Presence of a GLIPR1 family member in the mouse epididymis

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The CAP superfamily includes four distinct groups: the cysteine-rich secretory proteins (CRISPs), the human glioma pathogenesis-related 1 (GLIPR1) proteins, the peptidase inhibitor 15 (Pi15) proteins, and the Golgi-associated pathogenesis-related 1 (GAPR-1) proteins. All members of the CAP superfamily are characterized by the presence of the Pr-1 domain in the N amino terminal region, two signature sequence motifs within this domain, and a hinge region containing four cysteines. The isolation and proteomic analysis of mouse sperm lipid rafts revealed the presence of two GLIPR1 family members, GLIPR1l1 and GLIPR1l2. Based on our reports on the role of CRISP proteins in different events of fertilization, in the present work we have cloned and characterized both Glipr1l1 and Glipr1l2 as a first step to study their potential role in the fertilization process. A sequence homology comparison revealed that all Glpr1 genes exhibit 30-80% identity and 50-90% homology. The study of the deduced protein sequences revealed that GLIPR1L1 is predicted to have a short C-terminal region lacking both a transmembrane domain and a glutamate rich domain (ERD) predicted to be present in the C-terminal region of GLIPR1L2. Studies of RT-PCR tissue screening showed high testicular expression for Glipr1l1 and expression in both the testis and caput epididymis for Glipr1l2. Semi-quantitative RT-PCR of Gliprl1 in the testis and of Glipr1l2 in both the testis and the epididymis showed a decrease in mRNA expression from day 0 (birth) to day 7, and a gradual increase thereafter reaching maximum levels at 60-70 days. Glpr1l1 and Glprll2 were cloned in E. coli, fused to His-tag. When the positive clones (selected by PCR screening) were subjected to IPTG induction, recombinant proteins with the predicted molecular weights of 30 kDa and 42 kDa for Gliprll1 and Gliprll2, respectively, were detected by SDS–PAGE analysis. In summary, we have characterized the tissue and developmental expression of Glipr1l1 and Glipr1l2 as well as successfully cloned and expressed the corresponding proteins. The availability of these recombinants proteins will allow the production of specific antibodies to investigate the potential role of GLIPR proteins in the maturation, capacitation and fertilization processes.

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Keywords: GLIPR1, epididymis, sperm, testis.

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Sperm Associated Antigen 11C expression along rat epididymis and its regulation by steroid hormones

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Introduction/Aims: Several genes encoding antimicrobial proteins have been reported in the epididymis, including the Sperm Associated Antigen 11 (*Spag11*) gene, which is located within a beta-defensin cluster in the human, rhesus, bovine and rodent genome. In these species, *Spag11* gene presents a complex genomic structure and mRNA splicing pattern originating different transcripts that code for several protein isoforms. One of these isoforms, the SPAG11C, is known to be expressed in the epididymal epithelial cells, and to act as a potent antibacterial agent in vitro. Its physiological role, however, has not been determined yet. The present work was designed to gain insights into SPAG11C cellular distribution along adult rat epididymis and its regulation by steroid hormones. **Methods:** Epididymides from male Wistar rats (90 days old) were isolated. Immunohistochemistry (using an anti-SPAG11C antibody) and in situ hybridization assay (using a *Spag11c* single-DIG labeled LNA probe of 22 nt) were performed. Proper negative controls were used. SPAG11C regulation by steroid hormones was evaluated in epididymides collected from rats submitted to sham-operation, surgical castration or bilateral adrenalectomy. SPAG11C immunofluorescence studies were also performed with sperm collected from testis and epididymal regions (initial segment/caput, corpus and cauda) from control rats. **Results and Discussion:** Immunohistochemistry showed SPAG11C-positive staining in the nuclear/perinuclear/supranuclear region and in the cytoplasm of epithelial cells along the entire epididymis. SPAG11C-positive staining was also revealed in few interstitial cells and in the smooth muscle layer surrounding the epididymal tubules and blood vessels from caput and cauda epididymis, in some nerve fibers present in the cauda region, and in spermatozoa located in the lumen of epididymal tubules. In situ hybridization confirmed *Spag11c* mRNA in the same epididymal cell types where SPAG11C immunolocalization was detected (except spermatozoa), confirming their ability to synthesize this protein product. SPAG11C immunofluorescence was detected in the head and principal piece of the flagellum of isolated testicular and epididymal sperm. Moreover, changes in the dynamics of SPAG11C immunolocalization in the head of the spermatozoa were observed during its transit from the testis to the cauda epididymis, suggesting a role for SPAG11C in events related to sperm maturation. *Spag11c* gene regulation by androgens was evidenced by in situ hybridization, which indicated a reduction of Spag11c mRNA in caput and cauda epididymis from 7- and 15-day castrated rats. Castration-induced changes on SPAG11C immunolocalization, however, depended on the epididymal region and cell type analyzed. In fact, SPAG11C expression in the epithelia and smooth muscle from caput epididymis was significantly reduced in 7- and 15-day castrated rats. In the cauda region, SPAG11C immunostaining was already reduced in the smooth muscle cells of tissues from 7-day castrated rats, while changes in the epithelia were significantly evidenced within 15 days of androgen deprivation. Glucocorticoids did not seem to play a significant role in *Spag11c* gene modulation, since adrenalectomy (7 and 15 days) did not change SPAG11C immunodistribution along the epididymis. **Conclusions:** We demonstrate for the first time that the expression of a beta-defensin-like gene, such as SPAG11C, is not restricted to the epithelial cells in the epididymis. Moreover, region- and cell-specific transcriptional and post-transcriptional events may be involved in the androgen regulation of this gene in the epididymis. Collectively, our data provide new perspectives into the search of SPAG11C physiological role in the epididymis and maturing sperm.

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Keywords: SPAG11, host defense, epididymis.

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Expression of the ATP-binding cassette transporter ABCG2 in the bovine epididymal duct and spermatozoa

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During the epididymal transit, testicular spermatozoa undergo modifications to acquire their fertilizing ability. One of the biochemical events suggested to be involved in this is the association to the sperm plasma membrane of stabilizing factors, like cholesterol sulfate. ABCG2 is a member of the ATP-binding cassette membrane transporter family involved in the efflux of sulfate conjugates and cholesterol. Its presence has been reported in several cells and tissues including testis, as well as murine and ejaculated bovine spermatozoa. Based on these findings, it is proposed that ABCG2 is present in the bovine epididymal spermatozoa and it has a cholesterol sulfate transporting activity. To corroborate the first hypothesis, the objective of this work was to evaluate the presence of ABCG2 in the spermatozoa and the epididymal duct. All the studies were carried out with material from sexually matured bulls obtained from the slaughterhouse. Immunochemical ABCG2 localization studies on spermatozoa from different epididymal regions revealed the presence of the protein on the acrosomal cap. Western blot assays confirmed the presence of expected molecular weight protein (~70 KDa). The membrane localization of the transporter was confirmed by nitrogen cavitation experiments on cauda epididymal spermatozoa. Localization studies done on testicular tissues and spermatozoa also showed the presence of the protein on the acrosome. ABCG2 expression was evaluated on tissues from the testis and different epididymal regions by immunohistochemistry, Western blot and semi-quantitative analysis, and Real-Time PCR. Protein and mRNA expression showed an increase from caput to proximal cauda with a decrease at the last cauda epididymal region. The protein was localized mainly in the apical plasma membrane of the cauda epididymal epithelial cells. Western blot assays and semi-quantitative evaluation done on protein extracts from membranous vesicles showed an increase in ABCG2 expression from caput to cauda epididymosomes with a really strong signal in membranous vesicles from seminal plasma. Spermatozoa-membranous vesicles co-incubation experiments were carried out. Subsequent Western blot assays and semi-quantitative analysis demonstrated that the amount of ABCG2 in caput spermatozoa does not change after caput, corpus or cauda epididymosomes co-incubation as well as in cauda spermatozoa after co-incubation with cauda epididymosomes. However, there was a significant decrease in the amount of the protein in cauda spermatozoa after co-incubation with membranous vesicles from seminal plasma. These results indicate that ABCG2 is present in the acrosomal region of bovine spermatozoa in the testicular output and in the epididymal epithelium and its apocrine secretion (membranous vesicles). The decrease in the amount of the transporter in the cauda epididymal spermatozoa after co-incubation with membranous vesicles from seminal plasma suggests a role for them in post-ejaculatory maturational processes.

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Keywords: bovine, ABCG2, ABC transporters, spermatozoa, epididymis.

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Spermatozoa mediated regulation of the epididymal gene expression

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Introduction: Epididymal gene expression is characterized by strong tissue-, segment- and cell-specificity, leading to checkerboard-like expression patterns for many genes. It is well established that androgens and unknown testicular factors present in efferent duct fluid (lumicrine factors) regulate gene expression in the epididymis. This regulation seems to be region-specific. Initial segment specific genes are mainly regulated by lumicrine factors whereas caput specific genes by androgens. However, the basis of lumicrine regulation is still poorly known. **Aims:** The aim of this study was to analyse whether the spermatozoa themselves or spermatozoa associated factors regulate gene expression in the epididymal epithelium. **Methods:** For studying the lumicrine regulation of epididymal gene expression, we have used Inhα-HSV-TK mouse model, in which germ cells are totally ablated when mice are treated with famcyclovir. In this model, Leydig cells function normally producing normal amounts of testosterone. Since hormonal levels of famcyclovir treated Inhα-HSV-TK mice are normal, we could distinguish the effects of sperm-associated and Leydig cell-derived lumicrine factors on regulation of epididymal gene expression. The effects of lack of sperm on gene expression in the initial segment have been analysed using Illumina's Mouse WG-6 v2 Expression BeadChip arrays, Gorilla pathway analyses and real-time RT-PCR. **Results:** The germ cell ablation in the testis resulted into down-regulation of 448 genes in the initial segment. Highly down-regulated genes contained many initial segment specific genes that have been previously known to be regulated by lumicrine factors, such as *Rnase10*. In addition, 248 genes were significantly up-regulated. Gorilla pathway analysis of the differentially expressed genes revealed that the biological processes mostly affected by the lack of sperm in the epididymis were the lipid biosynthesis process and the regulation of kinase activity. **Discussion:** Previous studies have demonstrated that especially initial segment of the epididymis requires testicular factors other than testosterone to maintain its functions. However, the identities of those factors have remained elusive. Our work demonstrates *in vivo* that spermatozoa themselves or sperm-associated factors regulate the expression of large groups of genes expressed in the initial segment. Further, as the epididymal maturation of the spermatozoa include modification of sperm plasma membrane lipid and protein composition, our data suggest that spermatozoa regulate epididymal genes important for the epididymal sperm maturation. **Conclusions:** Our results demonstrate that spermatozoa or sperm-associated factors are able to regulate epididymal gene expression *in vivo*. Furthermore, based on our results, lipid biosynthesis and regulation of kinase activity are the most-affected processes by the lack of sperm.

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Keywords: spermatozoa, regulation, epididymal gene expression.

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The LXR knockout mouse: a new model for dyslipidemia-induced post-testicular male infertility

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Introduction: Knockout mouse models showed that lipid signaling is critical for male reproductive capacity, and more particularly for sperm post-testicular maturation processes and, consequently for sperm function. Liver X receptors (LXR), nuclear receptors for oxysterols, are of major importance in systemic and cellular cholesterol homeostasis, and are thus good candidates to play a role in gamete maturation and function. **Specific aims**: Using a knockout mouse model for both LXRα (Nr1h3) and LXRβ (Nr1h2), the *lxrα;β-/-* animals, we have investigated the importance of cholesterol homeostasis in epididymal function. *Lxrα;β-/-* male mice aged over 9 months show testicular and epididymal phenotypes associated with complete infertility that were previously described by our group and others. Data presented below summarize the more recent advances made to better understand the roles of LXR in post-testicular sperm maturation. **Methods:** Lipid accumulation was evaluated by oil-red-O staining on 7µm thick cryosections; protein localization was performed using immunohistochemistry associated with peroxidase-dependent staining on 7µm thick paraffin sections; apoptosis was evaluated using a TUNEL method; mRNAs of the *de novo* cholesterol synthesis pathway were quantified using qPCR with SYBR green detection method, sperm viability was examined using an eosin/nigrosin coloration method and sperm acrosome was detected by staining with PNA-Alexa fluor. **Results**: First, the study demonstrates a new function of the proximal epididymis apical cells in cholesterol trafficking. Caput segments 1 and 2 apical cells of *lxrα;β-/-* mice were filled-up with cholesteryl esters due to the loss of ATP-binding cassette transporter A1 expression (ABCA1, a transcriptional target of LXR), involved in cholesterol efflux. This loss was associated with an increase in apoptosis. ATP-binding cassette transporter G1 (ABCG1) and scavenger receptor B1 (SR-B1), two other cholesterol transporters, showed little if any modifications, and *de novo* synthesis pathway was not affected either. The second part of the work demonstrated that the epididymis was an early target of cholesterol toxicity under dyslipidemic conditions. Briefly, an overload in dietary cholesterol led to complete infertility in young dyslipidemic *lxrα;β-/-* male mice that otherwise were fertile. Dietary-induced infertility resulted essentially from post-testicular defects impacting the fertilizing potential of spermatozoa. Spermatozoa of cholesterol-fed young *lxrα;β-/-* animals were found to be dramatically less viable and motile and highly susceptible to premature acrosome reaction. In addition, we provide evidence that this lipid-induced infertility is associated with the early appearance of a regionalized epididymis phenotype that otherwise would occur only in aging LXR-deficient males. This phenotype is characterized by peritubular accumulations of cholesteryl ester lipid droplets in smooth muscle cells lining the epididymis duct, leading to their transdifferentiation into foam cells that eventually migrate through the duct wall, a situation that resembles the well known inflammatory atherosclerotic process. Furthermore, the testis function of these animals is not affected, pointing out the particular sensitivity of the epididymis to dietary-induced cholesterol imbalance. **Discussion/Conclusions**: Epididymal sperm maturation is of primary relevance for fertilizing abilities and this organ appears to be very sensitive to cholesterol homeostasis changes when LXRs are invalidated, creating dyslipidemic conditions. The work presented here clearly demonstrates that complete infertility may have an epididymal-only origin without affecting testicular function. It also establishes the high level of susceptibility of spermatozoa epididymal maturation to dietary cholesterol overload and could partly explain reproductive failures encountered by young dyslipidemic men as well as aging males wishing to father.

Keywords: male infertility, sperm, cholesterol homeostasis.

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Implications of hormone levels and transepithelial calcium transport alterations in the formation of epididymal stones in roosters

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Epididymal lithiasis is a reproductive dysfunction described in roosters, which is associated with early loss of fertility and is characterized by the formation of luminal stones rich in calcium in the efferent ductules. In birds, the efferent ductules are involved in both testicular fluid and calcium reabsorption. These functions are essential for sperm concentration and maturation and are under control of several hormones, including androgens and vitamin D3. We recently showed that androgen receptor (AR) and vitamin D3 receptor (VDR) are overexpressed in the epididymal region of the affected animals, pointing out that a hormonal imbalance and/or alterations in efferent ductules fluid or calcium reabsorption could be involved in the origin of epididymal lithiasis. However, it is well known that the efferent ductule reabsorptive functions are also under control of estrogens and its receptors ERα and ERβ. Estrogens also regulate the expression of proteins involved in calcium reabsorption, such as TRPV6 and calbindin D-28K. Thus, the present study aimed to investigate the expression of ERα and ERβ as well as TRPV6 and calbindin D-28K in the epididymal region of affected roosters. Additionally, we addressed the concentrations of estrogen, vitamin D3 and androgen in the epididymal region, testis and plasma of affected animals. Fragments of tissue were processed for immunohistochemical and Western Blotting assays to investigate protein expression, whereas hormone concentrations in tissue and plasma were measured by ELISA. It was shown that estradiol levels were increased (95%) in the epididymal region of affected animals, whereas plasma levels decreased by 30%. Conversely, vitamin D3 concentrations were decreased (86%) in the epididymal region and increased 11 fold in the plasma of affected animals. No alterations in testicular estradiol and vitamin D3 measurements were detected. Testosterone levels were decreased in testis, epididymal region and plasma of affected roosters when compared to non-affected animals (80, 84 and 60% respectively). Regarding protein expression, both ERα and ERβ were found to be expressed in the epididymal region. ERβ was widely expressed in the epithelium of all tubular components of the epididymal region, as well as in some connective tissue cells. ERα was found to be strongly expressed in the efferent ductule epithelium, whereas the epididymal duct showed a moderate staining. In affected roosters, ERβ expression increased significantly in the epithelium of all segments analyzed, contrasting with ERα protein which was not altered in epididymal tissue. TRPV6 was found to be strictly expressed at the apical membrane of the non-ciliated cells of the efferent ductules epithelia, whereas calbindin-D28K was expressed in the cytoplasm of the epithelia of efferent ductules and epididymal duct. Both proteins were increased in the epididymal region of animals affected by lithiasis. Taken together, our results suggest that alterations in both hormonal levels and transepithelial calcium transport may be related to the formation or development of the luminal calcium stones.

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Keywords: efferent ductules, estrogen receptors, epididymal lithiasis, roosters, calcium transport.

