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I Workshop on Male Reproductive Biology*

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05-07 November 2007

* Scientific content, writing and style of the Abstracts were reviewed by the organizers.

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CONTENTS*

	Pages
Editorial	A4
Overviews	A5 – A12
Updates	A13 – A16
Poster Session	A17 – A70

*For searching author name, filiation or subject, use the search tool of your pdf software.

Editorial

Wilma De Grava Kempinas, Maria Christina Werneck Avellar

I Workshop on Male Reproductive Biology, 05-07 November 2007

The I Workshop on Male Reproductive Biology took place from November 5-7 2007 at the Institute of Biosciences of the São Paulo State University (UNESP) at Botucatu, São Paulo, Brazil, under the coordination of Dr. Maria Christina W. Avellar (Federal University of São Paulo, UNIFESP-EPM) and Dr. Wilma De Grava Kempinas (UNESP-Botucatu). During the event, basic, applied and advanced biotechnological topics in male reproductive biology, with emphasis on the molecular and cellular aspects of the testis and epididymis and sperm/oocyte interaction, were discussed. The event gathered 121 researchers - professional and also graduate and undergraduate students - active in the area of male reproductive biology in research centers and universities throughout the country. Of a total of 13 invited speakers, 2 came from the United States (Dr. Barry T. Hinton and Dr. James Tsuruta) and 1 from Argentina (Dr. Patrícia Cuasnicu). The international guests led the sessions called "Updates in Reproductive Biology", while the Brazilian speakers presented their research groups in the "Overview of Male Reproductive Biology in Brazil" sessions. An important outcome of the Workshop was that Brazilian scientists had the opportunity to discuss their research results and establish international cooperation. Besides other contributors, the event was funded by UNESP, UNIFESP, the State of São Paulo Research Foundation (FAPESP), the National Council for Scientific and Technological Development (CNPq), and the Coordinating Body for the Improvement of Postgraduate Studies in Higher Education (CAPES). The Workshop was also sponsored by the Fogarty International Center. As discussed in the closing session and based on the participants' positive evaluation expressed during and after the event, the next Workshop should happen at a date and place yet to be announced. This initiative was shown to be unique on the national scene on account of gathering researchers in the area of reproductive biology and attracting new talent for the scientific work and development of human resources for this research area in Brazil.

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Overviews

Reproduction Biology Group of Institute of Biosciences, Humanities and Exact Sciences of São Paulo State University – IBILCE/UNESP*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Taboga SR. Reproduction Biology group of Institute of Biosciences, Humanities and Exact Sciences of São Paulo State University-IBILCE/UNESP. *ARBS Annu Rev Biomed Sci* 2008;10:A6. Created and recognized by CNPq (Brazilian National Research and Development Council) in 1991, the Reproduction Biology Group – IBILCE/UNESP is coordinated by Drs Sebastião Taboga and Classius de Oliveira, and congregate different research areas of animal reproductive biology, such as: *Prostate Biology*, *Experimental spermatogenesis*, *Comparative Reproduction Biology* and *Reproductive Biology Genomic* respectively by supervision of Drs Sebastião Taboga, Rejane Góes, Classius de Oliveira and Maria Elizabete Jorge Amaral. In this presentation will be focused only about the *Prostate Biology*. This area is constituted by 3 effective research members, 3 Brazilian and 2 International collaborators, 3 post doctoral researchers, 5 graduate (4 PhD and 1 MSc) and 5 undergraduate (scientific initiation) students and 2 biologists promote the technical assistance for the research group. Our initial studies were realized using the Wistar rats as experimental model and evaluated the effects of castration on the ventral prostate. These studies revealed modifications in the elastic system (*Prostate* 1997;32:27-34), collagens (*Tissue Cell* 1997;29:163-70) and smooth muscle cells phenotypes to contractile for synthetic activities (*Tissue Cell* 1997;29:163-70; *Prostate* 2000;45:253-8; *Cell Biol Int* 2005;29:809-16). Using the same experimental design of surgical castration we recently described that matrix metalloproteinase MMP-7 is related with the remodeling of prostatic tissue during the regression process (*Cell Biol Int* 2007;31:1173-8). Employing a novel experimental model since 1999, the Mongolian gerbil (*Meriones unguiculatus*, Gerbilinae, Criscetidae) our laboratory have been develop several experimental systems for evaluating the effects of the surgical and chemical castration on the gerbil ventral prostate. The chemical castration employed at this moment include: flutamide (Góes *et al.*, *Micron* 38(3):231-6), cyproterone acetate (*Cell Biol Int* 2007;31:235-45), finasteride (*Differentiation* 2004;72:198-208). Several steroids therapies have been used too, such as 17-b-estradiol (*Int J Exp Pathol* 2007; *in press*), testosterone cypionate (*Cell Biol Int* 2007;31:235-45; *Anat Rec A* 2006 288:1190-200). Aging related alterations in the gerbil prostate have been described and the hormonal imbalance may be responsible for these alterations (*Anat Rec A* 2006;288:723-33; *Int J Exp Pathol* 2007; *in press*). A sub area in the rodent prostate biology studied in our laboratory is the presence of the functionally active prostate gland in the female. In the Mongolian gerbil, the female prostate was described at the structural and ultrastructural levels (*Cell Biol Int* 2004;28:335-44) and the comparison with the male one the studies were employed (*Tissue Cell* 2003;35:447-57). In addition, the testosterone therapy in the adult female promotes an increase of this gland (*Biol Reprod* 2006 75:370-9), and the aging promotes several prostatic disorders such in the male one (*Anat Rec A* 2007; *in press*). The gerbil male and female prostate has been an excellent model for the prostate biology studies due the peculiar anatomy of this gland complex in the male (*Anat Rec A* 2007;290:1233-47) and the plasticity of this gland during hormones therapy in the female (*Anim Reprod* 2006;3:3-18). Our current interest on this research area is to evaluate the morphofunctional differences in the female prostate during estrus cycle, including the studies on androgen and estrogen receptors, and the evaluation of the autochthonous and induced cancer in the aged male and female prostate.

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Prostate Cell Biology: Laboratory of Extracellular Matrix

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Carvalho HF. *Prostate Cell Biology: Laboratory of Extracellular Matrix. ARBS Annu Rev Biomed Sci* 2008;10:A7. The androgen dependency of the prostate for development, growth and maintenance of the differentiated state is remarkable. Strong evidences of this are the facts of almost absolute absence of the gland in AIS (androgen insensibility syndrome) patients, that eunucs never develop prostate cancer and the strong reduction in prostatic activity and weight loss after either chemical or surgical castration. As a matter of fact, castration is a prime measure to refrain prostatic cancer growth, with the inconvenient that androgen-independent recurrence is frequent. We have been interested in the gland remodeling after castration, focusing on stroma-epithelium interactions and stromal remodelling. We have early shown a remarkable behavior of the basement membrane after castration (*Cell Biol Int* 1996;20:809-19) and assumed these changes take place in an attempt to accommodate a progressively smaller number of epithelial cells. Next we have shown that collagen fibers are reorganized during the castration induced remodeling and that this is at least in part due to the activation of smooth muscle cells (*Prostate* 2000;45:253-8), which assumes a more synthetic function departing from a predominately contractile phenotype (*J Androl* 2004;25:50-6; *Cell Biol Int* 2005;29:809-16; *J Androl* 2007; 28:777-83). We have determined that simultaneous androgen deprivation and high concentration estrogen administration modifies the kinetics of cell death in the regressing prostate. These analyses have suggested that androgen deprivation and high estrogen triggers different apoptotic pathways, but resulted in the same amount of epithelial reduction by the seventh day after beginning of treatment (*Braz J Med Biol Res* 2005; 38:487-97). This led us to suggest that further reduction of the epithelium requires stromal remodelling which would depend on new roles attributed to the estromal cells as well as the remodelling of the extracellular matrix. To check this hypothesis, we have examined the expression of MMPs and heparanase-1 in the rat ventral prostate after castration and detected a peak of MMP-2, MMP-7 and MMP-9, and a concomitant increase in heparanase-1 by the 10th day after surgery. To check whether this increased metalloproteinase and glucosidase activity were associated with further loss of epithelial cells, we followed the rate of epithelial cell apoptosis up to the 14th day after castration. To our surprise, we have identified a peak of epithelial cell death, secondary to the classical peak found 3 days after castration. It became clear that this second peak is dependent on the activation of the MMPs and heparanase, which degrade the extracellular matrix, in special the basement membrane and induces anoikis and subsequent epithelial cell death. It might also be possible that cleavage of the extracellular matrix liberates cryptic molecules that induces cell death. The present series of results demonstrate that stromal-epithelium interactions are hallmarks of the post castration physiology.

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Laboratory of Biology and Toxicology of Reproduction and Development – ReproTox

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Kempinas W De G. Laboratory of Biology and Toxicology of Reproduction and Development – ReproTox. ARBS Annu Rev Biomed Sci 2008;10:A8. The studies carried out at the Laboratory ReproTox aim to investigate the effects of potentially toxic agents on the male and female reproductive tracts, on the fertility of the treated animals and the development of litters, using mostly rodent experimental models. The activities of the Laboratory ReproTox started in 1988 and, since then, projects were developed assessing the reproductive toxic effects of heavy metals, pesticides, plant extracts and drugs. We also focus on the role of the sympathetic innervation on the neuroendocrine control of the male reproductive tract, the effects of stress on sexual behavior and fertility of rats, and the effects of chemically-induced diabetes and high-calorie-diet-induced obesity on male reproduction. Techniques used for this include histopathological analysis of the reproductive organs, immunohistochemistry, *in utero* artificial insemination, radioimmunoassay for hormonal dosages, 2D electrophoresis, ELISA, sperm counts, morphology and motility. The effects of drugs during gestation are evaluated using classical techniques of experimental teratology. Working in the lab is a group of post-docs, as well as both graduate and undergraduate students. We maintain research collaboration with other universities in Brazil and with scientists of the Reproductive Toxicology Division, NHEERL – US EPA. Special attention is given to the epididymal biology and toxicology and the sperm maturation processes. Recent studies demonstrated that some chemicals exert deleterious effects on the epididymis, independent of the testis. In the past 10 years our lab has been involved in studying the influence of altered sperm transit time through the epididymis on sperm parameters and fertility of rats. The results have shown that accelerated sperm transit through the epididymis, as provoked by some endocrine disruptors, compromises sperm maturation, resulting in decreased sperm quality, including fertility. Toxicology of the epididymis is a relatively new facet of male reproductive toxicology. Research in this field is inherently difficult because to identify toxicant-induced alterations in the structure and function of the epididymis it is necessary to control experimentally for any confounding factors, such as testicular lesions produced by the agent. For this, unique *in vivo* and *in vitro* methodologies need to be developed and applied. There is evidence that sperm quality in humans and other animals has diminished, in parallel with increased adverse trends in male reproductive health. It is speculated that these events may be manifestations of changes in environmental influences that have presented over the past 50 years. It is reasonable to hypothesize that if semen quality is declining in men around the world, that this may be at least in part due to environmental factors that compromise the process of epididymal sperm maturation. Studies on the toxicology of the epididymis not only elucidate exposures that target this organ, but also foster a better understanding of the normal structure and function of the organ. This may benefit the treatment of certain idiopathic infertile men, as well as lead to the development of novel, nonhormonal male contraceptives that irreversibly target sperm maturation rather than the process of spermatogenesis. At present, we are interested in investigating the epididymis as a target organ for environmental chemicals during reproductive development. The studies mentioned above have been funded by national, state and institutional foundations, specifically CNPq, CAPES, FAPESP and FUNDUNESP, as research grants and scholarships for students.

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Laboratory of Cellular Biology*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

França LR de. Laboratory of Cellular Biology: ARBS Annu Rev Biomed Sci 2008;10:A9. The Laboratory of Cellular Biology is relatively young and was created in 1995. In the past ten years we have developed very productive scientific interactions with several research groups, mainly from the USA, Argentina, Japan, and Europe (particularly The Netherlands, France, Belgium, and Scotland). Our main research interest is related to the testis function and the male reproductive tract in mammals and fish. Specifically, we are investigating the following topics: 1) comparative spermatogenesis in mammals, including wild, domestic and laboratory species; 2) hormonal regulation of the testis and the regulation of Sertoli and Leydig cells proliferation; 3) testis function in teleost fish (mainly tilapia and zebrafish which are excellent experimental models); 4) toxicology of the male genital tract; and 5) germ cells transplantation in fish and mammals. More recently, we also have been studying the influence of leptin and mild caloric restriction on the male genital tract development and fertility, using rats as an experimental model. Concerning the studies related to the testis function in mammals we have a broader interest that includes: a) investigations of Sertoli and Leydig cells proliferation, mainly through cyclins, gonadotropins (FSH and LH), androgens and thyroid hormones; b) the establishment of puberty and sexual maturation; c) characterization of the seminiferous epithelium cycle and the duration of spermatogenesis; d) and Sertoli cells and spermatogenic efficiencies (daily sperm production per gram of the testis). Most of the aforementioned studies for mammals are also performed in fish that are, however, kept at different temperatures. Probably, the most important approach that we are currently developing is the germ cells transplantation, an excellent and fascinating research tool developed in 1994 by Brinster and colleagues. This technique has been utilized to investigate reproductive biology, mainly the aspects related to spermatogenesis and stem cell biology, offering also great potential for studies involving biotechnology, transgenic animals, and the preservation of genetic stocks of valuable animals or endangered species. Although germ cells transplantation is well characterized in mammals, such as mice and rats, there is no study utilizing this approach for fish or other lower vertebrates. In this regard, we are evaluating the suitability of adult tilapias as recipients for syngenic or xenogenic germ cell transplantation, and all necessary approaches for spermatogonial transplantation in fish were standardized recently in our laboratory. The results from tilapia-to-tilapia transplants showed the presence of transplanted germ cells developing up to sperm. Regarding xenogenic transplantation, ongoing studies in our laboratory are also showing that frog (*Rana catesbeiana*), tucunaré fish (*Cichla monoculus*), and rat germ cells are able to colonize and to develop in the tilapia testes. Therefore, tilapias represent an excellent experimental model to investigate the stem cell biology and the testis function in fish and other vertebrates. Moreover, this technique could be also utilized as a potential approach for fish bioengineering, and/or preservation of genetic stocks of endangered fish species or fish strains carrying commercially valuable traits.

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Laboratory of Biology of Reproduction

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Martinez FE. *Laboratory of Biology of Reproduction*. *ARBS Annu Rev Biomed Sci* 2008;10:A10. The objectives are to describe the structures and relations of the male and female genital system organs and the alterations of in their structures when treated with ethanol. The discussion dates from the beginning of the 1920s, when clinical gonadal atrophy in chronically alcoholic men with no experimental support was observed. In the 1970s, chronic alcoholics with severe reproductive dysfunctions in the absence of cirrhosis were recognized. From the 1980s and 90s, the literature describes ethanol (alcohol) consumption either as directly or indirectly effecting reproduction by decreasing circulating androgen levels. The group collaborated on the concomitant double action, directly and indirectly. Alcoholism changes the structure of prostate ventral lobe, coagulating gland, vesical transition epithelium, prostate lateral lobe, seminal vesicle, uterine tubes, endometrial epithelium, testis, epididymis, uterine horns, ovary, vas deferens, sex accessory gland ducts and others. There are similarities between ethanol alterations and stress on reproduction. Is there any reproductive response pattern associated with drugs (ethanol) and stress (maternal separation)? Conclusions: 87% of variables show positive and negative interactions; ethanol interacts with stress (maternal separation) through the same or similar action mechanism; 8% of variables show no interactions, while 8% are independent of treatment and 92% show interactions with ethanol or stress. **Key words:** Ultrastructural, prostate ventral lobe, experimental chronic alcoholism, coagulating gland, vesical transition epithelium, prostate lateral lobe, seminal vesicle, uterine tubes, endometrial epithelium, testis, epididymis, acrosome formation, uterine horns, spermatogenesis's kinetics, morphometric analysis, ovary, urethra, vas deferens, pelvic and penile urethra, sex accessory glands's ducts, spermatogenic cycle length, spermatogenic efficiency, castration, vasectomy, ductal system => rats, Mongolian gerbil and *Calomys callosus*. **Researchers:** Camila C. D. Almeida-Francia, Patrícia F. F. Pinheiro, Wilson de Mello Júnior, Sérgio Pereira (UNESP), Tânia M. Segatelli (UFMG), Valéria H. A. C. Quitete (UNICAMP) e Marcelo Martinez (UFScar). **Students:** Undergraduate: Caroline G. C. Toledo, Cristiane C. Centrone, Midyan D. Giastali, Edgar V. Oliveira. Graduate: Fabiana Marconsini, Suelen A. Oliveira, Luiz G. A. Chuffa, Rafael Kremer, Giovana R. Teixeira, João P. A. Amorim, Otávio Augusto Martins, Raquel Dominiconi.

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Laboratory of “Receptors and Transduction Mechanisms” and “Pharmacology of Reproduction”

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Pereira OCM. Laboratory of “Receptors and Transduction Mechanisms” and “Pharmacology of Reproduction”. *ARBS Annu Rev Biomed Sci* 2008;10:A11. The researches developed in our laboratory involve investigations of reproductive aspects and sexual behavior in response to perinatal and adulthood hormonal manipulations as consequence of the utilization of drugs, treatments, and adverse conducts that interfere with the reproductive hormones. Thus, the process of hypothalamic sexual differentiation and the long-term effects of these drugs/manipulations on reproductive physiology and sexual behavior are studied, with emphasis on endocrine disruptors and some other drugs that interfere with estrogen action in their receptors (*Annu Rev Biomed Sci* 2003;5: 87-94). In birds and mammals, the sex is determined for two distinct processes: Sexual Determination - during the embryonic development, following the sexual determination, the gonads, through discharge of little quantity of hormones of reproduction will guarantee the phenotype of male or female at birth, as well as will actuate in specific areas to permit the sexual differentiation of the central nervous system; and Sexual Differentiation - in mammals, before the sexual differentiation, the hypothalamus is organized as female type. The sexual differentiation begins with the genetic determination of the gonads, which, once completed, will determine the sex of the brain (*Brain Res Bull* 1997;4:487-95). In males the hypothalamus needs to be defeminized and masculinized to guarantee a normal reproductive function. This process depends on an abrupt discharge of testicular testosterone that occurs at perinatal period. However, it is not androgen *per se* that is responsible for masculinizing the brain. It is necessary to have the conversion of androgen to estrogen via cytochrome P450 aromatase. This enzyme is increased in the preoptic-hypothalamic area, in perinatal period. In this sense, exposure to testosterone or its metabolites during this period is critical for masculinization and defeminization of sexual behavior, the establishment of gonadotropin secretion patterns, and also for various morphological indices. In the absence of testosterone or its metabolites, sexually dimorphic structures and functions are feminized (*Dev Neurosci* 1997;19:430-7). Several experimental models in development in our laboratory like this, early lactation milk deprived pups, perinatal estrogen exposure, aromatase inhibitor in perinatal life, neonatal inhibition of the estrogen receptors, neonatal inhalatory anesthetic exposure, 5alpha-Redutase2 inhibition in perinatal life, neonatal androgenic supplementation, prenatal stress, and perinatal corticosteroid exposure have shown interferences in the hypothalamic sexual differentiation. In the course of brain differentiation, these conducts and/or exposure to some drugs in the perinatal period that apparently do not endanger the neonate, may lead to later side effects. Thus, reproductive function may be impaired by interferences in the process involved on estrogen exposure during the perinatal life. Then, reproductive function may be impaired by interferences in the process involved on estrogen exposure during the perinatal life of males. In conclusion, perinatal exposure to drugs which may be considered as endocrine disruptors, some treatments, and some adverse conducts may induce to an incomplete masculinization and defeminization of the central nervous system of the animals. Alterations in these processes, if present, generally are perceived only at puberty or adult reproductive life. These later alterations may include anomalies in the process of fertility and in sexual behavior or by adulthood, could provide interesting long-term development aspects as those observed in the skeletal musculature during physical fitness and in response to ergogenics.

