis and epididymis (Testis (g): CON=1.91±0.06, FEN=1.64±0.05*; Epididymis (mg): CON=704±23.75, FEN=631±13.99*). It was also verified that treated rats presented a reduction in daily sperm production (10^6/testis/day: CON=37.24±2.08; FEN=25.72±1.49*) and in cauda epididymal sperm number (10^6/organ: CON=203.8±15.4, FEN=131.6±9.64*). On the other hand, the treatment did not altered plasma testosterone levels as well as sexual behavior and fertility after natural matings and AI. The results of fenvalerate quantification revealed, in the treated group, high concentrations of insecticide residues in the epididymis, testis, brain and liver. The histological aspects of the testis and epididymis were similar in both experimental groups. Moreover, fenvalerate, at the doses tested, did not present estrogenic activity in vivo. It was concluded that fenvalerate, in these experimental conditions, was retained in reproductive organs and was spermatotoxic, since it reduced sperm production and storage, but this alteration was not sufficient to compromise fertility by virtue of the high reproductive efficiency of rodents.

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Anabolic/androgenic response of reproductive organs from male rats submitted to different protocols of training

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Introduction

Physical exercise can modify the pattern of release of the sexual hormones in the male. This alteration may be interesting to obtain a more effective response to training. The aim of this study was to evaluate the effects of the two protocols of exercise (continuous and alternated) and the response to an anabolic androgenic steroid (AAS) on accessory reproductive organs.

Materials and Methods

At 75 days of age, male Wistar rats were divided into sedentary (S) and trained (T) and were treated with vehicle (V) or nandrolone (N), 5mg/kg, 2/week, during 6 weeks. Training was performed by jumping into water (4 sets, 10 repetitions, 30-second rest, 50% to 70% body weight load, for 5 consecutive days/week (T5), or 3 days/week (T3) with at least 1 day of rest between each training. Body weight (BW) was evaluated at beginning and end of treatment. The accessory reproductive organs - levator ani muscle (LAM), seminal vesicle, and seminal vesicle secretion - were isolated and measured. The ANOVA and a posteriori test of Bonferroni was performed to compare average ± SEM among groups, $p<0.05$.

Results and Discussion

All results are shown in table 1. The BW at the end of treatment showed significant reduction in the animals that were submitted at the two protocols of exercise as well as nandrolone treatment when compared to SV. The variation of the body mass from beginning until the end of the treatment was smaller in both groups that were submitted to exercise plus nandrolone. The seminal vesicle mass increased in T3N in relation to SV, SN, and T5V. Both exercise protocols reduced the secretion in the seminal vesicle while the nandrolone restored this values. The response of the LAM to the Nandrolone was significantly increased in the T5N when compared to T5V.

Table 1. Effects of the daily exercise, exercise with one day of rest and AAS response in BW and reproductive organs.

	BW (g)	BW Variation during treatment (g)	Seminal vesicle (mg)	Seminal Vesicle Secretion (mg)	LAM (mg)
SV N=5	525.65 ±16.18A	106.65 ±18.29A	230.38 ±13.24A	571.25 ±42.40A	381.70 ±18.41ABC
SN N=5	461.74 ±9.35B	79.08 ±5.03AB	231.79 ±11.04A	633.16 ±34.56A	421.63 ±24.05BC
T5V N=5	466.59 ±17.05B	56.14 ±12.61AC	189.07 ±12.37A	383.58 ±30.95B	309.60 ±8.36A
T5N N=5	385.87 ±7.23C	9.13 ±8.85C	253.97 ±19.35AB	558.93 ±31.10A	414.22 ±25.37BC
T3V N=5	425.67 ±16.68BC	26.58 ±10.60 BC	252.48 ±6.38AB	262.48 ±47.11B	314.52 ±35.83AB
T3N N=5	396.74 ±14.67C	8.66 ±15.58C	332.88 ±32.84B	641.78 ±150.60A	318.40 ±30.41ABC

Different letters indicate statistical differences among the groups ($p<0.05$) by Bonferroni test.

These results showed different responses according to the protocols of exercise in terms of androgenic/anabolic activity in relation to the sedentary group.

Rats from training with rest between each session showed a significant reduction in body weight in relation to sedentary animals, indicating a more effective action in terms of reducing corporal mass than rats submitted to continuous training. The mass of androgenic/anabolic organs from rats submitted to both training did not show differences, while nandrolone reverted the quantity of secretion in training animals probably by compensation in terms of testosterone reduction induced by intensive training. More studies will be performed to prove this hypothesis.

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Neonatal androgenic supplementation: later effects on skeletal muscle and response to an anabolic androgenic steroid

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Introduction

In mammals, at birth the testosterone released from the testis is metabolized in the hypothalamus by aromatase enzyme to estrogen, which is responsible to warrant the masculinazation and defeminization of the SNC (2). The aim of this study was to supplement Wistar male rats at birth with testosterone propionate (TP) to evaluate the development and later effects in the body weight and in the skeletal muscle in the absence and presence of treatment with an anabolic androgenic steroid (EAA), in supraphysological doses commonly abused by athletes (1).

Materials and Methods

At birth, Wistar male rats received TP, 0.1 □g, sc, (SP) or vehicle (CT) (2). The body weight (BW) was evaluated at 22 and 75 days old. At 75 days animals received nandrolone decanoate (N), 5 mg, 2/week, during 6 weeks or vehicle (V). At the end of the treatment the BW of the animals were measured. In this day they were killed and the Soleus (SOL) and extensor digitorum longus (EDL) muscles were isolated and the muscular fiber area was measured. The Student´s "*t*" test was performed to compare average±SEM between two groups and ANOVA and a posteriori test of Bonferroni was performed to compare 4 groups, $p<0.05$.

Results and Discussion

At 22 days, there was not any difference in BW between groups (unshowed data) but at 75days BW(g) of the SP was less than CT (CT 395.13 ±4.16, n=32, SP 369.47±5.21** n=28, p<0.01). Despite the fact that after the treatment with nandrolone all groups showed lower BW than CTV (CTV 525.65±16.18 n=8(A), CTN 461.74±9.35 n=9(B), SPV 458.60±13.73 n=7 (B), SPN 429.15±7.03 n=8(B), $p<0.0001$), only SPN showed reduced in the enhance of BW in this period in relation CTV (CTV 106.65± 18.29 n=8(A), CTN 79.08±5.03 n=9(AB), SPV 92.69±7.63 n=6 (AB), SPN 56.53±17.36 n=8(B), $p<0.05$). Though there weren't significant differences in the fiber area (□m^2), SOL and EDL muscles showed differences in response to treatments. The EDL showed a slightly enhance in response to neonatal supplementation with testosterone and in nandrolone treatment (CTV 4053.93±717.06 n=3, CTN 4855.41 ±715.42 n=5, SPV 4546.02±679.17 N=4, SPN 5182.97±219.74 n=4, $p>0.05$), while SOL didn´t show a pattern of change (CTV 6099.17±311.37 n=3, CTN 5775.19 ±327.70 n=5, SPV 5649.75±492.83 N=4, SPN 5694.46±656.16 n=4, $p>0.05$). The reduction in BW of SP group at 75 days could be related to a more anabolic activity in these animals. Due the fact that during treatment with EAA only SPN showed significant reduction in the enhancement of BW in relation to CTV, we believe that there was more proliferation of androgens receptors in these animals. The different response of the two muscles at the treatment can be an indicative of this hypothesis. The EDL have predominantly more fast twitch fibers, that respond more to EAAs, while SOL have more slow fibers, that respond less to it. More studies to evaluate the response of exercises will be performed.

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Effects of mild calorie restriction on fertility, testosterone levels and testicular gene expression in mice with altered GH/IGF-I axis

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Introduction

Male reproductive functions are controlled by the hypothalamus which stimulates the pituitary to secrete gonadotropins, that will in turn act on the gonads. The hypothalamic-pituitary-gonadal axis, the somatotropic axis and the nutritional status are interrelated. Calorie restriction (CR) extends lifespan and the reproductive period, but supresses the axes mentioned above. Most of CR studies apply restriction of 40-50% that compromise reproduction. Thus, we hypothesized that a milder CR (20%) would not be deleterious for reproduction and that CR effects may be influenced by alterations of the somatotropic axis. To test this hypothesis, we evaluated the effects of 20% CR on fertility, testosterone levels and testicular gene expression in mouse lines in which growth hormone (GH) signaling is blocked (GH receptor knockout GHR-KO mice), normal or increased (transgenics MT-bovine(b)GH and PEPCK-bGH mice).

Material and Methods

Male mice were kept under 12h dark/12h light, 22 ± 2°C, with free access to water. Feeding groups were as follow: *ad libitum* (AL) and CR (20% less food, compared to AL). **Exp. 1 - CR starting earlier in life:** GHR-KO (KO) and normal (N) littermates: CR since 2-3 months of age, fertility test at 6 months, sacrifice at 8-9 months; PEPCK-bGH (Tg) and N: CR since 3 months, fertility test at 7 months, sacrifice at 11 months. **Exp. 2 - CR starting later in life:** GHR-KO (KO) and N: CR since 7 months, fertility test 11 months, sacrifice at 13 months; MT-bGH (Tg) and N: CR since 8-9 months, sacrifice at 11-12 months. Testosterone levels in plasma and in testis homogenates were measured by radioimmunoassay. Real-time reverse-transcriptase polymerase chain reaction was carried out using testis total RNA to quantitate mRNA expression of insulin-like growth factor (*IGF1*), aromatase (AROM), androgen receptor (*AR*), luteinizing hormone receptor (*LHR*), follicle-stimulating hormone receptor (*FSHR*), 3β-hydroxysteroid dehydrogenase/isomerase (*HSD3*) and cytochrome P450c17 (*CYP17*).

Results and Discussion

Mild (20%) CR did not affect fertility, only phenotype effect was evident, since KO and Tg performed poorer than N. Testosterone levels in plasma and in testes were highly variable among individuals, no significant effect of diet or phenotype was noted. In GHR-KO line Exp.2, testicular AROM mRNA levels wee higher in KO animals compared to N. This may be leading to a higher rate of conversion of testosterone to estradiol in these mutants. *AR* mRNA levels were increased in the KOs (Exp.2), compared to N, and by CR in the MT-bGH line. This may represent a compensatory mechanism, attempting to maintain normal function in the testis. The CR increased *FSHR* gene expression in the GHR-KO line (Exp.2), perhaps representing a response to a putative reduction in circulating FSH levels in this group, since it has been demonstrated that FSH negatively regulates the levels of its own receptor (1). No diet effect was observed in testicular expression of the genes *IGF1*, *LHR*, *HSD3* and *CYP17*. Altered activity of the GH/IGF-I axis had major impact on the parameters analyzed. This preliminary study of selected parameters of reproductive function encourages speculation that mild regimens of CR can produce longevity benefits without the impairing reproduction.

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Altered reproductive parameters in obese adult male rats

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Introduction

Obesity is growing rapidly in male population, and it has been associated with lower sperm counts and hormonal imbalances (1). Due to the difficulty to study obesity-induced reproductive complications in men, experimental models have been used. According to one of these models neonatal rats are treated with monosodium glutamate (MSG) becoming obese through the development. The objective of this work was to evaluate reproductive parameters of male rats treated neonatally with MSG.

Materials and Methods

Obesity was induced through MSG administration (4mg/g body weight), n=08, subcutaneous route, in neonatal period of male Wistar rats (postnatal days 2, 4, 6, 8 and 10). Rats of the control group (CON, 2.0% NaCl body weight), n=10, received saline solution at the same postnatal days (2). In adult age (120 days), the following parameters were analyzed: Lee index, a parameter of obesity (cube root of body weight (g) x 10 / naso-anal length in mm), body weight, absolute and relative weights of reproductive organs (testis, epididymis, prostate and seminal vesicle), germ cell counts, plasma testosterone level and testicular and epididymal histopathology. Results, expressed as mean ± SEM, were considered statistically different at $p<0.05$ (*).

Results and Discussion

Obesity was confirmed by a Lee Index of 0.398. Male obese rats presented a significant reduction in absolute and relative weights of the testis, epididymis, prostate and seminal vesicle, as well as in plasma testosterone level. Moreover, experimental obesity lead to a decrease in sperm counts, as follows: number of mature spermatids in the testis (10^6/organ; CON = 262.99 ± 9.88; MSG = 134.70 ± 7.91*), daily sperm production (10^6/testis/day; CON = 43.13 ± 1.62; MSG = 21.90 ± 1.24*), number of sperm in the caput/corpus epididymidis (10^6/organ; CON = 172.15 ± 5.65; MSG = 76.43 ± 5.60*), number of sperm in the cauda epididymidis (10^6/organ; CON = 335.16 ± 37.46; MSG = 126.13 ± 22.12*), sperm transit time in the cauda epididymidis (days; CON = 7.9 ± 0.46; MSG = 5.7 ± 0.84*). The histological aspects of the testis and epididymis were similar in control and MSG-treated group. The present study, besides validating the model of induction of obesity by monosodium glutamate administration in neonatal life of rats, showed that obesity provoked reproductive disorders in the male reproductive tract. More studies are necessary to investigate the physiopathological mechanisms involved in the reproductive alterations induced by obesity.

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Impairment of reproductive development of male rats exposed to a hyperglycemic intrauterine environment

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). A diabetic intrauterine environment may interfere with fetal development, causing disturbances in adulthood as cardiovascular disease and insulin resistance (2). This study aimed to evaluate the reproductive development of adult male rats exposed to a hyperglycemic intrauterine environment.

Materials and Methods

In order to experimentally induce diabetes female rats (90 days old) received an intravenous injection of Streptozotocin (40mg/Kg body weight). A group of 24 rats, assigned as control group, received citrate buffer, in the same experimental conditions. Seven days later glicemy was measured and rats presenting concentrations of 200 mg/dl or higher were considered severely diabetic and included in the study (n=59). Then, all females were mated overnight with normal rats. Every morning, vaginal smears were examined for the presence of sperm, which was considered day 0 of gestation. After delivery, sexual development of the male pups was assessed by the determination of the time, in days, of testicular descent and balano-preputial-separation, indicative of the onset of puberty, sperm counts, testosterone plasma levels, histopathology of the testis and epididymis, and number of Sertoli cells. Results are expressed as means ± SEM, and the differences were considered statistically significant at $p<0.05$.

Results and Discussion

The body weights of the pups from diabetic mothers on postnatal day 3 were significantly lower that pups of control rats (6.29±0.37; 9,80 ± 0.43, in grams, respectively), evidencing intrauterine growth retardation. The onset of puberty was delayed in the offspring of diabetic dams, as shown by the times of testicular descent and preputial separation (22.89 ± 0.44; 45.13 ± 0.36, respectively) when compared with controls (19.93 ± 0.19; 42.83 ± 0.39). Plasma glucose levels (mg/dl) of 90 days old male rats were about the same in both groups (103.00 ± 0.39, offspring of diabetic rats; 103.00 ± 2.37, offspring of control rats). Sperm counts in the testis and caput/corpus epididymis were similar in both groups. However, in the cauda epididymidis, the sperm number was significantly decreased in the offspring from diabetic mothers (137.80±10.55) when compared with control rats (188.08±5.34). The same occurred in relation to the sperm transit time (in days) in this region of the epididymis (4.09±0.26), when compared with control rats (4.95±0.15). There were no differences in testosterone plasma levels, testicular and epididymal histological aspect, and number of Sertoli cells**.** This study has shown that the exposure to a hyperglycemic intrauterine environment impairs the reproductive development of male rats.

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Evaluation of the mechanisms causing infertility in hyperglycemic rats

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Introduction

Hyperglycemia induced by diabetes is usually related to some kind of sexual dysfunction in men as well as in experimental animals. One of the most used methods to chemically induce hyperglycemia in rats is the administration of Streptozotocin (STZ). Previous studies in our laboratory (1) demonstrated reduced fertility and impairment of other reproductive parameters in STZ-induced diabetic rats. The aim of the present study is to evaluate possible mechanisms involved in these reproductive disorders, with special reference to the role of testosterone.

Materials and Methods

Adult male rats (90 days-old) were divided into 3 groups: hyperglycemic (n=7) whose rats received a single injection of STZ (40mg/Kg body weight); hyperglycemic plus testosterone (n=9), whose rats received a testosterone-filled subcutaneous implants 7 days after STZ injection; control (n=10), whose rats received only the vehicle (citrate buffer). Three weeks after STZ injection the rats were sacrificed and materials were collected for the following analysis: sperm counts in the testis and epididymis, body and reproductive organs weights, and *in vitro* vas deferens isometric contractions.

Results and Discussion

While prostate and seminal vesicle weights were diminished, testicular weight was increased in STZ-treated rats. Testosterone replacement recovered these parameters to normal values. Testicular sperm counts showed no differences between experimental groups. On the other hand, there was a decrease in the sperm number in the cauda epididymis in both groups injected with STZ. Vas deferens contractility *in vitro* analysis demonstrated that diabetic animals, with or without testosterone replacement, are more sensitive to noradrenaline than control animals, possibly because of a lower production of the neurotransmitter by the central nervous system or a greater amount of recaptation of it at the neuromuscular junction region, which would contribute to the erectile dysfunction caused by diabetes.

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Effects of paternal cigarette smoke on fertility and sexual behavior

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Introduction

Some investigations have proposed a detrimental effect of smoking on sperm concentration, sperm motility, and percentage of morphologically normal spermatozoa. The adverse effects of cigarette smoke on Leydig cell function in animals have been also reported. The aim of the current study was to evaluate, in an animal model, the effects of paternal exposure to cigarette smoke on fertility and sexual behavior.

Materials and Methods

Male Wistar rats at 6-week-old were divided into: control group (G1; n=10) and cigarette smoke-exposed for 9 weeks group (G2; n=10; nicotine 0.6 mg). Rats in G2 were exposed to 1260 cigarettes during the 63-day period, at the rate of 20/day. After finishing the treatment the males, were housed in a large cage with fertile untreated female rats (2 females/male). Vaginal smears were examined daily for the presence of spermatozoa, until every female was pregnant or until day 15. On day 20 of pregnancy, the rate of pregnancy, the number of implantation sites, alive fetuses, dead fetuses, reabsorption sites, and corpora lutea were recorded. The rates of pre- and post-implantation were also quantified. For evaluation of sexual behavior the male rats received Testosterone propionate (1 mg/day, sc) 3 times a week, during 2 weeks. The sexual behavior was observed during 30 min and the parameters were recorded.