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Lysosomal proteins could be transported by alternative pathways in rat epididymal cells

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Introduction: The mammalian epididymis is involved in the maturation of gametes and its integrity and functionality depends on steroid hormones. The epididymal epithelium secretes proteins into the lumen, which could be involved in sperm maturation. In the epididymis of mammals, the secretion is enriched in acid hydrolases, an unusual fact, because these enzymes are normally confined to the lysosomal environment. In most cell types, the normal distribution of lysosomal enzymes is mediated by receptors that recognize mannose-6-phosphate (MPRs). Two forms of these receptors have been described so far, the cation-dependent (CD-MPR) and cation-independent (CI-MPR). In some cell types, the CD-MPR participates in the secretion of lysosomal enzymes, while the CI-MPR is involved in endocytosis of ligands. From previous results, we observed an increase in the expression and secretion of procathepsin D in rats deprived of androgens (castration), indicating that these events could be regulated by steroid hormones. Furthermore, it is known that the gene encoding Cathepsin D contains an element of response to estrogen, indicating that these hormones regulate the expression of the enzyme. Although procathepsin D is carried by the MPRs in most cell types, alternative routes have been proposed for transport to lysosomes. For example, in some cell types, procathepsin D complexes with prosaposin (a soluble lysosomal protein), which are transported to lysosomes or released into the extracellular medium through the sortilin receptor. **Aims:** From this evidence, we wonder if the transport and secretion of procatepsin D are mediated by CD-MPR or sortilin or both in epididymal cells and whether this mechanism is regulated by estrogenic hormones. We proposed to study the incidence of Sortilin and CD-MPR in the secretion of procathepsin D in epididymal cells subjected to hormonal changes. **Methods:** A cell line derived from rat epididymis (RCE-1, generously provided by Dr. D. Cyr), was used in the experiments. The cells were grown in D-MEM-F12 medium. They were then subjected to treatment with estradiol or with its antagonist, tamoxifen. In some experiments, hormone treatment was combined with NH_4Cl. After 48 hr of treatment, the media were collected and cells were homogenized to study the expression of proteins. Co-immunoprecipitation assays were performed to detect prosaposin in the extracellular medium and to observe if this protein formed complexes with procathepsin D. **Results and Discussion:** We observed that procathepsin D and prosaposin are complexed in the extracellular medium and these complexes are increased in the RCE-1 cells treated with estradiol. In addition, treatment with estradiol induced an increase in the expression and secretion of cathepsin D. Moreover, NH_4Cl caused a reduction in the secretion of procathepsin D and an increased intracellular retention of the enzyme. This retention could be justified by the increased expression of sortilin due to estradiol, which is powered by the acidotropic amine. All this suggests that increased secretion of the enzyme due to estradiol is not "by default" and that alternative routes for the transport of cathepsin D (sortilin mediated) would be involved, which are regulated by estrogens. Although the expression of CD-MPR was not affected by estradiol, its involvement in intracellular trafficking of the enzyme in RCE-1 cells can not be ruled out. Unexpectedly, NH_4Cl caused a greater secretion of prosaposin in RCE-1 cells, and probably this is explained by an alteration in the turnover of this protein. Based on these results, we decided to directly investigate the incidence of sortilin on transport and secretion of procathepsin D in epididymal cells. For this, we designed an experimental model on RCE-1 cells, with silenced sortilin-gene, by using siRNA. This knock down resulted in an increase in the expression of CD-MPR and prosaposin, and a redistribution of both proteins. However, procathepsin D showed no major changes in intracellular distribution, suggesting that the enzyme could be transported either by sortilin or CD-MPR. In conclusion, this study shows that some proteins of epididymal cells could be transported by alternate routes which are regulated by hormones.

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Keywords: epididymis, lysosomal enzymes, receptors, hormones.

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Genome-wide mapping of *in vivo* androgen receptor binding sites in mouse epididymis[#]

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Epididymal structure, function and gene expression depend on androgen signaling through the androgen receptor (AR). However, most *in vivo* direct AR target genes in epididymis remain unknown. Here we globally mapped the AR binding sites using ChIP-seq in mouse caput epididymis where AR is highly expressed. AR bound selectively to 19,377 DNA regions, the majority of which were located in intergenic and intronic regions. Motif analyses showed that 94% of the AR binding regions harbored consensus androgen response elements (AREs) enriched with potential coregulator binding motifs that include the previously reported NF1 sites and novel AP-2 sites. ChIP-qPCR assays revealed that NF1 and AP-2 occupied most of the detected AR binding regions, indicating the combinatorial regulation of NF1, AP-2 and AR. AR binding regions were associated with an active chromatin marker, histone H3 acetylation and exhibited transcriptional regulatory activities. Unexpectedly, AR binding regions showed only limited conservation across species regardless of whether local sequence similarity or presence of ARE motifs were used as the metric for conservation. Further analyses illustrated that AR target genes were extensively involved in diverse biological themes, including lipid metabolism, tight junctions, MAPK signaling pathway and sperm maturation. Our data also suggested novel mechanisms of AR regulation at individual genes, such as Crisp1 and Fkbp5. Overall, our studies provide a useful resource of global binding sites for AR, enabling a more profound understanding of AR-regulated important physiological processes such as sperm maturation in a tissue-specific manner and suggest new insights for regulation mechanisms of AR under physiological conditions.

Keywords: androgen receptor, gene regulation, epididymis.

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Characterization of a dendritic cell network in the murine epididymis

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Introduction: The establishment and maintenance of male fertility requires close interactions between the reproductive, endocrine and immune systems. Yet, the mechanisms that prevent the development of an autoimmune response against spermatogenic cells and mature spermatozoa, while maintaining the ability to fight pathogens and cancer cells, are still not well understood. These mechanisms have been studied mostly in the testis, probably largely underestimating the immune responses that take place in the epididymis. Immune cells have been observed in the epididymis, but the precise nature of these cells and their immunological properties remain to be elucidated. Dendritic cells, which are key regulators of immune functions in numerous organs, have never been identified in the epididymis. **Aims and methods**: The present study aims at characterizing immune cells that form a dense peritubular network in the epididymis, using a combination of microscopy, flow cytometry and in vitro antigen presentation techniques. **Results**: We describe an unexpectedly extensive network of stellate cells in the murine epididymis expressing CD11c-EYFP and CX3CR1-GFP, two reporter proteins that have been widely used to visualize dendritic cells. Based on their dendriform morphology, their intimate interactions with the epididymal epithelium, their phenotype and antigen-presenting capabilities, we named these cells "epididymal dendritic cells" (eDCs). CD11c and CX3CR1-positive cells populate the base of the epithelium in the entire organ, but intraepithelial dendrites are extremely abundant in the initial segment. In this region, 3D reconstructions of image stacks showed that most, if not all, epithelial cells appear to be in direct contact with a DC, and each DC extends dozens of dendrites toward the lumen. In the more distal regions (corpus and cauda), DCs are exclusively peritubular. We isolated eDCs and characterized CD11c+ CD103+ and CD11c+ CD103- cells, two populations that have been shown to exhibit distinct functional properties in organs such as the gut and the lung. All CD11c+ cells express CX3CR1 and MHC class II. Interestingly, CD103+ eDCs express DC-SIGN and langerin, which are involved in infection by viruses. CD206-positive macrophages do not express CD11c, they are exclusively interstitial and do not exhibit the stellate morphology of CD11c+ cells. The macrophage marker F4/80 is expressed by a subset of CD11c+ cells as well as some CD11c- cells. Finally, in accordance with their high level of expression of MHC and co-stimulatory molecules, we show that FACS-sorted CD11c+ eDCs take up, process and present ovalbumin to activate the proliferation of OT-I and OT-II T cells in vitro. **Conclusion**: the murine epididymis contains a dense network of dendritic cells, together with other cells from the mononuclear phagocyte system. All these cells are ideally positioned to play a major role in male reproductive physiology and pathophysiology, possibly by regulating the fragile balance between tolerance to maturing spermatozoa and defense against pathogens. Unraveling DC function in the unique environment constituted by the epididymis will contribute to better understanding the mechanisms of tolerance and autoimmunity in general.

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Keywords: epididymis, dendritic cells, macrophages, immune response.

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Impact of oxidative stress on Peroxiredoxin 1 and 6 in rat epididymis and epididymal spermatozoa

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Introduction: Peroxiredoxins (PRDXs) are enzymes with dual role as scavengers of reactive oxygen species (ROS) and as modulators of ROS signaling. We previously demonstrated that PRDXs are dose-dependently oxidized after exposition of human spermatozoa to H_2O_2, resulting in the formation of disulfide bridges and high molecular mass complexes. PRDX 1, 4, 5 and 6 are differentially localized in sperm subcellular compartments (head, acrosome, mitochondrial sheath and flagellum). PRDX 1 and 6 are diminished and highly oxidized in spermatozoa from infertile men. **Aims:** The aims of the present study were 1) to determine the localization of PRDX1 and 6 in epididymis and spermatozoa and 2) to determine whether PRDX 1 and 6 levels and oxidized status are altered by oxidative stress induced by tert-butyl hydroperoxide (*t*-BHP) in male rats. **Methods:** Adult Sprague-Dawley male rats were treated with *t*-BHP or saline (controls) by i.p. injection for 2 weeks. After the animals were euthanized, epididymis and liver were retrieved and frozen until RIPA protein extraction. Localization of PRDXs were determined by immuno-histochemistry in paraffin-embedded sections. PRDX 1 and 6 expression (reducing conditions) and oxidized status (non-reducing conditions) were determined by SDS-PAGE and immunoblotting. Lipid peroxidation (LP) was determined by the 2-thiobarbituric acid reactive substances (TBARS) assay. Spermatozoa from the cauda epididymis were also obtained to determine levels of lipid peroxidation and oxidized PRDXs. **Results and Discussion:** Immunohistochemistry of epidydymis (caput and cauda) sections revealed that PRDX 1 and 6 are expressed in principal basal, narrow and halo cells, but not in clear cells. In the caput epididymis, PRDX 6 was found also in the nucleus of principal cells. Spermatozoa from *t*-BHP-treated rats have higher levels of TBARS and of oxidized PRDX 1 and 6 and lower motility compared to controls ($P < 0.05$). These results suggest that spermatozoa from treated rats have impaired function and reduced antioxidant protection. Moreover, PRDX 1 and 6 levels were higher in *t*-BHP-treated rats compared to those from controls ($P < 0.05$), probably due to an increased residual cytoplasm. We observed an increase of PRDX 1 and 6 expression in caput and cauda epididymis, respectively ($P < 0.05$) and a trend of increase of PRDX 1 in cauda epididymis in *t*-BHP treated rats compared to controls. Levels of oxidized PRDX-6 were increased in the cauda epididymis of t-BHP-treated rats compared to controls ($P < 0.05$), suggesting enzymatic inactivation. LP was similar in epididymis from control and treated rats. Together these data suggest that oxidized PRDXs levels are a more sensitive marker for oxidative stress compared to LP. The over expression of PRDX 1 and 6 in the epididymis did not prevent the deleterious effects of oxidative stress on epididymal spermatozoa. The increased levels of oxidized (and inactive) PRDX 6 may contribute to the decrease of the antioxidant protection in the epididymis. **Conclusions:** The oxidative stress generated by *t*-BHP treatment affected the antioxidant protection during epididymal maturation causing deleterious effects on spermatozoa. PRDX 1 and 6 in epididymal spermatozoa were oxidized and unable to protect these cells against oxidative stress damage. These findings may help to better understand the causes of infertility in reproductive malignancies associated to oxidative stress such as varicocele or idiopathic infertility.

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Keywords: reactive oxygen species, oxidative stress, peroxiredoxins, rat epididymis, rat spermatozoa.

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Molecular mechanisms involved in the association of CRISP1 to sperm during epididymal maturation

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Cysteine-Rich Secretory Protein 1 (CRISP1) is synthesized in the rat epididymis and associates with the sperm surface during maturation. Our previous results support the existence of two populations of CRISP1 in sperm: one loosely-associated that is released during capacitation, and one strongly bound which remains after capacitation and participates in fertilization. However, the mechanisms underlying the association of these populations remain unknown. Considering the high concentrations of zinc (Zn^{2+}) in the epididymal lumen, in the present work we investigated the potential involvement of this cation in the association of CRISP1 to sperm during maturation. For this purpose, caput epididymal sperm were incubated with biotinylated CRISP1 in the presence or absence of 1mM Zn^{2+} evaluating the association of the protein to the cells by flow cytometry. Results revealed that only the cells exposed to the cation exhibited an increase in fluorescence that was dependent on CRISP1 concentration and could be blocked by EDTA. To examine the localization of the bound protein on the cells, both caput and cauda sperm exposed to biotinylated CRISP1 in the presence or absence of Zn^{2+} were analyzed by epifluorescence microscopy using avidin-FITC. While a faint labeling was observed in the absence of Zn^{2+}, caput and cauda sperm exposed to the cation exhibited a clear staining in the tail and in both the tail and the head, respectively, suggesting differences in the ability of the sperm plasma membrane to associate CRISP1 during maturation. No changes in CRISP1 tryptophan fluorescence spectrum were detected when the protein was exposed to increasing Zn^{2+} concentrations indicating that the presence of the ion would not affect CRISP1 conformation. To evaluate the potential formation of CRISP1 complexes induced by the presence of Zn^{2+}, the epididymal protein was incubated with the cation *in vitro* and then subjected to native gel electrophoresis and Western blot using the polyclonal antibody anti-CRISP1. Under these conditions, a high molecular weight complex was detected. To investigate the possible formation of such a complex *in vivo*, epididymal fluids were analyzed by electrophoresis in native gels and Western blot. Results revealed the presence of a high molecular weight band not detected in fluids pre-treated with EDTA. When the high molecular weight band was extracted from the nitrocellulose membrane by SDS and re-analyzed by SDS-PAGE and Western blot, a band of 32kDa was detected confirming the presence of CRISP1 in the fluid complex. Altogether, these results suggest that a molecular complex between CRISP1 and Zn^{2+} might be involved in the *in vivo* association of CRISP1 to sperm during epididymal maturation.

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Keywords: CRISP1, zinc, sperm, epididymis, maturation.

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Influence of age in the expression of estrogen receptor ERα, sodium-hydrogen exchanger 3 (NHE3) and aquaporin-9 in efferent ductules of rats

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Introduction: The efferent ductules comprise the male tract segment that connects the rete testis to the epididymis. These ductules are responsible for reabsorption of most of the fluid coming from the testis, thereby concentrating sperm prior to their entrance in the epididymis, favoring the process of sperm maturation and storage. The reabsorption function involves coupling of Na^+ and water transport from the lumen toward the basolateral membrane. This apical transport is mediated by sodium-hydrogen exchanger 3 (NHE3) and aquaporin-9 (AQP9), respectively. There is evidence that disturbance in the reabsorptive function of efferent ductules may result in fluid build-up and testis swelling followed by atrophy and subsequent infertility. Estrogens acting via specific receptor ERα play an important role in the regulation of efferent ductule reabsorption, by modulating NHE3 and AQP9, which are key proteins in this process. Although the importance of estrogens in male fertility is now recognized, it remains unknown whether aging alters the expression of ERα or proteins modulated by the estrogen responsive system, such as NHE3 and AQP9. **Aims**: The purpose of this study was to investigate possible changes in epithelial morphology and expression of ERα, NHE3 and AQP9 in efferent ductules of adult and senescent rats (6, 12 and 24 months). **Methods**: The study will be performed by using immunohistochemistry and Western blotting assays. **Results**: It was observed that the body weight and weight of the epididymis associated with the efferent ductules did not vary with age. Structural changes in some ductules were observed in aging rats, including reduction in tubular diameter and increase in the thickness of the peritubular smooth muscle layer, especially in the terminal region of the efferent ductules. The immunohistochemistry and Western blotting assays showed that after 12 months of age, there was a significant reduction in ERα, NHE3 and AQP9 levels, especially in older animals. **Conclusions**: These results point out that reduction in estrogen response and key proteins involved in efferent ductule reabsorption function may contribute to age-related reproductive disorders, including decrease in sperm count and quality.

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Keywords: efferent ductules, Estrogen receptors, aquaporin, NHE3.

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CRES protein is associated with amyloid-type structures in mouse epididymal spermatozoa

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Introduction: CRES (cystatin-related epididymal spermatogenic, *Cst8*) is the defining member of a reproductive subgroup within the family 2 cystatins of cysteine protease inhibitors and is present in the sperm acrosome as well as synthesized and secreted by the proximal caput epididymal epithelium. Several cystatins self-oligomerize including cystatin C (*Cst3*) that forms protein deposits associated with Icelandic-type cerebral amyloid angiopathy. We have shown that CRES is also a highly amyloidogenic protein in vitro as evidenced by its organization into defined oligomeric structures that ultimately transition into fibrils. Furthermore CRES is present within epididymal lumen as monomeric as well as high molecular mass complexes suggesting CRES may also form amyloid in vivo (Von Horsten et al. 2007). Western blot analysis of spermatozoa showed that the monomeric 14 kDa and N-glycosylated 19 kDa CRES proteins disappear as spermatozoa migrate from proximal to distal epididymis with only low levels of the 14 kDa CRES protein detected in cauda spermatozoa yet immunofluorescence analysis detected CRES in the sperm acrosome from both caput and cauda spermatozoa (Syntin et al. 1999). These studies suggested that during maturation CRES may transition into higher ordered structures that may not be readily detected by SDS-PAGE. **Aims:** We hypothesize that during maturation CRES may form amyloid-like structures within the sperm acrosome that function during fertilization. The goals of our current study were to determine if amyloid-like structures are present in sperm and whether CRES is associated with these structures. **Methods:** Percoll® purified mouse spermatozoa were prepared from the testis and five epididymal regions: proximal caput, mid caput, distal caput, corpus and cauda. Negative stain electron microscopy (EM), dot blot analysis, and the use of conformation-dependent antibodies (A11 and OC) in indirect immunofluorescence analysis were used to identify amyloid-type structures in spermatozoa. Protein aggregation disease (PAD) ligand linked to magnetic beads (Seprion Inc), designed to recognize any protein in the amyloid conformation was used to isolate amyloid structures from spermatozoa. **Results and discussion:** Dot blot analysis of whole sperm showed the presence of both oligomeric (A11 antibody) and fibrillar (OC antibody) forms of amyloids. Indirect immunofluorescence demonstrated like CRES, A11 and OC immunoreactivity were associated with the sperm acrosome in methanol treated spermatozoa. In addition A11 also showed strong immunoreactivity in a small punctuate structure near the sperm neck as well as midpiece and flagella staining. Partially solubilized caput and cauda spermatozoa were incubated with PAD reagents and proteins were eluted from beads by 0.75% SDS followed by Laemmli buffer. Western blot analysis demonstrated that CRES belonged to the PAD eluted proteins from both caput and cauda spermatozoa suggesting that CRES was present in an amyloid conformation. Negative stain EM was also carried out to examine the structure of the proteins that bound to the PAD beads. Long fibrillar (caput) and branched (cauda) structures characteristic of amyloid were detected in extracts from both sperm populations. Studies are ongoing to determine by immunogold negative stain EM whether CRES is associated with these structures. **Conclusions:** Taken together, our studies suggest that amyloid-type structures are present within sperm including the sperm acrosome and that CRES may contribute to the formation of these structures.