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Avellar MCW, Lazari MFM, Porto CS. Section of Experimental Endocrinology, UNIFESP-EPM. ARBS Annu Rev Biomed Sci 2008;10:A12. The Section of Experimental Endocrinology, Universidade Federal de São Paulo, develops research on several aspects of male reproductive biology in different animal species: primates, rodents, bull and snakes. Molecular, pharmacological and immunohistochemical studies have been used to define the mechanisms by which hormones (neurotransmitters, androgens, estrogens, glucocorticoids, gonadotropins, relaxin and growth factors) control physiological aspects of the male reproductive tract, such as gametogenesis, gene expression and signal transduction. The role of the autonomic neurotransmission and associated receptors (α_1 -adrenoceptors and muscarinic acetylcholine receptor subtypes) and the influence of steroid hormones (androgens and estrogens) on these receptors have been assessed in testis, epididymis, seminal vesicle and prostate from rodents and primates. One branch of this research line has also focused on molecular and pharmacological aspects of α_{1A} -adrenoceptor splicing variants expressed in the male reproductive tract of primates but not in rodents. Comparative studies with gonadotropin receptors have also been conducted to help the understanding of structure-activity relationship. A snake FSH receptor with several interesting structural features has been cloned. Furthermore, studies on the function of relaxin in the rat male reproductive tract have also been conducted. cDNA microarrays have been used to assess the effects of estrogen and antiestrogen (fulvestrant) on gene expression in efferent ducts and epididymis. Recent research has also focused on the nongenomic effects of estrogen via the G protein-coupled receptor (GPR30) present in cultured rat Sertoli cells and tissues from rat male reproductive tract and its role in epidermal growth factor receptor transactivation. A systematic analysis of the impact of changes in glucocorticoid plasma levels on the histology, gene expression and host defense mechanisms in the rat epididymis has been also conducted. Host defense mechanisms in the male reproductive tract have become a subject of investigation due to a research collaborative program established between UNIFESP-EPM and The Laboratories for Reproductive Biology, University of North Carolina at Chapel Hill, USA, supported by Fogarty International Center. These studies have focused on the characterization and mechanisms of regulation of genes coding for antimicrobial peptides in the epididymis from primates, rodents and bull, as well as on innate immune mechanisms that protect the male reproductive tract from infection by pathogenic microorganisms, using rat as experimental model. The Section of Experimental Endocrinology is currently staffed by 3 Associate Professors, 1 post-doctoral fellow, two technicians, 7 graduate students and 4 undergraduate students. Research and fellowships are also supported by FAPESP, CAPES, CNPq and Fogarty International Center. The members of the group also maintain active scientific collaboration with other laboratories from Brazil and USA.

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Updates

Molecular Mechanisms Involved in Gamete Interaction

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Cuasnicu PS. Molecular Mechanisms Involved in Gamete Interaction. ARBS Annu Rev Biomed Sci 2008;10:A14. Mammalian sperm-egg interaction is a multistep process involving sperm penetration through the cumulus oophorus that surrounds the egg, binding to and penetration of the zona pellucida (ZP), and fusion between the sperm plasma membrane and the oolema. Although the structural and physiological aspects of gamete interaction have been extensively studied, during the past years efforts have been made towards elucidating the molecular mechanisms involved in this process. Evidence indicates that many of the fertilization events occur through the interaction of complementary molecules localized on the surface of both gametes. Biochemical and molecular approaches identified several molecules as mediators of gamete interaction, and the participation of few of them has been more recently confirmed through the use of KO models (i.e. SED-1 and ZP2/ZP3 for sperm-ZP interaction, and Izumo and CD9 for gamete fusion). The results from these genetic approaches also suggest that there must be other as yet unidentified components contributing to the fertilization process. For many years, our laboratory has been working on the functional role of protein DE, a sperm epididymal protein described by our group and also known as CRISP1 for being the first member of the Cysteine Rich Secretory Protein (CRISP) family. Evidence supports the involvement of DE/CRISP1 in both sperm-ZP interaction and gamete fusion through its binding to complementary sites on the ZP and egg surface, respectively. Recent results also support the participation of the testicular homologue of DE, known as Tpx-1 or CRISP2, in gamete interaction through its interaction with the same egg binding sites than DE/CRISP1. The results from KO mice for each of these proteins will provide important information for a better understanding of the molecular mechanisms involved in the fertilization process.

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Developmental Aspects and Gene Expression in the Epididymis

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Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Hinton BT. Developmental aspects and gene expression in the epididymis, Department of Cell Biology, University of Virginia School of Medicine. ARBS Annu Rev Biomed Sci 2008;10:A15. It is very clear that tubular morphogenesis is a fundamental process during the genesis of many important organs, including: brain, heart, kidney, lungs, intestine and the reproductive tracts of many species. The epididymis is certainly no exception in that the survival of mammalian species depends upon this organ being fully functional. Hence, the formation (tubulogenesis), elongation and subsequent folding (morphogenesis) of the Wolffian/epididymal duct must be highly coordinated with its function. At day E14 in the mouse the Wolffian duct is approximately 1mm and will grow to over 1000 times its length. This elongation coupled with its needs to grow within a defined space demands an extraordinary coordinated series of morphogenic events. We suspect that it is a combination of cell proliferation, cell shape changes, cell rearrangements, fluid secretion, apoptosis that could contribute to duct elongation and coiling. Recent studies suggest that several transcription factors play a key role during tubulogenesis including *Gata3*, *Pax2*, *Pax8*, *lim1*, *Emx2* and *hox* genes. Androgens are also critically important for Wolffian duct development and recent studies suggest that they act to stabilize and maintain the integrity of the Wolffian duct during development. Fundamental studies directed towards understanding the processes of Wolffian duct tubulogenesis and morphogenesis in mammals are needed. It has been well established that the functions of the adult epididymis are also highly dependent upon the presence of androgens. In addition, estrogens and testicular luminal fluid factors, for example, Fibroblast Growth Factors, regulate the functions of very specific regions of the epididymis. Studies, especially the use of gene arrays, have focused on understanding the regulation of genes that are expressed throughout the epididymis in the hope of identifying specific genes that are important for epididymal function and thus male fertility. Alternative approaches to identify important genes include the generation of gene knock out animals as well as forward screening approaches. For example, using a gene knock out strategy, the orphan tyrosine kinase receptor *Ros1* was discovered to play a key role in male fertility. Another approach to understand the role of specific genes in the epididymis has been to use *in vivo* electroporation. This approach utilizes a technique that allows for the transfer of plasmids, RNAi and morpholinos, for example, into very specific sites along the epididymal duct. A plasmid is microinjected into the epididymal interstitium or lumen of a defined region, then a pair of tweezer-type electrodes are positioned over the injection site and the plasmid electroporated. Our laboratory has electroporated several plasmids including a dominant negative construct of a member of the PEA3 transcription factor family into the initial segment of the rat epididymis to identify downstream targets of the transcription factor. We have also used this technique to identify downstream signaling events following *in vivo* electroporation of dominant negative constructs of growth factor receptors. This technique may also prove useful in rapidly identifying genes that play a role in male fertility and thus identify putative targets for the development of a male contraceptive.

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Spermatogenesis and Gene Expression in the Testis

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Tsuruta JK. Spermatogenesis and gene expression in the testis, The Laboratories for Reproductive Biology, University of North Carolina at Chapel Hill. ARBS Annu Rev Biomed Sci 2008;10:A16. The testis provides a unique environment for the mitotic proliferation of spermatogonia, the meiotic recombination of DNA in spermatocytes and the differentiation of the haploid spermatid. The structure of the testis and the organization of the seminiferous epithelium by tight junctions impact spermatogenesis in part by the formation of distinct compartments: the interstitium between seminiferous tubules which contains testosterone-secreting Leydig cells, the basal compartment of the seminiferous tubule where spermatogonial proliferation occurs and the adluminal compartment of the tubule where germ cells undergo meiosis and differentiation. This theme of compartmentalization can even be extended longitudinally down the length of each seminiferous tubule where distinct cohorts of germ cells have their transit through spermatogenesis precisely controlled; this kinetic control results in a repeating pattern of fourteen different associations of germ cells or "stages of spermatogenesis" in the rat (twelve in the mouse). The Sertoli cell is the somatic cell that spans the basal and adluminal compartments of the seminiferous epithelium. This gives the Sertoli cell the ability to regulate the environment within these two compartments along the length of the tubule, providing it an opportunity to regulate spermatogenesis. Many techniques are available to study spermatogenesis but particular emphasis will be placed on those that facilitate the study of paracrine interactions between Sertoli cells and germ cells. Examples will be given illustrating the use of Sertoli cell cultures, isolation of purified germ cells, co-culture of Sertoli and germ cells, synchronized testes and the culture of seminiferous tubule fragments. The relative merits of Northern analyses, RNase protection assays, targeted cDNA/oligo macro arrays, gene chips, *in situ* hybridization and real-time RT-PCR will be presented as they relate to spermatogenesis. Our recent studies with Sertoli cells and vitamin A-deficient testes will be used to illustrate specific considerations for experimental design and data analyses when using real-time RT-PCR to study the regulation of gene expression during spermatogenesis.

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Poster Session

3 β -diol, an Estrogenic Metabolite of Dihydrotestosterone, Modulates the Expression of Estrogen (er β) and Androgen Receptors in the Rat Ventral Prostate*

Fernanda Dettori Guedes¹, Polyana Helena Coelho¹, André Gustavo de Oliveira¹, Germán Arturo Bohórquez Mahecha¹, Rex Allen Hess², Cleida Aparecida de Oliveira^{1†}

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Abstract

Guedes FD, Coelho PH, Oliveira AG, Mahecha GAB, Hess RA, Oliveira CA. 3 β -diol, an Estrogenic Metabolite of Dihydrotestosterone, Modulates the Expression of Estrogen (er β) and Androgen Receptors in the Rat Ventral Prostate. *ARBS Annu Rev Biomed Sci* 2008;10:A18. 3 β -diol (5 α -androstane-3 β -17 β -diol) is a metabolite of the potent androgen dihydrotestosterone (DHT), which is found in high concentration in the prostate gland. The conversion of DHT to 3 β -diol is mediated by 3 β -hydroxysteroid dehydrogenase (3 β -HSD), an enzyme also highly found in the prostatic tissue. There is a growing body of evidence that 3 β -diol is not an inert metabolite of DHT, but an active hormone. Differing from DHT, the 3 β -diol does not bind to androgen receptors (AR), but rather to estrogen receptors ER β . It is noteworthy that the prostate concentration of 3 β -diol (10pmol/g) is 100-fold higher than estradiol, thus indicating that it may be the major local ligand of ER β . ER β has been implicated in several biological functions in the prostate, including cell proliferation, apoptosis and differentiation. Most importantly, it has been shown that these ER β effects play important role in protecting the prostate against abnormal growth. Despite the evidence showing a close relationship between ER β and prostate physiology and pathologies, the mechanism of local ER β modulation is still a matter of debate. Moreover, studies related to physiological effects of 3 β -diol/ER β complex are still scarce. Based on the high concentration of 3 β -diol and considering that its receptor ER β is widely expressed in the prostate, we hypothesized that 3 β -diol would be involved in local functions, such as modulation of ER β expression. To test this hypothesis is the primary aim of the present study. For comparison, the effects of the 3 β -diol on AR modulation was also investigated. Adult male rats were submitted to castration followed by replacement with estradiol (400mg/day), DHT (5mg/day) or 3 β -diol (3mg/day). For the investigation of ER β and AR protein levels, ventral prostates were frozen in liquid nitrogen or fixed in neutral buffer formalin to perform Western blotting or immunohistochemistry assays, respectively. Both immunohistochemical and Western blotting analyses showed that after bilateral castration ER β and AR protein levels were decreased. DHT was the most effective inducer of AR expression whereas it induced just partial recovery of ER β . Conversely, replacement with 3 β -diol induced the highest levels of ER β , but was less effective in recovering the AR expression and the gland structure. Estradiol had just minor effects on the receptors expression. The results offer evidence that one functional role of 3 β -diol in the prostate may be autoregulation of its natural receptor, ER β .

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Analysis of the Y Chromosome and MtDNA in Bovines from National Breeds for Identifying the Animals Origin

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Abstract

Issa EC, Jorge W, Sereno JRB, Egito AA. Analysis of the Y Chromosome and MtDNA in Bovines from National Breeds for Identifying the Animals Origin. *ARBS Annu Rev Biomed Sci* 2008;10:A19. The keen interest regarding the Y chromosome in the reproductive biology of bovine males is due to the fact it locates the possible factor that causes infertility or genes that when absent may lead the animal to azoospermia, as in humans. Little is known about the relation of Y chromosome and the bovine fertility. Factors that control the spermatogenesis are in the region Yq11.23, known as AZF (Azoospermia Factor). Currently, it is known the existence of, at least, three regions of AZF – all located at the long arm of Y chromosome – whose micro-deletions are responsible to the absence of: (a) Sértori's cells (AZFa); (b) spermatogenesis (AZFb); and (c) post-meiotic maturation process (AZFc). According to DIMEO et al (2005), the SRY (the sex determining region in the Y chromosome) is located at the distal portion of the long arm of Y chromosome in taurines and at the distal portion of the short arm of Y chromosome in zebu cattle. Oppositely, the infertility in bovines is not related only with deletions in Y, but also with numeric and structural mutations as the 1/29 Robertsonian translocation. The current bovine breeds belong to two groups: the group that presents members with hump and abundant dewlap (*Bos taurus indicus*), and acrocentric Y chromosome; and the group which members do not present hump or neither abundant dewlap, but present meta or submetacentric Y chromosome. Thus, the existence of those two morphological presentations of Y allow us to verify if a certain animal or breed, which is under suspicion of being resultant of inter-crossing between those two subspecies, present taurines or zebuines paternity. Moreover, the mitochondrial DNA (mtDNA), besides influencing the productive characteristics of bovines, has also been used for detecting the maternal origin of the animals. The mtDNA polymorphism allows the demonstration of the participation of taurus or zebu females in the formation of the breeds. In this stage of the work, the objective is to determine, within national bovine breeds, the paternal origin (Y chromosome) and maternal origin (mtDNA). Lymphocyte cultures were performed for analysing the Y chromosomes of the following breeds: Pantaneiro (12), Curraleiro (6), Crioulo Lageano (2), and Junqueira (4), all provided by EMBRAPA Pantanal (Corumbá) and CENARGEN (Brasília). The mtDNA of the twelve animals of Pantaneiro breed was also analysed. The results have demonstrated that Pantaneiro and Curraleiro have presented both forms of Y, indicating that the paternal participation of *Bos taurus indicus* in the formation of some animals, therefore, attesting the crossing with zebus in the formation of Pantaneiro. These ones have presented mtDNA of *Bos taurus taurus*, therefore, indicating that the participation of zebus, in this breed, was paternal, entirely. The small sample analysed from the other breeds has not allowed any conclusion.

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Aquaporin-9 is Differently Expressed in the Dog and Mongolian Gerbil Epididymis

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Abstract

Domeniconi RF, Orsi AM, Justulin Jr LA, Beu CCL, Felisbino SL. *Aquaporin-9 is Differently Expressed in the Dog and Mongolian Gerbil Epididymis*. *ARBS Annu Rev Biomed Sci* 2008;10:A20. Recent studies have identified proteins called aquaporins (AQP) related to the fast water permeability in some biological membranes. AQPs are small, intrinsic membrane proteins that are present in many cell types involved in fluid transport (*Am J Physiol Renal Physiol* 200;278:F13). AQPs had been identified in the male reproductive tract, being their localization species-specific and region-specific. In view of the importance of the luminal fluid to sperm maturation and integrity of the spermatozoa, it is important to study the distribution of the AQPs throughout the spermatogenic way. The aim of this study was to examine the expression of AQP9 in epithelial cells in the gerbil and dog epididymis, comparatively. Samples from adult mongrel dog and Mongolian gerbil male reproductive tracts comprising fragments of the initial segment, caput, corpus and cauda of the epididymis were obtained. Immunohistochemistry procedures were used to show AQP9 localization and distribution. AQP9 reactivity was less intense in the dog caput and increased in the corpus and cauda epididymis, showing a pattern similar to that described for rats (*Biol Reprod* 2001;65:384) and humans (*Am J Physiol* 1999;277:F685). In gerbil, the reactivity was similar to those observed for dog, however a strong reaction in the caput epididymis was also observed. AQP9 was absent in the clear cells of the gerbil epididymis cauda promoting a discontinuous reaction along the epithelial apical brush border, as described for other authors for rat and human (*Biol Reprod* 2006;74:427). In this study, clear cells were not observed in dog cauda epididymis in agreement with related by Chandler et al. (1981). The results confirm the presence of AQP9 in gerbil and dog principal cells in different regions of epididymis. Thus the AQPs are species-specific and region-specific, suggesting activity variations related with the fluid and solute absorption throughout male excurrent ducts. Investigations of AQP biology could be relevant to clinical studies of the male reproductive tract, as well as to technologies for assisted procreation.

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Cadmium in Low Doses Increases Cell Proliferation and The Incidence of Neoplastic Lesions in Rat Ventral Prostate*

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Abstract

Lacorte LM, Delella FK; Justulin-Junior LA; Almeida AA; Godinho AF; Amorin RL; Felisbino SL. *Cadmium in Low Doses Increases Cell Proliferation and the Incidence of Neoplastic Lesions in Rat Ventral Prostate. ARBS Annu Rev Biomed Sci* 2008;10:A21. The benign prostatic hyperplasia and the adenocarcinomas are the two main prostatic lesions that appear in men after the fifth years old. However, besides the androgenic hormones unbalance, other important causes, such as chemical agents exposure, arise as important factors in the lesions etiology and in the prostate carcinogenesis. Cadmium, an environmental contaminant, present in the cigarette smoke, has been described as prostate carcinogen in higher doses. Investigated if the exposure of a low dose of cadmium in an important phase of prostatic growth (puberty) could induce changes in the proliferation of adult epithelial cells and than be related with the appearing of neoplastic lesions in this gland. Male Wistar rats (2-months-old) (n=15), received by drink water a 10 ppm dose of cadmium (CD). Another group of animals (n=15), control group (CT), received filtered water. After 30 days, the animals were sacrificed by overdose of sodic pentobarbital. The bloody plasma and the ventral lobes pairs from each animal were collected. The lobes were weighted and, randomly, the right and left lobes were separated for espectrofotometry analysis of atomic absorption, to determine the quantity of cadmium present in the prostates of the CD and CT groups or for histological process of Paraplast embedding. Paraplast sections were submitted to Hematoxylin-Eosin (H&E) coloration for view general the morphology and to the immunohistochemistry for the Proliferating cellular nuclear antigen (PCNA) to determine the cellular proliferation index. The neoplastic lesions incidence was determined by a Pathologist (RLA). There were no significant differences between CT and Cd animals and prostate weights; The bloody concentration of cadmium in CD were significantly higher than in the CT (17.0 ± 0.58 ug/l and 0.0, respectively). The concentration of cadmium in the CD prostates were 2 times higher than the CT (0.12 ± 0.009 mg/g and 0.06 ± 0.007 mg/g, respectively). The cellular proliferation index from CD prostates was significantly higher than CT prostates epithelial cells ($0.62\% \pm 0.49$ and $0.19\% \pm 0.22$ respectively). The incidence of the neoplastic lesions in the CD was 5 times higher than the CT (100% X 20%, respectively), in which prostatic intra-epithelial neoplasia of low and high grade were the most frequents. Our results suggest that cadmium, even in low doses, could accumulate in the prostate, to increase the proliferation of epithelial cells and can represent an important initiator/promoter of prostatic lesions.