Results and Discussion

There was reduction in the pregnancy rate of normal females mated with cigarette smoke male rats when compared with control males (Table 1). In spite of this, the data used to estimate fertility rates showed that females mated with cigarette smoke males exhibited a significant increase in pre-implantation loss (G1= 0(0.68-8.66); G2= 8.33(3.73-30.98)* $p<0.05$. The post-implantation loss was not significantly altered. Data from male sexual behavior evaluation are shown in Table 2. The exposure cigarette smoke induced a delay in the latencies to the first mount and number of postejaculatory intromission. No alteration was observed in the total number of ejaculations.

Table 1. Fertility capacity observed in pregnant control female rats mated with control and cigarette smoke male rats

male rats	tot number in control female rats (2 females/male)				
	pregnant females	corpora lutea	alive fetuses	reabsorption sites	implantation sites
control (n=10)	20 100%	235 (11.75)	207 (10.35)	235 (11.75)	235 (11.75)
cigarette smoke (n=10)	15* 75%	195 (13.06)	139 (9.26)	235 (11.75)	235 (11.75)

Values in parentheses as mean number/female *$p<0.05$ by Fisher's exact test

Table 2. Effects of exposure cigarette smoke on sexual behavior of male rats

Parameters	Groups	
	control N=10	cigarette smoke N=8
Latency to first mount (s)	7 ± 4.35	51.57 ± 11.43*
Number of mounts with intromission	2.50 ± 0.50	2.60 ± 0.68
Latency to first intromission (s)	44.70 ± 13.13	56.17 ± 7.29
Number of intromission	24.60 ± 2.52	21.50 ± 4.12
Latency to first ejaculation (s)	488.60 ± 56.99	507.40 ± 156.42
Number of postejaculatory intromission	38.9 0± 5.31	18.83 ± 4.11*
Number of ejaculation	3.10 ± 0.23	2.53 ± 0.62

Data are means ± S.E.M. *$p<0.05$ Student's *t*-test.
Two animals from cigarette smoke were not able show copulatory behavior

These results suggest that cigarette smoke may affect the sperm fertilizing process. This effect is probably mediated through damage in spermatogenesis and/or direct influences on spermatozoa as well as due to a decrease libido and/or erectile dysfunction.

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Sexual behavior evaluation of male wistar rats treated with *Cheiloclinium cognatum* (Meirs.).

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Introduction

Bacupari, *Cheiloclinium cognatum* is a Brazilian "Cerrado" plant from the Hipocrateaceae family. In same classification system this taxonomical family is included as Hippocrateoideae sub-family of Celastraceae. This one has several species that have shown male antifertility effects, such as *Tripterygium wilfordii, Catha edulis* and *Austroplenckia populnea*. Based on chemotaxonomy characteristics *C. cognatum* was selected for this study with the aim to evaluate the plant effects on male sexual behavior.

Materials and Methods

Adult male rats (350 g body weight) were treated with dichloromethanic extract made from *C. cognatum* leaves, 500 mg/kg/day, orally, for 30 days (n=9). Soy oil was administered to the control animals (n = 9). At the endl of experiment the rats were submitted to sexual behavior evaluation. Each animal was placed in a wooden cage with frontal glass for 10 min before introducing a sexually receptive female that received hormonal treatment with estradiol cypionate 24 hours before the evaluation.

The following measures were recorded: mount, intromission and ejaculation latencies; number of mounts and intromissions preceding the first ejaculation; intromission latencies post-ejaculation; and total number of ejaculations. If the male did not mount within the next 10 minutes, the test was considered negative. Mann-Whitney non-parametric test was used for statistics assays of the results ($p<0.05$).

Results and Discussion

Treated animals showed an increase of latency for first mount and in the mount number before first ejaculation. However these results did not affect the sexual potency, because the number of ejaculations, and all the other parameters, were similar between the groups (table 1).

Table 1. Sexual behavior evaluation from animal treated with dichloromethanic extract made from *C. cognatum* leaves. 500 mg/kg/day, orally; during 30 days, and control group that received soy oil as vehicle. Data are reported as mean ± SEM. * $p<0.05$

Parameters	Control	Treated
Latencies for (s)		
First Mount	12.7 ± 5.2	32.6 ± 10.4 *
First Intromission	45.3 ± 19.0	67.0 ± 20.0
First ejaculation	538.0 ± 91.7	716.0 ± 112.5
Intromission PE	781.0 ± 94.7	980 ± 113.9
Number of		
Mount BE	2.0 ± 0.8	4.0 ± 0.9*
Intromission BE	16.0 ± 3.1	13.0 ± 2.4
Ejaculations	3.0 ± 0.3	3.0 ±0.4

PE: Post-ejaculation; BE: before first ejaculation;
(s): seconds

The chemotaxonomical approach permits the data presented in this study to be compared with other plants of the same botanical family. Treatment with *A. populnea* produced an increase of sexual interest of the animals, without affecting sexual potency (1). Studies with *T. wilfordii* did not show alterations in potency and libido in men who consumed this plant species for treatment of rheumatic arthritis (2). However, the use of *Catha edulis* was associated with an increase of men's libido without increase of sexual potency (3). Different of the other plants, *Cheiloclinium cognatum* affect the beginning of sexual behavior, although did not affect sexual potency of animals.

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Fertility evaluation of male rats treated with *Cheiloclinium cognatum* (Meirs.) A. C. Sm.

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Introduction

Bacupari, *Cheiloclinium cognatum* is a Brazilian "Cerrado" plant from the Hipocrateaceae family. Previous studies showed an antispermatogenic effect of dichloromethanic extract made from *C. cognatum* leaves. Adult male *Wistar* rats treated during 35 days with 500 mg/kg./day showed a significant reduction of sperm daily production (DSP), 30% less than control group (1). So, the aim of this study was evaluate the effect of *Cheiloclinium cognatum* on male rat fertility.

Materials and Methods

Adult male rats (weighing 350 g) were treated with dichloromethanic extract made from *C. cognatum* leaves, 500 mg/kg/day, orally, for 30 days (n=9). Soy oil was administered to the control animals (n=9). At the end of the experiment the rats were mated with virgin females (2 female:1 male). The day when spermatozoa were found in the vaginal smear was considered day 0 of gestation. On the 20th day of pregnancy, all females were anaesthetized under ethyl ether and killed. After removing the uterine horns, the number of implantation sites, live fetuses, dead fetuses, resorption sites, and corpora lutea were recorded. The proportions of females with pre- and post-implantation losses were quantified, and the mean rates calculated. The pre-implantation loss was calculated as the difference between the number of corpora lutea minus implantation sites x 100/number of corpora lutea, and the post-implantation loss as the number of implantation sites minus live fetuses x 100/number of implantation sites. Sexual ratio was calculated by the number of male pup/female pup. Mann-Whitney non-parametric test was used for statistical analyse assays of the data ($p<0.05$).

Results and Discussion

Fertility parameters showed an increased on pre-implantation loss in female fecundated by treated male with *C. cognatum*. The placenta weight was reduced in compare with control group (see table 1).

Table 1. Fertility parameters from animal treated with dichloromethanic extract made from *C. cognatum* leaves. 500 mg/kg/day, v.o.; during 30 days, and control group that received soy oil as vehicle. Data are reported as mean ± SEM. * $P<0.05$

Parameters	Control	Treated
Female fecundated	9	10
Live fetuses	77	87
Dead fetuses	0	0
Sexual ratio	1± 0.0	1 ± 0.0
Pup body weight	2.6 ± 0.3	2.4 ± 0.3
Placenta weight	0.4 ± 0.0	0.4 ± 0.01*
Pre-implantation loss	9. 9± 2.8	20.9±4.6*
Post-implantation loss	10.7± 2.0	10.8±2.8

Abnormal sperm production or minor sperm viability can explain the increase of pre-implantation loss (2,3). *Cheiloclinium cognatum* reduced DSP, however it did not affect sperm morphology (1). Thus, the possibility of a disturbance in the maturation of spermatozoa should be considered. The effect of *C. cognatum* on spermatogenesis can affect spermatozoa maturation and might explain the reduced fertility observed in treated males.

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Sterilization of dogs with intratesticular injection of a zinc–based solution – clinical and semen evaluation

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Introduction

Chemical sterilization can be a solution to control pet overpopulation and/or to owners who search for non-surgical methods to interrupt male reproduction (1). A zinc-based solution that is pH neutralized in BioRelease Technologies physiological vehicle (B.E.T.Labs, Lexigton, Kentucky, USA) was developed as a chemical sterilant for intratesticular injection in male dogs. The aim of this study was to evaluate the clinical safety of a single intratesticular injection of zinc-based solution, in causing sterility of male dogs evidenced by semen and clinical analysis.

Materials and Methods

Fifteen mongrel dogs were assigned to 3 groups (5 dogs/group). Group 1, the control placebo, was injected with saline solution. Group 2, which consisted of animals ranging from puberty to 1 year old and Group 3, animals ranging from 2 to 4 year old, were injected with zinc-based solution, in six different doses (0.2 to 1.0mL). The dosage was based on testicular width (10 to 27mm), determined by a caliper. General attitude, ability to walk, scrotal alteration (pain, swelling, dermatitis) and rectal temperature were evaluated on days 1 to 3 post-injection and weekly until the end of the experiment. Semen analysis was performed on day 0, 4 and 7 post-injection and weekly until the end of the experiment. Each dog had blood samples taken for hematology, renal and hepatic function at days 0, 2, 8 and monthly until orchiectomy.

Results and Discussion

No biting/licking was recorded after the injection in the treated animals. Transient testicular swelling was reported in the treated animals in the first three days after injection. Scrotal pain when the testis were manipulated was not observed in any animal of either group. By one month, the testicles in the treated animals decreased 7 to 10% in size. One dog (Group 3) developed mild scrotal irritation and dermatitis 30 days after the injection. Housing conditions (wet, cement flooring) were also considered a contributing factor in the development of the lesion. The use of zinc-based solution, which is neutral in pH and is part of the composition of the male reproductive fluid and tissue (2), is thought to contribute to the absence of local reaction reported in this investigation. At 60 days post-injection, 8 of 10 of the treated animals were azoospermic. The two non azoospermic dogs (Group 3) had live sperm in the ejaculate and both animals were found to be oligospermic ($<10x10^6$ spermatozoa/ml). There were no significant differences between treated and control groups for the clinical parameters evaluated and values for the parameters were within reference ranges for domestic dogs. In conclusion, intratesticular injection of zinc-based solution is effective in impaired spermatogenesis in dogs without causing clinical adverse effects.

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Growth rates of follicle membrana granulosa in goats

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Introduction

In mammals, studies on membrana granulosa in ovarian follicles are important to characterize follicular dynamics and for the success of reproduction technologies. Proliferation of granulosa cells is mainly responsible for follicular development in small and large follicles. Although morphologic structure and growth rate of membrana granulosa were widely investigated in bovine (1), there is a lack of data in goats.

Materials and Methods

Five cycling crossbred Saanen x local goats of similar ages were used. In all animals estrus was synchronized using 45mg FGA vaginal sponge for 11 days, 300IU eCG and 50 µg cloprostenol 48 h prior to sponge removal. One ovary was removed by unilaterally ovariectomized and fixed in Bouin's solution. Immediately colchicine (1 mg/kg body weight i.v.) was administrated. Two hours after colchicine treatment goats were sacrificed and the remaining ovaries were also fixed**.** Ovaries were dehydrated in ethanol and embedded in paraffin wax. Every 5th section sliced (8 µm of thickness) was stained with periodic acid Schiff (PAS) and Harris´ hematoxylin. The follicles were classified on the basis of the size: < 0.5 mm, 0.5 – 1 mm and > 1 mm. In twenty follicles of each class prophase and metaphase figures were counted and granulosa cells were classified in three types according to (1): A) columnar cells, basal cells adjacent to the basal lamina; B) rounded cells, intermediate position and c) flattened cells, cells near to antrum. The mitotic index was determined as described by (2). Data was analyzed by the GLM procedure of SAS (SAS, Inc., USA). Comparison between means was performed by the Duncan test. Values were expressed as mean ± SEM

Results and Discussion

Follicles between 0.5–1 mm exhibit a higher ($P < 0.05$) mean mitotic index (3.04 ±0.53 %) when compared to < 0.5 mm (0.95 ±0.12 %) or > 1 mm (0.95 ±0.19 %) follicles. The mitotic index for the different classes of follicles in ovaries treated with colchicine shows a pattern similar to that reported for the cow (3) and for the ewe (4). Decrease of growth rate in this ultimate class probably was caused by large heterogeneity of size analyzed (1 to 2.7 mm). Literature reported a significant reduction of mitotic index for follicle with 1.5 mm of diameter (3) (4). Findings showed that in opposite follicular classes (< 0.5 and > 1 mm), growth rates were similar ($P > 0.05$) between morphologic types cells of membrana granulosa. Mean of mitotic index was 0.08 ± 0.02 % and 0.04 ± 0.01 % respectively. By contrast higher value ($P < 0.05$) of growth was observed in the follicles from 0.5 to 1 mm for columnar (0.84 ± 0.12 %) and rounded (0.84 ± 0.10 %) shaped cells. In this follicular class flattened cells of granulosa layer show a lower ($P < 0.05$) mitotic index (0.23 ± 0.10 %). Collectively these results demonstrated that proliferation of membrana granulosa is subordinate to the follicular size and exhibit evident regional differences across the membrane granulosa. In goats as reported in bovine (1) morphologic phenotype of granulosa cells reflects their differentiation levels and dynamics of the follicles.

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Plasmatic levels of oestrone sulphate in estrous cycle of young mares

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Introduction

Some attempts to predict the time of ovulation through estrogens concentrations was made. The peak of oestrone sulphate can be detected between 24 to 48 hours before ovulation, starting to decline at moment of ovulation until 24 hours later (2). The accuracy of oestrone sulphate to predict the time of ovulation is controversy and some authors consider its level constant during all estrous cycle (3). The objectives of the present study were to characterize the hormonal profiles of oestrone sulphate in relation to day of cycle and correlate the levels of oestrone sulphate with ovulation time.

Materials and Methods

Nine BH (Brazilian Jump Horses) young mares between 12 and 24 months of age and weighting 370 to 430 kg were used. They were maintained at the Experimental Station of Zootechny stalls, at Colina (20º43'05"S, 48º32'28"W), SP, Brazil. The ovarian activity was evaluated by using a teaser stallion and by ultrasound exam using the Scanner 480 VET (Pie Medical, Holand) equipped with a 5MHz, real time B-mode, linear array and rectal transducer. Blood samples of all mares were collected daily during a complete estrous cycle, for hormonal analysis, by RIA, of progesterone (kit of DPC, Diagnostic Products Co., Los Angeles, CA, USA, Coat-A-Count), oestrone sulphate and estradiol (Kit DSL Diagnostic Systems Laboratories, Inc., Texas, USA). The inter and intra-assays coefficients were < 20%. Repeated measures ANOVA with Tukey-Kramer multiple comparisons post test and linear regression were performed using GraphPadInstat V 3.00 (1).

Results and Discussion

The basal evels of oestrone sulphate varied from 0.15 to 0.81ng/mL, with mean±S.E.M.= 0.43±0.02ng/mL. The peak of the oestrone sulphate concentration was between the days D-4 and D-1, and ranged from 1.11 to 1.63ng/mL (1.27±0.23ng/mL, mean±S.E.M.; $p<0.001$). It was observed a correlation between the peak of oestrone sulphate and the higher folicular growth at days -6 and -2 before the ovulation ($r2=0.93$; $p=0.0072$). The peak of oestrone sulfate characterizes the peri-ovulatory phase. Analyzing the oestrone sulfate plasma concentration it was observed a low variability between the individuals and that the homogeneous behavior of the animals corresponded to the estrous cycles. The maximum values at the peak were observed two days before the ovulation. It is possible that, after the peri-ovulatory LH release, the oestrone sulfate concentration decline and the ovulation occurs.

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Synchronized ovulation protocols in nelore breed cows by the reutilization of the progesterone device

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Introduction

Estrus detection is the main factor that influences artificial insemination (AI) in bovine. Ovulation synchronization protocols and artificial insemination in fixed time (AIFT), with the use of a second dose of GnRH, simultaneous to AI, resulted in a satisfactory conception rates. The pregnancy rates with the use of intravaginal progesterone devices, in the interval of the first dose of GnRH and $PGF_{2\alpha}$, were satisfactory. The use of the estradiol benzoate, replacing GnRH, in order to reduce the protocol cost, resulted in a similar pregnancy rates. eCG application plus $PGF_{2\alpha}$ injection, to improve follicular development, has resulted in a satisfactory pregnancy rates with considerable repeatability. To optimize the costs of AIFT, the use of the progesterone devices by the second time resulted in a satisfactory pregnancy rates with high repeatability.

Materials and Methods

Sixty Nelore lactating cows were allocated into four treatments: T1 - use of a progesterone device (CIDR®) plus the injection, im, of 2 mg of estradiol benzoate at day 0, removal of CIDR at day 8 and injection, im, of 300 IU of eCG plus 25 mg of $PGF_{2\alpha}$ and AI 48 hours after the CIDR removal with simultaneous injection, im, of 25 μg of GnRH; T2 - similar to T1, with the injection of 1 mg of BE, im, at day 9, replacing the second GnRH dose, and AI 50-56 hours after CIDR withdraw; T3 and T4 were identical to T1 and T2 respectively, but reutilizing the CIDR® device. Device asepsis after its first use was done. At insertion time, the cows were weighed (PV) and its body condition scored (CC). At the start of treatments the presence of corpus luteum was registered and ovarian follicles were measured (small - FP <6 mm, medium - FM from 6.1 to 8.5 mm and dominant - FD> 8.5 mm).

Results and Discussion

Body weight and body condition of the cows were uniform in all treatments. The presence of follicles of 6.0; 6.1 to 8.5; and, above 8.5 mm in diameter were uniform. The corpora lutea showed a normal distribution. The cyclic and in anestrous cows were uniformly distributed. Pregnancy rates for the animals submitted to T1, T2 and T4 were 53.3, 40.2 and 33.3%, respectively, and were higher than in T3 (0.0%). In general, in the AI protocols, cows with synchronized ovulation showed pregnancy rate of 40.2%. Pregnancy rates in all treatments were not influenced by the body weight, days postpartum or body condition. The asynchronism between GnRH injection and pre-ovulatory wave of LH may not be the influential factor in the results of T1, since GnRH was injected at AI time and the pregnancy rate was 53.3%, in despite of the similarity between T3 and T1 in which the only difference was the reutilization of CIDR. Although the animals from T4 were using a reutilized CIDR, their pregnancy rate of 33.3% was similar to the results in (1), which showed a pregnancy rate of 38.5; 23.1; and 41.0% for the first, second and third use of CIDR, respectively. The protocols of T1 and T2 showed satisfactory results. The postpartum anestrous was controlled by the hormonal protocols.