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Keywords: epididymis, spermatozoa, amyloid, cystatins, CRES.

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Reproductive parameters and fertility of male rats with delayed puberty

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Currently, the exposure to environmental contaminants is considered one of the factors responsible for alterations in child development, resulting in pubertal precocity or delay. Many chemical compounds with domestic, industrial and agricultural use, known as endocrine disruptors, have shown hormonal activity. The aim of this study was to investigate whether a delay in puberty installation (represented as delay in prepucial separation - PPS) affects reproductive parameters in pubertal and adult male rats. Pregnant Wistar rats (n = 11) were treated by gavage with 500mg/kg/day of dibutyl phthalate (DBP) from gestational day 12 to 21, to promote PPS delay. The control group (n = 11) received corn oil. Starting on postnatal day 30 the male pups were evaluated for PPS. On postnatal day 55 (puberty) and 90 (sexual maturity) one pup per litter was sacrificed to determine serum levels of testosterone, reproductive organ weights, sperm counts and histopathology of testis and epididymis. On postnatal day 90, another pup per litter was sacrificed to investigate sperm quality using *in utero* artificial insemination (AI), by inseminating 5 millions sperm per uterine horn. Data were compared using Mann-Whitney or Student´s t tests ($P < 0.05$). The mean age of PPS (days) was delayed in the DBP-treated group compared to the control. There was no alteration in testosterone serum levels in both ages. At puberty, the prostate weight of the treated animals was reduced compared with controls. The daily sperm production was reduced in the testis of the pubertal rats, and this alteration remained in the adult rats. On the other hand, while pubertal rats showed decreased sperm reserves in the cauda epididymis and accelerated sperm transit time, no alteration was observed in these parameters in sexually mature rats. The histopathology of testis revealed a high number of tubules with abnormalities, especially epithelium vacuolization, in DPB-treated rats, at both ages. In the DBP-treated animals malformations were found in the caput epididymis, as already reported in the literature, after exposure to a high dose of DBP. The sperm quality assessed by AI was not altered, despite a quantitative reduction in the fertility potential in the DBP-treated group. We concluded that, in this experimental model, the delay in pubertal onset did not compromise the fertility of rats.

Keywords: puberty, rat, phthalate, reproduction, epididymis, sperm, fertility.

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Expression of estrogen receptors ERα and ERβ in the efferent ductules and epididymis of *Artibeus lituratus* during the reproductive and regressive periods

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Estrogens modulate the primary function of the efferent ductules, which is testicular fluid reabsorption. However, the role of estrogens in the epididymis is still a matter of debate. The functions of estrogens are mediated by estrogen receptors ERα and ERβ. Information about the occurrence of ERs in the male genital system is limited to human, some domestic animals and rodents, but rarely in wild species. A recent study in the large fruit-eating bat *Artibeus lituratus* showed seasonal variation in the expression of ERα and ERβ in the testis, but did not describe the excurrent ducts. Further investigation using this seasonal model presenting natural variation in the ER expression would be helpful to bring new information about the role of estrogens in the male reproductive tract, without the inconveniences of genetically modified, castrated or drug treated animals. Therefore, the present study aims to investigate the cellular distribution of ERα and ERβ in efferent ductules (ED) and epididymis (EP) of *Artibeus lituratus,* during reproductive and regressive periods. Fragments of testis, ED and EP were used for morphological, morphometrical, as well as immunohistochemical and Western blotting assays for detection of ERα and ERβ. It was observed that parallel to testicular regression, there were remarkable changes in the ED and EP during the regressive period (December to April), including significant reduction in the epithelial height and diameter of the ED and EP. The reduction in ED diameter was about 16% in the proximal region and 11% in the distal region. The decrease in tubular diameter in the EP was more conspicuous, being on the order of 51, 45, 47 and 31% in initial segment, caput, corpus and cauda, respectively. The reduction in ED epithelial height was 14% in the proximal region and 19% in the distal region, whereas in EP the decrease was 55% in the initial segment, 40% in the caput, 29% in the corpus and 13% in the cauda. Spermatozoa were absent in the lumen of regressed EP. During the reproductive period, strong ERα immunoreactivity was observed in the nuclei of efferent ductule non-ciliated cells. In the epididymis just a few basal cells showed moderate positivity for this receptor, except in the cauda, where peritubular smooth muscle cells were also positive for ERα. During the regressive period, the strongest positivity for ERα was still found in the efferent ductule nonciliated-cell, however moderate staining was also detected in the ciliated cells and some peritubular and stromal cells. ERα was more extensively expressed along the regressed epididymis, being detected in most epithelial and peritubular smooth muscle cells. The epithelial cells showed a gradient of staining decreasing from proximal to caudal ducts, whereas the intensity of the smooth muscle cells were similar along the epididymal duct. ERβ was widely detected in both efferent ductules and epididymis, apparently with stronger staining intensity in the initial segment and head of the epididymis. There were no detectable differences in the ERβ staining pattern and intensity during the reproductive and regressive periods. In conclusion, there was differential expression of ERα and ERβ along the *A. lituratus* male genital tract, however just the pattern of ERα staining was changed along the reproductive cycle, being more widely expressed in the regressed epididymis.

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Keywords: estrogen receptors, *Artibeus lituratus*, efferent ductules, epididymis.

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Castration induces changes in expression and distribution of receptors in rat epididymis. Implications on cathepsin D secretion

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Introduction: It is well known that the epididymal epithelium (which is stimulated by steroid hormones) is involved in the process of sperm maturation through its secretory activity. High concentrations of some acid hydrolases have been found in the epididymal lumen, an intriguing fact considering that these enzymes should be confined to an intracellular compartment. Moreover, it was confirmed that some enzymes are secreted into the epididymal lumen in response to androgenic hormones. In other cells and tissues, acid hydrolases are selectively transported to lysosomes via mannose -6-phosphate receptor (MPRs), although alternative routes of transport have been described. **Aims:** In this work, we investigated whether the MPRs (or alternative pathways) regulate the transport and secretion of lysosomal enzymes in the rat epididymis, and whether these mechanisms are altered by changes in levels of steroid hormones. **Methods:** As a model of hormone deprivation, we used adult Sprague-Dawley rats, which were surgically castrated and sacrificed 48 hours later. The epididymal caput and cauda were finely chopped and left to stand for 30 min. The supernatant was collected, and the fluid was separated from sperm by centrifugation. The remaining tissue was used as a source for the study of MPRS, prosaposin and enzymes. Expression of proteins was studied by Western blot and distribution was observed by immunohistochemistry. **Results and Discussion:** In castrated animals, we observed that the expression of cation-dependent MPR (CD-MPR) increased in the caput and cauda of epididymis, with respect to controls. In addition, we observed CD-MPR that was redistributed to the apical region of the epithelium in castrated rats. Consistent with a possible involvement of the CD-MPR in lysosomal enzyme secretion, we observed an increase in procathepsin D in the epididymal fluid from castrated animals, accompanied by a redistribution of the enzyme to the apical region of epithelium. Because the transport and secretion of procathepsin D can follow an alternative route in other cell types, we intended to study the possible association of procathepsin D with the expression and transport of the lysosomal protein prosaposin and its receptor sortilin in the epididymal tissue of castrated rats compared with controls. We confirm, for the first time that procathepsin D and prosaposin are complexed in the epididymal fluid and that, in turn, these complexes increases in the castrated animals. As with the CD-MPR, there was a redistribution of prosaposin and sortilin to the apical region of the epithelium due to castration Furthermore, we observed a correlation between the expression of prosaposin and its receptor sortilin, and that both proteins are increased in caput but decreased in cauda of castrated animals. However, interestingly, castration induced an increase in the secretion of prosaposin in both regions of the epididymis. In conclusion, this study showed that the expression of CD-MPR and sortilin could be influenced by hormonal changes in the epididymal epithelium, and consequently induce an alteration in the normal transport of some lysosomal proteins.

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Keywords: epididymis, lysosomal enzymes, receptors, hormones.

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Inhibited protein tyrosine phosphorylation in sperm from CRISP1 knockout mice

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Mammalian sperm become competent to fertilize only after undergoing a series of changes that occur during their transit through the male and female reproductive tracts known as maturation and capacitation, respectively. Epididymal protein CRISP1 associates with the dorsal region of rat sperm during epididymal maturation. While a substantial amount of CRISP1 is released during capacitation suggesting its role as a decapacitating factor, part of the protein remains on sperm surface and participates in both sperm-zona pellucida interaction and gamete fusion by binding to egg-complementary sites. In agreement with this, capacitated sperm from *Crisp1*$^{-/-}$ mice recently generated in our laboratory exhibited a significantly lower ability to interact with the zona pellucida and the oolemma. However, contrary to what it is expected for a decapacitating factor, tyrosine phosphorylation levels were significantly lower than in controls suggesting that CRISP1 could play a regulatory role during capacitation different from that originally expected. In view of previous reports indicating that the presence of CRISP1 during rat sperm capacitation inhibits protein tyrosine phosphorylation, we studied the effects of CRISP1 on *Crisp1*$^{+/-}$ and *Crisp1*$^{-/-}$ sperm capacitation. Results indicated that the presence of the protein did not modify protein tyrosine phosphorylation levels in any of these populations. The decrease in tyrosine phosphorylation levels in CRISP1 mutant sperm were also detected by indirect immunofluorescence as judged by the faint fluorescent labeling in both midpiece and principal piece of *Crisp1*$^{-/-}$ sperm compared with the strong staining observed in *Crisp1*$^{+/-}$ sperm. Since it has been demonstrated that tyrosine phosphorylation is downstream in a cAMP/PKA pathway, we investigated whether cAMP is involved in the observed reduction of tyrosine phopshorylation by exposing *Crisp1*$^{-/-}$ sperm to a cAMP analog (db-cAMP) and a phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine, IBMX). Results evaluated by Western blot, showed that, under these conditions, there was a reversion in the phosphorylation pattern of *Crisp1*$^{-/-}$ sperm supporting the existence of a lower content of cAMP in mutant sperm. This possibility was confirmed by the significant reduced levels of cAMP determined by radioimmunoassay in both C*risp1*$^{+/-}$ and *Crisp1*$^{-/-}$ sperm. Together, the results indicated that the inhibition of tyrosine phosphorylation in sperm lacking CRISP1 could be attributed to the lower levels of intracellular cAMP generated during capacitation. Although the molecular mechanisms underlying cAMP reduction are still unknown, at present we are investigating whether these observations are related to the reported ion channel regulating ability of CRISP proteins. Considering that sperm acquire both CRISP1 and the ability to undergo capacitation-induced tyrosine phosphorylation during their transit through the epididymis, these studies suggest that the lower levels of tyrosine phosphorylation in capacitated *Crisp1*$^{-/-}$ sperm might be due to the lack of association of CRISP1 during epididymal maturation.

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Keywords: CRISP1, sperm, capacitation, epididymis.

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Antiestrogen fulvestrant regulates the expression of androgen receptor in rat epididymis

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Introduction: The epididymis is a highly specialized tissue of the male excurrent duct system, which plays an important role in sperm maturation, and can be functionally divided into four distinct regions: the initial segment, caput, corpus and cauda. The functional segmentation of the epididymis is regulated by androgens and growth factors, and this is reflected at the molecular level by complex region-specific gene expression. Estrogen and the classic estrogen receptors (ESR1 and ESR2) are also present in the epididymis; the role of estrogen in epididymal function, however, is still not completely understood. The antiestrogen fulvestrant (ICI 182,780), which does not modify testosterone and 17β-estradiol plasma levels in male rats, but impairs estrogen action on ESR1 and ESR2, can regulate the expression of androgen receptor (AR) and ESR1 in different cell types within the male reproductive tract. **Aims:** This study was proposed to further investigate the role of estrogens during pubertal development of rat epididymis, by analyzing the effect of the treatment with ICI 182,780 on the expression of steroid receptors in this tissue. We now report the effect of ICI 182,780 on the expression of AR in the caput, corpus and cauda of the epididymis. **Methods:** Thirty-day-old rats were treated once a week for 2 months with corn oil (control group) or fulvestrant (ICI 182,780) (10 mg/rat, s.c.). Total RNA was extracted from caput, corpus and cauda of the epididymis. Transcripts for AR were evaluated by real time PCR. Western blot for AR was performed with total tissue extract. Negative controls were performed with antibody pre-adsorbed with blocking peptides. **Results:** In the adult rat (90 day-old), testosterone concentration was higher in the caput than in the cauda of the epididymis and the estradiol levels were similar in both regions. The levels of mRNA for AR were similar among three regions from control animals (90 day-old), but the AR protein level was lower in the caput than in the corpus and cauda of the epididymis. Fulvestrant did not modify testosterone and estradiol levels in the caput of the epididymis. In the corpus and cauda, fulvestrant decreased the testosterone levels by 34 and 47%, respectively, and increased the estradiol levels by 178 and 100%, respectively. Fulvestrant did not change the mRNA for AR, but significantly increased the AR protein levels in the three regions of the epididymis: 3.5-fold in the caput, 1.3-fold in the corpus and 1.6-fold in the cauda. **Conclusion:** Fulvestrant up-regulates AR expression in the epididymis through post-transcriptional mechanisms. Furthermore, the results suggest the involvement of differential signaling mechanisms in regulating and/or mediating the actions of estrogen in the different regions of the epididymis. This regulatory diversity is probably important to control region-specific sperm-related functions.

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Keywords: epididymis, rat, androgen receptors, Fulvestrant.

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Influence of two different extenders on cryopreservation of bovine epididymal sperm

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Introduction: The recovery of spermatozoa from the epididymal cauda allows the preservation of germoplasma of valuable animals after an unexpected death. However, it is known that the *in vivo* fertility of bovine epididymal sperm tends to be lower than ejaculated sperm. The reasons of these differences may be related to variations in cell surface characteristics and low motility of epididymal sperm. Therefore, the use of a specific extender for the cryopreservation of bovine epididymal sperm may improve its fertility. **Aim:** The aim of this study was to evaluate the effect of different freezing extenders on viability and post-thaw motility parameters of frozen bovine epididymal sperm. **Methods:** For this experiment, 28 testicles were transported from the abattoir to the laboratory at 5°C. Each epididymal cauda was flushed with a skim milk-extender–Botu-Sêmen™ (BS) and then the samples were incubated either without or with Sperm Talp (SP) at 37°C for 10 minutes. After that, the samples were centrifuged at 600 x*g* for 10 min. The supernatant was removed and the pellets were re-suspended in two egg yolk extenders, Tris extender (TRIS) or Botu-Bov™ extender (BB). Straws were cooled at 5°C for 20 minutes, frozen 6 cm above nitrogen level for other 20 minutes then immersed into nitrogen. The straws were thawed at 46°C for 20 seconds and evaluated for motility parameters. Therefore, four groups were evaluated: BSBB (incubated and then frozen with BB), BSTRIS (incubated and then frozen with TRIS), SPBB (incubated with SP and frozen with BB), and SPTRIS (incubated with SP and frozen with TRIS). Motility parameters were analyzed after thawing by CASA (HTM—IVOS 12, Hamilton Thorne Research, USA). In addition, plasma membrane integrity was evaluated by fluorescent probes carboxyfluorescein diacetate and propidium iodide. All parameters were analyzed by ANOVA with Tukey's test using GraphPad InStat Version 3.06, t*o* identify the significant differences ($P < 0.05$). **Results and Discussion**: On post-thaw samples, no differences ($P > 0.05$) were observed in plasma membrane integrity between BSBB, BSTRIS, SPBB, SPTRIS groups. Though, differences were observed on Progressive Motility ($27,86 \pm 12,01^a$ *versus* $46,39 \pm 12,73^b$); Straight Line Velocity ($63,25 \pm 8,42^a$ *versus* $72,68 \pm 6,71^b$); Linearity ($44,07 \pm 4,38^a$ *versus* $48,36 \pm 5,08^b$) and Rapid ($43,93 \pm 20,03^a$ *versus* $64,18 \pm 17,43^b$) between BSBB and SPBB, respectively. For the groups BSTRIS and SPTRIS, differences were observed only on Progressive Motility ($29,86 \pm 1\ 5,23^a$ *versus* $43,71 \pm 15,49^b$) and Beat Cross Frequency ($24,75 \pm 3\ ,78^a$ *versus* $27,75 \pm 3,07^b$), respectively. These results are probably justified by the presence of certain substances in Sperm Talp that inhibit phosphodiesterase activity, leading to a subsequent increase of intrasperm cAMP levels. Cyclic adenosine monophosphate (cAMP) seems to be responsible for the sperm motility, which may have enhanced the motility of sperm recovered from epididymal cauda after thawing. No differences ($P > 0.05$) were observed between BSBB and BSTRIS; or between SPBB and SPTRIS groups. **Conclusions**: The results of the present study allowed concluding that, the incubation of recently recovered epididymal bovine sperm in Sperm Talp prior to cryopreservation improves the post-thaw motility sperm parameters. Both freezing extenders, Tris and Botu-Bov™ can as well be used for the cryopreservation of bovine epididymal sperm. More studies are required to investigate the effects of Sperm Talp on fertility.

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Keywords: bovine, cryopreserved epididymal sperm, freezing extender.