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Can *Heteropterys aphrodisiaca* Infusion Protect Rat Testes from Ciclosporine Induced Damages?*

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Abstract

Monteiro JC, Predes FS, Matta SLP, Dolder H. Can *Heteropterys aphrodisiaca* Infusion Protect Rat Testes from Ciclosporine Induced Damages? ARBS Annu Rev Biomed Sci 2008;10:A22. Cyclosporine A (CsA) has powerful immunosuppressive properties and is widely used in organ transplant therapy, improving graft survival rates, and in the treatment of some auto-immune diseases. However, it has a number of undesirable side effects, such as decrease in serum and testicular testosterone levels, as well as damage to testicular citoarchitecture. Stimulant and aphrodisiac properties have been attributed to the plant, *Heteropterys aphrodisiaca*, known as “nó-de-cachorro”. Data from the literature suggest that the root extract can increase body and testicular weight, the diameter of seminiferous tubules and individual and total volume of Leydig cells in rat testis. The present work was undertaken to study the association of the CsA and the medicinal herb in adult Wistar rats, evaluating the testis ultrastructure. Twenty-four animals divided into four groups were used: I-control (sham); II- CsA; III- CsA plus *H. aphrodisiaca* infusion; IV- *H. aphrodisiaca* infusion. CsA was administered at a dose of 15 mg/kg/day and *H. aphrodisiaca* at a dose of 0.5 ml of the infusion, prepared with 25 g of roots/100 ml boiling water. The treatments were administered daily by oral gavage, during 56 days. Rats were anesthetized and perfusion-fixed. The testis were removed and weighed, then processed for electron microscopy using standard techniques. In the CsA-treated rats, the Sertoli cell showed numerous cytoplasmatic vacuoles and abundant lipid droplets. Germ cells appeared degenerated, the round spermatids exhibiting nuclei with expanded pouches and vacuoles apparently due to swelling of the endoplasmic reticulum. Late spermatids were deformed and exhibited irregular acrosomes, while a large accumulation of residual cytoplasm released by spermatids was observed in the apical part of the seminiferous epithelium. Although the Leydig cell organelle reduction was observed, analysis of steroid dependent sexual organs suggests continued steroid synthesis. *H. aphrodisiaca* caused loss of germ cell attachment and expanded intercellular spaces between these cells and Leydig cells with an increased quantity of mitochondria and smooth endoplasmic reticulum. The modifications found for the testis in the simultaneous treatment with *H. aphrodisiaca* and CsA were a combination of the alterations identified for each individual treatment, however, germ cell degeneration and Leydig cell organelle reduction, as in the CsA-treated rats, were not found in these animals. The administration of *H. aphrodisiaca* infusion to CsA-treated rats diminished nearly all the CsA-induced damage in the ultrastructure of the testis. This suggests that *H. aphrodisiaca* infusion may be used combined with CsA to reduce CsA-induced testis injuries.

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Characterization of Cellular and Regional Distribution of Components of Ubiquitin-Proteasomal System Along the Rat Efferent Ductules and Initial Segment of the Epididymis*

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Abstract

Victor-Costa AB, Hess RA, Oliveira CA. Characterization of Cellular and Regional Distribution of Components of Ubiquitin-Proteasomal System Along the Rat Efferent Ductules and Initial Segment of the Epididymis. *ARBS Annu Rev Biomed Sci* 2008;10:A23. Intracellular protein degradation in eukaryotic cells occur through two main routes: lysosomal and proteasomal pathway. The lysosomal system is primarily involved in degrading extracellular and transmembrane proteins that are taken up by endocytosis. Conversely, the 26S proteasome complex is implicated in degradation of intracellular misfolded or short-lived proteins, which are targeted for degradation after labeling by ubiquitin. Among the targets for proteasomal degradation are the steroid receptors, such as estrogen receptor ER α . We have previously found that in the efferent ductules (ED), a segment that expresses the highest concentration of estrogen and ER α in the male tract, the ER α expression was reduced by the antiestrogen ICI182,780 and by exogenous estrogen. This *in vivo* effect occurred simultaneously with a transient increase in the amount of lysosome, raising the possibility that these organelles could be involved in the ER α degradation. Nevertheless, several *in vitro* cell models have shown that, instead of lysosomes, the ligand-induced degradation of ER α occurs through the ubiquitin-proteasomal pathway. Except for the testis, information related to the occurrence of components of the ubiquitin-proteasomal system in the male genital tract is still lacking. Considering the essential role of the ED in the maintenance of male fertility, this information becomes important to clarify the mechanism governing the local ER α modulation. The purpose of this study was to investigate the expression and cellular distribution of proteasome and ubiquitin in the rat ED. Initial segment of the epididymis (IS) was also studied for comparison. Fragments of ED and IS of adult Sprague-Dawley rats were processed for immunohistochemical detection of proteasome 20S and ubiquitin. The intensity of immunoreaction was estimated by computer assisted image analysis. Proteasome and ubiquitin were strongly expressed in the nuclei of nonciliated cells in the ED epithelium, whereas ciliated cells were moderately stained or negative for both components. Positivity for proteasome was also detected in the proximal region of the ED cilia. A gradient of proteasome and ubiquitin staining was seen in the ED epithelium, which decreased from the proximal to terminal region. The levels of proteasome and ubiquitin in the ED terminus were 32% and 41% reduced compared to the proximal ductules, respectively. Positive staining for proteasome and ubiquitin were also detected in the epithelium and connective tissue of the IS. In the epithelium there was moderate positivity for both components in the nuclei and cytoplasm of principal cells. Conversely, the basal, apical and some narrow cells showed intense nuclear staining. Slight staining for proteasome in the apical cytoplasm was also found in the apical cells. Distinct cellular, subcellular and regional distribution of components of the ubiquitin-proteasomal pathway in the epithelial cells along the ED and IS suggests differences in proteolytic activity possibly reflecting specific role in each segment.

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Characterization of Estrogen Receptors (ER α , ER β), Vitamin D3 Receptor and Androgen Receptor Expression in the Testis and Epididymal Region of Roosters Affected by the Epididymal Lithiasis*

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Abstract

Oliveira AG, Dornas RAP, Mahecha GAB, Oliveira CA. Characterization of Estrogen Receptors (ER α , ER β), Vitamin D3 Receptor and Androgen Receptor Expression in the Testis and Epididymal Region of Roosters Affected by the Epididymal Lithiasis. *ARBS Annu Rev Biomed Sci* 2008;10:A24. Epididymal lithiasis is a dysfunction characterized by the formation of calcium-rich stones in the epididymal region of roosters, associated with decreased serum testosterone and loss of fertility. The segment most affected by the lithiasis is the efferent ductules (ED), which, in birds, are responsible for the reabsorption of significant amount of calcium, in a process that follows the reabsorption of luminal fluid. Therefore, we hypothesized that the lithiasis could result from local impairment in the calcium and/or fluid homeostasis, culminating in the luminal stone nucleation. It is known that ED fluid reabsorption is modulated by estrogen. On the other hand, transepithelial calcium transport depends on Vitamin D3 and its receptor VDR, but estrogens and estrogen receptors (ER α and ER β) also take place. All these receptors are found in the ED of roosters, thus raising the possibility that they may be involved in the mechanism of calcium stone formation. This study aims to investigate possible changes in the pattern of ER α , ER β and VDR in the testis and epididymal region of roosters affected by epididymal lithiasis. To evaluate the potential impact of testosterone reduction, change in the expression of androgen receptor (AR) was also investigated. Fragments of the testis and epididymal region of adult roosters, affected and non-affected by the lithiasis, were used for immunohistochemistry, Western Blotting and morphometrical studies. There were no changes in the expression of AR, ER β and VDR in the testis of the affected animals compared to non-affected. The proportion of seminiferous tubules, interstitial tissue and Sertoli cells were also similar when animals of both groups were compared. However, in affected animals there was an increase of 3 fold in the proportion of Leydig cells. The epididymal region of roosters consisted of the rete testis, proximal and distal ED, connecting and epididymal ducts (EP). Western blotting analysis showed that ER β , VDR and AR expression was increased in the epididymal region of affected roosters, whereas there was no difference in ER α levels. The immunohistochemical studies revealed that the ER β immunoreaction was increased in all segments composing the epididymal region of affected animals, whereas AR was increased only in the EP and VDR was slightly increased in the distal ED. It was noteworthy the increase in VDR-positive cells in the stromal tissue of affected animals, especially in the mononuclear cell infiltrates commonly found close to the ED. ER β , VDR and AR protein levels were altered in specific segments of the epididymal region of roosters affected by epididymal lithiasis, suggesting that these hormones may be involved in the development/progression of the disease.

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Comparative Distribution of Androgen (AR) and Estrogen Receptors ER α and ER β in the Testis and Male Excurrent Ducts of Big Fruit-Eating Bat *Artibeus lituratus**

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Department of Morphology, Federal University of Minas Gerais, MG, BRAZIL

I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Oliveira RL, Oliveira AG, Nogueira JC, Mahecha GAB, Oliveira CA. Comparative Distribution of Androgen (AR) and Estrogen Receptors ER α and ER β in the Testis and Male Excurrent Ducts of Big Fruit-Eating Bat *Artibeus lituratus*. ARBS Annu Rev Biomed Sci 2008;10:A25. Androgens play important role in the maintenance of male fertility, its actions being mediated by androgen receptors (AR). However, the aromatization of androgens to produce estrogens is an alternative pathway for the modulation of male reproductive functions. The biological actions of estrogens are mediated by estrogen receptors ER α and ER β . Despite the recognized importance of estrogen in the male reproduction, studies related to ERs distribution are restricted to rodents, domestic animals and a few primate species, including man. According to these studies, ERs present variable distribution in the male reproductive tract depending on the species considered. In general, ER α expression is restricted to testicular Leydig cells and efferent ductules epithelium, whereas ER β are more widely distributed in the testis, excurrent ducts and accessory sexual glands. Nevertheless, studies regarding the distribution of ERs and even AR in the male genital tract of wild animals are restricted to few species, and data addressing this issue in Chiroptera is still missing. Chiroptera shows a number of reproductive peculiarities, including higher affinity of the sex steroid binding protein (SBP) for estrogen than androgen, which differs from most mammals and points out that Chiroptera species may be a good model for investigating the role of estrogen in male. In this study, we investigated the expression and cellular distribution of ER α , ER β and AR in the testis, efferent ductules (ED) and epididymis (EP) of the big fruit-eating bat *Artibeus lituratus*, a species largely distributed in South America and which may act as important reservoir of rabies virus. Fragments of testis associated with the ED and EP were fixed in neutral buffered formalin, embedded in paraffin and used for immunohistochemical localization of ER α , ER β and AR. ER α immunoexpression was found limited to the nuclei of non-ciliated cells of the efferent ductule epithelia of *A. lituratus*. ER β was expressed in the Leydig cells, myoid cells, Sertoli cells and some germ cells within the testis, as well as in the epithelial cells, peritubular cells and some connective cells of the efferent ductules and epididymis. Strong positivity for AR was found in the Sertoli cells, apparently in a stage-dependent pattern, whereas a slight reaction was seen in Leydig and myoid cells. In the ED, the AR immunoreaction was more intense in the nuclei of non-ciliated than in ciliated cells, whereas in the epididymis the corpus region showed stronger staining than the other segments. The cell distribution of AR and ER β presently shown was in agreement with the pattern found for most mammalian species, whereas the ER α distribution was in close similarity to that found for primates, including man, but differed from rodents and most domestic species. There was differential expression of ER α and ER β along the *A. lituratus* male genital tract. The efferent ductules were unique in expressing both subtypes of ER, whereas only ER β appeared to be target for estrogen action in the other segments.

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Comparison of Morphophysiological Aspects of the Rat Male and Female Prostate*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Taboga SR, Biancardi MF, Góes RM, Vilamaior PSL, Santos FCA. *Comparison of Morphophysiological Aspects of the Rat Male and Female Prostate*. ARBS Annu Rev Biomed Sci 2008;10:A26. The prostate is not a gland exclusive to the male reproductive system since it is also found in females of several mammals. During the male and female prostatic morphogenesis, the formation of prostatic buds is a constitutive process, but the branching and outgrowth of these buds is regulated by androgens. In female rats, the prostate morphology is different from that observed for human and gerbils, and little is known about the factors that regulate their physiology. This study compares the morphological patterns of the ventral prostate of male and female Wistar rats. Sixty males aging 1-12 weeks and 10 adult females (12 weeks) were employed. Five females received injections of testosterone cypionate (T - 1mg/Kg/every 48 hours) up to 7 days. The male and female prostates were processed for light microscopy. The total blood of them was collected for testosterone serologic analyses. Sections were stained by haematoxylin-eosin, subjected to immunohistochemistry for detection of androgen receptor (AR), and submitted to stereological analyses. In the control females and young males, the epithelial cells shift from cuboidal to columnar and this compartment presents epithelial budding regions. The luminal compartment was absent or little developed. In treated females and adult males, the secretory epithelium became more developed, showing a great Golgi region. The stereological data indicate that the androgenic stimuli caused statistically significant changes ($p < 0.05$) in the proportion of tissue compartments of the female prostate, in a dynamic similar to that observed during the male prostate postnatal development. In the treated females, there was an increase in the epithelium (control $16.2\% \pm 0.7\%$; treated $37.4\% \pm 1.8\%$) and lumen proportion (control $1.5\% \pm 0.2\%$; treated $40.4\% \pm 2.3\%$), with a consequent decrease in the stroma percentage (control $82.3\% \pm 0.5\%$; treated $22.2\% \pm 0.9\%$). Testosterone serum dosage indicated that the control female hormone levels are similar to those observed in young males (control females 0.3 ± 0.05 ng/mL; 2 week males 0.4 ± 0.05 ng/mL), and that testosterone levels of the treated females are equivalent to the adult male (treated females 4.9 ± 2.2 ng/mL; 12 weeks males 3.9 ± 1.2 ng/mL). Strong immunohistochemical staining by AR were observed in prostate epithelial cells of control female and young male rat, and in the stromal and epithelial cells of the adult male and female rats treated by T. These results indicate that the Wistar adult females have a prostate similar to the young male rats. However, in the androgens presence, this gland presents similar secretory capacity to that observed in adult male rats. In the male organism, androgens are related to the differentiation, growth and maintenance of prostate secretory activity. Thus, these data suggest that androgens play a similar role in the rat female prostate.

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Differential Effects of Estrogenic Metabolites on the Expression of Androgen Receptor in the Rat Efferent Ductules and Initial Segment of the Epididymis*

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Abstract

Picciarelli-Lima P, Oliveira AG, Mahecha GAB, Oliveira CA. *Differential Effects of Estrogenic Metabolites on the Expression of Androgen Receptor in the Rat Efferent Ductules and Initial Segment of the Epididymis. ARBS Annu Rev Biomed Sci 2008;10:A27.* Testosterone is an androgen that can act either as hormone or as hormone precursor, which can be converted to dihydrotestosterone (DHT) and estradiol (E2). Both testosterone metabolites play important role in the maintenance of the male tract morphophysiology. Nevertheless, it is now known that DHT can be further metabolized to 3 β -diol (5 α -androstane-3 β -17 β -diol), an estrogenic compound which does not bind to the androgen receptor (AR) but rather to estrogen receptors, especially ER α . Despite the wide expression of ER α in the male genital tract, few are known about its biological function. Data revealing that 3 β -diol is an alternative ligand for ERs raised the possibility that estradiol may not be the only estrogenic molecule to play a role in the male tract. Indeed we recently found that, in the efferent ductules (ED), 3 α -diol was equal to E2 in its ability to maintain the expression of the water channel aquaporin-9 (*Reprod Biol Endocrinol 2006;4:51*). These results prompted us to compare the effects of these estrogenic metabolites in other key protein involved in the maintenance of the morphophysiology of the male genital tract. The present study aim to extend the investigation about possible role of 3 α -diol and estradiol in the rat male tract by comparing their effects on the androgen receptor expression in the ED as well as in the initial segment of the epididymis (IS). The ED and IS were chosen because both express high levels of ER α , whereas ER α is assumed to be expressed in the ED but not in the rat IS. Bilateral castration and hormonal replacement with 3 α -diol (3mg), estradiol (400 μ g), DHT (5mg) or corn oil (control), was performed in adult male Wistar rats. The AR expression in the ED and IS was investigated by immunohistochemistry followed by a quantitative estimation of the immunoreaction, using computer-assisted image analysis. Positive nuclei were traced and the mean pixel intensity was determined for traced areas, subtracting the background values. AR expression was detected in the nuclei of ciliated and nonciliated cells of the ED as well as epithelial cells of the IS. Immunoreaction for AR was stronger in the IS than in the ED epithelium. Stromal cells of both segments were also AR-positive. Castration caused reduction in the AR levels in both segments analyzed. Estradiol replacement was able to increase the expression of AR in the IS but had no effects on the ED. On the other hand, 3 α -diol as well as DHT was able to recover the AR expression in the ED and IS. The differential effects of estradiol and 3 α -diol on the AR expression in the ED and IS reaffirmed the assumption that, by binding ER α , the 3 α -diol may serve as an alternative estrogenic molecule playing a role in the male reproduction.

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Differential Effects of Fulvestrant and Anastrozole on Gene Expression in Efferent Ductules of the Rat*

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Abstract

Gomes GRO, Yasuhara F, Siu ER, Porto CS, Lazari MFM. *Differential Effects of Fulvestrant and Anastrozole on Gene Expression in Efferent Ductules of the Rat*. ARBS Annu Rev Biomed Sci 2008;10:A28. Estrogen plays an essential role in male fertility. Mice that lack the aromatase gene have a defect in the germ cells that causes infertility, and mice that lack the estrogen receptor ER α are infertile because of impaired fluid absorption in the efferent ductules. Chronic treatment with the steroidal antiestrogen ICI 182,780 (fulvestrant) causes abnormalities in the efferent ductules that appear similar to those seen in mice that lack ER α . In a microarray analysis we found that 2 months of treatment with fulvestrant altered the expression of several genes related to ion transport as expected in the rat efferent ductules. However, some genes upregulated by estrogen were also upregulated by fulvestrant treatment, including metalloproteinase 7 (MMP7). Since MMP7 is involved in tumor cell invasion and cancer metastasis, it is important to clarify the effect of estrogen blockade on MMP7 expression. Aim: 1) to confirm the effect of fulvestrant on MMP7 expression (mRNA and protein) in the rat efferent ductules; 2) to compare the effects of the steroidal antiestrogen fulvestrant and the aromatase inhibitor anastrozole on mRNA levels for MMP7 and ER α , and on the morphology of the efferent ductules. Male Wistar rats were treated with fulvestrant (10 mg/animal s.c., once a week for 2 months) or anastrozole (0.1 mg/kg/day for 15 days, p.o.). The efferent ductules were removed. The tubules were fixed in Bouin's fixative, embedded in paraffin, cut in 5 μ m sections, and stained with hematoxylin or Masson trichrome. Images were captured with a Nikon light microscope with a digital camera, and analyzed with Image Pro-Express software. Total RNA was extracted from efferent ductules with TRIzol. MMP7 expression was evaluated by Northern blot and immunohistochemistry. For comparison between the effects of fulvestrant and anastrozole on gene expression, MMP7 and ER α mRNA levels were evaluated by semi-quantitative RT-PCR. Fulvestrant increased MMP7 mRNA and protein. Fulvestrant also caused dilation of the lumen of the efferent ductules, reduced the thickness of the epithelium and altered the extracellular matrix morphology. On the other hand, fulvestrant did not significantly affect the mRNA levels of ER α , whereas anastrozole significantly increased the ER α mRNA, but did not significantly affect the mRNA levels for MMP7, and had no substantial effect on morphology of the efferent ductules. Fulvestrant and anastrozole differently affect MMP7 and ER α expression in efferent ductules of the rat. We speculate whether some effects of fulvestrant result from its agonist activity on GPR30. This G-protein coupled receptor contributes for estrogen action in several tissues and is also present in the efferent ductules.