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Estrus synchronization and female ovine fertility employing different doses and route of prostaglandin F 2 α aplication

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Introduction

Results of two sequential experiments are presented which investigated the viability of the reduction of the recommended dosage of prostaglandin F2α for estrus synchronization in ovine by intramuscular injection (IM) and intrasubmucosavulvar (ISMV). Estrus synchronization is an important instrument for concentration of lambing and reduction of the number of service days at artificial insemination (AI). So, Prostaglandins have been employed for estrus synchronization in ovine since they act as luteolitic agent (1,2).

Materials and Methods

The experiment was conducted in a farm located in the southeast region of Bahia State, northeast of Brazil. There were employed 84 female ovine without wool of the Santa Ines breed randomly selected for weight, BCS, age, pos-partum status and free from genital infection. All had lambed between 45-60 days from March to April the fall favorable reproductive station; they were cycling and were raised at pasture. Two experiments were done where the females were distributed in similar bases after randomization, before receiving a prostaglandin, cloprostenol, (CIOSIN-Schering-Plough-SP, BR). Experiment 1 – composed by 42 ovine females, being 21 distributed into two treatments (T-1.1 and T-1.2) were it was used the following doses: 250 μg of PGF2α and the control treatment which received a physiologic solution (NaCl 0.9%) respectively, both administered by IM injection in the leg. Experiment 2 – the same procedure was used, except for the dose and route of application which were 125 μg of PGF2 α by IMSV route and 0.5 mL of the physiologic solution 0.9% of NaCl in both treatments (T-2.1 and T-2.2 – controls) respectively. The animals were observed for estrus occurrence and mounting activity with the help of a male previously painted in the chest and check for their fertility by clinical and andrologic exam and sex drive in a proportion of 1:5.3 females. The data obtained were submitted to statistical analyses employing qui-square to find the percentage of female that showed estrus and lambed. (Fertility = lambed female/total of treated female).

Results and Discussion

It was observed a high percentage of females presenting estrus in both experiments when using 250 μg by IM or 125 μg by ISMV injection, 19/21 (90.5%) and 18/21 (85.7%), respectively ($P>0.05$) while in the control groups, few animals showed estrus 6/21 (28.6%) and 5/21 (23.8%), for experiment 1 and 2, respectively ($P>0.05$). Comparing the treated groups to the control group it was observed a significant statistic difference ($P<0.01$). In the lambing rate it was not seen statistical difference in treated groups 16/21 (76.2%) and 17/21 (80.9%), respectively ($P>0.05$). On the other hand, the control groups presented 6/21 (28.6%) and 5/21 23.8% for treatment 1 and 2, respectively which was statistically significant when compared to the treated groups ($P<0.01$). This difference can be attributed to a higher estrous behavior presented by the treated groups, a higher ovulation rate and a higher lambing rate which is according to the data reported (2) who indicated the possibility of a reduction in the dose of prostaglandin.

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Ultrasonographic screening of the ovulatory response in saanen goat

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Introduction

Real-time ultrasonography (RTUS) has been used to study the ovarian structures in goats, that allows the visualization of follicles and *corpus luteum*. Thus, it is an alternative method when compared with laparoscopy and laparotomy, being a non-invasive, accurate and reliable technique. Furthermore, it allows to perform successive examinations without causing detrimental effects such as post-surgical adherences and infections to the animals. In this respect, the aim of this work was to evaluate the ovulatory response in embryo donor goats by ultrasound scanning in comparison with laparotomy referential method.

Materials and Methods

Twenty-three adult Saanen goats had their estrous synchronized with the insertion of intra-vaginal progestagen sponges [60 mg medroxyprogesterone acetate (Progespon®, Buenos Aires, Argentina)] for 10 days and a single dose of 75 µg of cloprostenol (Prolise®, Buenos Aires, Argentina) on 8th day. To induce superovulation, six decreasing doses of pFSH (Folltropin®, Ontario, Canada) totalizing 200 mg were given. Also, 100 mg of GnRH (Fertagyl®, Boxmeer, Netherlands) were injected 36 hours after progestagen withdrawal to obtain synchronized ovulations. The does were mated by bucks of proven fertility. The ovulation rate was determined by the couting the *corpus hemorragicum* in both ovaries through the use of a B-mode ultrasonograph connected with a transrectal 6.0-8.0 MHz linear probe.

Results and Discussion

In all ovaries (n=46), the total number (mean ± s.d.) of *corpus hemorragicum* per ovary was 7.18 ± 0.96 by RTUS and 9.60 ± 1.70 by laparotomy, without significant differences, which are in agreement with other studies (1). In the present study, no higher incidence of ovulations was registered the in right ovary when compared to the left, which differed from other previous reports in which the right ovary is responsible for more ovulations in cows (2). For the probability of detecting the *corpus hemorragicum* exact number in each ovary, the correlation coefficient was high (r^2=0.86) and positive between two methods. The results show that RTUS is an important and non invasive method which can be an useful tool to select donors for embryo transfer programs after submitted superovulatory treatment.

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Oocyte quality is influenced by fetal sex

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Introdution

In vitro fertilization (IVF) is a reproductive technique that enables embryo production using donor cows that do not respond to superovulation treatments, pregnant animals, immature or aged cows. Oocyte quality and *in vitro* maturation can influence embryo development and be related to poor fertility rates observed in this process. Hormone levels in different phases of the reproductive cycle are responsible for oocyte health and can influence its quality for aspiration and *in vitro* maturation (1, 3). This work was aimed to investigate pregnancy, fetal sex, and corpus luteum (CL) position influence over oocyte quality after follicular aspiration

Materials and Methods

Oocytes (n = 981) were obtained from 49 genital traits (34 pregnant, 14 male fetus, 14 female fetus, 6 unsexed and 15 not pregnant) of slaughterhouse cows. The *cumulus*- oocyte complexes (COC) were transported in saline solution (NaCl 0,9%) to the laboratory. Follicular aspiration (follicles with diameter between 3-8 mm) was done using 20 ml syringes with 40x12 mm needles. Oocyte evaluation was done using a stereozoom microscope with 80X magnification. Oocyte were classified according to the number of cell layers, cytoplasm homogeneity and cumulus cells adhesion, in grades ranging from I to V (Grade I = excellent; Grade V = degenerated) (1). Data were analyzed by ANOVA using the SAS software (2).

Results and Discussion

Pregnancy and corpus luteum position (ipsilaterallis) had a significant effect in the oocyte quality. Oocytes recovered from ovaries presenting the CL and from pregnant genital traits showed better quality in relation to that without the CL or non-pregnant uterus ($p<0.05$). Progesterone levels should be related to oocyte quality. Female fetus pregnancies showed higher proportion of grade I oocytes in relation to male fetus pregnancies (63.31% x 36.69%, $p = 0.039$). *In* vitro cultured oocytes enriched with testosterone exhibited higher levels of cellular apoptosis and disturbances in nuclear and cytoplasm maturation. Anti-mullerian hormone inhibits oocyte meiosis *in vitro.* It is possible that androgens influence *in vivo* decrease oocyte quality (3). Androgen metabolism during gestation is not completely elucidated but it seems that an increase in androgen concentrations in the maternal blood due to the presence of a male fetus should influence the pool of primordial follicles disrupting oocyte development. These results suggest that fetal androgen production should have deleterious effects to the oocyte. In female fetus gestations the recovery of high number of grade I oocytes probably occurred because androgen concentrations were lower than in male pregnancies.

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Imature domestic cat oocytes obtained from fresh or stored ovaries can be used for in vitro sperm-binding assays in felids

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Introduction

In vitro sperm-binding assays (SBA) have been used for evaluating the fecundating potential of felid spermatozoa after sperm cryopreservation. In vitro matured (IVM) oocytes either fresh or after been stored under refrigeration in salt-solutions for a few months have normally been used (1,2). This approach limits the use of ovaries obtained after ovario-histerectomy due to the small number of oocytes available for IVM from each ovary and the need to collect the oocytes within 6 h of surgery. In this study we investigated the feasibility of using imature domestic cat oocytes obtained either from fresh, refrigerated or frozen ovaries to evaluate domestic cat sperm function after thawing in SBAs.

Materials and Methods

Oocytes were obtained following ovario-hysterectomy at local veterinary clinics, stored in PBS with antibiotics (penicilin/streptomycin) at 4°C and processed within 6 h after surgery (FRE) or after been kept in PBS at 4°C for 24 h (REF), or at −20°C for a minimum of 24 h (FRO). Cumulus-oocytes complexes (COCs) were removed by mincing ovaries in 75 mM sodium citrate. To remove cumulus cells, COCs were incubated in 75 mM sodium citrate for 15 min (37°C in air, 5% Co_2), followed by vortexing for 15 min (2). For the SBAs, groups of 5 to 10 oocytes were transferred to 90 µL droplets of Hams-F10, under mineral oil, and 10 µL of frozen-thawed sperm suspension (2 x 10^6 motile spermatozoa / mL) from different protocols of cryopreservation were added to each droplet. After 18 h co-incubation (37°C in air; 5% Co_2), oocytes were washed in Hams-F10 to remove loosely attached spermatozoa, fixed in paraformaldeide (2%) and Triton-X (0.04%), and stained with bisbenzimida H-33342 (HOESCHST; 5 µg/mL). The proportion of oocytes with at least one spermatozoa bound to the zona pellucida (Binding Rate) and the total number of spermatozoa bound per oocyte were determined under fluorescence microscopy. Differences between treatments for the binding rate were detected by Fisher's Test and for the mean number of spermatozoa/oocyte by ANOVA, followed by Tukey test for $p<0.05$.

Results and Discussion

A total of 826 oocytes were analysed and, irrespective of the sperm treatment, the binding rate and number of spermatozoon bound per oocyte obtained for FRE oocytes (93%; 6.2 $\pm$ 0,3; n=384) were higher than those for REF (71%; 4.5 $\pm$ 0.3; n=207) or FRO (73%; 3.5 $\pm$ 0,2; n=235) ones, respectively. Data for REF and FRO oocytes did not differ of each other. Results obtained in this study for FRE immature oocytes are similar to previous data using IVM oocytes[1,3] suggesting that immature oocytes can be sucessfully used to assess the ability of spermatozoa to accomplish the binding to the zona. Oocytes either from cooled or frozen ovaries can also be used, although binding rate and number of spermatozoa bound per oocyte were compromised by both methods, as described for canine oocytes. Although aditional studies are needed to refine the method, the use of immature oocytes for SBAs is promissing for studies in felids.

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Effect of prolonged undernutrition on primordial follicle populations in goats

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Introduction

Nutrient availability is a fundamental regulator of ovary function activity as well as follicular development in goats. The literature regarding the nutritional influence in these species is limited to the nutritional effect as a regulator or moderator of the terminal folliculogenesis process. It is well know that ovulation rate is directly related to the nutritional state. Nevertheless, changes in the ovulation rate are consequence of nutritional effect on the follicular recruitment, which is the ultimate step of the primordial pool initiation and development. Aim of this work was investigate the effect of prolonged undernutrition on early preantral follicular population in goats.

Materials and Methods

Ten cycling crossbred Saanen x local goats with similar live weight and ages were submitted for six months to undernutrition (70% of maintenance) (1). Five goats chosen randomly were refed (150% of maintenance) for six weeks. In all animals estrus was synchronized using 45mg FGA vaginal sponge for 11 days, 300IU eCG and 50μg cloprostenol 48 h prior to sponge removal.

After collection, the ovaries were fixed in Bouin's solution. They were then dehydrated in ethanol and embedded in paraffin wax. Every 5th section sliced (8 μm of thickness) was stained with periodic acid Schiff (PAS) and Harris´ hematoxylin. The preantral follicles were classified into three types or classes namely, primordial, transitory and primary. Estimates of the number of normal and degenerated follicles counted in all sections were done by the Fractionator method. The effect of nutrition (food restriction, refeeding) was analyzed by the GLM procedure of SAS (SAS, Inc., USA). Comparison between means was performed by the *t* test. Before statistical analysis, number of follicles was transformed to log. Values were expressed as mean ± SEM.

Results and Discussion

From results obtained in this study, it was demonstrate that prolonged period of undernourishment in goats caused an increase of degenerate primordial follicles and a substantial reduction of activation of primordial cohort. In underfed group, it was observed a greater ($P < 0.05$) frequency of degenerate follicles in primordial class (1545 ± 570.58 vs. 315 ± 103.08) and transitory class (720 ± 167.73 vs. 285 ± 83.02). Also the number of follicles in transitory stage was widely reduced in undernourished females (6145 ± 2578.58 vs. 24330 ± 4485.74; $P < 0.05$). Concerning the nutritional influence on early folliculogenesis, some authors (2) (3) (4) have indicated that undernutrition caused a substantial change of preantral follicular population. Severe undernutrition for medium period mainly induces an increase of oocyte death (2) or a reduction of depletion process in the primordial pool (4). So far, maintaining follicular reserve from degeneration, in undernourished animals, can be interpreted as a survival strategy, which occurs by the sensibility of the nutritional flow in the ovary.

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Emergence of corpora lutea premature regression in superovulated moxotó goats with stimulated energy balance

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Introduction

In goats as well other ruminants, superovulatory response can be enhanced by stimulation of energy balance and for this purpose several strategies have been used (1) (2). However, in superovulated animals the main limiting factor is still the presence of the corpora lutea premature regression, being usually associated with a decrease of viability in embryo recovered (3). Therefore it appears important to verify the relationship between protocols of energy stimulation and occurrence of corpora lutea premature regression in superovulated goats.

Materials and Methods

Seventeen Moxotó goats with mean ± SEM live weight of 26.60 ± 4.08 kg and 3.29 ± 0.69 years old were synchronizated with 60mg MPA vaginal sponge for 11 days and 50µg cloprostenol, 48 h prior to sponge removal. During days 9-11 goats were superovulated using 120 mg pFSH. The number of corpora lutea (CL) and presence of premature regression (CL´s) were verified by laparoscopy performed eight days after sponge removal. Moxotó goats were allocated in three energy treatments: Feed (n = 5): diet with 150% maintenance (1.5 x M); Propylene (n = 5): diet with 1.5 x M plus administration of 80 mL/goat/d of propylene glycol during the hormonal procedure; Insulin (n = 7): diet with 1.5 x M plus three injections of insulin (0.2UI/kg BW/d) at the same time of FSH treatment. The energy protocols effect was analyzed by the GLM procedure of SAS (SAS, Inc., USA). Differences among proportions or numbers were analyzed by Chi Square. Values were expressed as mean ± SEM.

Results and Discussion

No difference was observed between the groups for the responsiveness to the superovulation treatment ($P > 0.05$). However at laparoscopy, the insulin group shows a higher frequency number of regressed CL ($P < 0.05$) in respect to the other energy protocols. In goats treated with insulin CL´s was 17% of total CL observed (14/83), while 9% in propylene group (4/41). None CL´s was counted in animals only fed. The CL premature regression is often observed in goats, associated to progesterone levels reduction, three to four days after oestrous harming the viable embryos recovered (4). The CL premature regression causes are still not clear. However, a nutritional cause has been suggested in sheep, relating to lower levels of plasma glucose in CL regressed ewes than in those with normal CL´s (5). In the insulin group, the direct insulin administration might have originated higher plasma insulin levels, that, hence through insulin's hipoglicemic action, might have reduced blood glucose levels.

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Exposure to fenvalerate *in utero* and lactation does not interfere with the time of puberty installation and estrous cycle in rats

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Introduction

Fenvalerate is a synthetic pyrethroid insecticide used in agriculture and in the control of domestic insects, and the literature on its reproductive effects is scarce. The aim of the present work is to evaluate if the exposure *in utero* and lactational to fenvalerate interferes with the time of puberty installation and with the estrous cyclicity in female rats.

Materials and Methods

Pregnant female rats (T, n = 10) were treated with fenvalerate with daily doses of 40 mg/kg by gavage, from gestational day 12 until the end of lactation (critical period of development of the reproductive system of the offspring). Control rats (C, n = 9) received corn oil (vehicle) in the same experimental conditions. Starting on postnatal day 30, the female pups were observed to determine the day of vaginal opening, indicative of puberty installation (1). After vaginal opening, vaginal smears were collected, to determine the occurrence of the first estrous. When these females were 60 days-old the regularity of the estrous cycle was assessed for 15 days. Results are expressed as mean ± SEM and statistically significant difference is indicated by $p<0.01$**.

Results and Discussion

There was a significant reduction of the body weight in the litters of the treated group (Figure 1). On the other hand, the time of vaginal opening (days) (C: 41.17 ± 1.0; T: 41.22 ± 0.69), the day of the first estrous (C: 42.6 ± 1.03; T: 42.5 ± 0.78) and the regularity of the estrous cycle (Table 1) were similar in the two experimental groups.

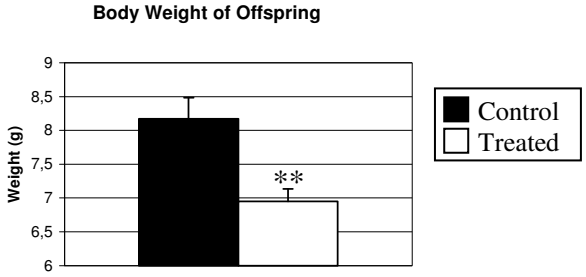

Figure 1. Body weigh (g) at birth of the offsprings from control and fenvalerate-treated groups.

Table 2. Estrous cycle of the offspring from control and fenvalerate-treated groups.

Phase	Control (n=9)	Treated (n=10)
Proestrous	3.88 ± 0.10	3.47 ± 0.22
Estrous	3.83 ± 0.11	3.74 ± 0.31
Metaestrous	3.06 ± 0.31	2.52 ± 0.18

The results indicate that fenvalerate, in these experimental conditions, although has detrimental effects on the intrauterine development of the offspring, did not provoke alterations in the time of puberty installation or on the regularity of the estrous cycle.