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Involvement of cystic fibrosis transmembrane conductance regulator (CFTR) in ATP-release and bicarbonate-secretion in epididymal principal cells

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Introduction: An acidic luminal pH in the epididymis contributes to maintaining sperm in a quiescent state during their maturation and storage. We showed that clear cells acidify the lumen via the proton pumping V-ATPase, and that luminal HCO_3^- or ATP induces V-ATPase apical accumulation in these cells, a process that increases proton secretion. CFTR is present in the apical membrane of epididymal principal cells and cystic fibrosis-associated CFTR mutations lead to male infertility in humans. Interestingly, using short-circuit current measurements, Wong and collaborators have previously shown that HCO_3^- is secreted via CFTR in epididymal epithelial primary cultures after activation with basolateral agonists. Bicarbonate secretion is thought to occur during sexual arousal to prime sperm before ejaculation. CFTR also participates in ATP secretion in a variety of epithelia, and we hypothesized that CFTR may have the dual property of mediating HCO_3^- secretion together with ATP-release in epididymal principal cells. **Aim:** The present study was performed to characterize HCO_3^- and ATP secretion in immortalized epididymal cell lines originating from the distal caput (DC2), which were kindly provided by Marie-Claire Orgebin-Crist and collaborators. **Methods and Results:** RT-PCR and Western-blotting experiments showed expression of several principal cell markers, including CFTR, the water channel AQP9, and other proteins potentially involved in ATP transport (ClC-3, pannexin) in these cells. The luciferin/luciferase assay was used to measure the concentration of ATP released into the culture medium by DC2 cells. Treatment of cells for 10min with forskolin (10 μM), PGE_2 (10 μM) or adrenaline (50 μM), which are known activators of principal cells and CFTR, induced a significant increase in ATP release. In addition, the forskolin-induced ATP release was blocked by pretreatment of the cells for 10 min with a CFTR-specific inhibitor, $CFTR_{inh172}$ (10μM), indicating the involvement of CFTR in ATP secretion in epididymal principal cells. Bicarbonate secretion from DC2 cells was measured using the ratiometric dye, BCECF, to measure intracellular pH (pH_i). The rate of pH_i recovery upon the removal of CO_2 and HCO_3^- from extracellular solutions was used as an indirect assessment of HCO_3^- secretion. Forskolin significantly increased the rate of pH_i recovery following CO_2 and HCO_3^- withdrawal. In addition, pretreatment with $CFTR_{inh172}$ abolished the forskolin-induced HCO_3^- secretion, supporting previous finding by the Wong group that CFTR is involved in epididymal HCO_3^- secretion. **Discussion and Conclusion:** These data suggest that CFTR mediates both ATP and HCO_3^- secretion in epididymal principal cells. We propose that stimulation of principal cells by basolateral paracrine factors during sexual arousal leads to subsequent activation of clear cells via ATP and bicarbonate that are secreted into the epididymal lumen. This would allow luminal pH to be restored to its resting acidic value. Our data also indicate the participation of CFTR in this crosstalk between principal cells and clear cells.

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Keywords: CFTR, ATP, bicarbonate, epididymis.

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Fertility of cryopreserved epididymal sperm from fertile and subfertile stallions

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Introduction: The recovery of sperm from the epididymal cauda allows the preservation of genetic material from valuable stallions that died suddenly or were unable to perform mating or semen collection. Therefore, subfertile stallions may also benefit from this technique, since epididymal sperm has no contact with the potentially deleterious secretions of accessory glands. Recent studies concerning the recovery and cryopreservation of epididymal sperm have been intensified. However, there are no studies in Veterinary Medicine on the viability and fertility of epididymal sperm from subfertile stallions. **Aim:** This study aimed to compare the *in vivo* fertility of ejaculated sperm from fertile (EJ-F) and subfertile stallions (EJ-SB) with epididymal cauda sperm from fertile (EP-F) and subfertile stallions (EP-SB). **Methods:** For this experiment, eight fertile and two subfertile stallions were used. Two ejaculates was obtained from each stallion, and then frozen using an egg-yolk based extender–Botu-Crio^{TM} (EJ-F and EJ-SB). A week after the last semen collection, the stallions were submitted to bilateral orchiectomy and each epididymal cauda were flushed with a skim-milk based extender-Botu-Semen^{TM} and then frozen with Botu-Crio^{TM} (EP-F and EP-SB). Sperm parameters were evaluated by CASA (HTM-IVOS 12, Hamilton Thorne Research, USA). In addition, plasma membrane integrity was evaluated by epi-fluorescence microscopy. For fertility trial, 41 cycles of fertile mares were used. Ovulation was induced with 2 mg of deslorelin acetate when the dominant follicle achieved the diameter of 35 mm. The inseminations were performed immediately after ovulation, with 800 x 10^6 frozen-thawed sperm in tip of uterine horn, ipsilateral to the preovulatory follicle using a flexible pipette. The pregnancy diagnosis was performed 15-day post-ovulation. All parameters were analyzed by ANOVA (SAS Institute Inc., Cary, NC, USA) followed by Tukey's test to identify the significant differences. Treatments were considered different if $P < 0.05$. **Results and Discussion:** The number of sperm obtained in Groups EJ-F; EJ-SB; EP-SB and EP-F was, respectively: 7.8 ± 4.69^b; 6.9 ± 3.37^b; $25{,}0 \pm 17.13^a$; $20.4 \pm 5\ .37^a$ x 10^9 sperm. The number of recovered sperm from ejaculates was lower than those recovered from the epididymal cauda in both fertile and subfertile stallions ($P < 0.05$). On post-thawed, sperm parameters for EJ-F and EP-F groups, respectively, were: Total Motility (TM): 66.3 ± 16.31^a *versus* 72.4 ± 6.86^a; Progressive Motility (PM): 33.0 ± 10.68^a *versus* 36.2 ± 8.44^a; Rapid (Rap): 53.9 ± 17.74^a *versus* $59.2 \pm 1\ 0.69^a$ and Plasma Membrane Integrity (PMI): 35.9 ± 6.11^a *versus* 41.4 ± 5.94^a. For subfertile stallions, sperm parameters for ejaculated and epididymal samples were: TM 7.7 ± 2.22^a *versus* 33.3 ± 2.06^b; PM: 1.8 ± 1.26^a *versus* 12.5 ± 3.32^b; Rap: 2.0 ± 1.41^a *versus* 19.3 ± 1.26^b and PMI: 22.3 ± 6.02^a *versus* 41.8 ± 10.59^a, respectively. Pregnancy rates were 61.5% (8/13); 92.3% (12/13); 0% (0/5) and 20% (2/10), for EJ-F; EP-F; EJ-SB and EP-SB, respectively. There were no differences on sperm parameters and fertility of ejaculated and epididymal sperm of fertile stallions ($P > 0.05$). Though, sperm parameters (TM, PM, Rap) of epididymal sperm were higher than ejaculated sperm on subfertile stallions ($P < 0.05$). According to some studies, the addition of seminal plasma from "bad-freezers" stallions on semen of "good freezer" stallions demonstrated a deleterious effect on sperm viability. Moreover, studies have indicated that seminal plasma proteins promote biochemical changes in the plasma membrane, which can affect the freezability and fertility of sperm cells. **Conclusions:** Based on these results, we can conclude that the sperm parameters and the fertility of epididymal cauda sperm are similar or better than ejaculated sperm. Indeed, more studies are required in order to identify possible deleterious effects of seminal plasma on sperm quality of subfertile stallions.

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Keywords: pregnancy rate, epididymal spermatozoa, stallion, cryopreservation, sperm viability.

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Effect of nandrolone decanoate and physical training on expression of aquaporin 9 in the adult rat epididymal epithelium

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Aquaporins (AQPs) are small intrinsic membrane proteins that are present in many cell types involved in fluid transport. AQP9 is a major apical water channel that is expressed in the male reproductive tract using sex steroid-dependent pathways. The classical therapeutic use of anabolic-androgenic steroids (AAS) is associated with the correction of male hypogonadism, stimulation of erythropoiesis and bone mineralization. The doses and combinations of these compounds used by athletes are typically in large excess (10–100-fold) of therapeutic doses. Many other adverse effects have been associated with AAS misuse, including endocrine disturbance and changes of hemostatic system and male genital system. The present study was designed to investigate the effect of high doses of exogenous androgen and physical training on expression of AQP9 in the adult rat epididymal epithelium. Twenty adult male Wistar rats were randomly allocated into 4 groups: Sedentary + vehicle (SV), Trained + vehicle (TV), Sedentary + AAS (SAAS) and Trained + AAS (TAAS). They received i.m. injections of nandrolone decanoate (ND) (5mg/Kg) or vehicle propyleneglycol (0.2mL/Kg) for 8 weeks and during the same period trained rats were submitted to resistance physical training, by jumping up and down in water carrying an overload. Sedentary and trained animals were anesthetized and sacrificed. The expression of AQP 9 in the adult rat epididymal epithelium was studied immunohistochemically. In accordance to specialized literature, in mammals the AQP9 localizes to the apical brush border of principal cells throughout the epididymis. In this study, in the initial segment and cauda epididymidis, the AQP9 staining presented stronger reactivity than corpus and caput. This is suggestive of major movement of water in these regions and could be related to a massive potential for movement of water and/or small uncharged molecules throughout the epididymis and especially within the distal cauda. The hormonal treatment with ND, independent of physical training, was determinant in the increase of the reaction intensity for AQP9 in all regions studied. Previous reports showed that AQP9 expression is regulated by androgens in the rat epididymis, with DHT being the principal androgenic mediator of AQP9 modulation in the initial segment. This region presented highest expression of 5α-reductase and it is the segment most sensitive to DHT. In contrast, another study showed that, in the cauda epididymis, AQP9 was regulated by testosterone. The similar metabolic pathway between endogenous and synthetic derivatives of testosterone could explain the reactivity differences observed in the AQP9 expression throughout epididymis in the groups treated with ND. In these animals the enzyme 5-α reductase converts ND to "dihydro" nandrolone which shows affinity for the androgen receptor. The groups submitted to the treatment with ND showed a stronger reaction that appears to be related to this conversion pathway of ND.

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Keywords*:* nandrolone decanoate, physical training, epididymis, aquaporin 9, rat.

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Immunolocalization of aquaporin 1 in rat epididymis after treatment with nandrolone decanoate supraphysiologic doses and resistance physical training

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The aquaporin (AQP) 1 is an important channel for the rapid absorption of the large amount of testicular fluid that occurs characteristically in the efferent ducts. The removal of water from the efferent ducts plays an important role in concentrating sperm in the initial segment of the epididymis and thus in providing a better interaction between the sperm surface and the secretory products of its epithelial cells. AQP1 is not regulated by anabolic androgenic steroids or oestrogens in the male reproductive tract of rat, but appears to be constitutively expressed. The anabolic androgenic steroids (AAS) and metabolites can cause changes in reproductive physiology and sperm quality. In epididymis and in the other male reproductive organs, the AAS bind to the androgen receptor complex, resulting in a cascade of events necessary for the formation of signaling factors that regulate cellular events. Reports about the influence of nandrolone decanoate (ND) and of physical training on epididymal structure have not received attention. So, this study investigated the effects of high doses of ND and PT on immunolocalization of AQP 1 in rat epididymis. Twenty male Wistar rats were randomly allocated into 4 groups: Sedentary (S) + vehicle (V) (SV), Trained (T) + V (TV), S + ND (SND) and Trained + ND (TND). They received i.m. injections of ND (5 mg/Kg) or vehicle propyleneglycol (0.2 mL/Kg) for 8 weeks and during the same period trained rats were submitted to resistance physical training, by jumping up and down in water carrying an overload. S and T animals were anesthetized and sacrificed and AQP1 expression was studied immunohistochemically. The AQP1 was detected in the peritubular cells of the initial segment and in the vascular channels of the intertubular space of the entire epididymis in the groups studied. The hormonal treatment with ND didn't influence the AQP1 expression. In the epididymis distal segments (corpus and cauda) of the rats submmited to a resistance physical training (TV and TND), independent of the hormonal treatment, a significant increase of AQP1 positive blood vessels was observed. In accordance to literature, physical training can promote capillary growth by stimulating the release of factors involved in angiogenesis. VEGF, vascular endothelial growth factor, is one of the critical factors in angiogenesis. A study with sedentary individuals and well-trained endurance athletes showed an association between increased circulating levels of VEGF and angiogenesis with physical training. Based on literature, the findings presented here show a possible relationship between physical training and an increase of blood vessels (angiogenesis), as demonstrated by an increase in AQ1 positive reactions.

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Keywords*:* nandrolone decanoate, physical training, epididymis, aquaporin 1, rat.

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High-fat diet consumption leads to impairment of sperm motility without affecting other reproductive parameters in rats

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Obesity is rapidly becoming a worldwide epidemic that affects children and adults, independently of economical and social conditions. Obesity is often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue. In an attempt to cause obesity in animals, several experimental models of obesity have been developed, and there is a consensus that intervention in the diet can provide a more physiological model of obesity. Studies have shown that high-fat diets are an important component in obesity etiology, since exposure to a high fat diet leads to body fat excess in several animals. Few works in the literature relate obesity with reproductive function. Thus, the aim of the present work was to investigate potential reproductive disorders related to obesity in adult male rats. The work was divided into two studies. In the first study Wistar rats (30 days old) were given a high-fat diet (HD) or standard chow (SD) for 15, 30 and 45 weeks. After each period of diet exposure, rats (n = 10/group/ food exposure time) were sacrificed by decapitation. Blood was collected from the ruptured cervical vessels for determination of serum leptin levels. The right testis, epididymis and vas deferens, ventral prostate and seminal vesicle were removed and weighed; testis and epididymis were used for sperm counts. In the second study the animals were exposed to high-fat diet or standard chow for a period of 15 weeks, long enough to increase body weight and serum leptin levels, which characterize obesity. Thereafter, the animals were euthanized and sperm were collected from the vas deferens for evaluation of sperm motility and morphology. Animals exposed to a high-fat diet showed a significant increase ($P < 0.05$) in body weight when compared to animals that received standard chow, in all periods of diet exposure. Besides, fat accumulation was higher ($P < 0.01$) in animals that ate a high-fat diet, and also the leptin serum concentration was higher ($P < 0.001$), as expected. Reproductive organs weight did not show any difference between HD and SD groups in the experimental periods. Similarly, no statistical alterations were observed either in daily sperm production or sperm number in the epididymis when HD and SD animals were compared. After 15 weeks of exposure to high-fat or standard diet, the sperm morphology showed no significant differences between groups. On the other hand, a significant decrease ($P < 0.01$) was observed in sperm with progressive movement, with consequent increase ($P < 0.01$) in sperm with non-progressive movement in HD animals. It was concluded that the protocol used in this work was able to provoke obesity in high-fat fed rats, leading to a reduction in sperm quality, without affecting other reproductive parameters.

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Keywords: obesity, high-fat diet, leptin, sperm count, sperm motility.

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Glioma Pathogenesis-Related 1-like protein 1 (GliPr1L1) in bovine testis and epididymis: Molecular aspect and potential role

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Using 2D gel electrophoresis and proteomic analysis of RAFT membrane domains isolated from cauda epididymal bovine spermatozoa, we have identified 2 protein spots with MW 25-28 kDa and a pI of 8.5. Liquid chromatography-tandem mass spectrometry identified these raft-associated proteins as GLiPr1L1: Glioma Pathogenesis-Related 1-like protein1. This protein belongs to a subgroup of the cysteine-rich secretory protein (CRISP). From a bovine testis cDNA library, we cloned the full length GliPr1L1 cDNA and we have produced an antibody directed against an overexpressed truncated form (a.a.23-220) of the protein. PCR analysis revealed that *GliPr1L1* is expressed in the testis and all along the epididymis with a higher expression in the caput. Western blot analysis revealed a similar distribution in tissues extracts. In the epididymal lumen, GLiPr1L1 was associated with the maturing spermatozoa and epididymosomes all along the excurrent duct but was undetectable in the soluble fraction of epididymal fluid. The protein was detectable as multiple isoforms with a higher MW form in the testis and proximal caput. Treatment of caput and cauda epididymal spermatozoa extracts with PNGase F revealed that N-glycosylation was at the origin of the multiple bands detected on Western blots. These results also suggest that the N-glycosylation moiety of GLiPr1L1 is processed during the transit in the caput. Cauda epididymal spermatozoa subcompartments were prepared following nitrogen cavitation and sonication. Western blots demonstrated that GliPr1L1 was associated with the sperm plasma membrane preparation. Raft membrane domains were prepared by discontinuous gradient centrifugation of detergent extracts of caput and cauda epididymal spermatozoa. GliPr1L1 was immunodetectable in the low buoyant density fractions where RAFTs are distributed along the gradient. GLiPr1L1 is glycosyl phosphatidyl inositol (GPI) anchored to both caput and cauda spermatozoa as demonstrated by the ability of PIPLC (phosphatidylinositol specific phospholipase C) to release GliPr1L1 from intact sperm cells. By indirect immunofluorescence (IF), GliPr1L1 was localised on the equatorial segment, the neck, and at the end of the principal piece of cauda epididymal spermatozoa. Preliminary data indicate that GLiPr1L1 is also immunodetectable in detergent extracts of ejaculated human spermatozoa. The association of the GliPr1L1 to membrane RAFT domains of spermatozoa and the fact that it is GPI-anchored suggest a possible role of this sperm protein in cell signalling and/or oocyte interaction. Work is in progress to define the function of GLiPr1L1 in sperm physiology.

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Keywords: bovine, GliPr1L1, raft domains, spermatozoa, epididymis.