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Differential Regulation of the Expression of Epidermal Growth Factor (EGF) and Its Receptor (EGFR) along Rat Epididymis with Sexual Maturation*

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Abstract

Silveira-Neto AP; Patrão MTCC; Avellar MCW. Differential Regulation of the Expression of Epidermal Growth Factor (EGF) and Its Receptor (EGFR) along Rat Epididymis with Sexual Maturation. ARBS Annu Rev Biomed Sci 2008;10:A29. Epidermal growth factor (EGF) is a polypeptide found in a variety of mammalian species. The biological actions of EGF include stimulation of cellular proliferation, growth and differentiation of mammalian tissues as well as its participation with androgens during male sexual differentiation. The presence, function and regulation of EGF and its receptor (EGFR) in the rat epididymis, however, have not been determined. Thus, the aim of the present work was to investigate the expression of EGF and EGFR (mRNA and protein) along epididymis of rats in different stages of sexual maturation. Epididymis from immature (40 days), young adult (60 days) and adult (120 days) rats were used for RT-PCR and immunohistochemistry. Epididymis (n=4) were dissected and divided into initial segment (IS), caput (CP), corpus (CO) and cauda (CD) to perform RT-PCRs with total RNA (5 µg) and specific primers against rat *Egf*, *Egfr* and *Gapdh* (used as internal control) mRNA. Longitudinal paraffin sections (6 µm) of the entire epididymis (n=3) were used in immunohistochemistry with the following antibodies: (1) *anti-EGF*, against the precursor and mature forms of rat EGF; (2) *anti-EGFR*, against rat EGFR and (3) *anti-pEGFR*, against the active conformation of rat EGFR (pEGFR, phosphorylated receptor). Specific PCR products for *Egf* and *Egfr* were detected in IS, CP, CO and CD rat epididymis from all ages. Densitometric analysis indicated that *Egf* mRNA levels did not change during the course of sexual maturation, while a significant reduction in *Egfr* transcript abundance was observed in the CP, CO and CD epididymis from adult rats when compared to the other ages. Immunohistochemistry confirmed EGF, EGFR and pEGFR expression along rat epididymis. Immunostainings were highly dependent on the epididymal region analysed and were observed in epithelial cells, interstitial cells and spermatozoa. Qualitative differences in the pattern of immunostainings of EGF (IS and CP) and EGFR (CD) were observed in tissues from the immature rats when compared to the other ages. All immunostainings were significantly decreased when experiments were performed with primary antibody preadsorbed with blocking peptide. The results indicate that EGF and EGFR (mRNA and protein) are differentially expressed along epididymis of rats in different stages of sexual maturation. The immunodetection of pEGFR also revealed that basal levels of activated EGFR is present in epididymal cells from developing rats, suggesting a role for EGF in the regulation and maintenance of epididymal function. Taken together, the results indicate that the regulation of EGF and EGFR expression along rat epididymis occur at both transcriptional and post-transcriptional levels. The physiological relevance of these events for epididymal function will need further investigation.

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Effect of Forced Swimming Stress from Pre-Puberty on Reproductive Parameters of Pubertal Male Rats*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Nolasco Silva AM, Anjos NR, Arcadepani Silva T, Martini S, Acrani S. *Effect of Forced Swimming Stress from Pre-Puberty on Reproductive Parameters of Pubertal Male Rats. ARBS Annu Rev Biomed Sci 2008;10:A30.* Both physical and psychological stress may interfere with the male reproductive capacity of several species. Activation of the hypothalamic pituitary–adrenal axis by stressors is a probable mechanism for the inhibition of male reproductive functions through inhibition of the hypothalamic–pituitary–testicular axis (Ann N Y Acad Sci 824:1, 1997). The purpose of the present study was to investigate whether forced swimming stress applied during puberty affects reproductive parameters of pubertal male rats. Pre-pubertal male *Wistar* rats (35 days of age) were housed in a controlled environment since weaning (temperature 23±2°C, light 12-h) and had free access to laboratory chow and tap water. The registers of body weight, consumption of ration and water ingestion had been carried through daily. Animals were submitted to forced swimming stress for 40 minutes, during 5 days in week, for 30 days, carrying a metallic ring of about 2% of their corporal weight on their tails. Swimming was carried through in a collective glass tank contends water in ambient temperature. The animals were anesthetized and killed with ethyl ether. The right testis, epididymis, prostate, seminal vesicle (without coagulating glands, with fluid) and adrenal gland were removed and their weights (absolute and relative to body weights) were determined. The right vas deferens was collected and washed with 1.0 ml of formol saline and sperm suspension was evaluated for individual sperm morphology. Two hundred spermatozoa (heads only or intact sperm) per animal were evaluated for head and/or flagellar defects by optic-microscopy. The project was conducted in compliance with ethical principles as approved by institutional guidelines (CEPA-UFTM). The stress group had not all presented significant differences in the ingestion of water and ration during the treatment when compared with the animal controls. The sperm morphology also did not present significant alterations in the two groups. The final body weights, the organ reproductive weight and the adrenal are in the table below. The swimming forced stress applied during the installation of the puberty did not promote significant alterations in the analyzed reproductive parameters.

Parameters	Control group (n = 9)	Stress group (n = 9)
Final body weight (g)	215.9 ± 7.2	208.4 ± 4.6
Testis (g)	1.03 ± 0.03	1.01 ± 0.03
Testis (g/100g)	0.48 ± 0.01	0.48 ± 0.01
Epididymis (mg)	243.9 ± 24.1	256.0 ± 17.3
Epididymis (mg/100g)	113.0 ± 9.8	122.8 ± 13.1
Ventral prostate (mg)	220.0 ± 30.0	230.0 ± 10.0
Ventral prostate (mg/100g)	103.7 ± 8.3	112.0 ± 5.6
Seminal vesicle (g)	281.0 ± 23.2	273.0 ± 21.0
Seminal vesicle (g/100g)	128.3 ± 7.1	132.2 ± 7.2
Adrenal	17.2 ± 1.1	19.3 ± 1.2

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Effect of Simultaneous *versus* Late Insulin Replacement on the Prostate Growth During Sexual Maturation of Rats*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Porto EM, Santos SAA, Felisbino SL *Effect of Simultaneous versus Late Insulin Replacement on the Prostate Growth During Sexual Maturation of Rats. ARBS Annu Rev Biomed Sci 2008;10:A31.* Besides adverse effects of diabetes on testicular functions are well established, there is relatively minor information about impact of diabetes on the accessory sex glands. Streptozotocin (STZ)-induced diabetes in rats provides a relevant model for studying human type I diabetic alterations in reproductive function. This study is aimed to investigate the effects of STZ-induced diabetes and the continuous or delayed insulin replacement on the rat prostatic lobes during the critical period of sexual maturation in rats, puberty. Prepubertal (40-days-old, 180g) male Wistar rats were divided 6 groups: control (C, n=10), Diabetic (D, n= 12) and Diabetic treated with insulin (DI, n=13). Diabetes was induced by administration of a single dose of STZ (40mg/kg body weight, intravenously). Only rats with blood glucose levels ≥ 250 mg/dl, after an overnight fasting, were considered diabetic. An insulin replacement (3U/100 g body weight, subcutaneously) was started three days (simultaneously) or twenty days (late) after STZ-administration. Diabetic, diabetic insulin-treated and age-matched control animals were killed by overdoses of pentobarbital after 20 days (i.e., day 61 of postnatal age: simultaneous replacement group) and 40 days of experimental period (i.e., day 81 of postnatal age: late replacement group). Blood were collected and frozen at -20°C for future analysis of the plasma hormone levels. Prostatic lobes were dissected out, weighted and immediately immersed in fixative or frozen at -80°C for future evaluation semi-quantitative western blotting, immunoprecipitation and zymography techniques for the analysis of matrix metalloproteinases (MMP-2, -9) and their inhibitors (TIMP-1, -2). Prostatic lobes fixed were embedded in Paraplast and sectioned in $5\mu\text{m}$ they will be analyzed morphometric and immunohistochemistry. Data were analyzed statistically using one-way ANOVA, Tukey-Kramer test. Data are expressed mean \pm SD. STZ administration resulted in a loss of body weight (g) that was prevented by insulin replacement (C=286.42 \pm 15.26; D=169.98 \pm 25.81; DI=272.45 \pm 21.86, simultaneous replacement group; C=346.66 \pm 26.78; D=210.86 \pm 39.80; DI=293.17 \pm 36.02, late replacement group). Plasma glucose levels (ng/dl) were significantly higher in STZ-injected rats than other groups (C=54.6 \pm 5.85; D=340.6 \pm 91.44; DI=43.0 \pm 14.98, continuous group; C=65.6 \pm 4.27; D=333.0 \pm 57.09; DI=38.2 \pm 3.96, delayed group). Diabetes caused reduction of prostate weight. However, the size of the prostate was restored to control values with both simultaneous and late insulin replacement. This initial data demonstrate the adverse effects of diabetes on the prostate growth during sexual maturation and are consistent with other reports suggesting that insulin may play an important role in the growth and development of the prostate.

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Effects of 30% Calorie Restriction on Biometric and Reproductive Parameters of Male Wistar Rats*

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Abstract

Rocha JS, Bordoni LS, França LR.. *Effects of 30% Calorie Restriction on Biometric and Reproductive Parameters of Male Wistar Rats. ARBS Annu Rev Biomed Sci 2008;10:A32.* There is a great interest nowadays in studying calorie restriction (CR), since its role in prolonging lifespan is well established in several species, from yeast to dogs, including some evidences in monkeys. Alternatively, its negative impact on reproduction is also reported, although seemingly negligible when CR is very mild. Rodents have been extensively used in CR approaches and show extension in lifespan as well as impairment of reproductive function generally proportional to the level of CR applied. To investigate the effects of 30% CR on several biometric and reproductive parameters during the post-natal developmental period in male Wistar rats until the age of 50 days (puberty establishment). Eight male Wistar rats were subjected to 30% CR starting at age of 24 days and ending at age of 50 days, at sacrifice. As the control group, eight age- and sex-matched animals had free access to food (*ad libitum* - AL). Animals were placed in wire-top plastic cages with free access to tap water, and subjected to 12h light/12h dark cycle and 22°C. Animals were weighed daily and food consumption of AL animals was recorded, so food amount could be adjusted for the CR group at a daily basis. Thirty percent restriction was achieved by giving CR animals 30% less food than the AL animals ate during the previous 24 hours. Starting at day 21, animals were checked daily for balano-preputial separation, to determine the day of onset of puberty. At sacrifice, several organs were collected and weighed. Weights and indices of the following organs were recorded: total body weight (BW), testis, seminal vesicles, prostate, epididymis, epididymal fat pads, thyroid, adrenals, heart, liver, spleen, kidneys, and pituitary. In the present study, CR had significant effect on BW, what is consistent with several other reports. From age 29 days on, BW of control and treated groups was statistically different, and final BW of CR animals were 26% lower than AL animals. In contrast, onset of puberty was not affected by diet in this study, contrasting with some reports in the literature. Absolute weights of seminal vesicles, prostate, epididymal fat pads, adrenals, heart, liver, kidney and pituitary were decreased ($p < 0.05$ or less) in CR animals. Nevertheless, organ weight relative to body weight (index) was affected by CR only for epididymal fat pads. There was no difference in testis weight, but GSI was significantly higher in CR animals, probably due to their reduced body weight. Thirty percent CR proved its effect in reducing body weight and absolute weights of several organs, although in general not significantly when organ weight is correlated with body weight. An interesting finding was that CR apparently did not affect the onset of puberty in the animals studied.

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Effects of a Hypercaloric Diet Ingested from Weaning on Sexual Behavior of Adult Male Rats

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Abstract

Machado Junior A, Lamano-Carvalho TL, Acrani S. *Effects of a Hypercaloric Diet Ingested from Weaning on Sexual Behavior of Adult Male Rats*. *ARBS Annu Rev Biomed Sci* 2008;10:A33. Obesity is considered a global epidemic (World Health Organization). In Brazil, demographic, socio-economic and epidemiologic changes had allowed transition in the nutritional standards with a gradual reduction of malnutrition and an increase in obesity (POPKIN BM & DOAK CM. *Nutr Ver* 56:106, 1998). An experimental model of obesity, well related to human reality, is the ingestion of a hypercaloric diet. The purpose of the present study was to investigate whether a hypercaloric diet ingested from weaning interferes with the sexual behavior of adult male rats. Adult (100 days-old) male *Wistar* rats were housed in a controlled environment since weaning (temperature 23±2°C, 12-h dark period starting at 10:00 a.m.) and had free access to laboratory chow and tap water. Treated animals received a hypercaloric diet (ESTADELA, D - São Paulo School of Medicine, UNIFESP, São Paulo, 2001) containing 4.79 Kcal/g while control animals were fed a normocaloric laboratory chow (NUVILAB, Curitiba, PR, Brazil) containing 3.78 Kcal/g. For analysis of sexual behavior (performed always from 14:00 p.m.) the males with no previous sexual experience were individually placed in the observation cage (56 x 35 x 31 cm with a glass front wall) 10 min before an adult receptive female in natural estrus was introduced. The parameters of sexual behavior (number and latency of mount, intromission and ejaculation) were recorded during a 30-min session with light provided by a 40-watt red lamp. The project was conducted in compliance with ethical principles as approved by institutional guidelines (CEPA-UFTM). Rats from the hypercaloric group (treated rats) did not exhibit an excess body weight gain (data not shown) probably due to a high metabolism and growth rate characteristic of this age but presented an increased epididymis fat (table) indicative of impairment in lipidic metabolism and fat storage. Treated rats exhibited also a significant increase in the number of mounts without intromission (incomplete mounts) and in the latency to first intromission, which in addition to a decreased number of ejaculations are indicative of impaired sexual performance. The present results show a negative interference of a hypercaloric diet ingested from weaning in the sexual performance of adult male rats.

Parameters	Control Group (n = 7 - 9)	Hypercaloric group (n = 8 - 9)
Epididymis fat (g)	1.80 ± 0.1	2.6 ± 0.3*
Latency to first mount (s)	21.8 ± 3.6	26.0 ± 10.9
Number of mounts without intromission	2.5 ± 1.2	3.9 ± 0.4*
Latency to first intromission (s)	54.0 ± 23.3	120.6 ± 48.0*
Number of intromissions	19.6 ± 3.7	12.4 ± 2.1
Latency to first ejaculation (s)	740.6 ± 146.3	522.0 ± 94.8
Post-ejaculatory mount latency (s)	1150.8 ± 83.7	842.2 ± 103.0*
Post-ejaculatory intromission latency (s)	1150.8 ± 83.7	842.2 ± 103.0*
Number of ejaculations	3.4 ± 0.2	2.5 ± 0.4*

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Effects of Different Steroids Treatments, Combined or Not to Vitamin Supplement, on the Testicular Morphology of Adult Rats Submitted to Forced Swimming*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Mendes LO, Alves RF, Camargo ICC, Silva GH. *Effects of Different Steroids Treatments Combined or Not to Vitamin Supplement, on the Testicular Morphology of Adult Rats Submitted to Forced Swimming*. *ARBS Annu Rev Biomed Sci* 2008;10:A34. The anabolic androgenic steroids (AAS) are included between the drugs more utilized of abusive and indiscriminate form by men and women, athletes or not, to enhance muscle strength and physical performance. The popular steroids, Deca-Durabolin® (nandrolone decanoate) and Durateston® (testosterone decanoate), are common in the environment of sports and usually are utilized in supraphysiological doses, combined between itself e with vitamin supplement. Studies have shown that AAS have adverse effects upon spermatogenesis and fertility in human and animals. Aim - To analyze the effects of steroids Deca-Durabolin (DD) and Durateston (DT), combined or not to L-carnitine® (L) vitamin supplement, on the testicular morphology of adult rats submitted to physical exercise. Forty male adult rats of Wistar lineage distributed in eight experimental groups (n = 5/group): control (physiological solution) and treated with DD, DT, DD+DT, L, DD+L, DT+L, DD+DT+L. The animals received a single dose of 7.5 mg/Kg of body weight of each steroid, weekly, intraperitoneal via, and vitamin supplement was administered in a single dose of 1 ml, weekly, oral via (gavage), during 8 weeks of treatment. The animals were weighed, the reproductive organs were dissected and the gonads were prepared by usual histologic routine (sections of 5µm-thick, embedded in Paraplast® and stained by hematoxylin and eosin, for light microscopy analysis. The body, testis and liver weight were not affect significantly by different treatments, but the epididymis, seminal glands, prostate and hypophysis weight presented significative influence of treatments. The testis of rats treated with DD, DT and DD+DT presented intense atrophy of seminiferous tubules, associated to loosen of peritubular sheath of myoid and presence of intraepithelial vacuoles. In the group treated with L, there was apparent atrophy tubular. The combination of steroids to vitamin supplement reinforced the deleterious effects on the testicular morphology. The treatment only or simultaneous with the steroids, combined or not to vitamin supplement, promoted alterations on the histologic structure of testis of adult rats to swimming.

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Effects of Maternal Exposure to the Antidepressant Fluoxetine on Sexual Behavior and Endocrine Aspects of Adult Male Mice*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Gouvêa TS, Campanini MZ, Favaro PN, Moreira EG, Morimoto H, Faria MJSS, Gerardin DCC. *Effects of Maternal Exposure to the Antidepressant Fluoxetine on Sexual Behavior and Endocrine Aspects of Adult Male Mice*. ARBS Annu Rev Biomed Sci 2008;10:A35. Brain sexual development and differentiation involve a complex series of events which begin during gestation and continue, at least in rodents, in the early postnatal period. An early modification of neuronal reactivity to hormones and neurotransmitters appears to be a key point of genetic imprinting in the developing brain. Alterations in these processes generally are detected only at puberty or adult reproductive life. During brain development, serotonin acts as a neurotrophic factor for monoaminergic neurons, which are known to modulate sexual behavior. Fluoxetine (FLX), a serotonin reuptake inhibitor, has been widely prescribed for depression during pregnancy and/or lactation. The use of FLX by mothers could disrupt brain development resulting in sexual behavioral alterations in their progeny. Evaluate the effects of maternal FLX exposure on sexual behavior and on endocrine parameters of male mice pups. Dams were treated daily by gavage with 7.5 mg/kg of FLX or water, from gestational day zero to post-natal day (PND) 21. Male pups had their anogenital distance evaluated on PND 1 and 120 and were tested for copulatory behavior (CB) and sexual incentive motivation (SM) on PND 100. In the CB test, latencies and numbers of mounts, intromissions and ejaculations were measured. In SM test, the animals were placed on an arena with two kinds of incentives – a sexual one (an estrous female) and a social one (a sexually experienced male). The experimental animals were separated from the incentives by a wire mesh, so they could see, hear and smell the incentive animals but not interact physically with them. The number of visits and the time spent visiting each of the incentives were quantified. A preference score was calculated (time spent with the sexual incentive divided by the total time spent visiting both incentives). On PND 120 testis, epididymis, seminal vesicle and pituitary were removed for wet weight determination and plasma for testosterone quantification by chemiluminescence. Data \pm SEM were compared by Student's t test with Welch's correction. Preference score data were compared by Mann-Whitney's test. In CB test there were no statistical differences between FLX-exposed and control groups ($p>0.05$). However, in the SM test, FLX-exposed mice made more visits (CON=29.2 \pm 2.9; FLX=37.4 \pm 2.2, $p<0.05$) and spent more time (CON=237 \pm 18; FLX=353 \pm 33, $p<0.05$) with social incentive. The preference score tended to be decreased in FLX group (CON=0.65 \pm 0.03; FLX=0.55 \pm 0.03, $p=0.088$). Anogenital distance, plasmatic testosterone concentration and reproductive organs wet weights were not affected ($p>0.05$). Maternal exposure to FLX during pregnancy and lactation disrupts sexual preference, but did not alter copulatory behavior as well as sexual endocrine parameters of adult male mice.