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Imprinting: chronic ethanol consume causes alterations in estral cycle and uterine tube epithelium of uch female rats

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Introduction

Alcoholism is the third most serious problem in the World Public Health and, currently, affects mostly women (1). Secretions from the uterine tube form a fluid that provides the necessary means for gamete maturation, fertilization and initial embryo development. Ethanol causes alterations in sexual hormonal levels resulting, through imprinting, irreversible modifications in the normal differentiation means of cells (2). UChA and UChB rat lineages are models for studies related to human alcoholism factors.

Material and Methods

This study analyzed the estrus cycle and epithelial cell of the uterine tube of UChA and UChB rat lineages, voluntary drinkers of ethanol, divided into three groups: 1) UChA voluntary drinkers of ethanol at 10% (6.05 g /kg/day); 2) UChB voluntary drinkers of ethanol at 10% (8.18 g /kg/day); Wistar rats (control). These female rats were 55 days old when treatment with ethanol started for, at least, 40 days. Estrus cycle was evaluated, during 20 consecutive days, through vaginal smear collected with cotton swabs, moist in saline solution and introduced in the rat vagina with a short rotation movement. The material collected on the swab was spread on a histological plate, fixed, stained and analyzed. Estrus females were selected for blood collection and organ processing using hormonal dose and microscopic techniques.

Results and Discussion

UChA and UChB groups presented irregular cycle. The number of cycles did not differ statistically in the studied group, but the time in each cycle phase varied, indicating that the duration of the same phase was different according to the analyzed group. UChA and UChB groups presented greater variation coefficient and, therefore, a bigger variability. The uterine tube intramural region (In) of treated rats presented vacuoles in the cytoplasm and dilated cisterns, nuclei with irregular shapes and lipidic drops. The isthmus region (I) presented microvilli with electron density alteration and irregular distribution of cilia. The ampulla (A) presented laminar bodies increase and lipidic drops in the cytoplasm and epithelium lumen. Infundibulum (Inf) and fimbria (F) regions of treated rats presented vacuoles and gaps, with and without content, non-preserved mitochondria, laminar bodies, lipidic drops and dilated cisterns. Nuclei with irregular shapes and dead cells in the epithelium lumen were observed. There was alteration in the cilium morphology. The degenerative process presented greater proportions when the UChA group was compared to the UChB one. It was concluded that ethanol alters the estrus cycle and the epithelium cell structure in the uterine tube. It is inferred that fluid alteration caused by ethanol consume affects the reproductive process of UCh female rats through imprinting.

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Gonadal morphology of perciformes fishes from Três Marias Reservoir, São Francisco River, Minas Gerais, Brazil

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Introduction

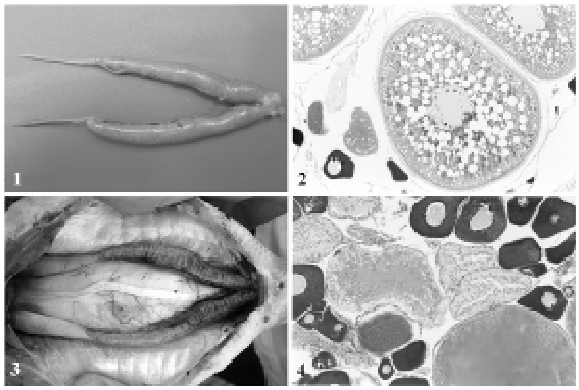
In the São Francisco river basin there are two families of fishes of the Perciformes order: Cichlidae e Sciaenidae. Cichlidae has diurnal habits, prefers lentic habitats and feeds on a variety of items. All cichlids are oviparous and build holes where deposit your eggs, and have parental care. In Três Marias reservoir two exotic species of Cichlidae, tucunaré and tilápia occur. Both have commercial and sport interests. The Sciaenidae, of marine derivation, recognized as corvinas, are piscivorous, prefer lentic habitats and have importance in professional fishing and are popular fish food.

Material and Methods

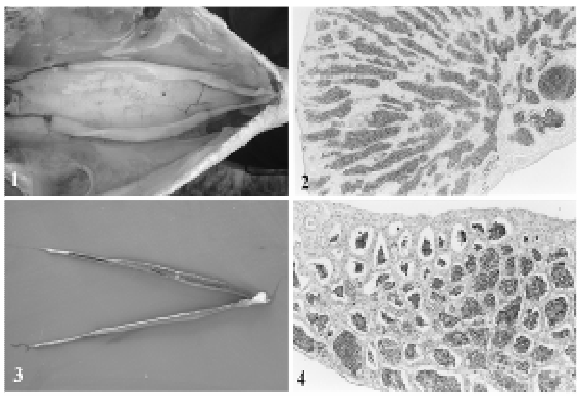
To study the gonadal morphology of Perciformes, we captured in Três Marias reservoir individuals of the following species: *Cichla monoculus*, *Geophagus brasiliensis*, *Oreochromis niloticus* and *Pachyurus squamipennis.* Fishes were dissected for macroscopical and microscopical analyses. Gonadal fragments were submitted to routine histological techniques.

Results and Discussion

Macroscopically, resting ovaries are flattened and translucent, in advanced maturation they reach maximum volume and yellowish coloration; spawned ovaries have reduced volume and hemorrhagic areas. Histologically, they present thick tunica albuginea and vitelogenic oocytes with lipid vesicles in ooplasm, characteristics of marine fishes, besides cortical alveoli and yolk globules. In *C. monoculus*, elliptic yolk globules were detected in vitelogenic oocytes. Testes are flattened and transparent when resting; they have white-milk coloration and maximum volume in advanced maturation, and flaccid and hemorrhagic when spent. Histologically, they have anastomosing tubules distributed radially towards the spermatic duct. The knowledge of gonadal morphology of Perciformes is important to determine the reproductive pattern of this group of fishes, supplying subsides to phylogenetic studies.



1: Ovary of *C. monoculus* in advanced maturation **2:** Vitelogenic oocytes with cortical alveoli and lipid vesicles in advanced maturation of *P. squamipennis.* **3:** Ovary partially spawned of *C. monoculus* **4:** Atretic follicle and postovulatory follicle in partially spawned ovary of *G. brasiliensis.* HE – 60X.



1: Testis in advanced maturation of *P. squamipennis* 2: anastomosing tubules of *C. monoculus* in advanced maturation. 3: Testis partially spent of *C. monoculus* 4: Testis partially spent of *G.brasiliensis.* HE – 2=30X, 4=50X.

Support: CNPq, FAPEMIG, CODEVASF, FIP/ PUC-Minas

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Comparative oocyte morphology and early development in three species of trahiras from the São Francisco River basin

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Introduction

Trahiras are piscivorous fish that has a broad geographical distribution in South America. They have an extended reproductive period, asynchronous oocyte development, multiple spawning, and low fecundity (1). Both parents construct a nest for spawning, mainly in shallow waters, and the male appears responsible for guarding the offspring. The purpose of this study was to provide a comparative analysis of oocyte morphology, embryogenesis and early larval development of trahiras from São Francisco River basin.

Materials and Methods

Mature specimens (*Hoplerithrinus unitaeniatus*, *Hoplias lacerdae* and *Hoplias malabaricus*) were submitted to hypophysation using crude extract of carp pituitary according to routine method from Hydrobiology and Hatchery Station of Três Marias (2). Stripping was performed by coelomic wall massage and the oocytes were fertilised immediately post-spawning. Following fertilisation, the eggs were incubated in funnel-type incubators with continuous water flow at 24º C. Embryonic development was monitored by hour. Larval development was recorded daily until complete resorption of the yolk sac.

Results and Discussion

Mature ovaries of the three species showed asynchronous oocyte development. The mean diameter of the mature oocytes was largest in *H. lacerdae*, followed by *H. malabaricus* and *H. unitaeniatus*. The eggs of the three species were yellowish and adhesive, containing carboxyl and sulphate radicals in the glycoconjugates of the zona radiata. Complex surface arrangement was identified in oocytes of *H. unitaeniatus* and *H. lacerdae,* while *H. malabaricus* oocytes had a simple surface pattern. Lectin histochemistry revealed different carbohydrate terminal residues in cortical alveoli, outer zona radiata and follicular cells of the three species. At the animal pole, the oocyte surface topography surrounding the micropyle was species-specific. The micropylar cell was ConA-positive, suggesting the presence of carbohydrates with mannose/glucose terminal residues that could have a role during fertilisation. Trahiras exhibited a prolonged embryonic and larval development compared to other Characiformes, a reproductive strategy used for increasing offspring protection. Early development proceeded most rapidly in *H. unitaeniatus*, followed by *H. malabaricus* and then *H. lacerdae,* which could have more developed parental care behaviour. An adhesive organ composed of secretory prismatic cells protruding from the cephalic region of the three erythrinid larva allowed them to attach to one another during development. Reproductive behaviour and early developmental strategies were similar in the three species, but the oocyte surface morphology suggests a close relationship between *H. unitaeniatus* and *H. lacerdae.*

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Gonadal morphology of siluriformes fishes from Três Marias Reservoir, São Francisco River, Minas Gerais, Brazil.

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Introduction

The Siluriformes order - fishes deprived of scale - has about 34 families and more than 2400 species, the majority of them living in freshwater and of great importance in commercial and sport fishing. The objective of the present work is comparatively to analyze the macro and microscopical morphology of ovaries and testes of the most abundant Siluriformes of Três Marias Reservoir.

Materials and Methods

Individuals of fourteen species were captured with gill nets in the Três Marias Reservoir: *Gymnotus carapo, Eigenmannia virescens, Sternopygus macrurus, Franciscodoras marmoratus, Trachelyopterus galeatus, Duopalatinus emarginatus, Pimelodus maculatus, Lophiosilurus alexandri, Cephalosilurus fowleri, Rhamdia quelen, Hypostomus francisci, Rhinelepis aspera, Pterygoplichthys etentaculatus* and the alien species, *Hoplosternum littorale.* The fishes had been dissected for macrocospic analysis of the gonadal morphology. For histological analysis, gonad fragments were fixed in Bouin's fluid and submitted to routine techniques.

Results and Discussion

The gonads in the resting stage are thin and translucent, gradually increasing their volume until the advanced maturation stage, when ovaries present yellowish coloration and testes, white-milky coloration. After spermiating or spawning the gonads became flaccid and hemorrhagic. The majority of the analyzed Siluriformes presented fringed testes with asynchronous development of the spermatogenic cells in each fringe. Some species presented spermatozoa embedded in acidophilic secretion which was also observed in the spermatic duct. The vitellogenic oocytes presented thin pellucida zone and high follicular cells in all analyzed species. In *T. galeatus*, an internal fertilizer, spermatozoa stored in the ovaries had been observed. The variations of the gonadal morphology supply important subsidies phylogenetic analyses.

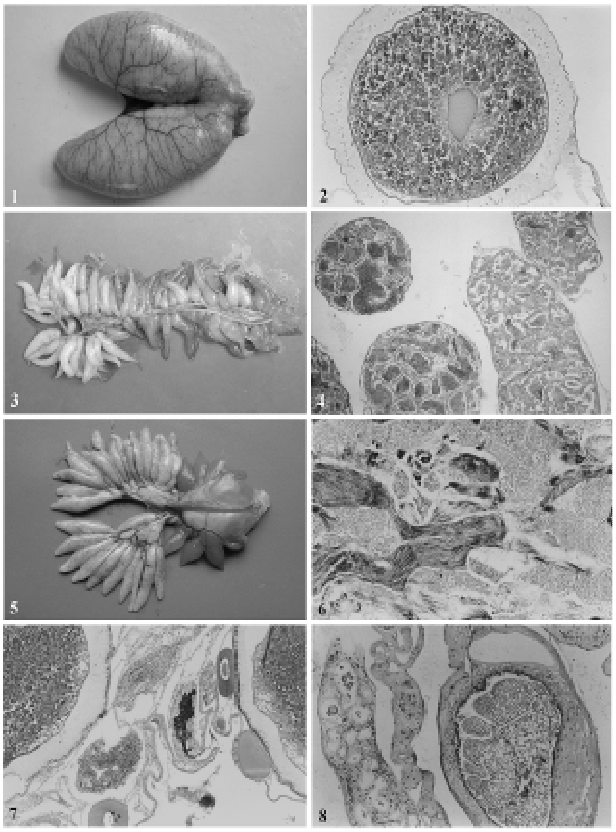

Fig. 1. 1: Ovary of *R. quelen* in advanced maturation. **2:** Vitellogenic oocyte of *R. quelen* with thin pellucida zone and high follicular cells. **3:** Testis fringed of *D. emarginatus.* **4:** Fringes of *P. maculatus* with asynchronous development of the spermatogenic cells. **5:** Testis fringed of *T. galeatus* with seminal vesicles. **6:** Elongated spermatozoa of *T. galeatus* in initial maturation. **7:** Spermatozoa stored in the ovaries of *T. galeatus.* **8:** Acidophilic secretion of *P. maculatus* in spermatic duct.
HE – 2-7=30X, 4=20X, 6-8=60X.

Support: CNPq, FAPEMIG, CODEVASF, FIP PUC Minas.

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Morphological study of postovulatory follicles involution in two different species of teleost

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Introduction

Postovulatory follicles are remains of ovarian follicles detected in fish ovaries after ovulation and spawning. They are histological indicators of reproductive success, being used in the determination of time and spawning frequency during the reproductive cycle of oviparous fish. Although they have no hormonal activity, studies on involution of these follicles are important for knowledge of the ovarian dynamic post-spawning. In the present work, morphological changes during involution of postovulatory follicles were analyzed in two species with different reproductive strategies: curimatã *Prochilodus costatus* with total spawning and lambari *Astyanax bimaculatus lacustris* with multiple spawning.

Material and Methods

Mature females of both species were submitted to induced spawning using crude carp pituitary extract. The spawned females were kept in concrete tanks with continuous water flow at 24-26°C. Samples of ovaries in advanced maturation and at different time intervals post-spawning were fixed in Bouin's fluid and processed by routine histology. Sections fixed in 4 % paraformaldehyde were processed for *in situ* TUNEL. Apoptotic index of granulosa and area of postovulatory follicles were performed using image analysis program. ANOVA followed by Tukey test ($p<0.05$) was used to compare means ± standard deviation.

Results and Discussion

The involution of the postovulatory follicles *P. costatus* and *A. bimaculatus lacustris* occurred similarly to *Leporinus taeniatus* [1]. Recently spawned follicles presented large and irregular lumen covered by a layer of hypertrophied granulosa cells and a few developed connective theca. Following, the follicular lumen was progressively occluding concomitantly to the increase of the thickness and vascularization of the theca. During the involution, apoptotic figures were frequently observed in the granulosa and theca through morphological analyses and TUNEL reaction. These cells with apoptosis characteristics were more numerous in the follicles in advanced regression. The area of the postovulatory follicles decreased and apoptotic index of granulosa increased in a time-dependent manner in both species. At later times, the area of the postovulatory follicles of *A. bimaculatus lacutris* decreased about 70% whereas *P. costatus* about 50%, suggesting a possible relationship between ovarian dynamic post-spawning and reproductive strategies. In addition, granulocytes were observed displacing toward the follicular lumen and also in the connective stroma, indicating an inflammatory response in ovary. In conclusion, the morphological changes occurring during involution of the postovulatory follicles are similar among species, indicating that fish ovary may be a model for tissue remodeling studies.

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Sexual steroids in the curimatã *Prochilodus argenteus* from São Francisco River, downstream from the Três Marias dam

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Introduction

Sexual maturation and spawning, in the most of the teleost fishes, are regulated by endocrine system, where sex steroids such as testosterone (T), 17β estradiol (E_2) and 17α hydroxyprogesterone (OHP) act on oogenesis and spermatogenesis. However, a wide variety of adverse environmental conditions may induce endocrine disruptions including sub-optimal temperatures, food supply, pH, pollutants and hydroelectric power plant. The impact of Três Marias dam on the reproductive activity of the migratory fish *Prochilodus argenteus* was demonstrated recently (1). The objective of this study was analyze the sexual hormone levels, gonadosomatic index (GSI), Fulton´s condition factor (K), fecundity, oocyte diameter and follicular atresia in mature *P. argenteus* captured in two stretchs of the São Francisco River, downstream from Três Marias dam: first 34 km immediately below the dam and 34 to 54 km after the confluence with the Abaeté River, a medium-sized tributary of the São Francisco River.

Material and Methods

Mature individuals of *P. argenteus* were captured between Jan/2005 and Feb/2006. Records were made of the total length (TL) and body weight (BW) of all specimens and gonadal weight (GW). Fulton's condition factor and gonadosomatic index were calculated for each specimen. Blood samples from caudal vein were obtained of each specimen for analyzing serum levels of T, E_2 and OHP through chemiluminescence, imunofluorimetry and radioimmunoassay, respectively. Gonad samples were fixed in Bouin's fluid, included in paraffin and processed for histological analyses.

Results and Discussion

Specimens from the stretch below the confluence with the Abaeté River showed significantly higher total length and body weight as compared to those from the stretch immediately below the dam. Mature females from the stretch below the confluence of the Abaeté River showed significantly higher levels of T, E_2 and OHP than those captured immediately downstream the dam. Fecundity, GSI and oocyte diameter were also significantly higher after the confluence with the Abaeté River. Follicular atresia affected vitellogenic oocytes predominantly in the stretch immediately downstream from the dam. In males, E_2, OHP and K were not significantly different, but T and GSI levels were higher after the confluence with the Abaeté River. These data indicate that *P. argenteus* has encountered adequate environmental conditions to the physiology of the neuroendocrine system at the stretch below the Abaeté River. Therefore, the environmental conditions influenced the steroidogenesis the *P. argenteus* in the São Francisco River, downstream from the Três Marias dam.

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Extracellular matrix and granulosa apoptosis in postovulatory follicles of *Prochilodus argenteus* (Teleostei)

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Introduction

Basement membrane is a specialized extracellular matrix that supports groups of cells, such as in epithelium. It creates an appropriated microenvironment for tissue development, influencing cell proliferation, differentiation and cell death. Apoptosis or programmed cell death is an energy-dependent process essential to tissue homeostasis, involving caspases that active endonucleases which degrade the DNA in the internucleosomal regions. In the mammary gland of mouse, the increase of apoptotic index of epithelial cells has been associated with the degradation of the basal membrane post-lactation (1). In the present study, the role of apoptosis (caspase 3), adhesion molecule (integrin β1) and basement membrane (collagen type IV) were analyzed during the involution of postovulatory follicles in the curimatã *Prochilodus argenteus*.