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Expression and hormonal regulation of Epididymal Protease Inhibitor (EPPIN) in the male rat reproductive tract

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Introduction and aims: EPPIN (epididymal protease inhibitor; official symbol: *SPINLW1*) is a cysteine-rich protein containing both Kunitz-type and WAP-type four disulfide core protease inhibitor consensus sequences. Previous studies have shown that human and mouse EPPIN originate from genes specifically expressed in testis and epididymis. EPPIN is present on the sperm surface in a protein complex that plays a role in sperm motility and protection. Contraceptive studies in non-human primates demonstrated that immunization with EPPIN results in effective and reversible male infertility, making EPPIN an interesting target for male contraception. The present study was designed to characterize the expression and cellular distribution of EPPIN in rat reproductive tissues and its in vivo regulation by androgens. **Methods:** Male Wistar rats (90 days; n = 5-6/group) were sham-operated (control) or surgically castrated (7 days). A group of castrated rats was treated daily with testosterone propionate (10 mg/kg, s.c., 6 days). Testis, efferent ductules, epididymis (initial segment/caput, corpus and cauda), vas deferens, seminal vesicle and prostate, as well as several non-reproductive tissues, were processed either for RT-PCR and real-time PCR studies to detect rat *Eppin* and *Gapdh* (internal control) transcripts or for Western blot and immunohistochemical studies using affinity purified anti-EPPIN antibody (negative control was antibody pre-adsorbed with recombinant EPPIN). Immunofluorescence studies were performed on sperm isolated from all epididymal regions. Real-time PCR data were quantified using $^{\Delta\Delta}C_t$ method and analyzed by ANOVA followed by Tukey test ($P < 0.05$). **Results:** In tissues from control rats, RT-PCR studies detected *Eppin* mRNA in testis, efferent ductules and all epididymal regions. Interestingly, *Eppin* mRNA was also observed in vas deferens, seminal vesicle and brain. Western blot analysis using anti-EPPIN antibody revealed the expression of EPPIN at different apparent molecular mass bands in testis (~25 kDa), initial segment/caput (~19, 25 and 38-46 kDa), corpus (~19 and 38 kDa), and cauda epididymis (~19 kDa), as well as in vas deferens (~19 kDa), seminal vesicle (~17 kDa) and brain (~22 kDa), indicating that EPPIN is differentially translated and/or processed in these tissues. In the epididymis, EPPIN-positive immunostaining was observed on luminal sperm and in principal and basal epithelial cells in a region-specific pattern. In the testis, EPPIN-positive immunostaining was found in Sertoli cells, primary spermatocytes, and both round and elongated spermatids. Immunofluorescence studies demonstrated that secreted EPPIN coats sperm in the postacrosomal, neck, midpiece and tail regions during their transit along the epididymis. Real-time PCR analysis revealed a significant decrease in *Eppin* mRNA levels in the initial segment/caput and cauda epididymis after castration. In parallel, immunohistochemistry of epididymal sections from castrated rats demonstrated a reduction in EPPIN-positive immunostaining in epithelial cells along this tissue. Testosterone replacement reversed the decrease in EPPIN expression levels induced by castration in both epididymal regions analyzed. **Conclusions:** Our results demonstrate that EPPIN expression in rat is androgen-dependent in the epididymis and more widespread than observed in other species. Further investigation may reveal additional physiological roles for EPPIN in the rodent male reproductive tract.

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Keywords: protease inhibitors, epididymis, rat.

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Acquisition of the ability to engage in sperm-zona pellucida interaction: the role of the CCT/TRiC complex

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Introduction: Although the molecular mechanisms that underpin sperm-zona pellucida interaction remain unclear, evidence from our laboratory has implicated a family of chaperones, including those of the CCT/TRiC complex (TCP1 complex / TCP1 ring complex), in the formation of a multimeric zona-receptor complex on the surface of mouse spermatozoa. **Aims:** The current study was undertaken to investigate the expression of this complex during the ontogeny of male germ cells through spermatogenesis, epididymal sperm maturation and capacitation. **Methods:** A panel of antibodies directed against the subunits of the CCT/TRiC complex was used in conjunction with immunolocalization, immunoblotting and biochemical assays to characterize the expression and functional significance of this complex in mouse spermatozoa. **Results and Discussion:** In testicular sections, the CCT/TRiC complex was detected in spermatogonia. However, this labeling pattern disappeared from the male germ line during spermiogenesis to become undetectable in testicular spermatozoa. Subsequently, these chaperones were detected in epididymal spermatozoa and in discrete structures within the lumen of the duct that we have previously termed, dense bodies. The latter appeared in the precise region of the epididymis (proximal corpus), where spermatozoa acquire the capacity to recognize and bind to the zona pellucida, implicating these structures in functional remodeling of the sperm surface during epididymal maturation. Consistent with this notion, analogous structures have been shown to harbor proteins (e.g. CRISP1, BPI, and additional molecular chaperones) that are acquired by spermatozoa during their epididymal maturation. The CCT/TRiC complex was subsequently found to become co-expressed with zona binding proteins on the surface of live mouse spermatozoa following capacitation in vitro and was lost once these cells had undergone the acrosome reaction, as would be expected of cell surface molecules involved in sperm-egg interaction. **Conclusions:** These data raise the intriguing possibility that the CCT/TRiC complex is intimately involved in the mechanisms by which mammalian spermatozoa both acquire and express their ability to recognize the zona pellucida.

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Keywords: dense bodies, CCT/TRiC, sperm-oocyte interactions.

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Glutamate-induced model of obesity altered reproductive parameters in adult male rats

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Obesity is growing rapidly in the male population, and it has been associated with lower sperm counts and hormonal imbalances. Due to the difficulty of studying 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 throughout development. The aim of this work was to evaluate reproductive parameters in a glutamate-induced model of obesity. Male rats were treated neonatally with MSG at doses of 4 mg/kg subcutaneously or with saline solution on postnatal days 2, 4, 6, 8 and 10 and then examined on day 120. Obesity was confirmed by the Lee index in all MSG-treated rats. Significant reductions in absolute and relative weights of testes, epididymis, prostate and seminal vesicle were noted in MSG-treated animals. Furthermore, this was accompanied by a significant decrease in plasma testosterone and follicle-stimulating hormone (FSH) concentrations, but not luteinizing hormone (LH). It is worth noting that in MSG-administered rats there was a fall in sperm counts, as well as reduction in seminiferous epithelium height and tubular diameter. However, stereological findings on the epididymis were not markedly changed by obesity. Data indicate that the neonatal MSG-administered model of obesity provoked significant alterations in reproductive function as evidenced by significant declines in male reproductive organ weights, sperm counts, hormonal concentrations and structural changes. Thus the use of MSG as an obesity model may serve as an important tool for determining the consequences of obesity in male reproductive functions and potentially reveal the mechanisms to ameliorate this disease.

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Keywords: obesity, monosodium glutamate, male reproduction, testosterone, sperm.

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Identification of serotonin vesicles in epididymis and testis in bat *Myotis velifer* during reproductive cycle

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Introduction: Reproduction in mammals is a complex process that is determined by the relationship of diverse factors, some external such as food availability, environmental conditions, and some internal, such as neuroendocrine regulation of sexual behavior mainly by the hypothalamic-pituitary-gonad axis which controls a variety of determinant reproductive functions in sexual behavior. The neurohormone serotonin is produced in blood, brain and intestine and now we know that it is involved in the reproductive system in mammals. The reproductive cycle of the bat *Myotis velifer* is interesting because it presents an annual testicular cycle that varies along environmental stations; when these animals are not in mating season, a testicular and epididymal regression occurs and spermatogenesis ceases, but in the days prior to mating, the testis becomes enlarged and spermatogenesis is reactivated. After spermatozoa generation, the sperm produced pass along the increased epididymis and are stored in it until the time of ejaculation. **Aims:** Serotonin is involved in reproductive system in mammals; this work was focused on detecting the distribution of serotonin in both epididymis and testis of bat. **Methods:** We used Falck-Hillarp histochemistry in order to detect indoleamines. Trapped bats came from the center of Mexico (state of Puebla); adult males were selected and both testis and epididymis dissected. The histochemical technique is based on a mixture of paraformaldehyde and glyoxylic acid incubated at 90°C. After incubating tissue sections on this mixture, we obtained representative photomicrographs using an epifluorescence microscope. **Results and Discussion:** Our results showed that in the non-reproductive stage a high signal of fluorescence was detected in the testicular cords (seminiferous tubules), suggesting the existence of serotonin. In contrast, in the reproductive stage serotonin was scarcely distributed, diminished the expression and the stain was distributed in Leydig cells and the basal membrane of some seminiferous tubules and in principal cells of some tubules of the epididymis. **Conclusion:** We conclude that serotonin is distributed and probably synthesized in both bat testis and epididymis and probably plays a main role in their annual testicular cycle.

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Keywords: bats, serotonin, epididymis, testicular cycle.

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Impact of different oily vehicles on testicular toxicity of di-butyl phthalate (DBP)

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Introduction: In rats, prenatal exposure to high doses of certain phthalate esters, such as di-butyl phthalate (DBP), can disrupt the development of male reproductive organs, including the epididymis. Although suppression of fetal testosterone production seems to be a key factor for induction of the "phthalate syndrome", the precise mode of action of these compounds remains partially unknown. For instance, there are reports indicating that phthalates could act either as pro-inflammatory, inducing the expression of cycloxygenases, or anti-inflammatory compounds, reducing the expression of cycloxygenases and their metabolic products (e.g., prostaglandins). On the other hand, production of eicosanoids (e.g., prostaglandins) with pro- or anti-inflammatory properties is largely related to the type of fatty acid used as substrate. For instance, omega-6 and omega-3 fatty acids usually originate eicosanoids that are predominantly pro-inflammatory and anti-inflammatory, respectively. In addition, the toxic response to DBP and other active phthalates seems to be highly variable depending on rat strain and laboratory conditions, and one possible component responsible for such variability could be the type of oily vehicle used in experimental studies. **Aims:** Evaluate the impact of different oily vehicles (corn, canola or fish oil) on the reproductive toxicity of DBP in male rats fetuses exposed *in utero*. **Methods:** Pregnant rats (n = 11-13/group) were treated with DBP diluted in different vehicles by gavage from day 13 to 20 of gestation. A total of 6 experimental groups were used - 3 control groups: 5 mL/Kg of oil (corn, canola or fish); and 3 treated groups: DBP 500 mg/Kg/day diluted in oil (corn, canola or fish). Dams were sacrificed on day 20 of gestation. The anogenital distance of male fetuses was measured with a digital calliper. The left testicles of one to three fetuses per litter were kept at -80^{o}C until measurement of intratesticular testosterone levels by enzyme immunoassay. **Results and Discussion:** All DBP treated groups exhibited a significant reduction in both anogenital distance and intratesticular testosterone levels, when compared to the groups that received the correspondent vehicles. There were no differences between the DBP groups, indicating that the three different vehicles used (corn, canola and fish oil) had no impact on testosterone production and anogenital distance, an external marker of androgen action. In addition, no significant differences were observed among the control groups that received only the vehicles. It remains to be determined whether these different vehicles can have any impact on other phthalate-induced alterations such as gonocyte multinucleation in fetal testis. Moreover, it is important to establish the impact of longer periods of supplementation of rats with oils containing high levels of omega-3 fatty acids (e.g., prior to pregnancy). **Conclusion:** The use of different oily vehicles (corn, canola or fish oil) had no impact on DBP-induced suppression of testosterone production in rat fetal testis.

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Keywords: phthalates, fatty acids, reproductive toxicity, rats.

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Therapeutic ultrasound treatment depletes epididymal sperm reserves

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Introduction: In contrast to imaging ultrasound, therapeutic ultrasound utilizes frequencies and powers that make it ideal for removing scar tissue or for treating muscle injuries. In the 1970s, the application of ultrasound was tested as a potential male contraceptive by M. Fahim with a custom-built instrument. Results indicated that a single application of therapeutic ultrasound to the scrotum could result in up to six months of reversible infertility. Sperm concentration in ejaculates was shown to be reduced by ultrasound but no investigation of epididymal histology was conducted. **Aims**: Determine if commercially available therapeutic ultrasound instruments can deplete epididymal sperm reserves. Determine if ultrasound treatment causes any gross changes in sperm motility or epididymal histology. **Methods**: Ultrasound was applied to the scrotum of anesthetized rats using a therapeutic ultrasound instrument (Sonicator 740, Mettler Electronics) with frequencies of 1 or 3 MHz and power of 1 to 2.2 watts/cm^2. The scrotum was suspended in a chamber filled with coupling medium. The chamber bottom was acoustically transparent to allow ultrasound to enter the coupling medium surrounding the scrotum. The temperature of the coupling medium was regulated using a temperature-controlled bath. Rats were sacrificed two weeks after ultrasound treatment. Testis and epididymis histology was evaluated using Bouin's fixed tissue. Testis and cauda epididymis spermatid counts were determined. Sperm from rat cauda epididymis were sonicated in a dual optical-acoustic-focus setup with 1 MHz pulses with lengths ranging from 1,000 to 10,000 cycles. Video data was recorded at 5,000 frames per second. **Results and Discussion**: Sham-treated rats had 200 to 300 million sperm per cauda epididymis. Treating with ultrasound at 3 MHz and 2.2 watt/cm^2 with coupling medium at 37C reduced sperm counts to 0.2 – 0.9 million sperm per cauda epididymis two weeks after treatment. The corpus and caput epididymis of treated animals had significantly lower numbers of sperm than sham-treated animals. In addition, portions of the epididymis had decreased tubular diameters, similar to that seen in castrated animals. The height of the seminiferous epithelium in ultrasound-treated rats decreased significantly due to a significant loss of testicular germ cells and this was reflected in significantly reduced counts of homogenization-resistant spermatids. Individual sperm exposed to ultrasound ex-vivo were observed to be extremely resistant to acoustic energy. In-vitro sonication of sperm up to ~ 10 Megapascals at 1 MHz did not permanently alter their motility after delivery of pulses. However, if microbubbles were introduced to the solution, cavitation caused permanent and terminal disruption of the cells within the field of view. **Conclusions**: Commercially available therapeutic ultrasound systems depleted epididymal sperm reserves sufficiently to warrant consideration of this treatment as a method of male contraception. Short-term, ex-vivo exposure to ultrasound did not affect sperm motility. However, further studies are warranted to determine if morphological changes in the epididymal epithelium ultimately affect sperm function.

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Keywords: ultrasound, motility, contraception, histology.

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Effects of pentoxifylline on cryopreserved equine epididymal sperm

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Introduction: The recovery of spermatozoa from the epididymal cauda and its cryopreservation represents a technological advancement in equine reproduction, since it is the last possibility to preserve genetic material from dead or deceased valuable stallions. It is known that equine epididymal sperm are able to fertilize the oocyte, resulting in satisfactory pregnancy results. However, most sperm are immotile after the flushing process. Biochemical studies have shown that the development of motility is associated with intrasperm pH, levels of cAMP and calcium. Therefore the addition of specific substances to flushing extender may stimulate motility of epididymal sperm. Pentoxifylline (PX) is known to inhibit phosphodiesterase activity in living cells, leading to a subsequent increase in intracellular cAMP levels. However, few studies have been conducted to investigate the effects of PX addition on cryopreservation of stallion sperm. **Aim:** The aim of this study was to evaluate the effects of pentoxifylline on frozen equine epididymal sperm when added prior to cryopreservation**. Methods:** For this experiment, 58 testicles from 29 two-year-old Brazilian Jumping Horses were used. Epididymal cauda of each stallion were separated and flushed with a skim milk-extender either without or with 7.18 mM of PX. The samples were analyzed by CASA (HTM–IVOS 12, Hamilton Thorne Research, USA), incubated at 37^{o}C for 15 min then centrifuged at 600x*g* for 10 min. The supernatant was removed and the pellets were ressuspended in an egg yolk extender–Botu-Crio^{TM}. Straws were cooled at 5^{o}C for 20 minutes, frozen 6cm above nitrogen level for other 20 minutes then immersed into nitrogen. The straws were thawed at 46^{o}C for 20 seconds and evaluated for motility parameters. Plasma membrane integrity was evaluated by fluorescent probes carboxyfluorescein diacetate and propidium iodide. In addition, tyrosine phosphorylation was evaluated as an indicator of sperm capacitation. All parameters were analyzed by unpaired *t* test using GraphPad InStat Version 3.06, through unpaired t test to identify the significant differences ($P < 0.0001$). **Results and Discussion:** On recently recovered sperm there were no differences ($P > 0.0001$) in plasma membrane integrity in samples treated without or with PX, though differences were observed on Total Motility (17.14 ± 14.03b *versus* 53.83 ± 21.92a); Progressive Motility (5.55 ± 6.04b *versus* 20.62 ± 11.26a); Path Velocity (76.79 ± 9.94b *versus* 103.76 ± 16.79a); Straight Line Velocity (60.31 ± 11.32b *versus* 77.03 ± 11.45a); Curvilinear Velocity (150.52 ± 25.03b *versus* 194.90 ± 30.19a) and Rapid (9.45 ± 8.91b *versus* 43.86 ± 22.19a) for control group and pentoxifylline group, respectively. According to some studies, the addition of PX on fresh semen enhanced motility sperm parameters possibly due its effects on increase of intracellular cAMPC. However, after post-thaw, no differences in sperm parameters and plasma membrane integrity were observed in samples treated without or with PX ($P > 0.0001$). These results are probably justified by the exhaustion of sperm energy substrate that occurs during cryopreservation process, leading to low levels of cAMP in post-thaw spermatozoa. Immunofluorescence labeling of cryopreserved equine epididymal sperm indicated that no differences ($P > 0.0001$) of sperm displaying tail-associated tyrosine phosphorylation were observed in samples treated without or with PX. **Conclusions:** The results of the present study allow concluding that, although the effects of PX addition on recently recovered epididymal sperm seems to be beneficial, its addition prior to cryopreservation has no significant effect on post-thaw motility parameters, membrane integrity or capacitation of cryopreserved equine epididymal cauda sperm. More studies are required to investigate the effects of PX on fertility.

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Keywords: pentoxifylline, cryopreserved epididymal sperm, equine.