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Effects of Zinc on Fertility and Seminal Quality of Male Rats Exposure to Cigarette Smoke

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Abstract

Garcia PC, Piffer RC, Kempinas WG, Rubio EM, Pereira OCM. *Effects of Zinc on Fertility and Seminal Quality of Male Rats Exposure to Cigarette Smoke. ARBS Annu Rev Biomed Sci 2008;10:A36.* Some investigations have proposed a detrimental effect of smoking on sperm concentration, sperm motility, and percentage of morphologically normal spermatozoa. The adverse effects of cigarette smoke on Leydig cell function in animals have been also reported. On the other hand, Zinc can be an antioxidant role and may be an important cofactor for the cellular division. To evaluate, in an animal model, the effects of Zinc on fertility and quality seminal of rats exposure to cigarette. Male Wistar rats at 6-week-old were divided in four groups (10/group): control (G1), cigarette smoke-exposed (G2: nicotine 0.6 mg), zinc control (G3: zinc chloride 25mg/kg- daily by gavage) and zinc/cigarette smoke (G4: zinc chloride 25mg/kg and cigarette smoke-exposed). Rats in G2 and G4 were exposed cigarettes during a period of 63 days. The spermatozoa were collected from testis and epididymis for evaluation of the number of spermatids, daily sperm production in the testis, sperm in the caput/corpus and cauda epididymis as well as the sperm transit time. In vas deferens were evaluated concentration, vitality, and morphology of spermatozoa. Data were compared by ANOVA (mean±SD); n=10 animals/group, p<0.05. The exposure to cigarette smoke during 63 days reduced the quality and quantity of spermatozoa. However the supplementation with zinc prevented the toxic effects of cigarette smoke - Spermatozoa x10⁶/organ: in testis (G1: 219.52±18.54^a/ G2: 201.89±12.67^b/ G3: 227.53±19.75^a/ G4:236.95±21.36^a), in caput and corpus of epididymis (G1: 99.30±13.96^a/ G2: 85.34±12.68^a/ G3: 156.94±16.21^b/ G4:148.29±20.15^b), and in cauda of epididymis (G1: 163.07±22.69^a/ G2:162.35±31.59^a/ G3: 271.88±38.82^b/ G4: 213.34±70.50^c). Sperm transit times/ days in: caput and corpus of epididymis (G1: 2.76±0.40^a/ G2: 2.56±0.39^a/ G3: 3.85±0.31^b/ G4: 3.95±0.40^b) and in cauda of epididymis (G1: 4.56±0.29^a/ G2: 4.97±0.67^a/ G3: 2.65±0.16^b/ G4: 2.91±0.14^b). Concentration of spermatozoa in vas deferens (G1: 66.20x10⁶±15.56^a/ G2: 56.00x10⁶±6.43^b/ G3: 144.60±17.90^c/ G4:127.30±27.03^c). Alive spermatozoa number (G1: 97.30±1.25^a/ G2:90.81±2.22^b/ G3:99.50±0.70^a/ G4: 97.80±1.47^a) and normal morphology spermatozoa percentage (G1: 97.90±0.99^a/ G2: 95.00±2.60^b/ G3: 98.10±1.28^a/ G4: 97.60±1.35^a). These results indicated that tobacco exposure induced a decreasing in the spermatid quantity and mainly through damage in the seminal quality. These features may be responsible by subfertility observed in a large number of smoking cigarette men. In addition, the results showed also that the supplementation with zinc prevented the toxic effects of cigarette smoke, and can be a potential agent in treatment of infertility induced by consumption of smoking cigarette.

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Estrogen Action Via the G-protein Coupled Receptor in Rat Sertoli cells*

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Abstract

Siu ER, Lucas TG, Lazari MFM, Porto CS. Estrogen Action Via the G-protein Coupled Receptor in Rat Sertoli cells. ARBS Annu Rev Biomed Sci 2008;10:A37. 17 β -estradiol (E2) plays an important role in the development, differentiation and growth of the male reproductive system (1-4). Our previous studies showed that the E2 effects on Sertoli cell function involves: translocation of the ER α and ER β to the plasma membrane mediated by the nonreceptor tyrosine kinase Src, activation of ERK1/2 and cell proliferation (5). In addition, E2 or ICI 182,780 (ICI) can also rapidly activate ERK1/2 after downregulation of both ERs in Sertoli cells, through unidentified ER-mediated mechanisms. The rapid action of estrogen can be mediated by membrane translocation of ERs or through proteins other than ERs, such as G-protein coupled receptor (GPR30), as previously shown in different cells (6-9). To study the expression of GPR30 in cultured Sertoli cells and the role of ICI to induce ERK1/2 activation. Primary culture of Sertoli cells was obtained from 15-day old rats (5). GPR30 expression was detected by RT-PCR and immunofluorescence. Cells were incubated with ICI, and phosphorylated ERK1/2 and total ERK1/2 were detected by Western blot. Additionally, ICI effects were also evaluated by estimating incorporation of [Methyl-³H]-thymidine after treatment with ICI. GPR30 (mRNA and protein) was detected in Sertoli cells. ICI induced a rapid and transient increase in ERK1/2 phosphorylation. Peak ERK1/2 phosphorylation levels occurred at 10 min (7- to 12-fold increase) with ERK1/2 activity returning to basal levels by 15-30 min. Cells treated with cAMP-dependent protein kinase A inhibitor H89 (20 mM, 2 h) maintained high levels of ERK1/2 activity for more than 30 min after E2 or ICI stimulation. An increase in [methyl-³H]-thymidine incorporation was observed after these treatments. The activation of ERK1/2 and [methyl-³H]-thymidine incorporation were blocked by pre-treatment with pertussis toxin (PTX, 100 μ M, 16 h) or the Src family tyrosine kinase inhibitor PP2 (5 nM, 30 min), before stimulation with ICI. EGFR kinase inhibitor AG 1478 (50 μ M, 15 min), metalloprotease inhibitor GM 6001 (200 nM, 30 min) and MEK1/2 inhibitor U0126 (100 nM, 30 min) also blocked ERK1/2 activation. These data suggest that E2-GPR30 signaling occurs through a PTX-sensitive pathway that requires Src-related tyrosine kinase activity. The activation of ERK1/2 induced by ICI is dependent upon transactivation of the EGFR via release of heparin-bound EGF. Furthermore, the activation of cAMP-dependent PKA is required to restore ICI-induced ERK1/2 activity to basal levels. References: 1. J Androl 18:602, 1997; 2. Mol Cell Endocrinol 26:246, 2006; 3. Ann N Y Acad Sci 1061:65, 2005; 4. Reprod. Biol. Endocrinol 3:51, 2005; 5. Biol Reprod *in press*, 2007; 6. Mol Endocrinol 16:70, 2002; 7. Trends Endocrinol Metab 16:362, 2005; 8. J Cell Physiol 207:594, 2006; 9. Biol Reprod 75:9, 2006; 10. Endocrinology 146:624, 2005; 11. Mol Cell Endocrinol 265-266:138, 2007

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Evaluation of Sertoli Cell Proliferation in the Transitional Region of the Seminiferous Tubules in Postpubertal Pigs

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Abstract

Avelar GF, Soares JM, Costa GMJ, Hess RA, Silva IJ, França LR. *Evaluation of Sertoli Cell Proliferation in the Transitional Region of the Seminiferous Tubules in Postpubertal Pigs. ARBS Annu Rev Biomed Sci 2008;10:A38.* Differently from mice and rats, in which Sertoli cells (SC) mitosis ends 2 to 3 weeks after birth, SC proliferation in pigs presents two postnatal peaks that occur during the first month (mo) of age and between 3 to 4 mo. However, in pigs and several other mammalian species, testis weight, total length of seminiferous tubules, and sperm production still increase after the animals reach full sexual maturity, without further increase in tubular diameter. These findings suggest that SC might still proliferate after the period they are expected to stop dividing. In fact, ongoing studies in our lab show immature SC present in the transitional region located between seminiferous tubules and *rete testis* (TR) in rats, retaining their mitotic activity up to 36 days after birth. In this regard, our aim was to investigate comparatively the SC proliferation in TR in pre- and postpubertal pigs. Twelve prepubertal (1, 3 and 4mo, n=4) and five postpubertal (6mo, n=5) crossbred pigs were used. Animals were orchietomized and samples from TR and albuginea parenchyma were obtained. Right testis was fixed in 5% glutaraldehyde, embedded in plastic and routinely prepared for histological and morphometric analyses. Left testis was fixed in NBF and embedded in paraplast. SC proliferation was assessed by immunohistochemistry and tissue culture. Testis sections were incubated with monoclonal mouse anti-human Ki67 and anti-human GATA-4, and counterstained with Mayer's hematoxylin. Small fragments of 105-day-old piglet testes were cultured for 4h in DMEM/F12 containing 1μCi/ml of [³H]thymidine, and embedded in plastic for autoradiographic analysis. The percentage of SC mitosis in TR was higher (p<0.05) than in albuginea parenchyma in 4mo pigs. As expected, proliferating immature SC in TR were observed in prepubertal pigs evaluated at 1 and 3mo. Also, immunohistochemistry and tissue culture evaluation showed that SC proliferation was occurring before puberty. These findings are in agreement with the literature and are related to the two postnatal phases of SC proliferation that occur in pigs. In addition, we also found several proliferating SC in postpubertal pigs TR. Since puberty in pigs usually takes place at approximately 4mo, this proliferation supports the hypothesis that the observed increases in testis weight and sperm production after pigs are fully mature are probably due to an extended period of SC mitotic activity in TR. The present results indicate that SC in TR is able to proliferate after puberty. In order to better understand these findings, we are now performing experiments searching for possible mechanisms related to the regulation of this important testis somatic key cell.

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Evaluation of the Effects of Acyline on the Evolution of Spermatogenesis in Recipient Mice Carrying Testis Tissue Xenografts*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Costa GMJ, Dobrinski I, França LR. *Evaluation of the Effects of Acyline on the Evolution of Spermatogenesis in Recipient Mice Carrying Testis Tissue Xenografts. ARBS Annu Rev Biomed Sci 2008;10:A39.* Germ cell transplantation success in mammals is highly correlated with the phylogenetic distance. In this regard, a new technique was developed utilizing grafting testis fragments from several newborn mammalian species into sexually mature nude mice. Besides establishing complete spermatogenesis, this approach maintains the testis structural integrity, allowing the study and manipulation of the testis and preserving the male germ cell line. However, whereas sperm production in porcine testis xenografts can reach spermatogenic efficiency similar to the donor species, bovine testis tissues grafted into mice resulted in inefficient spermatogenesis, and the most advanced germ cell type observed was elongated spermatid. Also, it was observed that seminal vesicles were overly large in the host mice, strongly suggesting that testosterone levels were higher than normal. To investigate the possible reasons for these findings in this study we aimed to perform a detailed evaluation of ectopically grafted bovine testis tissue, 7 months after xenografting. Our hypothesis was that excessive production of testosterone was responsible for the abnormal spermatogenesis in bovine testis grafts. In this regard, acyline, a drug that was previously shown to partially decrease GnRH and LH levels, was utilized to suppress serum and intratesticular testosterone in mice. Fragments of testicular tissue (0.5-1mm³) from 5 donor calves (one-week-old) were grafted under the back skin of castrated immunodeficient mice (n=12mice/donor). Half of the recipient mice were treated with 5 mg/Kg acyline (Jean River Group/NICHHD) every 2 weeks from 3 to 6 months after grafting. The remaining recipients were utilized as the untreated controls. All recipient mice were sacrificed and the grafts recovered 7 months after grafting in order to perform histological and morphometric evaluation. Total body weight was similar (p>0.05) in treated and control mice. Seminal vesicles in the acyline treated group were smaller (p<0.05) when compared with the control group, suggesting lower secretion of bioactive testosterone levels by the grafted tissue. Similar pattern was observed for testis graft weight, which was lower (p<0.05) in the treated group. The evaluation of the most advanced cells present in the seminiferous epithelium showed higher incidence of seminiferous tubules depleted of germ cells (Sertoli cell-only) and delayed germ cell development in the acyline treated group, compared with the control group. Instead of improving spermatogenesis efficiency, acyline had an even more deleterious effect on the development of bovine xenografts. This suggests that the striking higher levels of testosterone observed in mice that received bovine testis grafts may not be the responsible for the observed abnormal spermatogenesis.

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Evaluation of the Low Molecular Weight Fraction from the *Bothrops jararaca* Venom on Junction Dynamics of Mice Testis Seminiferous Epithelium*

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Abstract

Pannocchia MA, Gilio JM, Portaro FCV, Fernandes BL, Camargo ACM, Silva CA. Evaluation of the Low Molecular Weight Fraction from the *Bothrops jararaca* Venom on Junction Dynamics of Mice Testis Seminiferous Epithelium. *ARBS Annu Rev Biomed Sci* 2008;10:A40. A molecular mechanism of action for NO (nitric oxide) in spermatogenesis has been recently proposed, in which NO likely regulates tight junction (TJ) dynamics in the testis via the cGMP/protein kinase G, and adherens junctions (AJ), in part, via the NOS signaling pathway. These studies have clearly illustrated the interesting role of NOS and NO in Sertoli-germ cell AJ restructuring. Recently, we have shown that the low molecular weight fraction (LMWF) of the *Bothrops jararaca* venom causes a significant disruption of the seminiferous epithelium and inhibition of spermiogenesis in mice. In this work, we investigated if the disruption of seminiferous epithelium could be explained by the action of NO. Aim: Evaluate the level of NO and expression of NOS in mice testis treated with L-NAME, nitroprussiate and LMWF. Three male Swiss mice (35g) were treated with L-NAME (L group), sodium nitroprussiate (N group) or LMWF (5µg) in the left testis and vehicle in the right testis by intratesticular injection. On day 7, animals were killed and their testes were homogenized on ice with in a Tris-HCl 50 mM buffer, containing EDTA 0.1 mM, 2-mercaptoethanol 2mM, and phenylmethylsulphonyl fluoride 1mM, pH 7.4. Subsequently, 1% TCAm (4°C) was added and the samples were centrifuged for 7 min at 13,000 g. The nitric oxide levels were evaluated by nitrate and nitrite accumulations in the supernatant (30 µL) of total testis protein extract in a NO analyzer (NOA^{TM280}; Sievers Inc.). eNOS and iNOS expression were analyzed by western blotting of total testis protein extract using anti-eNOS and anti-iNOS (Zymed®). Group L showed severe degenerative changes of the seminiferous epithelium of the left testis, such as increased epithelium height and decreased lumen diameter of tubules. On the other hand, in group N, there were focal areas showing germinative cells distributed in the lumen, and a decrease in epithelium height. Morphological parameters of the LMWF group were significantly similar to group N. Levels of the NO of the left testis in the N and LMWF groups (326µM; 119µM) were higher than those in the L group (2µM) (P<0.01). On the other hand, levels of the NO of right testes in the three groups were not significantly different (P>0.05). To confirm this hypothesis, eNOS and iNOS expression are being analyzed by western blotting. Changes in NO levels in the testes of mice treated in the L-NAME and sodium nitroprussiate might be the cause for testes damage and disturbances of spermatogenesis as described in the literature. Moreover, we suggest that there are some compounds in the LMWF that increase the NO levels, promoting a disruption of mice seminiferous epithelium; opening new perspectives for the medical development of a male contraceptive.

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Fertility Evaluation of F1 Generation from Rats Treated with Bacupari

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Abstract

Sousa PV, Martins JS, Lavers MPN, Maia LO, Nery B, Costa MFO, Mazaro e Costa R. Fertility Evaluation of F1 Generation from Rats Treated with Bacupari. *ARBS Annu Rev Biomed Sci* 2008;10:A41. Bacupari (*Cheiloclinium cognatum* - Hippocrateaceae) is a Cerrado Brazilian plant. Studies have shown a decrease of diary sperm production in rats treated with dichloromethanic extract made from leaves of Bacupari; however these males were able to have viable pups. The offspring generation obtained from parents treated with Bacupari showed delays on neural development; nevertheless no differences were detected on their testicular evaluation. The aim of the study was to evaluate the effect of *Cheiloclinium cognatum* on fertility of the male offspring generation (F1) from parents treated with Bacupari. The parent generation was formed by male *Wistar* rats treated with dichloromethanic extract, made from leaves of Bacupari, during 30 days by 500 mg/kg/day; orally. (T, n=10), while the control group (C, n=10) received soy oil as vehicle. At the end of treatment the males were submitted to matting with virgins females not exposed to Bacupari. The offspring were obtained and when the male F1 reached adult phase (90 day old), the rats were mated with virgins females (2 female:1 male). The day when spermatozoa were found in the vaginal smear was considered day 0 of gestation. By the matting were obtained 10 female by control group (C), and 9 female by treated group (T). On the 9th day of pregnancy, all females were anaesthetized under ethyl ether and killed. After removing the uterine horns were recorded the numbers of: 1. implantation sites; 2. corpora lutea, 3. fetuses. From these parameters was calculated: pre-implantation loss. The Mann-Whitney non-parametric test ($p<0.05$) was used for statistical analysis. Fertility parameters showed an increased on pre-implantation loss in female fecundated by treated male with *C. cognatum* (C: 5.0 ± 3.0 ; T: $2.8 \pm 4.2^*$). Abnormal sperm production or decrease in sperm viability can explain the increase of pre-implantation loss (2, 3). But, *Cheiloclinium cognatum* did not reduce DSP or sperm morphology in the male F1 generation (1). Thus, an alteration in the maturation of spermatozoa should be considered, once the parental rats showed this alteration which could reach the offspring genetically. The effect of *C. cognatum* on spermatozoa maturation from parent may affect the F1 male generation and induce the decrease in fertility.

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GFP Immunodetection in Tilapia Testes and the Potential Use of These Cells in Fish Germ Cell Transplantation*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Batlouni SR, Lacerda SMSN, Campos Silva SM, Resende FM, Assis LHC, França LR. *GFP Immunodetection in Tilapia Testes and the Potential Use of these Cells in Fish Germ Cell Transplantation. ARBS Annu Rev Biomed Sci* 2008;10:A42. Germ cell transplantation is a fascinating technique that has been largely utilized in the past decade in mammals. This technique consists on the removal of the stem germ cells from a donor testis and their transfer into a recipient testis, where they will develop and form spermatozoa with the genetic characteristics from the donor. Aiming to investigate the germ cell transplantation in fish, all the necessary approaches to perform this technique were successfully standardized in our laboratory, utilizing donor spermatogonia labeled with the membrane dye PKH26. However, different from the nuclear or membrane markers, the usage of genetic markers permits that the developmental process of the transplanted spermatogonia may be evaluated in the recipient testes with some advantages, as follows: during the germ cell division process there is no loss of detection of the genetic marker; there is no need to label the cells with membrane dyes which may causes a loss of cell viability; membrane dyes may transfer to neighbor cells while genetic markers are stable. The aim of this study was investigate whether the GFP (Green fluorescent protein) could be detected through immunohistochemistry in the spermatogonia from GFP positive tilapia testes. Gonads from adults and from tilapia embryos carrying the GFP gene were analyzed by means of immunohistochemistry utilizing an anti-GFP antibody. As negative controls the omission of the primary antibody in the GFP positive samples was applied. In the testes of GFP adult and embryo tilapia germ cells were roughly labeled. Primary spermatogonia cytoplasm was entirely labeled, however a more intensive labeling was found in the area around the nuclear membrane. In the ovaries, germ cells were also broadly labeled. No specific staining was detected in negative controls performed. The obtained results demonstrated the GFP positive tilapia males, sent by Dr. Goro, may be used as potential donors of germ cell for further transplantations studies, since the presence of the GFP can be by far detected in their spermatogonia. The possibility of the usage of GFP positive spermatogonia will eventually facilitate the process of germ cell selection, once the germ cell labeling process won't be necessary. Moreover, the usage of a genetic marker in teleost fish transplantation, may improve the knowledge about the testicular function and the stem germ cell biology in this group of animals.