Materials and Methods

Mature females were submitted to induced spawning using crude carp pituitary extract. Ovarian samples, immediately after spawning and at various time intervals post-spawning were fixed in Bouin's fluid or in 4% paraformaldehyde and embedded in paraffin for the TUNEL reaction and immunohistochemistry analyses. Some specimens were also fixed in the modified Karnovsky solution, embedded in Epon-Araldite plastic resin and examined on transmission electron microscope. Apoptotic index of the granulosa and the immunohistochemically stained area for integrin β1 and collagen type IV in postovulatory follicles were evaluated by image analysis.

Results and Discussion

Immediately after spawning, postovulatory follicles had a broad lumen, low granulosa supported by continuous basement membrane and a thin connective theca. At 6 h and 1 day post-spawning, the granulosa was hypertrophied and theca was thickened. From 2 to 5 days post-spawning, disintegration and breakdown of basement membrane led to detachment of the granulosa into the partially occluded follicular lumen. In this time interval, apoptotic figures were frequently observed in the granulosa and theca. Immunohistochemistry reactions for integrin β1, collagen type IV and caspase 3 occurred only in the granulosa layer. In intervals from 2 to 5 days, weak staining was detected for integrin β1 and collagen type IV when compared to postovulatory follicles immediately after spawning. The areas stained for integrin β1 and collagen type IV decreased and apoptotic index of the granulosa increased in a time-dependent manner. These results showed the relationship between increase of granulosa apoptosis with decrease of the integrin β1 and degradation of the basement membrane during the involution of postovulatory follicles.

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Nuclear estrogen and progesterone receptors in the oviduct of heifers under natural and superovulated oestrous cycles

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Introduction

There are two types of estrogen receptors (ER), the alpha (ERα) and the beta (ERβ), the former being preferentially found in the oviduct. In the rat ERα is found along the mucosa and muscular layers at all phases of the estrous cycle (1). Under natural estrous cycles, 17β-estradiol (E_2) stimulates the production of both ER and progesterone receptors (PR) whereas progesterone (P_4) exerts an inhibitory effect on these receptors in several species (2). ERα and PR intensities within the bovine oviduct is greater at the beginning of the luteal phase (3). In heifers, superovulation did not have any effect upon the presence of both ER and PR within the oviduct (4).

The objective of the present study was to investigate possible relationships among circulating concentrations of E_2 and P_4 concentrations and ERα and PR within the oviduct of crossbred heifers at different phases of natural (physiological E_2 and P_4 concentrations) and superovulated (high E_2 and P_4 concentrations) estrous cycles.

Material and Methods

Oviducts from 22 crossbred heifers were examined for the presence of nuclear estrogen (ERα) and progesterone (PR) receptors at different phases (estrus, metaestrus and diestrus) of naturally occurring estrous cycles and estrous cycles during which superovulation was induced. Receptors were detected by immuno-histochemistry in the epithelial cells, connective tissue and muscular layer of oviductal infundibulum, ampulla, ampullary-isthmic transition and isthmus.

Results and Discussion

Epithelial ERα was found along the entire oviduct regardless of the cycle phase and of the circulating concentrations of 17β-estradiol (E_2) and progesterone (P_4). Epithelial PR was found mainly at the ampullary-isthmic transition and isthmus and more intensely at the estrus phase but their amount was not correlated with P_4 concentrations. ERα in the connective tissue was more abundant at the infundibulum and ampulla, regardless of the phase of the estrous cycle and of E_2 and P_4 circulating concentrations. PR in the connective tissue was found mostly at the ampulla, regardless of the estrous cycle phase but no correlations were found between amount and P_4 concentrations. ERα staining intensity in the muscular layer was similar at all phases of the estrous cycle and at all anatomical segments of the oviducts. However, PR staining was more intense at the isthmus during the metaestrus phase and it was negatively correlated with P_4 concentrations. In general, data from the present research suggest that P_4 exerts an inhibitory role upon ERα and PR. Also, no differences were found between animals subjected or not to superovulation.

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Antiluteolytic activity in bovine uterine microenvironment

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Introduction

Interferon-tau (IFN-t) is a conceptus secretory protein which interacts with the endometrium to block production of prostaglandin F2 alpha ($PGF_{2\alpha}$) and thereby maintain pregnancy in cattle. Biologic activity of IFN-t is usually measured by antiviral assays. However, antiviral activity is not well correlated with antiluteolytic activity, which is the ability to inhibit $PGF_{2\alpha}$ production (1). A bioassay was devised to measure the antiluteolytic activity of biological fluids. Assay consists in measuring fluids ability to inhibit phorbol 12 13 dibutyrate (PdBu)-stimulated $PGF_{2\alpha}$ production by bovine endometrial cells (BEND). Objective was to measure the antiluteolytic activity on uterine flushings obtained from cross-bred zebu cows on days 14, 16 or 18 of pregnancy or estrous cycle. It was hypothesized that uterine flushings obtained from pregnant animals had higher antiluteolytic activity than those obtained from cyclic cows.

Materials and Methods

Estrus was induced by a single injection of 530µg cloprostenol in cross-bred zebu, multiparous, non-lactating, cycling cows. Twelve hours after detection of estrous behavior (estrus=day 0) animals were randomly divided to receive (inseminated group-IG) or not (cyclic group-CG) artificial insemination. Semen from a single bull was used. After 14, 16 or 18 days after estrus, uterine flushings (250ml of Ringer's solution) were collected using a Folley catheter nº 24 from each animal and kept on ice. Animals from IG were considered pregnant whenever an elongated conceptus was recovered in the uterine flushing. Within each group, flushings from three animals in each day were pooled. According to procedures described in (2), BEND cells were seeded (4×10^4 cells) in 24-well plates and cultured to confluence. Cells were incubated in media containing 0, 0.005, 0.01, 0.05, 0.1, 0.5 or 1 µg/mL of total protein from each pool of uterine flushings, in the presence of 25 ng/mL PdBu, in triplicate, for six hours. Concentrations of $PGF_{2\alpha}$ in media samples were measured by radioimmunoassay. Percent inhibition of $PGF_{2\alpha}$ synthesis was calculated dividing the absolute production of each sample (PGFA) by the maximum $PGF_{2\alpha}$ production (observed in the presence of PdBu alone - PGFmax), so that % inhibition = (PGFA/PGFmax) x 100. The antiluteolytic activity of each sample was calculated as the reciprocal of the concentration of protein required to reach 50% of inhibition.

Results and Discussion

Following linear regression analysis of the % inhibition of $PGF_{2\alpha}$ production by the log of protein concentration in each sample, antiluteolytic activities were calculated for each pool. Activities were 7.09, 5.78 and 33.3 antiluteolytic units/µg of protein (UA/µg) for flushings collected on days 14, 16 and 18 of the estrous cycle respectively and 50, 13.5 and 45.4 UA/µg for pools of uterine flushings collected on days 14, 16 and 18 of pregnancy. It was concluded that uterine flushings of pregnant cows have a higher capacity to inhibit synthesis of $PGF_{2\alpha}$ than flushings of cyclic cows.

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In utero and lactational exposure to fenvalerate disrupts reproductive function in female rats

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Introduction

Fenvalerate is a synthetic pyrethroid insecticide used in agriculture, cattle raising and in the control of domestic insects, and its reproductive effects are little-known. Some studies already have proposed that fenvalerate is an endocrine disruptor, acting as an environmental estrogen. Knowing that the reproductive system of rats is more sensitive to the effects of toxic substances during fetal and neonatal periods, the objective of this work was to evaluate the possible late effects on the female reproductive function in sexual maturity, in female rats exposed to fenvalerate *in utero* and lactation.

Materials and Methods

Pregnant female rats (T, n = 10) were treated with fenvalerate with daily doses of 40 mg/kg by gavage, from gestational day 12 until the end of lactation (critical period of development of the reproductive system of the offspring). Control rats (C, n = 9) received corn oil (vehicle) in the same experimental conditions. The reproductive function was evaluated in the female offspring (in estrous) through the following parameters: weight and histological evaluation of the reproductive organs (uterus and ovaries, one pup per litter), sexual behavior, assessed by the number of lordosis in 10 mounts of the male, and fertility after natural matings. The reproductive outcome was evaluated on day 20 of gestation. Results, expressed as mean ± SEM, were considered statistically different when $p<0.05$*.

Results and Discussion

Fenvalerate exposure *in utero* and lactation provoked a decrease in the weight of the ovaries (C=139.34±3.10; T=103.14±3.25*), as well as in the number of pre-antral follicles and corpora lutea (table 1), suggesting decrease in the number of ovulations. On the other hand, although the sexual behavior was not altered by the treatment, the reproductive outcome after natural matings showed that there was an increase in the reabsorption number in the treated group (C=0.46±0.14; T=1.24±0.25*), with a consequent reduction in the number of fetuses (C=11.75±0.43; T=10±0.62*) that can be indicative of a genotoxic action of fenvalerate on the gamets (1). The histological aspects of the uterus of the treated and control rats were similar.

Table 1. Ovary structures in fenvalerate and control rats.

Structures	Control (n=5)	Treated (n=7)
Primary and primordial follicles	24.4±8.65	16.71±2.64
Pre- antral follicle	33.80± 4.15	19.43±2.65*
Antral follicle	23.20±4.97	15.57±4.06
Athretic follicle	2.80±1.16	4.28±1.94
Corpora lutea	16.20±0.97	12.86±1.01*

It was concluded that fenvalerate disrupted the reproductive function of female rats exposed *in utero* and lactation.

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Effects of the urban air pollution on some reproductive parameters. an experimental study in female mice.

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Introduction

Hazards to reproductive health have become prominent public health issues. A variety of factors are associated with reproductive system disorders including the environment air pollution. Disorders of female reproduction include reduced fertility, menstrual (estrous) disorders, spontaneous abortion, low birth weight, developmental defects. We have previously shown that air pollution may affect the reproductive health of female mice, with decrease in the number of viable fetuses and higher number of implantation failures (1). Numerous agents like dioxins and heavy metals have been shown to cause reproductive toxicity, disrupting the normal reproductive function. Many of these substances appear like constituents of the complex mixture that compose the air pollution. Considering this, the aim of this study was to evaluate if the exposure to ambient levels of the urban air pollution (Sao Paulo city) can affect the estrous cycle, time to mating, fertility and gestation index in female mice.

Materials and Methods

The experiments were carried out in downtown Sao Paulo where the source of air pollution is predominantly automotive. Two groups of 20 female mice (12 weeks old) were monitored. One group consisted of females maintained in clean chamber receiving filtered air (unexposed), and the other (exposed) in a polluted chamber receiving ambient air, both kept in the same conditions of temperature and humidity. The exposure protocol used the second generation of females mice born in the inhalation chambers to avoid, in the case of the unexposed group, an in uterus effect of the exposure to ambient air pollution. Besides the experimental groups, a group of 40 fertile males was used for mating. To investigate the estrous cycle, vaginal washes were recorded daily during two weeks prior to mating. According to the EPA Guidelines (2) the selected indices to evaluate the reproductive toxicity were: MATING INDEX (nº females mating/nº females cohabited) x 100; FERTILITY INDEX (nº pregnant females/nº female with vaginal plug) x 100; GESTATION INDEX (nº females delivering alive pups/ nº females with evidence of pregnancy) x 100; TIME TO MATING length of time required for each pair to mate after cohabitation.

Results and Discussion

The results are summarized in Table below.

	unexposed	exposed
Time to matting (M±SD)*	3.50±1.54	10.65±5.77
Days in estrus/period (M±SD)**	34.57±6.68	56.63±11.65
Mating index	100%	100%
Fertility index	55%	95%
Gestation index	55%	95%

(M±SD) = mean ± standard deviation; *p = 0.0001; **p = 0.03

An increased interval between initiation of cohabitation and evidence of mating in the exposed females suggests abnormal estrous cyclicity; the analysis of vaginal wash data provided information on the occurrence of persistent estrus in the monitored period. Although the mating index was 100% for both groups, the fertility and gestation indices were significantly different between the groups. These results indicate that the air pollution have a potential estrogenic activity or the ability to impair ovulation. This study showed that the ambient levels of the urban air pollution in Sao Paulo city elicit reproductive dysfunction in female mice.

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Diagnosis of *Neospora caninum* infection in bovine fetuses by hemi-nested PCR (Preliminary results).

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Introduction

Neosporosis is a parasitary disease that leads to abortion and decrease in productive and reproductive rates in infected herds. *Neospora caninum* is classified in the phylum Apicomplexa, Sarcocystidae family (1). Dogs are one of the definitive hosts of this parasite and shed oocysts in feces. Bovines are intermediate hosts and get infected by ingesting feed and water contaminated with oocysts. However, the main transmission route in bovines is the vertical one, when the fetus is infected by hematogenous route, leading to abortion or birth of congenitally infected, weak or normal calves. Seroepidemiological surveys in Brazil showed that occurrence rates range from 7.7% to 87.5%, with dairy herds showing the greatest frequency. The objective of this study was to detect the presence of *N. caninum* by hemi-nested PCR in aborted bovine fetuses sent to the Centro de *Pesquisa e Desenvolvimento de Sanidade Animal do Instituto Biológico* for differential diagnosis of infectious causes of abortion

Material and Methods

From January 2005 to July 2006, a total of 124 bovine fetuses from several Brazilian states (São Paulo, Paraná, Minas Gerais, Mato Grosso do Sul, Goiás, Mato Grosso, Rio de Janeiro and Pernambuco) were analyzed; 50% of these samples came from São Paulo. Brain, heart and liver samples of the fetuses were submitted to the hemi-nested PCR according to BASZLER et al. (2), for the detection of *N. caninum* DNA. Samples were macerated in a Stomacher® and DNA was extracted using guanidine isothiocyanate (Trizol LS® InvitrogenTM). The pNC-5 region was selected as the target sequence for DNA amplification. Pairs of primers used were outer Np4-Np7 and inner Np6-Np7, amplifying fragments of 275 pb and 227 pb, respectively. Amplified products were analyzed in 2% agarose gel electrophoresis stained with 0.05% ethidium bromide and visualized by ultraviolet light.

Results and Discussion

The frequency of fetal and placental infection in the samples submitted to PCR was 2.5% (3/124), two positive fetuses from dairy herds, four to six months of age, and one placenta of a four-month pregnant beef cow. As for the distribution of positive results per state, all samples – 4.48% (3/62) - came from São Paulo. Fetal age ranged from four to six months, similar to what was reported in the literature. However, the frequency of infected fetuses observed was lower than the one reported by other authors. *N. caninum* fetal infection rates in Brazil vary due to the kind of breeding management, diagnostic method used and environmental hygiene. Similar diagnostic survey performed in aborted fetuses by official animal sanity laboratories in Ireland from 2000 to 2005 showed 1.9% (52/2735) positive samples (3). Hemi-nested PCR showed low frequency of *N. caninum* in aborted fetuses in the population studied.

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Annual variations of pyroglutamyl aminopeptidase activity in the vagina and vas deferens of the snake *Crotalus durissus terrificus*

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Introduction

In *C. d. terrificus* occurs the storage of viable sperm in the female genital tract during the interval between autumnal courtship/mating and spring ovulation (1,2). This sperm storage during the winter seems to be an adaptive feature since secondary vitellogenesis is not completed by the time mating occurs (3). Components of semen, vas deferens, uterus and/or vagina could act on the preservation of fertile spermatozoids during this long-term period. Certain aminopeptidase (AP) enzymes have been related to the reproductive function in mammals. Particularly, the broad-spectrum pyroglutamyl AP (PAP I) cleaves the TRH analog, the fertilization promoting peptide, a known regulator of mammalian sperm function (4). This study evaluates the activity of PAP I in vagina (VG) and vas deferens (VD) of *C. d. terrificus* throughout the year.

Material and Methods

VG and VD were withdrawn by laparotomy. To clean these tissues stretching and compression were made followed by microperfusion with phosphate buffer saline (PBS). The tissues were then homogenized in 10 mM Tris/HCl buffer with or without 0.1% Triton X-100, to obtain respectively soluble (S) or solubilized membrane-bound (M) fractions, after ultracentrifugation. Lactate dehydrogenase (LDH) activity and protein content were spectrophotometrically evaluated.

PAP I activity was fluorometrically measured by beta-naphthylamine released (picomol/min/mg protein) from the reaction between samples and L-pyroglutamic acid-beta naphthylamide.

Results and Discussion

LDH activity was much higher in S than in M, which confirms the efficient separation of these fractions.

Levels of M PAP I in both VG and VD did not significantly vary along the year. The peaks of S PAP I in VG (554±69, n=9) and VD (529±91, n=4) occur respectively in winter (postmating period and sperm storage in female) and summer (premating period and peak of spermatogenesis). During the other seasons the levels of S PAP I did not differ significantly and were between 165-300 in VG and 67-271 in VD. Seasonal variations on catalytic activity in these tissues demonstrate that PAP I plays a role on the reproductive function of *C. d. terrificus.* The pattern of these variations suggest that PAP I may be part of the mechanism shared by male and female to keep the long term viability and sperm capacitation in this snake.

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Sexual differentiation and first gonadal maturation of commercial fishes from São Francisco River basin, Brazil

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Introduction

Sexual differentiation is a labile process in some fish species, dependent of endogenous sex steroids associated to environmental factors, mainly temperature (1). This plasticity enables production of monosex populations with commercial value and constitutes the basis for the application of hormonal sex control techniques, providing a widely used tool for both fundamental and applied research (2). Age and size of first gonadal maturation provides subsidies for breeding and cultive of species important for aquaculture. The present study had the purpose of determining the age and body length that matrinxã *Brycon orthotaenia*, piau-verdadeiro *Leporinus obtusidens and curimatã-pacu Prochilodus argenteus*, commercial fishes from São Francisco River basin, reach sexual differentiation and maturity.

Materials and Methods

Fish born from induced spawning using crude extract of carp pituitary were cultivated for 2 years at Hydrobiology and Hatchery Station of Três Marias/CODEFASF. After hatching, larvae were stocked in fishponds, receiving commercial feeding (36% of crude protein) supplied two times at each day during 3-4 months. Following, 400-500 alevins/species were stocked together in earthen tanks (200 m^2), fed with commercial feeding (22% of crude protein) in ratio of 1.5-2.0% of body weight, supplied daily. For each species, 5 samplings of about 40 fishes were colleted until 2 years after hatching. Records of total length (TL) and body weight were obtained of each specimen. Gonads samples fixed in Bouin's fluid were embedded in paraffin and processed routinely for histology.