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Effects of atrazine on the structure of adult rat efferent ductules and cauda epididymis

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an herbicide used worldwide, which is now recognized as an endocrine disruptor, affecting the reproductive function in both genders of all groups of vertebrates. In our previous studies, it was demonstrated that Atrazine affects the expression of testicular 3β-HSD protein, which is a key enzyme in the steroidogenic pathway. The herbicide also decreased testosterone and increased estradiol levels in the plasma and testis of adult rats, parallel to histopathological changes in the testis architecture, including a transient increase in the testis weight and luminal dilation of the seminiferous tubules, followed by a significant reduction in weight after longer treatment, correlated with organ atrophy. These effects resembled those described for other toxicants as well as genetic and chemical disruption of estrogen receptor action, which presented testis atrophy resultant from dysfunction of efferent ductules and/or epididymis. Considering the significant imbalance in estrogen/androgen levels found after Atrazine exposure, the present study aimed to investigate alteration in the male genital tract downstream from the testis, to clarify whether or not the effects on the testis were primary or secondary to Atrazine exposure. For this purpose, we chose efferent ductules (ED), which are highly sensitive to estrogen action, and cauda epididymis (EP), which is dependent on androgen levels. Adult male rats were treated by gavage with Atrazine at 300 mg/Kg for 7 days, 200 mg/Kg and 50 mg/Kg for 15 days and 200 mg/Kg for 40 days. Control rats received vehicle (corn oil) at the same volume. Alterations in the ED and cauda EP were investigated by histological and morphometric analysis. Fragments of ED and cauda EP were fixed in glutaraldehyde, embedded in glycolmethacrylate, sectioned at 3 μm and stained with periodic acid Schiff (PAS), counterstained with hematoxylin. An increase was observed of 42 and 86% in the epididymis associated with the ED weight at the dosages of 200 mg/Kg/15d and 300 mg/Kg/7d, respectively, and a reduction of 28% after exposure for 40 days. Morphologically, there were remarkable changes in the ED, including great luminal dilation (215%), concomitant with reduction in epithelial height (49%) and the amount of lysosomes (80%) in epithelial non-ciliated cells. Epithelial vacuolization was also frequent. Longer exposition to Atrazine (200 mg/Kg/40days) resulted in further regression of the efferent ductules. On these ductules the lumen was mostly collapsed or filled with sperm. The epithelium presented remarkable sloughing of cells. Some ductules were surrounded by inflammatory infiltration rich in neutrophils. Neutrophils were also observed into the epithelium of the ductules or even in intraluminal positions. Sperm granuloma was seen in most animals treated with high doses of Atrazine. Changes in cauda EP included reduction in the luminal proportion (42%), accompanied by a discrete increase in epithelia (14%) but a great increase in the interstitial compartment (97%). No evident alterations were observed in the weight or morphology of the ED and cauda epididymis of animals treated with Atrazine at 50mg/Kg. In conclusion, we have demonstrated that Atrazine causes histopathological alterations in the male genital tract downstream from the testis, which potentially may lead to testicular swelling and subsequent seminiferous tubule atrophy. Together these results highlight the effects of Atrazine as an endocrine disruptor which may target different organs of the male genital system.

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Keywords: atrazine, efferent ductules, epididymis, endocrine disruption.

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The functional study of estrogen membrane receptor GPR30 in rat caudal epididymal epithelium

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Introduction: In male reproductive system, the secretion and re-absorption functions of epididymis is critical for the formation of the epididymal luminal micro-environment important for spermioteleosis. Estrogen has been reported to be able to intervene in the epididymal ion-transport within the male reproductive system. **Aims:** The objective of this study is to investigate the mechanism of ion transportation after the activation of GPR30, the membrane receptor of estrogen, in the rat caudal epididymal epithelium. **Methods:** Primary culture of rat epididymal epithelium, Short-circuit current (Isc) measurements, ELISA and RT-PCR. **Results and discussion:** The specific agonist of GPR30, G-1, is able to evoke an obvious short current increase on primary cultured caudal epididymal epithelium. The short current change could be partly inhibited with the incubation of either Cl^- or HCO_3^- -free solutions, while it could be completely abolished when both Cl^- and HCO_3^- are absent. These results indicate that the anion secretion consists of Cl^- and HCO_3. Moreover, the short current induced by G-1 is prevented under the pretreatment of CFTRi-172 which blocks CFTRs specifically, rather than DIDS, the inhibitor of the Ca^{2+}-activated Cl^- channel, suggesting that secretion of anion is probably conducted by CFTR. In addition the down regulation of adenyl cyclase resulting from the pretreatment of its inhibitor MDL12330A also eliminated the effect of G-1. Enzyme immunoassay showed that G-1 could increase intracellular cAMP concentration in epididymal epithelium, which may elicit the activation of CFTR. Moreover, the blockade of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC) by Bumetanide also prevents G-1 from evoking the short current increase indicating that NKCC is involved in the anion secretion induced by G-1. **Conclusions:** the above results indicate that activation of GPR30 is capable of elevating the intracellular cAMP concentration, consequently inducing the activation of cAMP-sentive Cl^- channel CFTR and anion secretion in the rat caudal epididymal epithelium.

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Keywords: estrogen membrane receptor, GPR30, Epididymal Epithelium, anion secretion.

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Distribution of different classes of plasma cells within the epididymal region of roosters affected by epididymal lithiasis

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Introduction: Epididymal lithiasis is an anomaly of roosters that is characterized by the occurrence of stones rich in calcium within the epididymal region and often results in a significant decrease in fertility of affected animals. Several studies have shown that, among the components of the epididymal region, this dysfunction affects especially the efferent ductules. ED is a segment responsible for the reabsorption of up to 90% of the testicular fluid as well as great amounts of calcium. Several injuries have been described within this segment, such as reduced epithelial height and folds as well as increased intracellular vacuolization. A noteworthy characteristic of the affected epididymal region is the presence of abundant mononuclear cell infiltrations that are localized especially adjacent to the efferent ductules. Within these infiltrations, it is interesting the presence of a great number of cells resembling plasma cells. Although the origin of epididymal lithiasis is still not determined, several hypotheses have been postulated to explain its origins, including an infectious agent or an autoimmune disease. **Aims:** Therefore, due to the presence of the mononuclear cell infiltrations and possibly the occurrence of abundant plasma cells, that could be related to a local immunological response, this study aimed to investigate the occurrence and distribution of plasma cells within the epididymal region of roosters affected by epididymal lithiasis. Additionally, we characterized these cells with respect to the immunoglobulin (Ig) subtypes secreted: avian IgY, IgM and IgA. **Methods:** For this purpose, fragments of the epididymal region from affected and non-affected animals were processed for immunohistochemical and Western blotting assays. **Results:** The results showed that in non-affected roosters, plasma cells are present in the connective tissue of all segments of the epididymal region with no evident differences between plasma cells secreting IgY, IgM and IgA. On the other hand, in the epididymal region of affected roosters the most abundant subpopulation of plasma cells was that positive for IgY followed by IgM and IgA. Compared to non-affected animals, the epididymal region of affected roosters showed a significant increase of 3-fold in the number of IgY-positive cells and of 43% in IgM-positive cells. The immunohistochemical results were confirmed by Western Blot analysis. **Discussion:** These findings are interesting considering that affected animals showed increased concentrations of estradiol and decreased vitamin D3 in the epididymal region, which are hormones involved, respectively, with pro-inflammatory and anti-inflammatory response. **Conclusion:** Although our results cannot determine which change is cause or effect, they do suggest that besides a hormonal imbalance and calcium reabsoption disruption, an inflammatory process may be associated with epididymal stones formation, as found in other pathologies characterized by tissue calcium deposition.

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Keywords: plasma cells, epididymal lithisasis, roosters, immunoglobulins.

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Stereological analyses of epididymis and hormonal serum dosage in rats treated with finasteride followed by the suspension of treatment

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It is currently estimated that 20% of infertility cases are related to male factors and epididymis may be an etiology of this infertility. Finasteride is used in the treatment of cancer and benign hyperplasia of prostate and androgenic alopecia. Although epididymis is an androgen-dependent organ and the extensive chronic use of finasteride in humans is increasing, few studies have been made concerning the effect of finasteride in the caput of the epididymis. Based on these assumptions, this study aims to describe the histological and stereological epididymal changes in rats (Sprague Dawley) caused by the treatment with finasteride (5 mg/kg/day) during 56 days and after 30 days of suspension of treatment. In the group treated with finasteride, there was a significant 3-fold increase of serum levels of testosterone (T) and dihydrotestosterone (DHT) serum levels had a significant reduction, about 60%, consistent with the values observed in humans subjected to the treatment with finasteride of androgenic alopecia. There was no histological alteration in the group treated with finasteride. However, stereological analysis (epithelium height, total area and volume of epithelium and lumen of epididymal duct) showed significant alterations only in one region of the caput, called R2. After suspension of treatment the T and DHT values returned to normal. In addition, morphometric-stereological alterations were recovered. These findings indicate that: 1) the 5 mg/kg/day of the finasteride mimics the hormonal serum levels alterations observed in the treatment of androgenic alopecia; 2) finasteride cause morphometric-stereologic alteration only in R2 region of caput which showed complete recovery after suspension of the treatment. 3) only one sub-compartment of the caput of the epididymis showed a significant response to treatment with finasteride, probably due to a specific gene expression pattern.

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Keywords: epididymis, finasteride, stereology, hormonal serum.

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Thiol proteins electrophoretic profile of stallion epididymal spermatozoa

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Thiol groups of cysteine residues represent redox centers that are implicated in protein structure, enzyme catalysis, signal transduction and regulation of transcriptional activity of different cells types. During epididymal transit, mammalian spermatozoa undergo sequential post-translational changes that confer upon these cells the ability to exhibit a mature motility pattern and the fertilizing potential. This process is accompanied by oxidation of sperm thiol proteins. In this work, we evaluate the thiol-disulfide status of stallion sperm proteins obtained from caput and cauda epididymis regions using 1D and 2D-PAGE. Spermatozoa, with and without 1mM DTT pre-treatment, were labeled with 2 mM monobromobimane (mBBr), a fluorescent thiol labeling agent, solubilized in 5% SDS, 1 mM DTT solution, sonicated and centrifuged at 13.000 x g (30 min). The supernatants were submitted to 12-20% vertical gradient polyacrylamide gel electrophoresis. For 2D-PAGE, mBBr-labeled sperm cells were resuspended in a lysis buffer containing 8M urea, 4% triton X-100, 30mM DTT and protease inhibitors, then sonicated and centrifuged (17.000 x g; 30 min). The supernatant proteins were precipitated with TCA/acetone, resuspended in a rehydration buffer and loaded "in gel" into immobilized pH gradient (IPG) 4-7 strips. Isoelectric focalization was carried out in an IPGphor system fulfilling standard protocols and the second dimension was done in 12% SDS-PAGE. 1D and 2D gels were photographed under UV light, and then, stained with coomasie blue. One dimensional SDS-PAGE of mBBr labeled sperm displayed fluorescent bands of ~ 107, 81, 71, 65, 58, 53, 45, 32 and 28 kDa in absence of DTT pointing to the existence of thiols in these proteins, being the 32 and 81 kDa the most sulfhydryled ones. Thiol proteins of 34 and 43 kDa present in cauda spermatozoa were not observed in caput spermatozoa; on the other hand, a 41 kDa protein observed in caput samples was not detected in cauda spermatozoa. Pre-treatment with DTT increased the mBBr labeling of some sperm protein obtained from the cauda epididymis region, indicating that these samples contain both thiol and disulfide groups. Estimation of the SS/SH ratio in samples from cauda and caput epididymal spermatozoa showed to be 2.2/1.0 and 1.4/1.0, respectively. Protein 2D-PAGE profile displayed some differences in samples obtained from the caput and cauda epididymal regions. In samples from caput epididymides, the major coomassie blue stained spots corresponded to 64 kDa (pI6.4); 59 kDa (pI6.4); 54 kDa (pI4.7-5.5); 43 kDa (pI6.2), 43 kDa (pI 4.8) and 32 kDa (pI6.8-7.0) proteins and the 52 kDa (pI 6.6), 35 kDa (pI 5.75-5.6), 34 kDa (pI 5.5), 32 kDa (pI 6.7) and 23.5 kDa (pI 4.7) were majorities in samples from cauda epididymis. A striking spot of 54 kDa (pI 4.9 - 5.4) in caput sperm samples appears more faintly stained on cauda epididymis spermatozoa gels; a 42.5 kDa (pI 4.8) spot was present only in caput spermatozoa. Others spots were detected only in mature spermatozoa [44 kDa (pI 4.6), 38 kDa (pI 5.3) and 24 kDa (pI 4.7) proteins]. Visualized under UV light, a 32 kDa (pI 7.0) spot was the major fluorescent protein in bidimensional gels loaded with caput epididymal sperm samples; a 32 kDa (pI6.7-7.0), 64 kDa (pI6.3-6.4), 52 kDa (6.6-7.0), 44 kDa (pI 6.0 - 6.2) spots were the most fluorescents in samples from the cauda epididymal region. After DTT treatment, an increase of all pre-existing spots occurs in samples from the cauda epididymis; new thiol-labeled spots were also observed. Electrophoretic separation of sperm thiol proteins from the caput and cauda epididymal regions showed that during maturation thiol oxidation occurs in many protein fractions and that the magnitude of these reactions differs among proteins.

Keywords: thiol protein, stallion spermatozoa, epididymal maturation, 2D-PAGE.

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Tryptophan catabolism along the kynurenine pathway in mouse caput epididymidis

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Introduction: In mammals, after protein synthesis, indoleamine 2,3-dioxygenase (IDO) is the main non protein metabolic route of the essential aminoacid tryptophan (Trp) leading to the generation of kynurenine metabolites. Intriguingly, IDO is constitutively and highly expressed in the mammalian epididymis contrary to most other tissues where it is induced following inflammatory situations. **Specific aims:** To gain insight into the role of IDO in the physiology of the mammalian epididymis, we studied both wild type and *Ido1-/-* deficient mice. **Results:** We first show that the lack of IDO activity drastically depletes the caput epididymis in kynurenines, demonstrating that IDO is the major kynurenine producer in this tissue. We also show that the absence of IDO expression in the caput epididymis of Ido-/-animals is not compensated for by other tryptophan-catabolyzing enzymes. In addition, we show that mouse caput epididymis is characterized by a high kynurenine to tryptophan (K/T) ratio confirming the high basal IDO activity in this tissue and revealing a peculiar but physiological inflammatory context. We demonstrate that amongst the various kynurenine downproducts, kynurenic acid (KA) is by far the most abundant kynurenine generated in wildtype mouse caput epididymis. Absence of IDO activity resulted in an increased in Trp content and was found associated with an increase in total protein content in caput epididymis extracts of Ido-/- animals. Contrary to what would have been logically expected, we show that increased caput epididymis protein content in Ido-/-animals is not due to enhanced protein synthesis but rather to impaired protein catabolism, mainly because of a deficient proteasomal pathway. Finally, we also present evidence showing that the caput epididymis epithelium is affected by IDO deficiency leading to the appearance of a caput regionalized phenotype and an increase in cauda epididymis sperm counts. Nevertheless, although the epididymal tissue is altered by lack of IDO expression it has no noticeable impact on sperm fertilizing ability. **Discussion/Conclusions:** These data provide support for IDO playing a hitherto unsuspected role in sperm quality control in the epididymis involving the ubiquination of defective spermatozoa and their subsequent removal.

Triiodothyronine (T3) as a possible regulator of epididymis function

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Introduction: The impact of thyroid disorders on adult male reproductive function has been controversial, despite the recognized effects of thyroid hormone (TH) on almost all organs and tissues. This is probably due to earlier studies that have shown that the testes were not metabolically responsive to TH, and present low expression of TH receptors. Nevertheless, TH receptors were shown to be present in the epididymis, mainly in the cytoplasmic compartment, which suggests that T3 might act nongenomically, and to significantly increase in number during hypothyroidism. **Aims:** Based on clinical evidence and recent studies suggesting that TH affects the reproductive function, we investigated whether TH regulates the expression of androgen receptors (AR) and 5α reductase in rats. This enzyme converts testosterone to its more biologically active form, the dihydrotestosterone (DHT), which participates on the sperm maturation in the epididymis. The TH effects on serum testosterone concentration were also evaluated. **Methods:** In this study we evaluated the effect of acute administration of T3 on the activity of epididymis in male Wistar rats that were thyroidectomized at 60 days-old and kept under methimazole and CaCl treatment for 20 days. After this period the animals received T3 intravenously at doses corresponding to 1X, 5X or 50X of the physiological dose (0.3 μg/100 g BW) or saline (TX), and were decapitated 30 min thereafter. Sham-operated animals were used as control. Total RNA was extracted by guanidine-phenol-chloroform method and 1 μg was submitted to RT procedure. The AR, 5 alfa reductase subtypes 1 (S5AR 1) and 2 (S5AR 2) as well as RPL19 and GAPDH mRNA (housekeeping genes) expression was evaluated by real time PCR (Sybr Green, Life Technologies). The serum TSH and testosterone concentration was measured by RIA. The results were statistically analyzed by ANOVA followed by Tukey HSD *post hoc test* for unequal sample sizes, considering statistical difference at $P < 0.05$. **Results and Discussion:** Thyroidectomy increased the serum TSH concentration and the acute administration of T3 did not change this parameter, as expected. Hypothyroidism caused an important reduction on serum testosterone concentration and an elevation on AR mRNA expression in all portions of epididymis (caput, corpus and cauda). The acute treatment with T3 produced a decrease in mRNA expression of AR mRNA in all portions of epididymis despite any observed alterations in serum testosterone. The S5AR 1 (all portions of epididymis) and S5AR 2 (caput and cauda) were also elevated during hypothyroidism, probably to maintain DHT production, despite the low testosterone. The expression of S5AR 1 and S5AR 2 was decreased by T3 treatments to the control values. Interestingly, the S5AR 2 mRNA expression on the corpus of epididymis was inversely correlated with the caput and cauda, for hypothyroidism and T3 treatment, denoting a possible local differential expression of this enzyme. **Conclusion:** These results were suggestive of a rapid action of TH on the expression of these important genes in the epididymis, indicating that it might exert a central role on sperm maturation and acquisition of sperm motility. However, further studies are warranted to determine if these alterations are also observed at the level of the protein expression.