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Ginkgo biloba Attenuates Cadmium-Induced Damage in Rat Testis*

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Abstract

Predes FS, Monteiro JC, Matta SLP, Dolder H. *Ginkgo biloba* Attenuates Cadmium-Induced Damage in Rat Testis. *ARBS Annu Rev Biomed Sci* 2008;10:A43. Cadmium (Cd) is a wide spread environmental pollutant, characterized by its toxicity in various organs (*Food Chemistry and Toxicology* 2004;42:1563) of humans and animals. There is evidence indicating the involvement of oxidative stress in Cd-induced testicular damage (*Molecular and Cellular Endocrinology* 2004;221:57). Several studies have shown that free radical scavengers and antioxidants are useful in protecting against Cd toxicity. Since *Ginkgo biloba* extract (GbE) is known to exert protective influences against the action of free radicals, we hypothesized that application of such an extract might prevent cadmium-induced damage in testis. Aim: Investigate the association of Cd and *Ginkgo biloba* extract (GbE) on the ultrastructural morphology of the testis of adult Wistar rats. Thirty Wistar rats were divided in five groups that received: Group 1: water; Group 2: GbE; Group 3: Cadmium chloride (CdCl₂) and water; Group 4: CdCl₂ and GbE. The GbE was administered daily in a dose of 100 mg/Kg BW by gavage for 56 days. A single dose of CdCl₂ equal to 3 µmol/Kg BW, was injected i.p. Groups 1 and 3 received water by gavage for 56 days. The animals were fixed by whole body perfusion with Karnovsky's Fixative and post fixed in the same solution for 24 hours. The tissues were post-fixed with 1% osmium tetroxide, dehydrated in acetone and embedded in epoxy resin for observation with a transmission electron microscope. In the control and GbE treated groups, testis consisted of normal seminiferous tubules and interstitium. In the Cd-treated group, Sertoli cell cytoplasm showed vacuolation, loss of cytoplasmic organelles and presence of large, lysosome-like vacuoles, with polymorphous contents. There was an increase in the intercellular space due to disorganization of germ cells in some tubules. This disorganization showed cells isolated at the basal compartment, namely, Sertoli cells, spermatogonia and preleptotene spermatocytes. The adluminal compartment lost germ cell attachment, resulting in expanded intercellular spaces between spermatocytes, spermatids where Sertoli cell prolongations are observed. Irregularly condensed chromatin and abnormal acrosomes were found in late spermatids. The Leydig cells showed a nuclear envelope with many deep indentations and dense cytoplasm, and poorly defined organelles such as SER and mitochondria. The administration of GbE after a single dose of CdCl₂ attenuated the ultrastructural changes of rat seminiferous epithelium and revealed that the GbE is capable of diminishing the damage to Leydig cells usually caused by Cd, such as diminished smooth endoplasmatic reticulum and irregular nuclear boundaries.

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Glucocorticoid Receptor Regulation by Endogenous and Synthetic Glucocorticoids and Colocalization with Microtubule Associated Protein 1B in the Rat Epididymis*

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Abstract

Silva EJR, Queiróz DBC, Honda L, Avellar MCW. *Glucocorticoid Receptor Regulation by Endogenous and Synthetic Glucocorticoids and Colocalization with Microtubule Associated Protein 1B in the Rat Epididymis*. *ARBS Annu Rev Biomed Sci* 2008;10:A44. Glucocorticoids (GCs) are steroid hormones that regulate several physiological functions in vertebrates. The actions of GCs are mediated by glucocorticoid receptor (GR), member of ligand-activated transcription factor superfamily which includes androgen receptor (AR). Curiously, the role of GCs in the epididymis, an androgen-dependent organ that promotes sperm maturation, is still poorly understood. Evaluate the effect of adrenalectomy (ADX) and dexamethasone (Dex) treatment on the expression of GR and AR in the rat epididymis. The expression of glucocorticoid-responsive genes (I β B α and IL-1 α) and the colocalization of GR with a neuronal marker (Microtubule-associated protein 1B, MAP1B) were also evaluated. Wistar rats (90 days) were sham-operated (S) or submitted to bilateral ADX for 1, 2, 7, 15 days (d). Rats were also submitted to ADX for 7 days and immediately treated with Dex (Chronic- CrDex, 5 μ g/kg, ip, daily or Acute- AcDex, 7 mg/kg, ip, 6 h). Plasma corticosterone levels were monitored by RIA. Caput (CP) and cauda (CD) epididymis were used in semi-quantitative RT-PCR using specific primers to GR, AR, I β B α , IL-1 β and the housekeeping gene GAPDH (internal control). Western blot (total protein extracts), immunohistochemistry and immunofluorescence (cryosections) with samples from S, ADX 7d, AcDex and CrDex rats were performed with antibodies against GR, AR and MAP 1B (negative controls with blocking peptides). Results were analyzed by ANOVA followed by Newman-Keuls or Tukey test ($p < 0.05$). RIA confirmed the reduction on corticosterone plasma levels in all ADX groups. Densitometric data of RT-PCR indicated that GR, AR, I β B α and IL-1 β mRNA levels were not altered by ADX. However, Dex treatments caused a reduction on GR, AR and IL-1 β and an increase on I β B α mRNA levels in CP and CD when compared to S and ADX 7d groups. Western blot revealed similar GR and AR expression when ADX 7d, AcDex and CrDex groups were compared. GR and AR immunostaining in CP and CD was detected in epithelial, smooth muscle and interstitial cells (nuclear, perinuclear and cytoplasmic localization). The reduction of GR staining in the nuclei of epithelial cells from CP and CD caused by ADX 7d was reversed in Dex treated groups. In addition, GR epithelial cytoplasmic staining was reduced in the CP of AcDex rats. AR immunostaining was similar among all groups. Curiously, GR-positive interstitial fibers were observed in the CD of all groups. Colocalization studies revealed that GR-positive fibers were also immunostained by MAP1B antibody, confirming the expression of GR on epididymal nerve fibers. Our results suggest that endogenous and synthetic GCs differentially regulated GR and AR at transcriptional and post-transcriptional levels in the rat epididymis. The presence of GR-positive nerve fibers also suggests that GCs might have a role in the neuronal modulation of epididymal functions.

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High-Fat Diet Induced Obese Rats Did Not Alter Quantitative Sperm Parameters in Testis*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Fernandez CDB, Bellentani FF, Nascimento AF, Cicogna AC, Kempinas WG. *High-Fat Diet Induced Obese Rats Did Not Alter Quantitative Sperm Parameters in Testis*. *ARBS Annu Rev Biomed Sci* 2008;10:A45. Obesity is rapidly becoming a worldwide epidemic that affects children and adults, independent of economical and social conditions. Obesity is often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue. Few works in the literature relate obesity and reproductive function. The aim of present work was to investigate eventual reproductive disorders related to obesity in adult male rats. Wistar male rats (30 days old) were randomly divided in two groups that received a standard chow, or a high-fat diet. After 15 weeks of diet exposure, animals were classified in control and obese group. Animals were anesthetized and killed by decapitation, and the right testis, epididymis and vas deferens, ventral prostate and seminal vesicle were removed and their weights (absolute and relative to body weight) were determined. The right testis, decapsulated and weighed soon after collection, were homogenized in 5 mL of NaCl 0.9% containing Triton X 100 0.5%, followed by sonication for 30 seconds. After a 10-fold dilution a sample was transferred to Newbauer chambers (4 fields per animal), proceeding a count of mature spermatids. To calculate daily sperm production (DSP) the number of spermatids at stage 19 was divided by 6.1, which is the number of days these spermatids are present in the seminiferous epithelium. Animals exposed to high-fat diet (has) showed a significant increase ($p < 0.05$) in body weight when compared to animals that received standard chow ($502.31 \text{g} \pm 8.41$ and $472 \text{g} \pm 6.86$, respectively). Besides, fat accumulation was higher ($p > 0.01$) in animals that ate high-fat diet, so this group should be considered as obese animals. Reproductive organ weight, both absolute and relative, did not show any difference between the groups. The number of mature spermatids in the testis and the daily sperm production did not show statistically significant difference between obese and control animals. Until now, we have observed that the obesity did not provoke alterations in some sperm parameters. Other parameters should be evaluated for better conclusions about the effects of obesity in the male reproduction.

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Histological and Stereological Analysis of Epididymis and Leydig Cells in Mice Submitted to Experimental Cryptorchidism and Orchidopexy*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Garcia PV, Arroteia KF, Joazeiro PP, Mesquita SFP, Pereira LAV. *Histological and Stereological Analysis of Epididymis and Leydig Cells in Mice Submitted to Experimental Cryptorchidism and Orchidopexy. ARBS Annu Rev Biomed Sci 2008;10:A46.* Cryptorchidism is a disease in which the testes are retained in the abdominal cavity, resulting in atrophy of testes and epididymis and the interruption of spermatogenesis. Orchidopexy restores testicular spermatogenesis in experimental and clinical procedures, but it is still unclear whether the histological changes in the epididymis can be reverted by orchidopexy. The aim of this study was to use stereological analysis to evaluate the testicular and epididymal caput changes in immature mice following experimental cryptorchidism and to determine whether the alterations could be reversed by orchidopexy. In addition, the number and nuclear volume of Leydig cells and the serum testosterone levels were determined to examine their correlation with the stereological changes in the epididymal caput. In this study we used seventy 15-day-old-mice (C57BL6) that were randomized into control groups; cryptorchidic mice group: mice subjected to uni and bilateral cryptorchidism and sacrificed after 90 days at the age of 105 days and orchidopexic mice group: mice subjected to uni and bilateral cryptorchidism followed by orchidopexy at 105 days of age and sacrificed at 195 days of age. Mice were perfused with 4% paraformaldehyde in 0.2 M phosphate buffered saline (pH 7.4); testes and epididymis were removed and processed followed by embedding in paraffin. The sections were stained with haematoxylin-eosin and examined by light microscopy. All stereological analyses (total volume and area of the epididymis and testes; volume and area of the tissue components of the epididymis and testes; number and nuclear volume of Leydig cells) were done using an image analyzer (Image Pro-Plus) and the serum levels of testosterone were measured using an electrochemiluminescence immunoassay-ECL and evaluated analytically by Modular E170 immunoanalyzer. The measurements were compared statistically using one-way analysis of variance (ANOVA) followed by Tukey's mean comparison test and significance was set at 5%. There was a significant reduction in all testicular and epididymal parameters analyzed in cryptorchidic mice, with a significant recovery in mice submitted to orchidopexy. In general, the parameters analyzed in orchidopexic testes recovered to within 55%-83% of the control values while in the epididymis the values recovered to 79%-100% of the control values. The reduction in the number and nuclear volume of Leydig cells in cryptorchidic and orchidopexic mice associated with normal serum testosterone levels suggested a compensatory mechanism in the activities of Leydig cells. These findings indicate that orchidopexy restores the histological and stereological alterations caused by cryptorchidism; these findings suggested that the testes are more temperature-sensitive than the epididymis caput and post-orchidopexic.

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Histological Assessment of Epididymis of Adult Rats Subjected to Treatment with Different Steroids, Combined or Not to Vitamin Supplement

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Department of Biological Sciences, Faculty of Sciences and Letters/UNESP, Assis/SP, BRAZIL

I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Silva GH, Camargo ICC, Alves RF, Mendes LO. *Histological Assessment of Epididymis of Adult Rats Subjected to Treatment with Different Steroids, Combined or Not to Vitamin Supplement. ARBS Annu Rev Biomed Sci* 2008;10:A47. The Deca-Durabolin® (DD) is considered the most popular androgenic anabolic steroid on the market. Other substance abusively used in Brazil is the Durateston® (D). Such steroids are widely used by athletes or nonathletes, adolescents or adults, in order to increase muscular mass and strength. Usually the use of steroids is associated to use of vitamin supplement, being the L-carnitine (Way 1750)® (L) one of the most popular. The indiscriminate use of steroids promotes several undesirable side effects on reproduction. However, is unknown the effect of association of steroids to vitamin supplement in the morphology of reproductive organs. Aim: To analyze the effects of different steroid treatments, combined or not to vitamin supplement, on the epididymal duct morphology of adult rats. Male rats of Wistar lineage were divided in 8 experimental groups (n=5/group): control (saline solution) and treated with DD, D, DD+D, L, DD+L, D+L and DD+D+L. Each androgen was administered intraperitoneally in a single dose of 7.5mg/kg of body weight, for week, and the vitamin supplement was administered in a single dose of 1mL, for gavage, weekly, all in 8 weeks of treatment. After this period, the animals were weighed, killed and the reproductive organs were dissected. The epididymis were separated from testis and prepared by usual histological routine, obtaining sections of 5µm-thick, embedded in Paraplast®, and stained by hematoxylin and eosin, for analysis in light microscopy. The results demonstrated that there was discreet alteration in epididymal duct morphology of androgenized rats in comparison with control group. In the initial segment, considering the proximal portion of the duct, which is related to absorptive function, presents a high epithelium with long stereocilia and is formed by principal, apical, basal and narrow cells, there was a higher frequency of apical cells in the animal treated with DD and narrow cells in the animal treated with D. In the middle segment, the clear cells were frequently present in the rats treated with D. In the androgenized groups, the interstitial tissue presented leucocytary infiltrated. In the animals treated only with the L supplement, the ductular morphology remained unchanged. The terminal segment, which is concerned with sperm storage, was not affected by treatments, in relation to cellular frequency and morphology of epithelium. However, the density of sperm in the lumen was smaller in the steroid treated groups and apparently similar in the group that received only the L supplement, in comparison to control group. The treatment of adult rats with different steroids combined or not to vitamin supplement decreased the stored sperm density and changed epithelial cells distribution along of the duct.

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IGF-IR Signaling and Its Interaction with the Androgen and Estrogen Receptors in the Ventral Prostate from Diabetic Mice (NOD) Following Hormonal Replacement*

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Abstract

Cagnon VHA, Fávaro WJ. *IGF-IR Signaling and its Interaction with the Androgen and Estrogen Receptors in the Ventral Prostate from Diabetic Mice (NOD) Following Hormonal Replacement. ARBS Annu Rev Biomed Sci 2008;10:A48.* Clinical and experimental studies have shown that diabetes led to harmful morphophysiological effects in the prostate by means of changes on the hypothalamic-hypophyseal-gonadal axis. Then, the aim of this study was to characterize the immunolocalization of the α estrogen, α estrogen, androgen and insulin-like growth factor-I (IGF-IR) receptors from diabetic mice after long-term of glycemical control and hormonal replacement. Moreover, it was intended to establish the possible correlation between diabetes and prostatic disorders. A total of 30 mice were divided into six groups, after twenty days of diabetic state: Control group received 0.01 ml/100 g of body weight dose of vegetal oil subcutaneously; Diabetic group had the same protocols of the control group; Diabetic-Insulin group received 0.2 mL/100 g of body weight dose of insulin; Diabetic-Testosterone group received 5 mg/Kg of body weight dose of testosterone cypionate, diluted in 0.01 mL/100 g of body weight of vegetal oil; Diabetic-estrogen group received 25 mg/Kg of body weight dose of 17 β - estradiol diluted in 0.01 mL/100 g of body weight of vegetal oil; Diabetic-Insulin-Testosterone-Estrogen received insulin, testosterone and estrogen, simultaneously, in the same concentration administrated to the other groups. After 20 days of treatment, all animals were sacrificed and samples from ventral prostate were processed to immunological and hormonal analyses. The results showed decreased serum testosterone levels and the lowest value was found in the diabetic group which grew in the following order Diabetic, Diabetic-Insulin, Diabetic-Testosterone, Diabetic-Estrogen and Diabetic-Insulin-Testosterone-Estrogen groups, as well as its hormonal receptor level. The serum estrogen level and its receptor had the opposite trend in relation to the testosterone. Moreover, the biggest IGF receptor localization was found in the diabetic group. The diabetic state compromised the prostatic hormonal balance, which is a crucial element to the maintenance of the functional activities of this organ. The association between insulin and sexual hormone replacement demonstrated positive features on the recovery of the hormonal imbalance provoked by diabetic state. Moreover, the diabetes could be considered a proliferative factor in the prostatic gland, demonstrated by increased IGF receptor level.

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Immunolocalization of the SP22 Sperm Protein in Japanese Quail (*Coturnix japonica*) - Preliminary Data

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Abstract

Yamato FN, Favareto APA, Nishida SM, Klinefelter GR, Kempinas WG. Immunolocalization of the SP22 Sperm Protein in Japanese Quail (*Coturnix japonica*) - Preliminary Data. *ARBS Annu Rev Biomed Sci* 2008;10:A49. Studies about the function of the sperm protein SP22 in the organism are still being done, but it is already known that exist positive correlation between the quantity of the SP22 and fertility index in mammals (*Biomed Sci* 1999;1:145). *J Androl* 2002;23:48 showed that the localization of this protein in the rat sperm changed in conformity with its maturation. In the rete testis, sperm was immunostained over the cytoplasmatic droplet and modestly over the tail. When the sperm reached the cauda epididymidis, SP22 was localized over the equatorial region of the sperm head. This protein is found in major quantity in the sperm, but was also detected in the female reproductive organs. In avian, no work was done yet. To verify the existence of the sperm protein SP22 and to immunolocalize its expression in Japanese quail sperm. A sexually mature male Japanese quail (7 months old) was decapited and their vasa deferentia were removed for sperm collection. Sperm was dispersed in sperm isolation buffer (SIB) and washed twice by centrifugation (2,000rpm, 5min, 4°C) in SIB with freshly added 0.2mM phenylmethylsulphonyl fluoride. Sperm was fixed (4% paraformaldehyde in Sorenson phosphate buffer) for 5 minutes. After an initial wash in DPBS (2,000rpm, 5min, 4°C), the pellet was resuspended and incubated for 1 hour at room temperature in affinity-purified anti-rSP22 Ig (1:1000). After another wash, the sperm was incubated with FITC conjugated rabbit anti-sheep Ig (1:50) for 1 hour at room temperature. After the last centrifugation, a small aliquot of the sperm pellet was put on a slide and coverslipped with Vector's fade retardant mounting medium. Images were analyzed and captured by fluorescent microscopy (Olympus BX6 1) using Image-Pro Plus® (version 6.0, Media Cybernetics, Silver Spring, MD, USA) software. In the present study, SP22 was identified for the first time in avian sperm. The immunolocalization analysis using anti-rSP22 Ig revealed the presence of the protein and its probable distribution in the head and middle piece of the spermatozoa from Japanese quail, similar to several mammal species (Klinefelter et al., 2002). However, the specific localization of the SP22 needs to be confirmed by sperm cellular morphology studies of the quail, since it is still poorly understood. The confirmation of the presence of SP22 in quail stimulates the investigation of the correlation between this protein and fertility potential of animals of this specie.