Results and Discussion

Sex differentiation in fish takes place months or years after hatching in most neotropical fish species of commercial value. In present study, sexual differentiation occurred between 3 and 8 months after hatching, between 11 and 17 cm TL. During testicular differentiation, spermatogonia were organized in cysts into the seminiferous tubules. Testicular maturation happened earlier than ovarian maturation, beginning 1 year after hatching to *B. orthotaenia* and *L. obtusidens*. Ovarian differentiation was detected by germ cells in meiosis and lamellar organization. At 10-11 months old, ovaries have perinucleolar oocytes in completely organized lamellae. Oocytes with cortical alveoli were observed in *L obtusidens* with 11 months old. As the majority of teleosts, the species studied showed gonochorism as sexuality pattern and the majority of the females of *L. obtusidens* and *P. argenteus* were mature 2 years after hatching, wih 23-29 cm TL. About 90% of the alevins of *B. orthotaenia* differenced for males, suggesting possibly a gonadal differentiation process susceptive to temperature. Males of this species were mature at first year, with 17-23 cm TL and mature females were not detected. Females and males of *P.argenteus* showed high fertilization rate (80-90%) after induced spawning by hypophysation.

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Support: CNPq, FAPEMIG, CODEVASF

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Gametogenesis and reproductive biology of *Metynnis maculatus* Kner, 1858 (Pisces, Characidae) from Porto Colômbia Reservoir, Grande River Basin, Minas Gerais/São Paulo.

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Introduction

The determination of fish reproductive dynamic parameters allow us to verify the strategies that these species use on their living environment, to produce a maximum of offspring and provide important information to assure specimens perpetuate and survival.

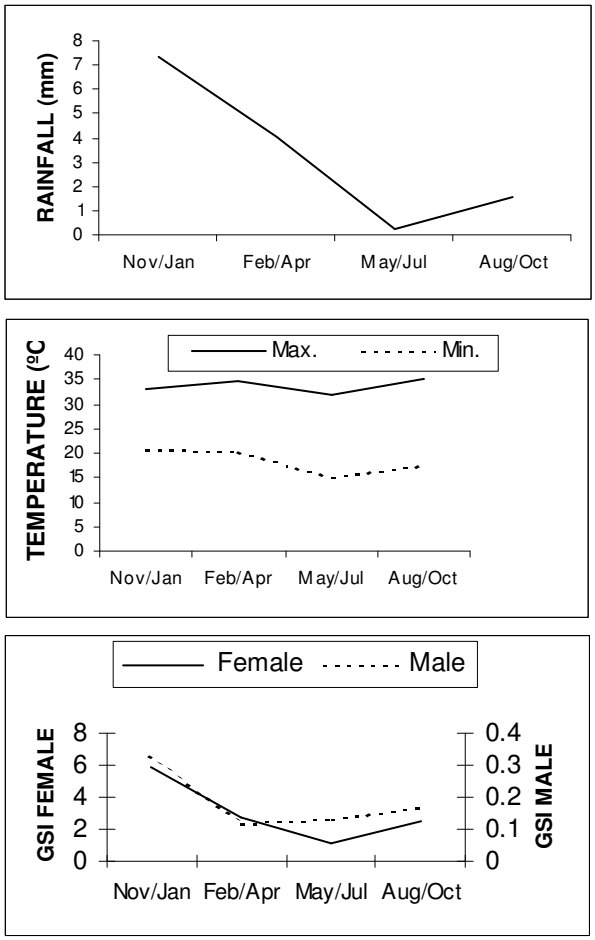
The aim of this work was to describe the gametogenesis and reproductive biology of *M. maculatus* through the characterization of germ cells, by determining the gonadal maturation stages and the biological indexes: gonadosomatic, hepatosomatic, stomach repletion, coelomic fat, condition factor and the relationship of reproduction with environmental factors (1).

Materials and Methods

Adult specimens of *M. maculatus*, comprising 58 males and 96 females were captured quarterly in Porto Colômbia reservoir, from November 2001 to October 2002, by using gill nets. From each specimen we registered the standard length; and the weights of the body, gonads, liver, stomach and coelomic fat. The biometric data obtained were used for calculating the gonadosomatic, hepatosomatic, stomach repletion, and coelomic fat indexes, as well as the Fulton condition factor. Fragments of gonads were fixed in Bouin's fluid and submitted to histological routine techniques.

Results and Discussion

The morphology of gonads of *M. maculatus* is similar to other teleosts of the sub-family Serrasalminae. In the vitellogenic oocytes, the zona pellucida is thick, composed of two layers and the follicular cells are cubic, similar to the others Serrasalminae. The reproductive period is extended, the spawning is fractioned and peaks of gonadosomatic index of males and females occurred from November to January, coinciding with higher values of temperature and rainfall in the region. During the reproductive period, feeding activity is reduced, and fat reserves are consumed, indicating by lower values of stomach repletion and coelomic fat. Males are smaller than females, indicating sexual dimorphism in *M. maculatus.*



Quarterly values of gonadosomatic index (GSI) of *M. maculatus* males and females, temperature and rainfall measurements in Porto Colômbia reservoir between November/2001 and October/2002.

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Gametogenesis of four fish species from Porto Colômbia Reservoir, Southeastern Brazil: histological histochemical and histometric, study.

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Introduction

Gametogenesis of fish shows characteristic patterns for each group and features for each species. Thus, information about gametogenesis of Brazilian fish still insufficient. This study makes comparative analysis of gametogeneis of four teleost species from Porto Colômbia reservoir, Grande River basin, Brazil.

Materials and Methods

Five to ten fishes, for each sex, with advanced maturation gonads, of the following of species were captured: *Metynnis maculatus* (pacu-prata) Characiformes, *Megalancistrus aculeatus* (cascudo-abacaxi) – Siluriformes; *Cichla monoculus* (tucunaré) and *Satanoperca pappaterra* (acará) - Perciformes. Gonad fragments were collected and fixed in Bouin's fluid, and submitted to routine histological techniques. The following histochemical techniques, were used: periodic-acid-Schiff (PAS), salivar amylase following of PAS, Alcian blue pH 2,5 and pH 0,5, Sudan black B and Ninhydrin-Schiff. For histometric study, 10-20 microscope slide for each species, using a micrometric ocular coupled to a light microscope, the following measurements were determined: oocyte diameter, follicular cell height, zona pellucida thickness and spermatogenic cells nucleus diameter.

Results and Discussion

Oocytes resulting from oogonias proliferation and differentiation were classified into four development stages: initial perinucleolar, advanced perinucleolar, previtellogenic and vitellogenic. Based on the histological characteristics, six types of cells were identfied during the spermatogenic development: primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Neutral glycoproteins were detected in the cortical alveoli of oocytes of *S. pappatera* and *C. monoculus* while carboxylated acid glycoconjugates and sulfated acid glycoconjugates were found in *M. maculatus* and *M. aculeatus.* In the four species, the yolk globules contained neutral glycoproteins and lipids while the zona pellucida contained only neutral glycoproteins. Carboxylated acid glycoconjugates were detected in follicular cells of *M. maculatus* and neutral glycoproteins in the other species. The Perciformes *S. pappaterra* and *C. monoculus* showed in the cytoplasm clear vesicles containing neutral lipids - features of fishes with marine ancestry.

Tabela 1: Histometrical gametogenesis parameters of *Metynnis maculatus* (M.m) *Megalancistrus aculeatus* (M.a), *Satenoperca pappaterra* (S.p) and *Cichla monoculus* (C.m) from Porto Comlômbia reservoir, southeastern Brazil.

	M.m	*M. a*	*S. p*	*C. m*
Ø nucleus G1	5.77±0,81	5.2±0,42	6.84±08	7±0,9
Ø nucleus G2	4.43±068	3.9±0.28	4.6±0.72	5.09±0.59
Ø nucleus C1	3.12 ±0,37	3.12±0,37	3.45±0.57	4.45±0.79
Ø nucleus C2	2.36±0,37	2.39±0,39	3.2±0,55	3.3±0.48
Ø nucleus T	1.76±0.54	1.69±0.32	2.13±0.34	1.82±0.38
Ø nucleus Z	1.63±0.56	1.03±0.22	1.84±0.29	1.4±0.44
Ø O4	839.95 ±145.96	2136.15 ±423.89	728.28 ±145.74	398.4 ±49.49
Height CF	2.38±1.25	6.2±2.29	3.33±1.3	9.61±1.28
Thickness ZP	7.77±1.38	6.42±1.7	6.35±3.03	1.72±0.52

Ø= diameter G1= primary spermatogonia, G2= secondary spermatogonia, C1= primary spermatocytes, C2= secondary spermatocytes, T= spermatids, Z= spermatozoa, O4= vitellogenic oocyte, CF= follicular cell, ZP= zona pellucida

Large oocytes are related with long embryonic development and they are typical of species with parental care. In fishes, the diameter of spermatozoon head varies in agreement with micropylar diameter, where the fertilizing spermatozoon penetrates. Morphological and histochemical features of oocytes and spermatogenic cells of the studied species provide an important basis for phylogenetic studies, knowledge of fertilization and embryo initial development.

Support: Furnas Centrais elétricas SA, CNPq, FAPEMIG, PROBIC- PUC Minas.

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Reproductive biology of Astyanax fasciatus (Curvier, 1819) (Characidae: Tetragonopterinae) in Furnas Reservoir, Brazil

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Introduction

The reservoir of Furnas, Grande River, high Paraná River basin, has been subjected to anthropogenic activities which cause alterations in the physical, chemical and biological characteristics of its waters. Dejects of domestic and industrial sewage *in natura*, contamination by pesticides and the water use for tourism are source of preoccupation for environmental agencies. The lambari *Astyanax fasciatus* is an abundant species in the Furnas reservoir. Besides its ecological importance in the ecosystem, it also supports fishing and is also recommended for biomonitoring essays. The aim of this study was to analyze the reproductive biology of *A. fasciatus* in Furnas reservoir and the impact of the anthopic activities on its gametogenesis.

Material and Methods

The experiments were conducted in two phases. Firstly, it was determined the reproductive period, type of spawning and histological characteristics of the reproductive stages. Bimonthly collections were made during a reproductive cycle, in a total of 209 fishes. In the second phase, in order to evaluate the impact caused by the environmental pollution on the gametogenesis, 117 specimens were captured during the reproductive period in four sites of the reservoir: Barranco Alto (polluted by pesticides), Fama (tourism), Boa Esperança and Guapé (domestic sewage) and a reference site (Turvo) with minor anthropogenic interference. For hystological analyses, sections of the gonads were fixed in Bouin´s fluid and processed by routine technique. The quality of the water in each site was assessed by the following parameters: temperature, oxigen, turbidity, pH, electrical conductivity and heavy metals.

Results and Discussion

The species presented a prolonged reproductive period and fractional spawning. The peak of the reproduction was coupled to the rain season and high temperatures. Comparing the impacted sites, most males and females were in reproductive activities in all sites, except Fama. At this site, more than 70% of the specimens were sexually resting and the maturing females had reduced IGS and oocyte diameter. High turbidity and elevated concentrations of aluminum and tin were also detected in the water at Fama. In conclusion, anthropic activities can affect the reproduction of *A. fasciatus* in the reservoir of Furnas. These results may be helpful to conservation and management programs of the reservoir native fish fauna.

Support: CNPq, CAPES, FAPEMIG and CODEVASF.

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Reproductive biology of tucunare *Cichla monoculus* (Pisces: Cichlidae), an alien species in Itumbiara Reservoir, Southeastern Brazil

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Introduction

The introduction of alien fishes has caused great changes in the fish fauna composition in many places. These introductions modify local ecological conditions by altering the reproduction, growth and development of native species, as well as causing hybridization and introduction of diseases and parasites (1). Considering that tucunaré *Cichla monoculus* is an alien picivore to Itumbiara reservoir and that reproductive studies of fishes outside their natural habitat is a basic parameter for understanding their adaptation and dispersion throughout the ecosystem, the current research intends to analyze the reproductive biology of this species.

Materials and Methods

From December 2004 to November 2005, 361 *C. monoculus*, (182 males and 179 females) were captured in Itumbiara reservoir. From each sample, the following data were obtained: total length, standard length, total weight and visceral weight. The following biological indexes were then calculated: gonadosomatic (IGS), hepatosomatic (IHS), stomach repletion (IRE) and condition factor (K). Gonads were fixed in Bouin´s liquid and submitted to histological routines techniques. Gonadal maturation stages were established based on macro and microscopic morphology of the gonads and on the IGS variation. The spawning type was determined based on the histological characteristic of spawned ovaries and on the frequency of gonadal maturation stages (2).

Results and Discussion

During the study period, 182 males (50,4%) and 179 females (49,6%) were collected, showing that the sex ratio was approximately 1:1. The biggest total length, standard length and body weight values were found in males, indicating sexual dimorphism for the species, which is a typical Cichlidae pattern. The following maturation stages were established for both, males and females: 1= resting, 2= initial maturation, 3= advanced maturation/mature, 4A= partially spent. In the current research, totally spent fishes were not captured. Males and females in reproductive activity were found all year around and partially spent females were not found only in June/July. The long reproductive period, the absence of totally spent females and the histological characteristics of partially spent ovaries containing postovulatory follicles along with oocytes in all developing stages, indicate that *C. monoculus* is a multiple spawner. The length at first sexual maturation in males and females, suggested by the smallest advanced maturation/mature specimens, was 31.0 cm and 29.0 cm long, respectively. The IGS average values in males and females followed gonad maturation, increasing from resting to mature and decreasing in partially spent. The average female IHS presented the lowest values in advanced maturation/mature, indicating hepatic substance (vitellogenin) transference to the ovaries during vitellogenesis. Both males and females IRE presented the lowest values in advanced maturation/mature and in partially spent stages, indicating that the fish had eaten less food during their reproductive period. The K values for males and females presented discrete variation, suggesting that reproduction does not interfere in the health condition of C. monoculus.

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Support: Furnas Centrais Elétricas SA,
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The reproductive biology of *Rioraja agassizi* (Müller & Henle, 1841) (Chondrichthyes, Rajidae), in Southeast Brazil, SW Atlantic Ocean

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Introduction

The Rio skate *Rioraja agassizi* (Müller & Henle, 1841) (Condrichthyes: Rajidae) is endemic to the South-western Atlantic coast of South America (1) and occurs from coastal waters to depths of up to 130 m (2). Females deposit egg capsules with mean dimensions of 47x31 mm (3). Apart from the description of the egg capsule, the reproductive biology and cycle of *R. agassizi*'s are unknown.

Materials and Methods

Samples of *R. agassizi* were monthly collected in from March of 2005 to March of 2006 in the area between 23°37'S and 27°40'S (States of Rio de Janeiro, São Paulo, Paraná and Santa Catarina) at depth between 10-146 m. Reproductive variables recorded to asses sexual development were: clasper, siphon, nidamental gland and uteri length, diameter of testicular lobules and ripe ovarian follicles, uteri and ovary condition and gonad weight. Gonadosomatic (GSI) and hepatosomatic (HSI) indexes were calculated. A logistic curve was fitted to the relationship between the fractions of mature males to calculate size-at-maturity.

Results and Discussion

A number of 275 males and 1049 females were captured and sampled. Both gonad were pair and functional. According to the analysis of the reproductive variables, size-at-maturity resulted in 32 cm for the males and 40 for the females. Rip vitellogenic follicles ovulate with 2.0 cm of diameter. The GSI, HIS, follicular and lobular diameter significantly varied ($p<0.05$) thorough the year in both sexes. However, ovulation, egg-laying, and therefore mating, occur thorough the year, suggesting an annual reproductive cycle for this species, as observed in other rajids (4).

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Reproductive potential of tucunaré *cichla monoculus* (Pisces: Cichlidae), exotic fish, in the Três Marias Reservoir, São Francisco River, Minas Gerais

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Introduction

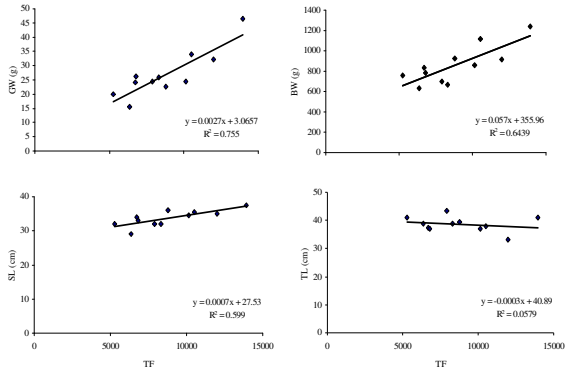
Tucunaré *Cichla monoculus* was introduced in the Três Marias reservoir, probably, in 1979/80. This exotic fish has importance in the commercial fishing and has caused decline of some endemic species. To analyze the tucunaré reproductive potential, the total and relative fecundity were determined.

Material and Methods

A total of eleven mature females were captured in October/2005. Total length (TL), standard length (SL), body weight (BW) and gonadal weight (GW) were obtained from each specimen. Fragments of gonads from all specimens were fixed in Bouin's fluid and subject to routine techniques for histological analysis. Larger and smaller diameters of 30 vitelogenic oocyte (O4) were obtained in histological sections. To estimate total fecundity, ovaries samples taken from its middle region were weighed and fixed in modified Gilson's fluid. After complete dissociation, the O4 were separated and counted using stereoscopic microscope. Relative fecundity was calculated with the total numbers of O4 by GW, BW, SL and TL. Pearson's correlation was done to establish the better parameter in the relative total fecundity determination.

Results and Discussion

Tucunaré females showed TL ranging from 33.0 to 43.5cm, and BW from 630 to 1.240g. Histology of O4 presented elliptic form, thin zona pellucida, prismatic follicular cells and ooplasm full of elliptic yolk globules, lipid vesicles and cortical alveoli. Average values of the larger and smaller O4 diameter were 1235.9 and 706.2µm, respectively. Total fecundity ranged from 5.263 to 13.944, with average of 8.792 oocytes. The best coefficient correlation was between total fecundity and GW (r = 0.86). Average relative fecundity by g of GW was 331.77. Literature data show differences in *C. monoculus* fecundity values, probably due to differences in animal size or on environment and ecological characteristics of reservoirs. The variation observed on total fecundity in the present study can be related to the multiple spawners of this species. The high correlation degree between total fecundity and BW show the efficiency of this method. These results are important to establish the estimate of size and dynamic population, besides reproductive prognostics of *C. monoculus* in the Três Marias reservoir.



Correlation of total fecundity (TF) of *Cichla monoculus* by: gonadal weight (GW), body weight (BW), standard length (SL) and total length (TL).