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Keywords: androgen receptor, 5 alpha reductase, thyroid hormone, epididymis.

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Pharmacological characterization of α_1-adrenoceptor subtypes in the rat distal cauda epididymis

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Introduction. Noradrenaline released from sympathetic varicosities within the cauda epididymis contracts the smooth muscle during the emission phase of ejaculation to propel the sperm towards the vas deferens. The α_1-adrenoceptor subtype(s) involved in the contractions of distal cauda epididymis in response to noradrenaline are unknown. **Aims**: The aim of the present study is to characterize the α_1-adrenoceptor subtypes involved in the contractions of distal cauda epididymis in response to noradrenaline using subtype-selective competitive antagonists. In addition, the α_1-adrenoceptor subtypes expressed in distal cauda epididymis were identified using binding assays with the selective α_1-adrenoceptor radioligand [^{3}H]Prazosin in tissue segments. **Methods**: *In vitro contraction studies.* Distal cauda epididymis segments (≈1 cm long) were isolated from adult rats (90 to 120 days, 350 to 450 g), trimmed of adherent tissues and had the luminal contents washed with the help of a syringe and needle. Distal cauda epididymis segments were mounted in organ baths for recording of isometric tension development. Cumulative concentration-response curves to noradrenaline were obtained in absence and presence of different concentrations of prazosin (α_1-adrenoceptor selective), yohimbine (α_2-adrenoceptor selective), RS 100329 and 5-methylurapidil (α_{1A}-adrenoceptor selective) and BMY 7378 (α_{1D}-adrenoceptor selective). Antagonist affinities (pK_B) were determined by Schild analysis. *Tissue segment binding.* For saturation analysis, rings of distal cauda epididymis (1.5 mm length) were cleaned by adherent tissues and incubated with increasing concentrations of [^{3}H]Prazosin (20 to 2000 pM). For competition analysis, the rings were incubated with increasing concentrations of non-radioactive competitors for 1 h and then incubated with 350 pM [^{3}H]Prazosin for 12-16 h at 4ºC. Non-specific binding was defined in presence of phentolamine 100 μM. **Results**. Noradrenaline induced concentration-dependent contractions of distal cauda epididymis (pD_2 of 7.1 ± 0.04, n = 22) that were competitively antagonized by prazosin (pK_B = 9.5), yoimbine (pK_B = 7.3), RS 100329 (pK_B = 9.8), 5-methylurapidil (pK_B = 9.0) and BMY 7378 (pK_B = 6.9). These results are consistent with the predominant activation of α_{1A}-adrenoceptors by noradrenaline in the contractions of the distal cauda epididymis. The specific binding of [^{3}H]Prazosin to distal cauda segments yielded a maximal binding capacity (Bmax) of 120 ± 14 fmol.mg^{-1} protein and dissociation constant (K_D) of 320 pM, consistent with the labeling of α_1-adrenoceptors. The specific binding of [^{3}H]Prazosin to distal cauda epididymis segments was displaced by prazosin, 5-methyl urapidil and RS 100329 according to the predominant labeling of α_{1A}-adrenoceptors. However, the competition curves for BMY 7378 were complex indicating the presence of a heterogeneous receptor population. **Discussion and Conclusions:** Although radioligand binding assays indicate the presence of a heterogeneous receptor population apparently composed of α_{1A}- and α_{1D} –adrenoceptors, the major α_1-adrenoceptor subtype involved in the contractions of distal cauda epididymis in vitro is the α_{1A}. This may explain, at least in part, the higher incidence of oligospermia observed in men treated with α_{1A}-selective antagonists. The predominant role of α_{1A}-adrenoceptors in the contraction of distal cauda epididymis indicates that this receptor subtype may be a target for further studies in the search of sexual hormone-independent male contraceptives.

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Keywords: α_1-adrenoceptors subtypes, distal cauda epididymis, contraction, tissue segment binding.

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Association of human CRISP3 with sperm and its behaviour during capacitation

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The cysteine-rich secretory protein (CRISP) family is a large group of secreted proteins characterized by the presence of 16 conserved cysteine residues. In mammals, four main groups have been described: CRISP1, synthesized by the epididymis, CRISP2, of testicular origin, CRISP3, with a wider tissue distribution, including reproductive and non-reproductive tissue, and CRISP4, also expressed in the epididymis. In our laboratory, we have studied the association of epididymal CRISP1 and testicular CRISP2 with human, rat and mouse sperm, observing that both proteins remain associated with sperm after capacitation and acrosome reaction and, in agreement with this behaviour, both molecules have a role in the fertilization process. Considering that CRISP3 is secreted by the epididymal epithelium and could also be involved in the acquisition of sperm fertilizing ability during maturation, in the present work we studied the association of human CRISP3 with sperm as well as its behaviour during capacitation as a first approach to investigate its potential role in fertilization. For this purpose, human sperm were subjected to different extraction treatments and the permanence of CRISP3 in sperm was evaluated by Western blots and indirect immunofluorescence (IIF) using a polyclonal antibody against human CRISP3. Results revealed the presence of two bands of 31 kDa and 29 kDa corresponding to the two already described forms of CRISP3 in protein extracts from fresh, untreated sperm. While the protein corresponding to the 31 kDa band was easily removed by washing the cells with PBS, the one corresponding to the 29 kDa band remained on sperm after their exposure to high ionic strength (0.6M NaCl), and was completely removed from the gamete only by treatment with detergent (1% Triton X-100) indicating the tight association of this protein to human sperm. Interestingly, the 29 kDa band was also detected in protein extracts from sperm that have already undergone both capacitation and calcium ionophore-induced acrosome reaction. Subsequent analysis of these sperm by IIF revealed the presence of fluorescent labelling in the acrosome and tail of capacitated sperm and in the equatorial segment of the acrosome-reacted cells. The existence of a strongly bound population of CRISP3 in human sperm and its permanence after capacitation and the acrosome reaction supports the potential participation of this protein in the fertilization process.

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Keywords: CRISP3, sperm, epididymis, capacitation.

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Effect of cadmium and *Arctium lappa* extract administration in rat epididymis

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Introduction: Cadmium (Cd) is a wide spread environmental pollutant, characterized by its toxicity in various human and animal organs. Although it is known that Cd is associated with damage to male reproductive organs of rats, there are few studies which have quantified the morphological alterations caused by this metal in the epididymis. *Arctium lappa* has been reported to protect many organs against toxicity and oxidative stress induced by harmful chemicals, therefore the therapeutic potential was tested against acute Cd intoxication in the epididymis of Wistar rats. **Aims**: To verify alterations in the epididymis caused by Cd and to examine the therapeutic potential of *A. lappa* using morphometric techniques. **Methods**: *A. lappa* (*Al*) root hydroethanolic extract (*Al*E) was administered by gavage in a dose of 300 mg/kg/day. Cadmium chloride ($CdCl_2$) solution was injected i.p. as a single dose of 1.2 mg/Kg BW. The control group was injected with a single i.p. injection of saline and received water by gavage. The *Al* group received A*l*E. The Cd group was injected with $CdCl_2$ solution. The Cd*Al* was injected with $CdCl_2$ and received A*l*E. Six animals of each group were sacrificed after either 7 or 56 days. The animals were fixed by whole body perfusion with glutaraldehyde 2.5% and paraformaldehyde 4% in PBS buffer 0.1 M, with pH 7.2 for 25-30 minutes and then post fixed in the same fixing solution for 24 hours. Historesin-embedded epididymis fragments were used for morphometric analysis with the Image Pro Plus software. ANOVA followed by Duncan's test was performed ($P < 0.05$). Significant data variations are enclosed in parenthesis. **Results and Discussion**: Epididymis weight was reduced in Cd7 (18%), Cd56 (31%) and Cd*Al*56 (37%). The proportion of ducts and interstitium of the epididymis caput and cauda did not change after 7 days in all treatments. However, after 56 days, the percentage of ducts of the caput was reduced only in animals treated with Cd (25%). In the epididymis cauda, a decrease of duct proportion in Cd (13%) and Cd*Al* (10%) groups was also observed, as well as an increase of the interstitium. The epithelium height increased significantly in the epididymis caput and cauda after 7 days. However, after 56 days, only in the epididymis cauda was this increase still observed. Light microscopy observations showed no changes in epididymal tissue of *Al* groups compared to controls after 7 and 56 days, as was corroborated by stereology. After 7 days, in both Cd and Cd*Al* groups, spermatozoa were not observed in the caput. However in the cauda, spermatozoa were present, as well as frequent testis cell debris. After 56 days, spermatozoa were absent and the quantity of cell debris was greater. These findings were supported by previous cadmium studies of Herak-Kramberger *et al.* (2000) which also observed epididymis atrophy, decrease in diameter of duct lumen and thickening of duct epithelium. **Conclusions**: Our findings showed that Cd causes important alterations in the rat epididymis and *A. lappa* extract was ineffective in protecting this organ.

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Keywords: epididymis, cadmium and *Arctium lappa.*

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Impact of prepubertal antiandrogen exposure on androgen receptor (AR) expression in the rat epididymis

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Introduction: Due to the widespread environmental and occupational exposure of the world population to endocrine disruptors, great attention has been given to these compounds, which can interfere with the physiologic action of endogenous hormones. Previous works have shown the effects of exposure to chemicals *in utero* and during adult life on the rat epididymis and reproductive health. However, few studies have focused on possible consequences on these parameters in chemical-exposed rats during prepuberty, when the epididymis undergoes important changes in its morphology, function and gene expression that result in the regional differentiation of the epididymal ducts. **Aims:** To evaluate the impact of prepubertal exposure to an antiandrogen endocrine disruptor on sexual serum hormonal levels and androgen receptor (AR) expression in the epididymis. **Methods:** Male Wistar rats, 21 days old, were divided into 2 groups: F (n = 10), which orally received 25 mg/Kg/day of flutamide (Sigma Aldrich) and C (n = 10), which received vehicle (corn oil). The treatment occurred from postnatal day (PND) 21 to 44. Animals were sacrificed on PND50. Body weight and reproductive organ wet weight were recorded. Blood was processed and serum LH, FSH and testosterone levels were evaluated by RIA. AR expression was assessed by Western Blot (WB) studies using total protein extracts (initial segment (IS)/caput, corpus and cauda epididymis) and an anti-AR antibody. Beta-actin antibody was used as internal control. Immunohistochemistry (IHC) was performed on paraffin-embedded epididymis to assess AR immunodistribution. AR antibody pre-adsorbed with respective blocking peptide was used as negative control. Statistical analyses were performed using Student´s t test and Mann-Whitney test, $P < 0.05$. **Results and Discussion:** Sexual serum hormonal levels were similar between C and F groups, probably due to the weak negative feedback exerted by testosterone on hypothalamic-pituitary-gonad axis in the immature rats. Prepubertal antiandrogen exposure did not change the rat body weight, but reduced the absolute tissue wet weight of the epididymis, ventral prostate, vas deferens and seminal vesicle. IHC revealed that prepubertal F treatment specifically induced a reduction in AR-positive immunostaining in the nucleus and cytoplasm of the epithelial cells from IS and caput epididymis, possibly representing changes in AR function. This change was slightly observed in cauda region epithelia. AR immunostaining of epididymal interstitial and smooth muscle cells was similar between C and F groups. WB assays indicated, however, an increase in AR expression in IS/caput, but not corpus and cauda epididymis, of the F group. **Conclusion:** The results indicate that prepubertal F exposure interferes with post-natal development of the epididymis by affecting AR expression in this organ at puberty. Further studies will be necessary to gain insights into the consequences of the changes induced by prepubertal antiandrogen exposure into AR function.

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Keywords: epididymis, antiandrogen, rat, prepuberty, AR.

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Effect of two commercial semen extenders on ostrich epididymal sperm viability

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Introduction: The ostrich, the biggest bird in the world, belongs to the ratite group. In some countries, the ostrich has been used as a protein source and, in the beginning of the 1990s this bird was introduced in Brazil and breeders started to commercialize their products. In spite of the increasing investment in ostrich breeding, there is a lack of scientific information related to the handling and reproductive behavior of this species. **Aims:** The objective of the present study was to evaluate the viability of storing epididymal sperm from ostriches using commercial extenders. **Methods:** For this experiment, semen samples obtained directly from the epididymis and deferens duct from 7 male sexually matured ostriches (African Black; older than 3 years old), collected from abattoirs. Sperm motility and progressive motility were microscopically evaluated after the addition of two commercial extenders (TCM 199[1] and TQC OVODYL[2]) and storage under 5 and 37ºC for 0, 2, 12, 24, 30, 42 hours. Data was analyzed using the Guided Data Analysis and Analyst of the SAS System for Windows V.8 (SAS Institute Inc., Cary, NC, USA, 2000). **Results:** Interactions between extender*temperature and extender*time were found on both motility and progressive motility. Semen samples diluted in TCM199[3] showed higher motility and vigor when compared to those diluted in TQC OVDYL[4] in both temperatures. On the other hand, depending on the extender used, differences were found between storage temperatures. While samples diluted in TCM199 showed better results when stored at 37ºC, when using TQC OVODYL, samples stored at 5º showed higher motility and progressive motility. Motility and progressive motility were maintained for a longer period when using TCM199. **Discussion and conclusion:** Based on the results presented in the present study we observed that epididymal sperm collected from ostriches can be maintained for up to 30 hours by using TCM199 to dilute the samples and store them under 37ºC.

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Keywords: ostriches, semen, epididymis, extenders.

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Characterization of basal cells during epididymal post-natal development

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We recently showed that basal cells act as both sensors that scan the luminal environment of the epididymis for the hormone angiotensin II, and transmitters that communicate their findings to neighboring clear cells via the production of nitric oxide (NO; 1). NO diffuses out of basal cells and acts locally on the cGMP signaling pathway in clear cells, to increase proton secretion. We, therefore, showed the presence of a novel crosstalk between basal and clear cells that controls proton secretion. We found that basal cells penetrate the blood/epididymis barrier and establish a new tight junction (TJ) with surrounding epithelial cells. We have, thus, uncovered how luminal signals are sensed and transmitted to proton-secreting clear cells to regulate luminal acidification, a mechanism that is critical to sperm maturation and viability. To determine whether the "apical-reaching" property of basal cells is acquired during sexual maturation, we followed here the appearance and behavior of basal cells in the epididymis during postnatal development. Immunofluorescence labeling for the basal cell markers claudin-1 (2), COX-1 (3) and keratin-5 (Krt5) showed that basal cells are absent from the vas deferens (VD) and epididymis at birth, and that they appear initially in the VD during the first post-natal week. During the second post-natal week, basal cells are also visible in the distal cauda epididymis, while they remain absent from other epididymal regions. Interestingly, even at such an early stage - at times when no sperm have yet arrived in the lumen - basal cells are already seen reaching out towards the lumen. 3D reconstruction of confocal Z-series optical sections of cauda epididymis double-stained for claudin-1 and the tight-junction protein, ZO1, showed that basal cells can reach the luminal border of the epididymis, where they appear to have opened the tight-junction barrier. These results indicate that basal cells acquire their luminal sensor property prior to puberty. In other pseudostratified epithelia, basal cells are believed to be progenitors of other epithelial cell types (4). We thus examined whether they might be progenitors of clear cells, which are also absent from the epididymis at birth (5). We found that the appearance of V-ATPase-labeled clear cells precedes the appearance of basal cells throughout the epididymis and VD during post-natal development. These results are not compatible with the hypothesis that basal cells are progenitors of clear cells. Previous studies proposed that basal cells might have immunological properties (6, 7). We, therefore, examined the relationship between basal cells and dendritic cells, which we recently showed are present in the epididymis (see abstract from Da Silva et al.). 3D confocal reconstruction of the epididymis from mice expressing CX3CR1-GFP (a reporter of dendritic cells) double-labeled for Krt5 showed that basal cells are distinct from the dense network of dendritic cells that populate the base of the epididymal epithelium. In summary, we showed that the apical-reaching property of basal cells does not depend on the appearance of spermatozoa or production of steroid hormones that occur during puberty. Thus, basal cells might be modulated by stimuli that are present before puberty. Alternatively, the body projections of basal cells might constitutively move to and from the lumen of epithelia. In addition, basal cells do not appear to be progenitors of clear cells and they are distinct from epididymal dendritic cells.

1) Shum WWC et al. Cell, 135:1108-1117, 2008; 2) Gregory M et al. Endocrinology, 142:854-863, 2001; 3) Leung GP et al. Mol Cell Endocrinol, 216:5-13, 2004; 4) Hong KU et al. Am J Pathol, 164:577-588, 2004; 5) Breton S et al. Histochem Cell Biol, 111:97-105, 1999; 6) Yeung CH et al. Biol Reprod, 50:917-926, 1994; 7) Holschbach C and Cooper TG. Reproduction, 123:517-525, 2002.

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The influence of anabolic steroids in clinical and laboratory parameters related to male fertility

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Infertility may be defined as the inability of a sexually active couple, without the use of contraceptive methods, to establish pregnancy after twelve months of unprotected intercourse. It affects up to 15% of couples in reproductive age. In a world population basis, these data show that 100 to 150 million people may have fertility problems. Several factors may contribute to a decrease in male reproductive potential, including anabolic-androgenic steroids (AAS) abuse. There are several ways in which the use and abuse of AAS may impact one's health. Impairment of male fertility is one of the less reported, but certainly, one that urologists should know better, since reversion of the majority of the dysfunctions caused by this misuse can be often achieved, at least among light and moderate users. In the middle of this decade, over 1.2 million individuals admitted AAS abuse in Brazil. The aim of this study is to analyze the influence of AAS abuse on clinical and laboratory parameters related to male fertility impairment. Charts of twenty-four AAS users from a single institution (median age = 30.5 y.o.) were reviewed and compared to a control group of 210 candidates of vasectomy surgery (median age = 35.68y.o.) from a major brazilian teaching hospital. Parameters analyzed included physical examination of the testicles, complete seminal analysis and basic hormonal evaluation. Mean sperm concentration among AAS users was $54.46x10^6$/mL, versus $110.1x10^6$/mL at the pre-vasectomy group (p=0.002). Mean sperm motility among steroid users was 48.13%, and 64.86% among the members of the control group (p=0.043). Mean Kruger morphology among steroid users was 1.8%, compared to 5.64% at pre-vasectomy group (p=0.001). Mean WHO morphology among AAS users was 8.6%, and at the control group was 19.53% (p=0.001). Mean serum testosterone among steroid users was 359 UI/L, and at the same time, the control group showed mean testosterone of 112.27 UI/L (p=0.003). Mean testicular volumes (left and right testicles) were also decreased among AAS users (18 cm³ versus 22 cm³ for left testis; and 17 cm³ versus 21 cm³ for right testis, with p=0.001 for both sides). In conclusion, this series reports that AAS use has a negative effect on seminal parameters, such as sperm concentration, sperm motility, sperm morphology, blood testosterone levels and testicular volume. Thus, the use of AAS in this cohort has apparently influenced the onset of male infertility.