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Impairment of Sperm Motility in Rats Exposed to a Mixture of Pesticizers*

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Abstract

Perobelli JE, Martinez MF, Fernandez CDB, Camargo JLV, Kempinas WG. *Impairment of Sperm Motility in Rats Exposed to a Mixture of Pesticizers*. *ARBS Annu Rev Biomed Sci* 2008;10:A50. In Brazil, the National Agency of Sanitarian Vigilance indicated that the local population has been constantly exposed to residues from different pesticides. Differently from experimental animals, in which the studies are done with single substances, humans are only seldom exposed to one chemical agent alone. Even though, few researches pay attention to this exposition to associated chemicals. Aim: To evaluate reproductive toxicity in male rats exposed to a mixture of pesticides at low doses (dichlorvos, dicofol, endosulfan, permethrin, dieldrin). It is important to address the fact that the five pesticides chosen belong to the group of the most detected environmental contaminants in several countries. Sixty male Lewis rats aged 6 weeks, weighing about 200g, were divided into 8 experimental groups: group 1 – negative control (n=8), received basal chow; group 2: low dose (n=10), received basal chow with the pesticides mixture, each pesticide in its NOEL dose for rats; group 3: effective dose (n=12), received basal chow with the pesticides mixture, in a concentration that corresponds to the LOAEL dose for rats to dieldrin and endosulfan, LOEL for rats to dicofol and LEL for rats to dichlorvos and permethrin; group 4 (divided into a, b, c, d, e): positive control, each subgroup of 6 animals was exposed to a chow containing each pesticide individually (dicofol, dichlorvos, permethrin, endosulfan, dieldrin, respectively), in a concentration similar to the effective dose (WHO/FAO, 1970; 1989; 1996, EPA, 1992; 1993). After 8 weeks of treatment, the animals were killed and evaluated by sperm morphology and motility and by germ cells counts in the testes and epididymis, to calculate the daily sperm production (DSP) and the sperm transit time through the epididymis. Data were expressed as mean \pm standard error of mean (SEM) or as median and interquartile intervals. Data were considered statistically significant when $p < 0.05$. There was no significant difference among the groups as to sperm morphology, DSP and sperm transit time through the epididymis. However, the number of motile sperm with progressive movement was significantly decreased in the groups 2, 3, 4a [30.00 (25.00 – 32.75), 27.50 (20.25 – 35.00), 28.00 (16.25 – 41.25), respectively] when compared to the control group 74.5 (56.0 – 77.5). This study has shown that the exposure to a pesticides mixture didn't interfere in spermatogenesis, but impair the sperm maturation without alter the transit time through the epididymis.

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In Utero and Lactational Exposure to Low Doses of Di-n-Butyl-Phtalate (DBP) and Its Effects on Initial Development of Male Litter

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Toledo FC, Guerra MT, Scarano WR, Kempinas WG. *In Utero and Lactational Exposure to Low Doses of Di-n-Butyl-Phtalate (DBP) and Its Effects on Initial Development of Male Litter. ARBS Annu Rev Biomed Sci 2008;10:A51.* Pthalates are esters used in the production of employed plastics in the industry and they are widely found as polluting substances in the environment. Accomplished studies demonstrated that DPB is a poisonous agent to the genital system in males and it has several effects on animals pre and post birth exposed. The objective of the present work is to evaluate the possible effects on the male reproductive function, in rats whose mothers were exposed to the dose of 100 mg/kg of DBP during pregnancy and lactation. Female Wistar pregnant rats were divided in two experimental groups: control (n=9) and treated (n=10). Females from treated group received daily doses of DBP (Sigma Chemical Co., St. Louis, Mo) (100 mg/Kg – o.v.) diluted in corn oil (vehicle), from 12nd day of gestation (DG 12) until the 21st postnatal day (PND 21), while females from control group received only the vehicle. At birth, male pups were weighted. On DPN 4 the anogenital distance (DAG) from male pups was measured with a paquimeter. On PND 13 the presence of areolas/ nipples was registered, with base in the presence or absence of the nipple or of a discoloration of the skin in the toracic area. The day of testicular descent also was determined, in male pups, starting from PND 15. The preputial separation, indicative of the installation of the puberty, was investigated starting on PND 33, and the criterion in met when the prepuce completely retracts from the head of the penis. Results were expressed as mean \pm SEM, and “t” of Student and qui-square statistical tests were used ($p < 0.05$). There was not significant difference between the experimental groups in relation to the corporal weight from male pups at birth (C: 7.30 ± 0.12 ; T: 7.49 ± 0.12). The dose of 100mg/Kg/day of DBP administered to pregnant female rats from DG12 until the end of lactation didn't provoke significant differences in absolute DAG, expressed in mm (C: 3.59 ± 0.05 ; T: 3.46 ± 0.06) and relative to body weight (C: 0.31 ± 0.01 ; T: 0.29 ± 0.01), and in the presence of areolas/nipples between the experimental groups. However, during the critical phase of sexual differentiation, interfered significantly with the age of testicular descent from male offspring (C: 17.44 ± 0.14 ; T: 18.65 ± 0.11 , $pd^*0.05$). There were not significant differences on the medium day of preputial separation (C: 33.29 ± 0.07 ; T: 33.12 ± 0.08). Results obtained until the moment show that DBP provoked a delayed on sexual development from male rats that were exposed during critical period of reproductive system development.

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Male and Female Gerbil Prostate: a Compared View about Structural and Quantitative Aspects between Sexes*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Custódio AMG, Campos SGP, Santos FCA, Rochel SS, Falleiros-Jr LR, Góes RM, Taboga SR. *Male and Female Gerbil Prostate: a Compared View about Structural and Quantitative Aspects between Sexes*. *ARBS Annu Rev Biomed Sci* 2008;10:A52. The prostate is an organ existing in male and female genital system and presents morphofunctional similarity. To evaluate this gland, experimental animals like rodent gerbil (*Meriones unguiculatus*, Gerbilinae: Muridae) can be employed. In this study we characterized the male and female gerbil prostate histological and quantitatively during young, adult and senile ages in order to better compare their biological behavior through androgenic levels of each sex. Sixty gerbils were analyzed being twenty animals for each age: young (1 month), adult (4 months) and senile (18 months). Thus structural (Hematoxylin-eosin), stereological (Weibel's multipurpose graticulate), morphometrical (epithelium height and smooth muscle cells thickness) and androgen serum level characteristics were obtained. The statistical tests were performed with Statistica 6.0 software. The quantitative results were expressed using the mean \pm standard deviation and the ANOVA and Tukey honest test for significance difference ($p < 0.05$). In the young animals the female prostate was composed by canalization alveoli forming a distinct lumen delimited by a developed epithelial compartment while the male prostate is an immature gland composed of same alveoli still in the process of glandular modeling. This difference was confirmed quantitatively with a bigger proportion of female prostate luminal. This morphological characteristic was not maintained in adult and senile female being that in male this compartment was more notable. According to stereological data, the stroma showed the biggest relative volume density in both sexes and ages. However, the smooth muscle layer was thicker around of the male prostate alveoli. In both male and female senil prostates the morphology was altered, showing an increased epithelium and desmoplasia resulting in an atypical tissue remodeling, resembling a histopathological profile. The androgenic level is particular to each ages and sexes modulating the structural features of the gland. The comparison between male and female indicated that there is precocious morphofunctional maturation in female prostate and this fact is probably due to the circulating levels of ovarian and adrenal steroids. The male prostate, however needs a high androgenic level to reach this functional phenotype what occurs only in adult age. On the other hand, the decline of the testosterone level is typical of senescence to both sexes coinciding with histopathological disarrangement. These analyses reinforce the use of this model for the comprehension of glandular morphofunctional aspects with special attention to senescence. Thus, the appreciation of this organ becomes relevant to avoid future discomfort to human health.

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Mechanisms Involved with Reduced Fertility in Streptozotocin-Induced Diabetic Male Rats*

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Abstract

Pontes DA, Fernandes GSA, Assumpção TA, Kempinas WG. *Mechanisms Involved with Reduced Fertility in Streptozotocin-Induced Diabetic Male Rats*. *ARBS Annu Rev Biomed Sci* 2008;10:A53. Diabetes is one of the most widespread diseases in the modern world, rendering many people hyperglycemic and infertile, most of them still in fertile age. Men in this situation usually present symptoms such as reduction of fertility, lack of libido, erectile dysfunction and retrograde ejaculation, but the mechanisms underlying these symptoms are not well established yet. The aim of this study was to evaluate the mechanisms related to the reduction of fertility observed in male rats experimentally rendered diabetic. *Experiment 1* - Male Wistar rats, 90 days old and weighing around 300g, were randomly allocated in 3 experimental groups: diabetic (streptozotocin 40mg/Kg BW), diabetic+testosterone (same protocol, receiving a silastic capsule implant of testosterone for 2 weeks) and control (vehicle). Three weeks after diabetes induction, the rats were killed, body and reproductive organs weights were kept and the following were collected: blood for hormonal assays, and the right testis and epididymis for sperm counts. *Experiment 2* - Another set of animals were used with the same protocol used for experiment 1; male rats were assessed as to sexual behavior parameters, while the left testis and epididymis were collected for histopathological analysis. Diabetic rats demonstrated statistically significant alterations, such as: lack of any parameter of sexual behavior (0%), reduction in plasma testosterone levels (control = 1.91 ± 0.53 ng/dL; diabetic = 0.32 ± 0.09 ng/dL), decreased body weight and epididymis, seminal vesicles, ventral prostate and vas deferens weights, loss of germ cells in the lumen and apparent epithelial disarrange in seminiferous tubules, and accelerated sperm transit time in the epididymis. These results together with the data from *Int J Androl* 2006;29:482-8 and *Anim Reprod* 2006;3:285 demonstrate that diabetic animals have reduced fertility. The data presented herein indicate that the mechanisms underlying the reduced fertility through natural mating observed in diabetic rats involve impairment of the spermatogenic process, as well as a deregulation of the male reproductive axis, together with evidence for problems in sperm maturation. Androgen replacement wasn't totally capable of reversing the damage caused by diabetes on the male reproductive system of adult rats.

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Morphometric Analysis of Seminiferous Epithelium and Daily Spermatic Production in Six Different Dog Breeds*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Soares JM, Avelar GF, França LR. *Morphometric Analysis of Seminiferous Epithelium and Daily Spermatic Production in Six Different Dog Breeds*. *ARBS Annu Rev Biomed Sci* 2008;10:A54. Morphometric analyses of the testis and the characterization of the different stages of seminiferous epithelium cycle in dogs are essential for understanding the reproductive biology in this group of mammals. This study is part of a broader approach, in which testicular parameters of ten different dog breeds (small, medium, standard and large) are being evaluated. The main goal of the present study is to comparatively investigate the structure of the testis, as well as the qualitative and quantitative details of the spermatogenic process in six different breeds of dogs. Animals: mongrel dogs, n = 8; pincher, n = 5; beagle, n = 5; pit bull, n = 9; poodle n = 12; and German shepherd, n = 6. Intratesticular injections of tritiated thymidine were performed in order to determine the duration of spermatogenesis, the testes were orchietomized at different time periods (1 hour, 14 days and 27 days) after injection. The testes were fixed by immersion in 4% buffered glutaraldehyde solution. Testis fragments were then embedded in glycol methacrylate, stained with toluidine blue-borate or PAS, and routinely prepared for histological and morphometrical analyses. An inverse correlation was observed between body weight and gonadosomatic index, whereas the frequencies of the eight stages remained quite similar in the six breeds studied. Data obtained from tritiated thymidine studies indicated that each spermatogenic cycle lasted approximately 13.7 days in mongrel dogs, poodles and beagles, but only approximately 12.9 days in the pit bull breed. There was a tendency for an inverse correlation between Sertoli cell number per gram of testis and Sertoli cell efficiency in all breeds studied. Interestingly, the sperm production was similar in most of the breeds, i.e., mongrel, pincher, beagle, pit bull and German shepherd, but statistically different in poodles. Certain breeds showed significant differences related to some specific parameters. For example, the poodle breed seems to have directed more energy to improve its sperm production, since this breed showed higher numbers of Sertoli cells per gram of testis and higher Sertoli cell and spermatogenic efficiency. On the other hand, breeds like pincher and specially pit bull seem to have invested more in the intertubular compartment, where androgens are produced. Although important, these are preliminary data, and more studies utilizing more breeds are necessary to allow a better understanding of the processes involved in dog spermatogenesis.

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Multiple Effects of Sibutramine on Vas Deferens and Seminal Vesicle Contractility and Its Relationship with Abnormal Ejaculation*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Nojimoto FD, Piffer RC, Kiguti LRA, Lameu C, Camargo ACM, Pereira OCM, Pupo AS. *Multiple Effects of Sibutramine on Vas Deferens and Seminal Vesicle Contractility and its Relationship with Abnormal Ejaculation*. *ARBS Annu Rev Biomed Sci* 2008;10:A55. Sibutramine is an inhibitor of noradrenaline and 5-HT reuptake largely used in the management of obesity. Although a fairly safe drug, postmarketing adverse effects of sibutramine were reported including abnormal ejaculation in men. This study investigates the effects of sibutramine on ejaculation and vas deferens and seminal vesicle contractility. Sexually experienced male Wistar rats received sibutramine (5; 20; or 50 mg kg⁻¹, i.p.) or vehicle and after 60min were exposed to receptive females for determination of ejaculation parameters. The vasa deferentia and seminal vesicles of untreated rats were mounted in isolated organ baths for recording of isometric contractions. Sibutramine 5 and 20 mg kg⁻¹ reduced ejaculation latency by approximately 48% whereas 50 mg kg⁻¹ increased ejaculation latency by approximately 50%. In vitro, sibutramine 3 to 100 μ M increased the basal contractility of the vas deferens. Sibutramine 3 to 30 μ M greatly increased the sensitivity of the seminal vesicle and vas deferens to noradrenaline, but at concentrations higher than 10 μ M there were striking depressions of maximal contractions. Depending on the doses, sibutramine either facilitates or inhibits ejaculation. Apart from its actions in the central nervous system, facilitation of ejaculation may result from augmented sensitivity of smooth muscles to noradrenaline and to membrane depolarisation while the delayed ejaculation observed with high doses of sibutramine may result from the reductions of intracellular Ca²⁺ concentrations.

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Quantification of SP22 Protein in Ram Sperm*

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Abstract

Favareto APA, Rodello L, Monteiro CD, Taconeli CA, Bicudo SD, Klinefelter GR, Kempinas WG. Quantification of SP22 Protein in Ram Sperm. *ARBS Annu Rev Biomed Sci* 2008;10:A56. Several studies have shown that the SP22 sperm protein is highly correlated with fertility. Recently, we immunolocalized the expression of SP22 on the equatorial segment of the ram sperm head and neck. This localization on the equatorial segment of the gamete head is similar in several species, and suggest the involvement of the protein on spermatozoa-oocyte interaction during fertilization process. Thereby, the quantification of SP22 can bring important data about fertility potential of the ram. The aim of this study was to quantify SP22 protein on ram sperm by ELISA and FITC immunostaining using anti-rSP22 Ig, and to determine the correlation between these methods. Semen was collected from mature Dorper (n=8) and Santa Inês (n=10) rams by an artificial vagina. Sperm were isolated from the fresh semen in Sperm Isolation Buffer. For immunocytochemistry analysis, isolated sperm were fixed (4% paraformaldehyde in Sorenson phosphate buffer) and incubated for 1 hour in anti-rSP22 Ig (1:200). After this, the sperm were incubated with FITC conjugated rabbit anti-sheep Ig (1:50) for 1 hour. Images were captured by fluorescent microscopy (Olympus BX61). SP22 staining of the equatorial segment of the sperm head was quantified using Image-Pro Plus® (version 6.0, Media Cybernetics, Silver Spring, MD, USA) software. For SP22 quantification by ELISA, proteins of the isolated sperm were extracted with 80 mM OBG in 10mM Tris and PMSF. Prior to ELISA, each extract was concentrated with 1mM Tris buffer, pH 7.2, by centrifugations in Ultrafree-4 centrifugation filter units. Protein concentration was determined using Bradford reagent and sample volumes containing 30µg protein were lyophilized. SP22 ELISA was performed as described by Kaydos et al. (2004). For comparison of results between the breeds, it was utilized Student t test. To determine the correlation between the SP22 quantification methods it was performed linear regression analysis. Differences were considered significant when $p < 0.05$. The ELISA values for SP22 were significantly correlated with the SP22 values determined by SP22 immunostaining analysis ($r^2 = 0.64$), validating this method. The equation for the regression line was $y = 1.07 - 12.45x + 182.74x^2$, where y is equal to SP22 immunostaining and x corresponds to ELISA. There was no significant difference between the breeds in SP22 levels accessed by ELISA or SP22 immunostaining ($p > 0.05$). Financial Support: FAPESP (06/54103-1).

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Rat Fetal Testis Histopathological Analysis after Phthalate Exposure from Gestation Days 12 to 20*

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Abstract

Scarano WR, Toledo FC, Guerra MT, Kempinas WG. *Rat Fetal Testis Histopathological Analysis after Phthalate Exposure from Gestation Days 12 to 20. ARBS Annu Rev Biomed Sci* 2008;10:A57. Certain Phthalate esters have been shown to produce reproductive toxicity in male rodents, in an age dependent way, being fetal animals more sensitive than neonates, which are in turn more sensitive than pubertal and adult animals. While testicular effects of phthalates in rats have been known for more than 30 years, recent attention has been paid to the ability of these agents in producing effects on the reproductive development of the male offspring following in utero exposure. The objective of this work was to evaluate the toxicity of the phthalate on the morphology of male Wistar rat fetuses' testes submitted to the Di-n-butyl-phthalate (DBP - 100 mg/kg) exposure from gestation days 12 to 20. Pregnant females were obtained through natural mating, being distributed in two experimental groups: Control and Treated. The females of the treated group received DBP (100 mg/Kg, by gavage) from gestation days (GD) 12 to 20, while the control group received only the vehicle (corn oil). On GD20, the females were anesthetized and submitted to laparotomy. The male fetuses were selected (2 per female) and the testes were fixed in Karnovsky solution, submitted to the historesin inclusion routine and H&E staining. The histopathological evaluation was accomplished in an Olympus microscope and the histological sections were subjected to the stereological analysis. After the histopathological analysis, clusters of Leydig cells in the interstitial compartment and the presence of germinative multinuclear cells in the seminiferous tubules were observed in the treated group. An increase in the relative volume of the interstitial component related to the seminiferous tubular component was detected through the stereological analysis ($C = 32.3\% \pm 4.5$ vs. $T = 47.6\% \pm 5.6$, $p < 0.05$). DBP at the administered dose presents effects on the rat fetal testicular structure, agreeing with the results obtained in treatments using high doses of DBP (> 250 mg/Kg).