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Reproductive traits of Jau: a threatened brazilian catfish

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Introduction

Knowledge on initial life history of most Neotropical migratory fish is limited. Migrant reproductive strategy in which spawning is associated to pulse uprising of the river (1) renders collecting ontogenic series in the wild a difficult task. Buoyant eggs are carried down into usually large floodplains where larvae, < 5mm in length, hatch within a few hours. For these reasons, eggs, embryos and larvae obtained under assisted spawning conditions are useful material for such studies. The migrant catfish jau *Zungaro jahu* (2) is one of largest (> 150 kg, body weight) Neotropical fishes and sadly threatened of extinction. Here, we present novel data, although limited, on reproductive traits obtained from induced spawning of jau kept in hatchery conditions

Material and Methods

We used 1 female jau in the reproductive period of 2004/2005 and another in 2005/2006, from a limited brood stock of the Energy Company of Minas Gerais (CEMIG). We used the hypophysation method (3) to induce spawning, which consists of carp pituitary extract injections, 'dry' oocyte fertilization, and egg incubation in funnel-type upwelling incubators (3).

Results and Discussion

The results are in Table 1. In general, our data on reproduction traits of the jau were similar to those available for other Neotropical catfishes (3), especially in regard to hour-degrees at spawning (HDS), gonadosomatic index (GSI) and egg diameter, which appear to be characteristics of the group. Despite of low GSI, jau is a highly fecund species (> 300,000 eggs/spawning/female; present study). Possible causes related to the large variation on fertilization rate between individuals are usually ascribed to female preparedness, environment and/or hatchery management. In this work, we were not able to identify the possible cause (s).

Table 1. Data (mean ±s.d., when applicable) from induced spawning of individual jaus kept in hatchery conditions in two consecutive annual reproductive periods (2004-2005 and 2005-2006) [values in parenthesis = number of observations]

Parameter	2004/2005	2005/2006
Male (body weight, kg)	8.5	6.0
Female		
Body weight (BW, kg)	15.0	9.2
Hour-degrees at spawning (HDS=sum of water temperature at each hour during hypophysation)	220	220
Weight of stripped ova (OW, g)	485	435
Gonadosomatic index (GSI=OWx100.BW^{1})	3.2	4.7
Eggs/g of ova (n)	663	582
Pre-hydration egg diameter (mm)	1.6 ± 0.04 (16)	1.6 ± 0.1 (41)
Post-hydration egg diameter (mm)	2.4 ± 0.1 (48)	2.7 ± 0.1 (66)
Fertilization rate (%)	91.8	26.6
Duration of embryogenesis (from fertilization to hatching, h)	13.3	11.0
Size of larvae at hatching (mm)	4.3 ± 0.2 (20)	3.7 ± 0.2 (20)

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Biological indexes of piaparas *(Leporinus obtusidens)* captured downstream from Funil Reservoir, Minas Gerais, prior to 'piracema'

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Introduction

Piapara (*Leporinus obtusidens*), as the majority of the species of tropical fish, migrate upward the river on the onset of the reproduction period (November to January). The displacement of hundreds of kilometers, during the migration, affects all the physiology of these fish, unchaining essential processes for the preparation of reproduction. Construction of dams has influenced the reproduction of these species once they are restrained to reach physiological preparation for reproduction. The objective of this study is to evaluate the reproductive characteristics, through biological indexes, of piapara *(L. obtusidens*) captured downstream from Funil reservoir, Minas Gerais.

Materials and Methods

L. obtusidens individuals were captured downstream from Funil reservoir in September 2006. Two collections had been carried out, with an interval of 15 days between them and a total of 13 animals were captured. The captured fishes were kept in box with ice and taken to the Physiology Laboratory of the Department of Veterinary Medicine of the Federal University of Lavras. The fish were measured and weighed. According to the weight, they were assigned to one of the following classes: 1) 950 to 1300g; 2) 1301 to 1651g and 3) 1652 to 2000g. Their gonads and liver were removed and weighed for identification of the sex and stage of gonadal maturation. The following indexes were calculated: gonadosomatic (GSI) and hepatosomatic (HSI). ANOVA and Tukey test were performed using the statistical program SISVAR (2001).

Results and Discussion

The average values for gonadosomatic and hepatosomatic indexes in the different classes of body weight are presented in table 1. There was no significant difference for the evaluated biological indexes between fish samples (P> 0.05).

Table 1. Average values of biological indexes in Piaparas prior of "piracema".

Classes of weight	GSI*	HIS**
1	0.70	0.57
2	0.73	0.61
3	0.67	0.54

*Significant differences at 6% level
** There were no significant differences

The GSI was greater in the lighter fish classes 1 and 2.This finding shows that in these classes it is occurring a gonadal development compatible to the period prior to 'piracema' when the animals initiate the physiological responses due to the climatic variations (1). On the other hand, the HSI did not present significant differences (P>0.05) between the weight classes, which demonstrates that despite the beginning of the gonadal development, the liver still does not respond metabolically to the climatic variations. This demonstrates that in the evaluated animals the process of vitellogenesis have not initiated yet. This study intends to analyze these parameters in earlier phases prior to the period of 'piracema'

Aknowledgments

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Influence of different period on reproductive characteristics of Curimba (*Prochilodus lineatus*)

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Introduction

The curimba (*Prochilodus lineatus*) is a reofilic teleost fish, pertaining to the Grande River basin, in Brazil. The reproduction in captivity is made by hypophysation technique using injection of pituitary extracts. The reproductive performance of this species in captivity has not been well elucidated yet. It may occur differences of performance in initial, middle and end of reproductive period due to environmental changes (temperature and photoperiod) and corporal conditions of fish. The aim of this study was to investigate the effects of the reproductive periods of curimba (*Prochilodus lineatus*), kept in captivity on some of its reproductive characteristics.

Material and Methods

The experiment was carried out in the Fish Reproduction Laboratory of the Environmental Station of Itutinga, CEMIG (Energy Company of Minas Gerais), Brazil, using reproductive fish able to receive injections of pituitary extracts in the years 2003, 2004 and 2005. Each year was divided into three reproductive periods: initial (November), middle (December) and end (January). The animals were weighted and divided into three weight classes: lightweight, 1600g for females and 1000g for males; medium weight, 1600 to 2199g for females and 1000 to 1499g for males and heavy weight, equals to or greater than 2200g for females and 1500g for males. The occurrence of males and females able to the reproduce and the weight at spawning were analyzed according to the weight of the brooders and the period of reproduction. Fertility rate and spermatic characteristics (sperm motility, motility duration, and semen volume and spermatozoa concentration) were analyzed in relation to the different reproductive periods. Spermatic characteristics were analyzed only in the last reproductive year. The data were analyzed using the statistic software SISVAR. (Variance Analysis System – 2003), and Scott-Knott test was utilized to compare means.

Results and Discussion

Females with medium and heavy weighs presented greater weight of spawning ($P<0.05$). Reproduction period did not influence spawning weight. The number of females able to reproduce was significantly greater ($P<0.05$) in the middle and in the end of the reproduction period. The presence of apt males was influenced by the interaction weight and time of reproduction ($P<0.05$). The males presented higher reproductive frequency in the end of the reproduction period, while middle weight and heavy males presented similar frequency in all periods. The frequency of females and males was influenced by year of reproduction indicating that environmental factors influenced the reproductive physiology of fish in this experiment. Similar results were obtained by (1), who concluded that rain is the best stimulus for fish spawning. The fertility rate was not influenced by reproductive period ($P>0.05$), being influenced by other factors. There were differences in spermatozoa concentration ($P<0.05$) only in 2005. Males in the end of the year presented greater number of spermatozoa/mL. It was also observed reduction in spermatic pathologies in this group.

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Alternative method of embryo transfer in buffalo – preliminary studies

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Introduction

Many efforts for embryo transfer in buffaloes (*Bubalus bubalis*) have been made, during the last years, by different groups of study, although the results have shown low efficiency in the super ovulation process. The main problem is the low rate of embryo recovery despite of the superovulation response, which indicates that the low efficiency in embryo recovery is not due to a problem of the buffaloes in responding to superstimulatory treatment. Generally what is observed is a reasonable number of corporal lutea which does not match with the number of structures recovered. In this way, the costs of super stimulatory treatment is not justified by the number of structures recovered. In an attempt to find an alternative method to promote embryo transfer in buffalo viable an alternative method was tried.

Materials and Methods

Three female buffaloes were submitted to a synchronization program of timed artificial insemination (TAI) without super stimulation treatment with the proposition of recovering the embryo from the induced ovulation. On day 0 the buffaloes were treated with 200 mg of gonadorelin (Profertil®) and received an intra vaginal device (CIDR®) containing 1.9 g of progesterone. In the day 8 the CIDR was removed and 500 UI of eCG (Folligon®) was given intramuscular. On day 10 the animals received an intramuscular injection of 100 mg of gonadorelin. Two TAI were done, one in the moment of ovulation induction with gonadorelin and a second 16 hours later on day 11. Seven days after the second TAI the uteri were flushed with 500 mL of DMPBS flush for embryo recovery. *Recipient preparation*: The recipient buffaloes were submitted to the same protocol as the donors except for the TAI. All buffaloes were submitted to ultrasound exams to evaluate the follicle before TAI and in the day of embryo transfer to evaluate the corpus luteum. After embryo collection they were washed in DMPBS flush and were stored in DMPBS with BSA 0.4% for 1 hour before inovulation. *Embryo transfer technique*: The fresh embryos were transferred nonsurgically into synchronized recipients on day 7 after ovulation induction.

Results and Discussion

This abstract reports the possibility of an alternative method of embryo transfer in buffaloes since the superstimulatory treatments has not resulted in acceptable index (1). In the day of TAI the donors presented the following follicle sizes: 1.9; 1.4 and 0.8 cm. The buffalo with the follicle of 0.8 cm presented some difficult to be inseminated. During the flushes only one embryo was recovered from the buffalo which presented the follicle of 1.4 cm at the insemination. Although the number of embryo recover was low, compared to buffaloes that are superstimulated it can be a promise of an alternative method of embryo transfer in buffaloes. As it is well known, in programs of TAI in buffaloes, the index presented is around 50 to 55% of conception rate. Considering that in this preliminary study it was observed a recover of 33.33% of the possible embryos, at the same time as one buffalo presented difficulty to be inseminated, this can be a viable method to perform embryo transfer in buffalo with a relative low cost compared to a superstimulatory program.

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Pregnancy rate after transfer of bovine blastocyst derived from in vitro culture of grade III morulae produced *in vivo*

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Introduction

Embryo quality constitutes an important factor which influences the success of an embryo transfer program. Low quality embryos (grade III) represent a considerable proportion of all the embryos produced after collection of superovulated cows. Usually, those embryos are rarely transferred into recipients because the pregnancy rate is low. Recently it was demonstrated that *in vitro* short-term culture of *in vivo*-derived grade III bovine embryos increases the development and quality of such embryos (1,2). However, there is no information if such "reconstituted" embryos are able to continue their development after transfer into recipients. The aim of this study was to evaluate if blastocysts arising from *in vitro* short-term culture of grade III bovine morulae produced *in vivo* can promote acceptable pregnancy rates when transferred into recipients.

Materials and Methods

Embryos of different stages and qualities were recovered from superovulated *B. taurus* and *B. indicus* donors. Control, non-cultured grade I morulae and blastocysts, and grade III morulae were transferred into crossbred recipient heifers previously synchronized with the donors. Grade III morulae were cultured in either phosphate buffered saline (with 10% bovine fetal serum) or in Holding Plus^{TM} milieus for 24 hours at 38.5 ^{o}C. After this culture period, the resulting blastocysts were morphologically classified (grades I, II and III) and transferred into recipients. Pregnancy diagnosis was carried out 60 days after transfer and data were analyzed by logistic regression, considering the stage and quality of embryo, milieu of culture, day of cycle of recipient, size of corpora lutea of recipient, and uterine horn deposition of the embryo.

Results and Discussion

Quality of the embryo was the only variable to shown significant effect on the pregnancy rate. The pregnancy rate of non-cultured grade I (morulae and blastocyst) and III (morulae), and cultivated blastocysts arising from grade III morulae was 63.0% (n=73), 18.4% (n=38) and 45.7% (n=46), respectively (P<0.05). There were no differences between non-cultured grade I embryos and cultured blastocysts. Pregnancy rates of cultivated blastocysts grade I (75.0%) and II (57.9%) was higher than grade III (21.0%) (P<0.05). It was concluded that the procedure of culture *in vitro* of low quality morulae before transfer into recipients can increase the efficiency of an embryo transfer program.

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Short term urea feeding to beef cows at different moment within a MOET protocol: quality of recovered embryo and plasmatic data

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Introduction

Feeding cows with high levels of urea may decrease their reproductive performance through a raise in plasmatic urea nitrogen (PUN), a metabolic end product of protein catabolism (1). This study aims to verify the effects of short term urea feeding on two different moment following a multiple ovulation embryo transfer program (MOET) in beef cows.

Materials and Methods

Animals: Thirty nine beef cows (471.7 ± 65.8 kg).
Design: Completely randomized design.
Treatments: WU: Without urea; **UB:** Urea before D0 (from D-5 up to D0 of MOET protocol) and **UA:** Urea after D0 (from D0 up to D5 of MOET protocol).
Diets: 3 kg of concentrate mixture (finely ground corn, soybean meal and mineral mixture) offered at 7:00 am + *Brachiaria brizantha* pasture (*ad libitum*). The only difference among treatments' diets was the presence of urea on both UB and UA concentrate (100 g of urea/animal/day).
MOET protocol: A commercial protocol was used, starting on D-8 (D0: injection of ovulation inductor and D7: embryo recovery).
Experimental period: Experimental period lasted 16 days. Cows received diets during 16 days, performing embryo recovery during the last day.
Plasmatic data: Blood samples were collected for later measurement of PUN and glucose on three days: -5, 0 and 5 of MOET protocol. The methodology used was enzymatic-colorimetric with commercial kits.
Embryo data: The evaluation of embryo quality was accomplished following IETS handbook (2).
Statistics: Regarding embryo data, orthogonal contrasts verified: effect of urea inclusion in diet [WU vs. (UB + UA)] and effect of the moment of urea inclusion in diet (UB vs. UA). For plasmatic data repeated measures ANOVA and Tukey test were used. For all statistic analyses, a significant level of 5% was used.

Results and Discussion

Regarding PUN levels it was not observed treatment effect except on D5 when UA presented significant higher levels than UB (29.22 vs. 20.75 mg/dl). No treatment effect was observed regarding glucose levels. For both measurements, WU did not differ from others. About embryo data (Figure 1), it was observed neither effect of urea addition nor the moment of urea inclusion in diet, despite Dawuda et al. (4) reported a significant decrease in yield and quality of embryos recovered from dairy cows fed urea from AI up to uterus flushing (7 days period).

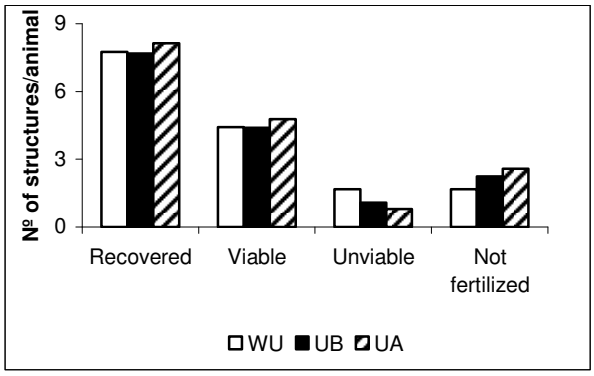

Figure 1- Evaluation of recovered structures

Conclusion

Feeding beef cows with 100 g of urea in 3 kg of concentrate/day in two different moments of a MOET protocol did not influence quantity and quality of embryos recovered.

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Type I IFN-related factors are constitutively present in the first trimester of pregnancy in the cow

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Introduction

The growth and development of the conceptus require progesterone and placental hormone actions on the uterus to the establishment and maintenance of gestation. Interferons (IFN) are cytokines that exhibit antiviral and antiproliferative activities. Atypical interferons (1) have been related to maternal recognition of pregnancy in ungulates. In this work in vivo placental interferon production was evaluated in the beginning of gestation in Bos taurus indicus and Bos taurus indicus x Bos taurus taurus cattle.

Materials and Methods

Samples of allantoic and amniotic fluids were collected from the placenta, concentrated 20 fold and antiviral activity was titrated in Madin Darby bovine kidney cells. Cytopathic effect in the monolayers was observed 48 h after challenge with vesicular stomatitis virus. Samples exhibiting high antiviral activity titers (> 1:120) were examined in scanning electron microscopy to avoid virus presence. To identify antiviral factors neutralization assays were done using polyclonal antibodies against IFNs –α, -γ and –τ. Bovine tumor necrosis factor (BoTNF) antiviral activity was also investigated. Checkerboard and residual activity tests, using mono and polyclonal antibodies against bovine alpha IFNs were done in order to examine structural-function relationship.

Results and Discussion

Amniotic and allantoic fluid supernatants showed antiviral activity in the first trimester of gestation. Out of nine samples submitted to anti-BoIFN neutralization assays, one showed to be antigenically related to rBoIFN-α1 (60 days) , another was neutralized by rBoIFN-τ antibodies (~30 days) and the remaining materials did not react with polyclonal antibodies against Bo-IFN- α, - γ or -τ.

Further, antiviral activity was not related to BoTNF-α. Virus particles were not observed through scanning electron microscopy, suggesting spontaneous production of antiviral factors. This antiviral activity might be related to type I interferons because of the physical properties observed and it seems that these factors are influencing placental membranes development. Production of placental lactogen is dependent of early IFN-τ priming and progesterone actions in the beginning of gestation (2). Alpha IFN in reproductive tissues in the cow is able to reduce epithelial cell numbers and probably has immunomodulatory effects in the uterus, preventing vertical infections between mother and fetus. Checkerboard and residual activity tests, using mono and polyclonal antibodies against bovine alpha IFNs, indicated that amino acids located at the N-terminus of helix A are relevant to neutralization of their antiviral activity.

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Antiviral factors are present throughout gestation in the cattle placenta and are poorly induced by NDV-virus

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Introduction

Interferons (IFN) have antiviral and antiproliferative properties and are present in placental tissues and fetal annexes in a number of species. They have been considered reproductive hormones in human and rats. In the cow, IFN-τ is responsible for maternal recognition of pregnancy (1). However, little is known about IFN production during gestation in cattle. In this study in vitro placental interferon production was evaluated throughout gestation in Bos taurus indicus and Bos taurus indicus x Bos taurus taurus cattle.