Keywords: anabolic-androgenic steroids, steroid users, male infertility, testis, spermatozoa, hormones.

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Expression investigation of RANTES and its receptors in epididymis

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Introduction: Regulated upon activation normal T-cell expressed and secreted (RANTES) a member of chemokines superfamily, is now known to have multiple and complicated effects. The recent studies demonstrated the presence of RANTES in male seminal plasma. However, the origin of RANTES in male genital tract is still unclear. **Aims:** The objective of this study was to investigate the expression and possible roles of RANTES in the male reproductive system. **Methods:** Six testes and epididymides were collected from three victims of traffic accidents aged 25–40 years. RT-PCR, in situ hybridization, immunohistochemical staining and immunofluorescence staining were employed to examine the distribution of RANTES and its receptors male gential track. Western blot was used to quantitate the levels of RANTES expression in BALB/c mouse epididymis on postnatal days. Immunofluorescence staining was applied to detect RANTES association with spermatozoa from adult mouse epididymis. **Results and Discussion:** The location of RANTES was restricted to ciliated cells of the efferent duct and apical, narrow and basal cells of epididymal ducts, in both human and mouse. RANTES–positive basal cells were only identified in the epididymal ducts in human. In addition, RANTES receptors CCR1 and CCR5 mRNAs were detected in human epididymis, and the immunoreactivity of CCR1 and CCR5 was found generally along the human epididymis. The signals of RANTES were first detected on day 28 and increased during mouse sexual maturation. We also observed that RANTES was bound on both normal and defective epididymal sperm but in different patterns. **Conclusions:** RANTES is constitutively expressed in the epididymis and secreted into the lumen of epididymis throughout sexual maturity, and differentially associates with viable and defective spermatozoa.

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Keywords: RANTES, epididymis, sperm, receptors.

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Regionalized expression of E-cadherin in caput epididymis of adult rat

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Introduction: Epithelial cadherin (E-cadherin) has been involved in several calcium-dependent cell-cell adhesion events; this molecule allows the maintenance of cell junctions and sustains the phenotype of cells of different epithelia. It's has been previously reported that there are higher levels of E-cadherin mRNA in the epididymis than in the testis in the human male. Furthermore, it has been described that different putative isoforms of E-cadherin are expressed in the principal cells of different segments of the epididymis, and that they have both an age- and androgen-dependent regulation. **Aims:** Characterize the distribution of E-cadherin in rat caput epididymis. **Methods:** We used immunohistochemistry to detect positive immunoreactivity in adult rat caput epididymis. **Results and Discussion:** We found that E-cadherin is mainly expressed in principal cells of some epididymal tubules of the whole caput epipidymis. Because androgen synthesis takes place in the principal cells too, we believe that a direct correlation between androgen synthesis and expression of E-cadherin may exist in caput epididymis, but further experiments must be realized to test this idea. **Conclusion:** It is possible that the identification of proteins that interact with E-cadherin and with the immature sperm along different segments in epididymis will contribute to the understanding of the molecular basis of fertilization and will aid in the diagnosis and treatment of infertility in human males.

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Keywords: E-cadherins, principal cells, caput epididymis.

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The ultrastructure of the lining epithelium of the cauda epididymis in the Mongrel dogs

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Mammalian spermatozoa leaving the testis undergo a series of structural, functional and biochemical changes during their descent through the epididymis when they gain the ability to move and to fertilize eggs. They are kept in an immotile state in the cauda epididymis where they are stored until the moment of the ejaculation. Thus, the cauda epididymis is a place for long-term storage of mature spermatozoa. Depression of their metabolism and motility as well as prevention of the acrosome reaction occur in this region. In this study, epididymis of four adult and sexually mature Mongrel dogs *Canis familiaris* were used. Examples from epididymis were processed for observation by transmission electron microscopy. The lining epithelium of the cauda epididymis of the Mongrel dogs is made up of four cell types: principal, basal, clear and apical cells. Abundant secretor units are observed in the supranuclear cytoplasm of columnar principal cells. The apical region of principal cells shows an endocytotic apparatus. The apical surface is covered by numerous stereocilia. The clear cells, arranged between the principal cells, are characterized by the presence of abundant vesicular elements and larger vacuoles in the apical cytoplasm. Basal cells were observed to contact the basement membrane and were rather poor in organelles. Apical cells were rarely. The presence of abundant vesicles and vacuoles in the clear cells was correlated with sites of fluid absorption. The well-developed Golgi complex and numerous cisternae of rough endoplasmic reticulum, that were noted in the principal cells, were suggestive of their ability to synthetize and secrete protein/glycoproteins. Thus, the present results suggest that cauda epididymis in the Mongrel dog exerts other morphological roles than storage of spermatozoa. The results are compared with previously published data in the cauda epididymis of other species, in an effort to understand the significance of the epididymis in sperm maturation.

Keywords: cauda epididymis, dog, ultrastructure.

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Short and long-term reproductive effects in male rats treated with the antineoplastic agent cisplatin during puberty

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Cisplatin (CP) is an antineoplastic agent widely prescribed against testicular cancer, which represents the most common cancer among young men of reproductive age. It is also applied to treat childhood and adolescent malignant neoplasms. However, no reports were found about reproductive effects caused by treatment during puberty in rats. Thus, the aim of this study was to evaluate short and long-term effects on reproductive endpoints of CP-treated pubertal male rats. Wistar male rats (45 days old) were assigned to 2 groups. Control (n = 42 saline 0.9%) and CP (n = 49, 1mg/kg of CP, 5 days/week, for 3 weeks, ip.). The study was divided into two phases. I) At 66 (post-pubertal age) and 140 (adult age) days old, rats were sacrificed by decapitation. Blood was collected from the ruptured cervical vessels for determination of serum testosterone levels by RIA. The right testis and epididymis were weighed and used for sperm counts, while the left organs were fixed and submitted to the paraffin wax inclusion routine. H&E stained testicular and epididymis sections were submitted to histomorphometric analysis. The TUNEL method was used to label apoptotic germ cells. Sperm were collected from the vas deferens for evaluation of sperm motility and morphology. II) At 66 and 140 days old, rats were mated with adult females for reproductive performance evaluation. Ten days after mating, males were euthanized and right testis was collected for testosterone concentration determination. Mann-Whitney test was used for statistical analysis. CP rats showed testicular histological alterations, such as loss (sloughing) of immature cells into the lumen and seminiferous tubules with few germ cell layers, vacuolization and acidophilic cells. These alterations were focal, scattered and observed more frequently in the post-pubertal rats. The number of TUNEL-positive seminiferous tubules was increased ($P < 0.05$), while epididymis weight, daily sperm production, sperm reserves in the cauda epididymis, tubular diameter, fertility potential and intratesticular testosterone concentration were reduced ($P < 0.05$) in CP-treated rats at post-puberty, but not in adulthood. On the other hand, the number of sperm with progressive movement was decreased ($P < 0.05$) in CP group at both ages. Testis weight, epididymal histology, sperm morphology and serum testosterone levels were comparable between groups ($P > 0.05$). These results show that cisplatin administration during puberty, in these experimental conditions, provokes alterations in several reproductive endpoints. The damages to spermatogenesis are partially recovered; Sperm motility, however, a characteristic acquired during transit through epididymis, is a long-lasting impairment in rats.

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Keywords: cisplatin, pubertal rat, spermatogenesis, sperm motility, recovery.

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Effects of maternal protein restriction on rat male reproductive parameters

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Protein restriction (PR) *in utero* or perinatally has been used as an experimental model of fetal programming for verifying effects on cardiovascular and renal functions at adulthood. It is well established that maternal PR in the early development affects pup birth weight and causes metabolic and physiologic alterations, but few studies investigated effects on reproduction. So, the aim of this study was to evaluate the effects of maternal PR in different periods of pregnancy or during lactation on reproductive parameters of rat male offspring. Wistar pregnant rats were divided into 6 experimental groups (n = 7/group): 1) Control, 2) Gestational (PR from gestational day 0 (GD0) to GD21), 3) Pre-implantation (PR from GD0 to GD5), 4) Embryonic (PR from GD6 to GD15), 5) Fetal (PR from GD16 to GD21), and 6) Lactation (PR from postnatal day 1 (PND1) to PND21). Pregnant mothers of control and PR groups were fed, respectively, with normoproteic (17% protein) and hypoproteic (6% protein) chow, according to the experimental design. The following parameters were evaluated in the male offspring: age of puberty onset, indicated by the day of prepucial separation; testis and epididymis weights and serum testosterone levels on PND55 (puberty) and PND90 (sexual maturity); sperm counts (daily sperm production per testis – DSP and sperm number in the epididymis), transit time, morphology and motility on PND90. Data were statistically compared using One-way ANOVA, followed by Tukey-Kramer test or Kruskal-Wallis test, followed by Dunn's test, $P < 0.05$. PR *in utero* or during lactation did not interfere with the age of prepucial separation, serum testosterone levels, sperm morphology and motility. The body weights on PND55 and PND90 were reduced in group 6 compared to the other groups. In this same group there was a reduction in the testis and epididymis weights at PND55, which did not persist until PND90. DSP was also reduced in groups 2 and 6 compared to control. On the other hand, sperm counts and transit time in the epididymis were similar between the groups ($P > 0.05$). We concluded that PR *in utero* and during lactation caused deleterious effects in reproductive parameters in pubertal and adult male rats.

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Keywords: protein restriction, fetal programming, male progeny, reproductive parameters, rats.

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Systematic mapping and functional analysis of a family of human epididymal secretory sperm-binding proteins

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Introduction: The mammalian spermatozoon negotiates the female reproductive tract, binds to and penetrates the zona, fuses with and transfers genetic material to the oocyte. It acquires this potential during transit through the epididymis, which secretes proteins that coat different sperm membrane domains. **Aims** To generate a database of human epididymal proteins as a basis for understanding sperm maturation and fertilization, diagnosing male infertility and designing human post-testicular contraceptives. **Methods:** Epididymides from six accident victims (fathers, 27-32 y) were collected from an organ transplantation programme and semen was obtained from volunteering fathers (23-30 y). Epididymal luminal fluid was released by mincing and the supernatant centrifuged to produce sperm-free luminal fluid. Sperm-free tubule segments were ground in liquid nitrogen for the tissue fraction. Proteins were precipitated with acetone and electrophoresed on non-linear pH 3-10 strips and 12% SDS gels. Excised spots were digested in-gel and peptides were analyzed by a Voyager DE-STR biospectrometry workstation or a 4800 MALDI TOF/TOF Analyzer . Database searches for MS and MS/MS were performed with Mascot and GPS Explorer programs against the SWISS-PROT and NCBInr database for *Homo sapiens,* respectively. Primers for RT-PCR of the epididymal genes were based on GenBank sequences. Rabbit polyclonal antibodies were generated to 2-3 epitopes of recombinant proteins or synthetic peptides of non-structural proteins and anti-sera or affinity-purified IgG prepared. They were used for western blots, immuno-histo- and -cyto-chemistry. PBS-washed donor spermatozoa were fixed before incubation with primary antibodies and FITC-labelled secondary antibodies. **Results and Discussion:** 2D-PAGE revealed >1720 spots, 1508 identified by MALDI-TOF/MS. There were 745 tissue proteins (117 structural) and 408 fluid proteins. Antibodies were raised against 619 of these proteins. Most of the proteins were associated with epithelial cells but regional expression varied. RT-PCR confirmed regional differences in expression of gene transcripts. Epididymal proteins were found at 15 types of locations on spermatozoa. All identified epididymal proteins were given a unified terminology: there were 174 secreted sperm-binding proteins; 234 secreted non sperm-binding proteins; 33 non-secreted sperm-binding proteins; 162 non secreted non sperm-binding proteins. Molecular weights were under 100 kDa (most between 20 and 50 kDa); isoelectric points ranged from 4.5 to 9 and they were located on the autosomes and the X-chromosome. Functional proteomic database searches revealed that the sperm-binding proteins were related to metabolism (32%); immune defense, anti-oxidation and molecular chaperoning (8%); signal transduction proteins (19%). Some antibodies and recombinant proteins showed effects on sperm function. **Conclusions:** This proteomic analysis revealed novel human epididymal proteins that may influence sperm function.

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Keywords: human epididymis, proteome, database localization, function.

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Study of the bactericidal permeability increasing protein (BPI) in the epididymis and sperm of mouse

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Introduction: Phase-specified gene expression in testis and regional specific expression in epididymis are thought to be related to spermatogenesis and sperm maturation. Bactericidal permeability increasing protein (BPI) is a super endogenous cationic antibiotic against infection. It was isolated initially from human neutrophil azurophillic granules and can also be detected in specific granules of eosinophils and on the surface of polymorphonuclear leukocyte. It inhibits or kills gram-negative bacteria selectively via high affinity binding to the bacterial cell wall LPS and neutralizes the endotoxin. Recent studies showed that, the murine BPI ortholog is expressed mainly under resting conditions; these results in testis and epididymis suggest that BPI has a certain relationship with spermatogenesis or sperm maturation. **Aims:** To provide clues to further explore possible roles of BPI in reproductive activities, this work focused on the expression pattern of mouse BPI gene in testis and epididymis, especially its spatial and temporal expression pattern in postnatal testis and regional specificity in the epididymis. **Methods:** Real-Time PCR, Prokaryotic expression, affinity chromatography, polyclonal antibody production, immunofluorescence staining and immune electron microscopy were used in experiments. **Results and Discussion:** Real-Time PCR analysis showed that mouse BPI mRNA was highly expressed in testis and epididymis, but not in prostate, seminal vesicle and coagulation gland. In epididymis, BPI mRNA was only expressed in caput epididymal epithelium and has higher expression level throughout postnatal development compared with same period testis. The BPI was expressed in an age-dependent pattern in mouse testis and epididymis at the mRNA level which peaked on postnatal day 60, which suggested that the level of BPI mRNA could be androgen dependent. Polyclonal BPI antibody was raised after rabbits were immunized with recombinant BPI N terminal protein, which was expressed in *E. coli* and purified by affinity chromatography. Immunofluorescence staining with the anti-BPI-N antibody revealed that, in mouse, BPI protein could be detected in the caput epithelium closed to corpus as well as at the spermatozoa surface of acrosome region. The positive immunofluorescence signal of BPI protein was also distributed in the acrosome matrix after induced by calcium ionophore A23187. On the other hand, some spermatozoa showed negative signal after the acrosome reaction, which suggested that the BPI protein was released or degraded. **Conclusion:** Murine BPI protein may play multiple roles according to its distribution at different part of spermatozoa. There is a possibility that BPI covered at the sperm surface and existed in the acrosome, and therefore might be involved in sperm maturation or sperm-egg interaction during fertilization.

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Keywords: bactericidal permeability increasing protein (BPI), epididymis, spermatozoa, acrosome.

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CFTR affects segment-specific cell surface expression of E-cadherin in mouse epididymis

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Introduction: Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) cause cystic fibrosis (CF), a disease with clinical manifestations in many vital organs including infertility in both sexes. However, the detailed mechanism by which defective CFTR affects the male reproductive system, particularly the epididymis, remains elusive. **Aim:** The present study aimed to investigate the role of CFTR in regulating the blood-epididymis barrier by examining the segment-specific cell surface expression of E-cadherin, which is important for the maintenance of apical junction complexes and epithelial polarity, in CFTR+/+, +/- and -/- mice. **Methods:** Epididymal segments, including caput, corpus and cauda, from three CFTR genotypes mice were examined morphologically and for E-cadherin expression by immunofluorescence staining. **Results:** Morphologically, the diameter of the epididymal duct of CFTR-/- mouse was found to be the smallest among the three genotypes, with notable atrophy of the luminal walls, indicating abnormal epididymal differentiation. In the caput, corpus, and cauda epididymis, E-cadherin was found to exhibit a segment-specific expression profile, being more expressed in the corpus and cauda segments than the caput. While E-cadherin was found to be predominantly localized to the apical-lateral surface of CFTR+/+ epididymal epithelium, its expression levels and localization in CFTR-/+ and -/- epididymides were altered with less immunoreactive signals found at the apical surface domain but enhanced accumulation of E-cadherin in the cytoplasm, which may be caused by the disrupted formation of junctional complexes, of which E-cadherin is an integral part. **Discussion:** These results indicate that absence of or defective CFTR may affect the stability of cell surface E-cadherin expression and thereby affects the epithelial junctional assembly, disruption of which may affect the integrity of epididymal epithelium and the luminal microenvironment. The abnormality in the blood-epididymis barrier due to CFTR mutations may contribute to the epididymal atrophy and infertility in CF patients. **Conclusion:** In addition to its previously demonstrated role as a chloride channel in regulating the fluid environment of the epididymis, the present findings indicate another important role of CFTR in normal epididymal differentiation and the maintenance of the adhesion junction in the epididymal epithelium, which is essential to epididymal functions and sperm maturation.

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Keywords: CFTR, epididymis, E-cadherin, infertility and cystic fibrosis.

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