Materials and Methods

Placental tissues (allantochorion, amniochorion, caruncles, cotyledons and endometrium) were harvested from pregnant uterus in slaughterhouses, cultured in Minimum Essential Medium (EMEM) and half of the samples were induced with the Newcastle Disease Virus (NDV). Aliquots were concentrated 20 fold and antiviral activity was titrated in Madin Darby bovine kidney cells. Cytopathic effect in the monolayer was observed 48 h after challenge with vesicular stomatitis virus. Samples exhibiting high antiviral activity titers (> 1:120) were examined in scanning electron microscopy to avoid virus presence. To identify antiviral factors neutralization assays were done using polyclonal antibodies against IFNs –α, -γ and –τ. Bovine Tumor Necrosis Factor (BoTNF) antiviral activity was also investigated.

Results and Discussion

Only allantochorion and amniochorion supernatants exhibited antiviral activity throughout gestation. Neutralization assays showed that antigenically the materials did not react with polyclonal antibodies against Bo-IFN- α, - γ or -τ. Further, antiviral activity was not related to BoTNF-α. Virus particles were not observed through scanning electron microscopy, suggesting spontaneous (constitutive) production of antiviral factors in the control group. The observed antiviral activity might be related to type I interferons because of the physical properties they showed and it seems that these factors are influencing placental membranes development. No difference ($p>0.05$) between antiviral activity of induced and non-induced tissues were verified, thus it was concluded that placental genes are poorly induced by the NDV. Higher titers ($p<0.05$) were observed in the last trimester of gestation, and apparently antiviral factors production is related to fetal membranes development.

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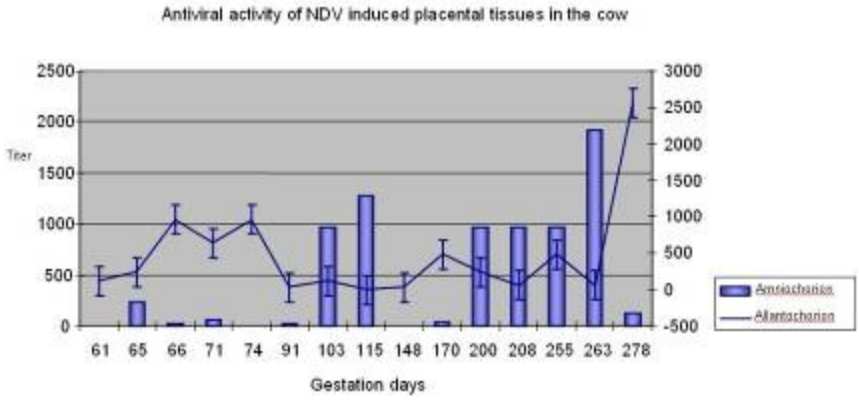

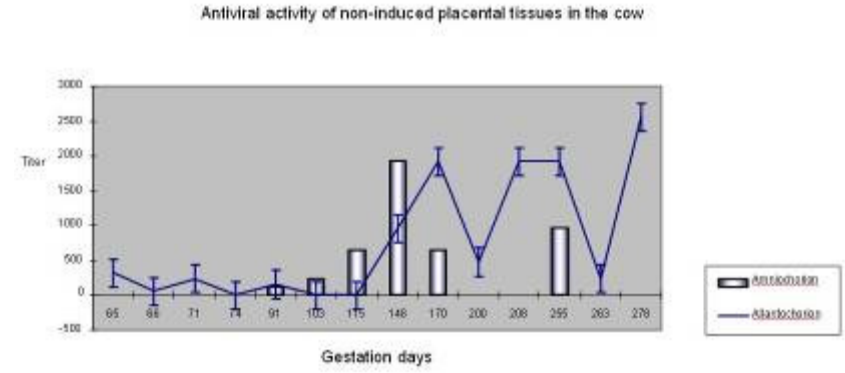

Maternal pre gestational and first stage of pregnancy exposures to air pollution are critical for low birth weigth in mice

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Introduction

The use of large extensive health related databases and continuous measurements of ambient pollutants provided sufficient evidences to support the concept that ambient levels of air pollution have adverse effects on fetal growth and gestational age. An important point in environmental toxicology relates to the effects of ambient levels of air pollution on the estimators of fetal development, such as birth weight. Recent reviews indicate that these results are observed in different parts of the world and exhibit consistency enough up to a point to conclude that these epidemiological associations are causal and not fallacious. Assuming that Sao Paulo is a large urban conglomerates with high level of air pollution and the causality of the epidemiological evidences relating air pollution and impairment of fetal development, the present study was undertaken to investigate the relative risk of low birth weight (**LBW**) associated to maternal pre-gestational and different gestational stages (**GS**) exposures to the ambient levels of urban air pollution.

Materials and Methods

Experiments were carried out in downtown Sao Paulo where the source of air pollution is predominantly automotive. Mice were raised and maintained in two inhalation chambers, one receiving urban ambient air (polluted, **P**) and the other receiving filtered air (clean, **C**). Seventy (12 weeks old) female mice were mated with fertile males and divided in 10 different exposure groups according to three gestational stages: GS1 from day 1 to 5 (pre-implantation); GS2 from day 6 to 10 (yolk sac placenta) and GS3 from day 10 to 19 (chorioallantoic placenta).The exposure protocols for the groups are shown in Table 1.

All the females were sacrificed in the 18th.day of pregnancy and the live fetus removed and weighed. It was carried out a linear regression to assess the exposure relative risks associated.

Results and Discussion

The results are summarized on Table 2.

Our findings show for the first time that maternal pre-gestational exposure (in real environmental conditions) is critical to increased risk for LBW in mice (11.17% higher). We found also that exposure at GS1 (and not during GS2 and GS3) is associated with an increased risk for LBW (5.4% higher), agreeing with epidemiological studies in humans (1).

Table 1. Differential exposures groups.

Groups	Pré-gestacional	Exposure Gestacional period		
		GS1	GS2	GS3
1	C	C	C	C
2	C	P	P	P
3	P	C	C	C
4	P	P	P	P
5	P	C	P	P
6	P	C	C	P
7	P	C	P	C
8	P	P	P	C
9	P	P	C	P
10	P	P	C	C

GS: gestational stage; C: clean chamber; P: polluted chamber.

Table 2. Relative risk for LBW according to the exposure.

Exposured period	Relative risk	p
Pré-gestational	11.178	0.001
GS1	5.403	0.020
GS2	1.650	0.199
GS3	1.342	0.247

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Pregnance as a protective factor of age-related proliferative lesions in gerbil female prostate

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Introduction

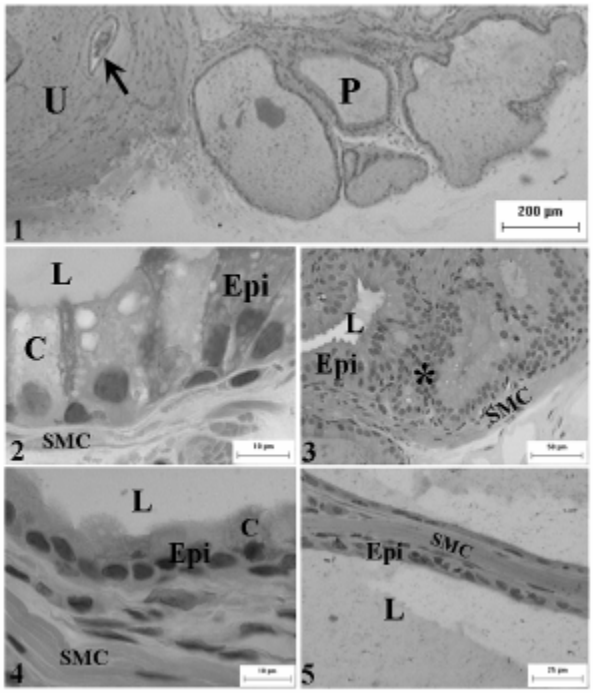
The female prostate shows several similarities with the basic structure and secretory activity of the male prostate (1). Tumor cells develop from secretory cells in both male and female prostate (2) and morphofunctional homologies were found between human and gerbil female prostate (3). In addition, this gland is under influence of steroids imbalance (4). This work objective was to compare, morphologically, adult and old gerbil female prostate in both pregnant and non-pregnant period with the aim to evaluate the influence of pregnancy on prostate gland structure.

Materials and Methods

Were used 20 female gerbils (*Meriones unguiculatus*), distributed in 4 groups: 5 adult pregnant animals; 5 adult non-pregnant animals; 5 old pregnant animals; and 5 old non-pregnant animals. After sacrifice, and tissue processing, the prostatic histological sections were subjected to hematoxylin-eosin (HE) staining for general and histopathological studies.

Results and Discussion

The gerbil female prostate shown a characteristic organization of glandular epithelium with basal and apical secretory cells, surrounded by a fibromuscular stroma with smooth muscle cells (SMC) and fibroblasts, immerses in extracellular matrix (Fig. 1). In all groups of animals were found some clear epithelial cells, suspecting neuroendocrine cells (Fig. 2 and 4). These cells are target of controversial discussions, and our morphological analyses just identify variations of phenotype, and cells markers are necessary to the cell type identification. This technique will be used in future works to further explanations. In addition, marked tissue and histopathological differences between groups were found. Adult non-pregnant/pregnant animals and old non-pregnant animals showed a normal high secretory epithelium (Fig. 2 and 3). Nevertheless, old pregnant animals showed very low secretory cells in epithelium, hyperplasic acini, an apparent high frequency of basal cells, and a more compact arrangement of stromal elements (Fig. 5). In addition, old non-pregnant female prostate shown several pathological non-proliferative lesions (Fig. 3). At the light of these qualitative results we can assume that pregnancy seems to prevent proliferative disorders in comparison to the senile non-pregnant females.



1) Pregnant gerbil adult female prostate gland: duct (arrow) urethra (U), prostate (P); **2)** Non-pregnant adult female gerbil: lumen (L), clear cell (C), epithelium (Epi) and smooth muscle cell (SMC); **3)** Non-pregnant old female gerbil: neoplasm (*); **4)** Pregnant adult female gerbil; **5)** Pregnant old female gerbil.

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Effects of *Citrus aurantium* essential oil on reproductive outcome of pregnant rats

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Introduction

Citrus aurantium L. (Rutaceae family) is a plant popularly known as bitter-orange or sour-orange. It is used on gastrointestinal disturbs treatment and presents diuretic and antispasmodic effects and action against taquicardy and rheumatism. More recently, this specie is being used as an important component of losing weight drugs. It was confirmed that its essential oil has an antibiotic effect against *Helicobacter pylori*. Although it has been very used popularly, no data was found in the literature about the effects of this plant on pregnancy. Thus, the aim of this work was to verify the effects of the essential oil of *Citrus aurantium* on reproductive outcome of pregnant rats.

Materials and Methods

Female rats were mated and vaginal smears were collected, and if sperm were detected, it was determined as day zero of gestation. Sperm-positive females were divided randomly into two groups: treated (T, n=12), whose rats received 500 mg/kg/day of *Citrus aurantium* essential oil, by gavage, from day 0 to 14 of pregnancy; control (C, n=12), that received tween following the same protocol. The oil administered to the treated group was extracted from fruit bark of the plant by hydro-distillation for 5 h using Clevenger-type apparatus. On day 20 of pregnancy rats were killed and the numbers of corpora lutea, implantation sites, resorptions, fetuses and fetal and placental weights were determined. From these results were determined pre- and post-implantation loss rates and placental index (1). Results were considered statistically significant at $p<0.05$.

Results and Discussion

The treatment with the essential oil did not interfere with body weight gain and food and water consumption of the rats. On the other hand, there was a significant decrease in placental weight (mean ± SEM: C= 0.53 ± 0.08; T= 0.46 ± 0.09) and placental index (C= 0.13 ± 0.02; T= 0.11 ± 0.02), without interference in fetal weight, which was similar in the two groups (C= 4.22 ± 0.43; T= 4.17 ± 0.32). The treatment also did not altered the parameters of the reproductive outcome (numbers of corpora lutea, implantations, resorptions and live and dead fetuses). Pre- and post-implantation losses rates were similar in the two groups (C= 15.72%; T= 12.50% and C= 6.72%; T= 3.40%, respectively). Thus, this study showed that exposure to *Citrus aurantium* essential oil, at the dose of 500 mg/kg/day, during preimplantation and organogenic periods, has no effects on the reproductive outcomes of pregnant rats.

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Early development of four commercial fishes from São Francisco River basin, Brazil

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Introduction

In fishes, early development includes stages of life history, which comprise eggs, embryos and yolk-sac larvae, being important for providing subsidies for aquaculture and reproductive biology studies. Egg surface ultrastructure is related to reproductive behavior and phylogeny (1), and carbohydrate molecules at oocyte surface play a role in fertilization and egg attachment (2). The present work analyzed egg surface ultrastructure, sugar residues in oocytes and embryo/yolk-sac larvae development of matrinxã *Brycon orthotaenia*, piau-verdadeiro *Leporinus obtusidens*, curimatã-pacu *Prochilodus argenteus* and dourado *Salminus brasiliensis*, commercial fishes from San Francisco River basin.

Materials and Methods

Fish were submitted to induced spawning by hypophysation at Hydrobiology and Hatchery Station of Três Marias/CODEFASF, and oocytes were fertilised immediately post-spawning. Following fertilisation, eggs were maintained in funnel-type incubators with continuous water flow at 24º C. Embryos were constantly monitored and larval development was recorded daily until total yolk sac reabsorption. Oocytes, embryos and larvae were analyzed by histology, carbohydrate histochemistry and scanning electron microscopy.

Results and Discussion

Histology and carbohydrate histochemistry showed similar characteristics in follicular cells, inner layer of zona radiata and yolk globules among all species. Cortical alveoli and outer layer of zona radiata were species-specific. The oocyte surface ultrastructure showed species specificity for parameters such as density and distance of zona radiata pore-canals in *B. orthotaenia*, *L. obtusidens* and *S. brasiliensis*, fibrillar net in *P. argenteus* and different micropyles in all species. These arrangements in egg surface such as fibrillar net and undecorated smooth zona radiata with visible pore-canals were also reported in neotropical freshwater teleosts that exhibited non-adhesive eggs and migratory behaviour (1). The embryonic and organ development were similar, with a brief embryogenesis, ranging from 17h to 21h, at 24-26 ºC. At hatching, the larvae were poorly developed in all species, showing no jaws, depigmented eyes and a big yolk sac. The total yolk sac reabsorption ranged from 2nd day post-hatching in *S. brasiliensis* to 5th day post-hatching in *L. obtusidens*, and larval mouth opened one day prior to total yolk sac reabsorption, stage that larvae depend on exogenous feeding (3). Egg surface and early development were similar between *S. brasiliensis* and *B. orthotaenia* and between *L. obtusidens* and *P. argenteus*, indicating phylogenetic proximity in these species.

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Gonadal maturation and embryonic development of Mandi-amarelo *Pimelodus maculatus* (Pisces, Pimelodidae) in captivity.

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Introduction

Mandi-amarelo has a wide distribution in Brazil and in various South America basins. It can reach 50 cm in length and 2 kg in weight. At the São Francisco river basin, mandi-amarelo is important in professional and sportive fishing.

Materials and Methods

To study gonadal maturation and embryogenesis of *P. maculatus*, fish were maintained in tanks at the Hydrological and Hatchery Station of Três Marias, CODEVASF-MG during one year. Bimonthly, samples of gonads were collected and fixated on Bouin´s fluid and then submitted to routine histological techniques: embedded in paraffin, sectoned at 5µm, and stained with hematoxilin-eosin. Fishes in advanced gonadal maturation were submitted to hypophysation with water temperature at 26ºC according to the station routine. Crude common carp pituitary extract (CCPE) were used as follows: 1) female fish: first dose = 0.8-1.0 mg of CCPE/kg of fish and second dose = 5.0-6.0 mg of CCPE/kg of fish; males: a single dose of 1.0-2.5 mg of CCPE/kg of fish. The interval between doses was 10-12 h. Injections were made in the celomic cavity or intra-muscular and fertilization was performed dry. Embryogenesis was observed at a stereoscopy microscope analyzing eggs samples collected from incubators with water temperature at 24ºC at intervals of 10 minutes until hatching.

Results and Discussion

In September-October, gonads were on initial maturation with ovaries contained perinucleolar and previtellogenic oocytes. Milky-white testes with the seminiferous tubules showing few quantity of sperm. In November-December, the gonads were at advanced maturation, maximum volume ovaries with predominance of vitellogenic oocytes. Testes with seminiferous tubules full of sperm. The following main embryogenic phases (Fig. 1) for *P. maculatus* with respective time after fertilization were registered: 2-8 blastomeres phase (20 minutes), blastula (2-5h), gastrula (5-9h), blastopore closure (7:30h), differentiation of embryonic layers and somite formation (9-13h), tail release (13-15h) and hatching (18h). Studies of gonadal maturation and embryogenesis provides important subsidy for handling and cultivation of potential fishculture species such as mandi-amarelo.

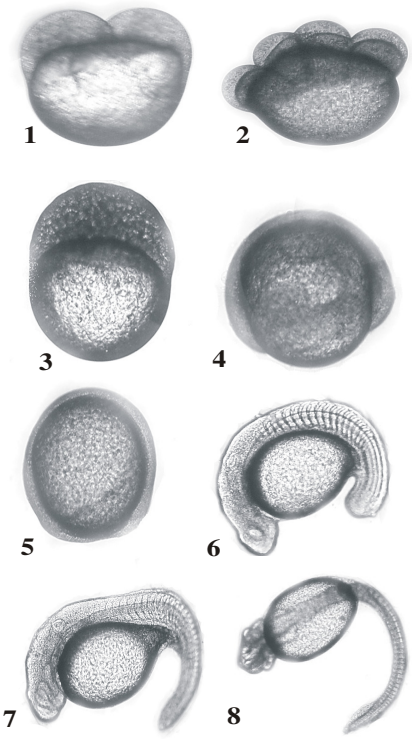

Fig. 1. Phases of the embryonic development of *P. maculatus*: (1) two blastomeres; (2) eigth blastomeres; (3) blastula; (4) gastrula; (5) blastopore closure; (6) differentiation of embryonic layers; (7) tail release; (8) hatching.

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