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Rat Germ Cells Can Colonize Tilapia Seminiferous Tubules and Form Elongated Spermatids: Evidences of the Fish Testis Functional Plasticity*

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Abstract

Lacerda SMSN, Batlouni SR, Resende FM, Silva RC, França LR. *Rat Germ Cells Can Colonize Tilapia Seminiferous Tubules and Form Elongated Spermatides: Evidences of the Fish Testis Functional Plasticity*. *ARBS Annu Rev Biomed Sci* 2008;10:A58. Germ cell transplantation is a fascinating technique that has been largely utilized in the past decade in mammals, aiming to investigate spermatogenesis and the germ stem cell biology. Although germ cell transplantation is well characterized in rodents, there are very few studies utilizing this approach for lower vertebrates. In this regard, a pioneer study involving germ cell transplantation in teleost fishes, using the Nile tilapia as an experimental model, was previously developed in our laboratory. In this former study, we successfully demonstrated the donor spermatogenesis development into transplanted tilapia recipient testis. Therefore, after we standardized the technique of germ cell transplantation in tilapia, our main objective in the present study was to investigate the viability of adult tilapias as a recipient model for germ cell transplantation using donor spermatogonias from vertebrates belonging to other classes. In this way, we utilized Wistar rats as the germ cell donor. The depletion of endogenous spermatogenesis in tilapias was successfully performed with two busulfan injections [18mg/kg/BW and 15mg/kg/BW (two weeks after the first injection)] in animals kept at 35°C. The germ cells to be transplanted were obtained from ten days old Wistar rats that had their testes enzymatically digested with hyaluronidase, collagenase, trypsin, and DNase. Spermatogonial germ cells were selected and enriched utilizing percoll gradient and differential plating. These cells were labeled with PKH26 (Red Fluorescent Cell Linker; Sigma) and mixed with a trypan blue solution to check the transplantation efficiency. Four adult tilapias received through the common spermatic duct a total of $\sim 10^7$ donor germ cells. Recipient fishes had their testes analyzed by light, fluorescence microcopies, 4, 5 and 7 weeks post-transplantation in order to investigate the eventual presence of rat germ cells in the tilapia seminiferous tubules. Four and five weeks post-transplantation rat spermatogenesis was identified in the tilapia testes by light microscopy. Rat spermatogenic differentiation following transplantation ranged from colonization to progression through meiosis with formation of elongated spermatids. In addition, the fluorescence microscopy analysis revealed the presence of PKH26 labeled germ cells seven weeks post-transplantation. Therefore, these surprising findings strongly suggest that the interactions between germ and testicular somatic cells are at a certain extent very flexible and functionally competent among species belonging to different vertebrate classes, even if they present a distinct pattern of spermatogenesis arrangement.

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Relaxin: an Autocrine/Paracrine Regulator of the Male Reproductive tract?*

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Abstract

Cardoso LCC, Nascimento AR, Siu ER, Lazari MFM. *Relaxin: an Autocrine/Paracrine Regulator of the Male Reproductive Tract? ARBS Annu Rev Biomed Sci 2008;10:A59*. Relaxin is a 6 kDa peptide, structurally related to insulin, with well known functions in the female reproductive tract (Endo Rev 25:205, 2004). The development of relaxin knockout animals revealed that relaxin is important for male reproduction as well (Lab Invest 83:1055, 2003). Relaxin null mice had decreased sperm maturation, increased collagen and decreased prostate epithelial proliferation. Our laboratory has previously identified the mRNA for the relaxin receptor RXFP1 in several tissues of the reproductive tract of the male rat (Reprod Biol Endocrinol 2007, 5:29), including testis, epididymis, vas deferens, seminal vesicle and prostate. In females, relaxin is produced by the ovary and uterus. In men relaxin is produced by the prostate and released to the semen. In other animals, testis (shark) or seminal vesicle (boar) may be the main source of the hormone. Information about the relaxin source in the male rat is controversial. mRNA has been detected in testis and prostate, while immunohistochemistry not always confirmed the expression of the protein. The aim of the present study was to analyze the expression of relaxin throughout the reproductive tract of the male rat and to identify a possible paracrine/autocrine role for relaxin by analyzing the co-expression of relaxin and its receptor. Tissues (uterus, testis, epididymis, vas deferens, ventral and dorso-lateral prostate, coagulating gland, seminal vesicle, kidney and adrenal) were removed from 120-day old rats. The presence of mRNA for relaxin was detected by RT-PCR. Total RNA was extracted with TRIzol and first cDNA synthesis was carried out with the Thermoscript cDNA synthesis kit (Invitrogen). GAPDH was the internal control. Expression of relaxin was assessed by Western blot, using an anti-relaxin antibody from Santa Cruz Biotech (N-16) that recognizes an epitope close to the aminoterminal region of the relaxin precursor. RT-PCR detected the transcript of the expected size for relaxin in uterus, caput and cauda epididymis, vas deferens, ventral prostate, coagulating gland, seminal vesicle and adrenal, but not in kidney and dorso-lateral prostate. Relaxin mRNA in testis was not always detected. Preliminary studies identified relaxin-like immunoreactivity in all the tissues except kidney and adrenal. The strongest reaction was seen in uterus, seminal vesicle, epididymis and vas deferens. The detected protein band had the correspondent size of the relaxin precursor (20 kDa), suggesting that the tissues may produce rather than only store the hormone. Analysis of the co-distribution of relaxin and RXFP1 transcripts indicates that testis, epididymis, vas deferens, seminal vesicle and prostate are potential tissues for a paracrine and autocrine action of relaxin. This may affect several events in male reproduction, such as spermatogenesis, fluid composition, extracellular matrix organization and apoptosis.

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Reproductive Aspects of Male Rats Exposed to Altered Perinatal Corticosteroid Levels*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Piffer RC, Garcia PC, Oba E, Rubio EM, Pereira OCM. *Reproductive Aspects of Male Rats Exposed to Altered Perinatal Corticosteroid Levels*. *ARBS Annu Rev Biomed Sci* 2008;10:A60. Corticosteroid administration has been widely employed for the obstetric treatment of pregnant women at risk of preterm birth. It is uncertain whether there is a correlation between high maternal corticosteroid levels and endanger of the testosterone peak in the critical period of brain sexual differentiation of male rats. To investigate the effects of prenatal betamethasone exposure on maternal and male pups corticosterone levels, immediately after delivery, and the long-term effects on the wet weight of reproductive organs, fertility, and sperm quality. Pregnant rats received (i.m.) 0.1mg/kg of Betamethasone (Sigma Co., USA) or saline on days 12, 13, 18, and 19 of pregnancy. Data were compared by Student's *t*-test (mean±SEM) or Mann-Whitney test [median (IQ₁-IQ₃)], n=10/group, **p*<0.05. Even though exposure to betamethasone did not alter the wet weight of adrenal glands of mothers after delivery, it led to increased corticosterone levels (323.75±39.52/461.70±23.24*, ng/ml). In male pups, the prenatal treatment with betamethasone led to reductions in the wet weight of the adrenal gland (1.83±0.13/1.32±0.08*, mg) and in corticosterone levels (83.61±7.04/58.92±7.58*, ng/ml) immediately after birth. In adulthood, the adrenal weight was unchanged in these pups. However, there were reductions in the corticosterone levels (339.53±28.32/210.36±33.65*, ng/ml), wet weight of testis (1.83±0.04/1.70±0.04*, g), and vesicular secretion (0.44±0.03/0.36±0.01*, g). For the fertility test, adult male rats were housed in a large cage with fertile untreated female rats (2 females/male). After 15 days of cohabitation, 80% of males exposed to betamethasone were able to mate, however the females mated with these males showed, on the 21st day of pregnancy, an increased rate of post-implantation loss [8.33 (8.33-11.11)/30.77 (25.00-42.86)*], reduced rates of implantation [100.00 (92.59-100.00)/86.98 (79.49-100.00)*] and fetal viability [100.00 (96.88-100.00)/75.00 (60.91-88.93)*], when compared with the control group. Prenatal exposure to betamethasone also altered sperm quality in vas deferens by significantly reducing sperm concentration x10⁶ (778±55.61/570±43.74*), and also the percentages of live [97.00 (97.00-98.00)/96.00 (94.25-96.00)*], mobile [33.50 (27.50-40.00)/28.00 (16.25-28.75)*], and morphologically normal spermatozoa [98.00 (97.00-99.00)/94.00 (93.00-95.00)*]. These results suggest that increased maternal corticosteroid levels induced by the prenatal treatment impaired the hypothalamus-pituitary-adrenal axis in male pups until adulthood and may have decreased endogenous testosterone levels during the critical period of brain sexual differentiation of male rats, subsequently impairing the sperm quality and capacity to generate viable descendants.

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Sperm Associated Antigen 11C (SPAG11C) Expression and Its Regulation by Surgical Castration and *In Vivo* LPS in Adult Rat Epididymis*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Queiróz DBC, Silva EJR, Honda L, Avellar MCW. Sperm Associated Antigen 11C (SPAG11C) Expression and Its Regulation by Surgical Castration and *In Vivo* LPS in Adult Rat Epididymis. *ARBS Annu Rev Biomed Sci* 2008;10:A61. The epididymis promotes maturation, storage and transport of the sperm from testis to the vas deferens. The Sperm Associated Antigen 11 (SPAG11) protein family was found to play an important role in epididymal innate immunity in addition to its role in sperm maturation. One of its isoform, SPAG11C, is highly secreted by epididymal epithelial cells and act as a potent antibacterial agent *in vitro*. Our aim was to gain insights in the regulation of this protein. Thus, the expression of Spag11C in the epididymis from rats surgically castrated or challenged *in vivo* with an infectious agent (lipopolysaccharide, LPS) was evaluated. 90-day old Wistar rats were sham operated or surgically castrated for 7 or 15 days. In another set of experiments, rats were injected with LPS from *E. coli* (1 mg/kg, i.v.) or sterile saline (control) and sacrificed 2h or 24h after treatment. The epididymis was removed, divided into caput (CP) and cauda (CD) regions. The tissues were frozen, embedded in tissue freezing medium and cryostat cut (8 µm) for immunohistochemical studies, using antibody specific to SPAG11C. Co-localization experiments with microtubule-associated protein 1B (MAP 1B, neuronal marker) antibody were performed by immunofluorescence. Immunohistochemical assays (n=3) indicated SPAG11C staining in the nuclear and cytoplasmic compartments of epithelial cells from both CP and CD regions. The results also indicated immunostaining of smooth muscle cells of the epididymal tubules and blood vessels, as well as in few interstitial nerve fibers in both epididymal regions. Co-localization experiments by immunofluorescence indicated that the SPAG11C-positive fibers were also immunostained by the MAP 1B antibody. LPS treatment for 2h (n=3) decreased the staining in the nuclei of epithelial cells in CP, an effect reverted after 24h of treatment to the immunostaining pattern observed in control tissue. In CD, 2 h LPS significantly decreased the staining detected in smooth muscle cells, an effect still observed after 24 h of treatment. While surgical castration for 7 and 15 days (n=3) induced a significant decrease in the smooth muscle staining in both CP and CD, the staining in the nuclei of epithelial cells decreased in CD 15 days postcastration. All immunostainings were significantly decreased when experiments were performed with the antibody previously incubated with specific blocking peptide. The results reveal a segment-specific regulation of SPAG11C expression in the rat epididymis by androgen and LPS treatment. We also show evidence that the expression of this protein is not restricted to epididymal epithelial cells, suggesting additional functions besides antibacterial activity.

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Spermatogonial Stem Cells Transplantation: Ultrastructural Evaluation of Lipid Bodies within Macrophages*

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Garcia¹, Gleydes Gambogi Parreira¹

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Dias FF, Melo RCN, Chiarini-Garcia H, Parreira GG. *Spermatogonial Stem Cells Transplantation: Ultrastructural Evaluation of Lipid Bodies within Macrophages*. *ARBS Annu Rev Biomed Sci* 2008;10:A62. The germ cell transplantation in mammals is a powerful technique providing great advances in infertility treatment and genetic improvement of agricultural species (*Biol Reprod* 1998;59:1360). However, the injection of donor cells seems to generate a local inflammatory process, specially represented by an influx of induced macrophages (*Tissue Cell* 1999;31:242). A distinguishing feature of inflammatory macrophages is the formation of cytoplasmic, non-membrane-bound lipid bodies (LBs) (*Tissue Cell* 2003;35:59), organelles involved in generating inflammatory mediators (*Pharmacol Ther* 2007;113:30,). The structure and function of testicular macrophages after germ cells transplantation are poorly characterized, as well as, the presence of LBs and their intracellular interactions within those cells had never been documented. Here, we have investigated the ultrastructure of testicular macrophages and LBs formed within these cells, after the spermatogonial stem cell transplantation. Donor mouse testicular cells were isolated and the cellular suspension was injected in sterile WCB6F1 W^v/W^v mouse seminiferous tubules (*Int J Dev Biol* 1997;41:111). Receptor animals were sacrificed at different times of transplantation to visualize the events occurred up to establishment of spermatogenesis. Testes fragments were collected by perfusion technique and fixed in a mixture of cacodylate-buffered 5% glutaraldehyde, post-fixed in reduced osmium and processed for evaluation by transmission electron microscopy (TEM). Migration of macrophages around the tubule was observed during different times post-transplant. Monocyte-like cells (testicular clear-cells) were visualized at the peritubular layer after 1 week of transplantation. At 1 month of transplantation, macrophages showing typical morphology could be seen in the seminiferous epithelium. After 3 months of transplantation, macrophages were seen phagocytosing sperm at the lumen of the tubule. Quantitative analyses performed in 4 distinct regions of the testicular tubule showed a significant increase of macrophage numbers within the epithelium and lumen. LBs were identified in both infiltrating macrophages and Sertoli cells. There was a significant increase of LB numbers within these cells after 1 to 3 months of transplantation. LBs showed a regular or irregular surface and were seen in close association with other organelles, including phagolysosomes and rough endoplasmic reticulum, as previously described by us (*Inflamm Res* 2006;55:342). In conclusion, we have demonstrated, for the first time, an increased LB formation within infiltrating macrophages induced by germ cells transplantation, a finding that may be related to phagosome maturation or formation of inflammatory mediators.

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Stereological Investigation of the Testis and Spermatogenic Cycle Length in Five Wild Rodent Species*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Cordeiro-Júnior DA, Costa GMJ, Talamoni SA, França LR. *Stereological Investigation of the Testis and Spermatogenic Cycle Length in Five Wild Rodent Species*. *ARBS Annu Rev Biomed Sci* 2008;10:A63. The Atlantic Forest is considered one of the most diverse and threatened biome in the world. The knowledge of reproductive biology and physiology is critical for conservation and species management, allowing also to prevent species extinction, and to utilize males in natural and artificial reproductive programs. Spermatogenic cycle length is a biological species-specific constant which is controlled by the germ cell genotype. Approximately 4.5 spermatogenic cycles are necessary in mammals for the completion of spermatogenesis, from type A stem spermatogonia up to the releasing of spermatozoa in the tubular lumen. The main objectives of the present study was to investigate the testis structure and to estimate the duration of spermatogenesis in five wild rodent species largely distributed in the Natural Reserve of Caraça located in the State of Minas Gerais, Brazil. Intraperitoneal injections of tritiated thymidine were performed at different time periods in order to determine the duration of spermatogenesis. The different stages of the cycle of seminiferous epithelium were characterized according to the acrosomic system. The testes were perfused-fixed in 4% buffered glutaraldehyde, embedded in plastic (glycol methacrylate) and routinely processed for histological and stereological analyses of the testis. The results found for *T. moojeni*, *O. nigripes*, *A. cursor*, *A. montensis* and *B. lasiurus* were, respectively: A) seminiferous tubules volume density (%): 97 ± 0.8 ; 96 ± 1 ; 96 ± 0.7 ; 97 ± 0.6 e 96 ± 0.4 . B) The duration of spermatogenic cycle and the total duration of spermatogenesis: 8.7 ± 0.2 and 39.3 ± 0.8 ; 7.7 ± 0.1 and 34.6 ± 0.2 ; 8.4 ± 0.2 and 37.8 ± 0.9 ; 8.4 ± 0.2 and 37.8 ± 0.9 and 7.8 ± 0.1 and 35 ± 0.5 days. C) number of round spermatids per Sertoli cell (Sertoli cell efficiency): 15.5 ± 1 ; 8.4 ± 0.2 ; 10.9 ± 0.8 ; 10.3 ± 0.7 ; 13.2 ± 0.5 . D) Daily sperm production per gram of testis (spermatogenic efficiency): 90 ± 6 ; 76 ± 6 ; 66 ± 4 ; 65 ± 4 e 80 ± 5 (million). These results showed that the volume density of seminiferous tubules found for all species are among the highest observed for the mammalian species already investigated. The duration of spermatogenesis, particularly for *O. nigripes*, was one of the shortest found for mammals investigated up to date. Mainly due the high percentage of the seminiferous tubules in the testis parenchyma and the short duration of spermatogenesis, the spermatogenic efficiency observed for the five species investigated in the present study was very high.

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Study of the Effects of β -glucan in Reproductive Performance of Male Mice Treated with Cyclophosphamide

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Kanno TYN, Sensiate LA, Lourenço ACS, Faria MJSS. *Study of the Effects of β -glucan in Reproductive Performance of Male Mice Treated with Cyclophosphamide*. ARBS Annu Rev Biomed Sci 2008;10:A64. The β -glucans are extracted mainly from fungus and cereal and they are described for their antioxidant proprieties. This polysaccharide is considered a functional food because they can control and modulate organic functions. The cyclophosphamide is a chemoterapic widely used in the treatment of cancer. This substance is related with lots of mutagenic and teratogenic effects. The objective of the present work was evaluating the effects of β -glucans related to the morphophysiological alterations caused by cyclophosphamide in the reproductive parameters of male mice. Male mice (*Mus musculus*) were randomly distributed in 8 experimental groups: Group 1 received PBS 0.1mL/10g of body weight (b.w.) for 3 consecutive days; Group 2 received cyclophosphamide 50mg/kg p.c. (b.w.) once in a week, Groups 3 to 5, received β -glucans for 3 consecutive days, in 100, 150 and 200mg/kg b.w. doses respective and the Groups 6 to 8, received β -glucan for 3 consecutive days with the doses above mentioned and one dose of cyclophosphamide in second day treatment. The animals received treatment for 6 weeks and at the fifth they were mated with female mice. Pregnancy was determined by the detection of a vaginal "plug", that was considered zero day of gestation. The female was euthanized by cervical dislocation and submitted to laparotomy at 17^o gestation day to verify fertility, implants number, post-implantation losses, and resorption /lethal dominant frequency. The data were analyzed by ANOVA followed by Tukey test. According to the statistic analysis, for the parameters fertility, number of implants and fetal viability it was not verify significant difference. As the same way, it was not observed differences for fetal weight and length. To verify damage in germinative cells, a test frequently used is the dominant lethal assay. This test is widely used to test mutagenic substances. In mating, the presence of implants sites is used as a criterion of insemination success. In this implants, the number of resorption is an indicative of genomic lesions that modify the genic expression taking the fetuses to death. Cyclophosphamide plus β -glucans do not cause alterations in the fertility and number of implants; however, cyclophosphamide increased the number of resorption. Higher incidence of pos-implantation losses and resorptions was verified on Groups 2 (41.20 ± 21.05 and 41.20 ± 21.05), and 8 (45.81 ± 31.56 and 45.81 ± 31.56) when they are compared with group 1 (10.41 ± 6.64 e 11.97 ± 6.15). β -glucans in dose 100mg/Kg administered in group 6 was more efficiently to prevent resorptions (34.76 ± 2.34), thus, it increased the viability. It was observed a percent reduction in damage of 46.46% in this group. The present findings indicate that β -glucan administered in adequate concentrations is capable to prevent damages on germinative cells. Therefore, it becomes a strong candidate for use on genetic damage prevention.

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Testicular Morphometrical Evaluation in the Adult Rats Treated with Different Steroids Combined or Not to Vitamin Supplement*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Alves FA, Camargo ICC, Silva GH, Mendes LO. *Testicular Morphometrical Evaluation in the Adult Rats Treated with Different Steroids Combined or Not to Vitamin Supplement. ARBS Annu Rev Biomed Sci 2008;10:A65.* The nandrolone decanoate commercial Deca-Durabolin is an anabolic utilized indiscriminately by youth and adults to increase physical strength and endurance. Other steroid very utilized is the Durateston, formed by combination of four compounds of testosterone. In the sports environment, usually these steroids are utilized simultaneously and combined to vitamin supplement, promoting some side effects. To analyze the effects of treatment simultaneous of steroids Deca-Durabolin (DD) and Durateston (DT), with the L-carnitine vitamin supplement (LC) on the testicular morphometrical parameters in adult rats. Forty adult rats of Wistar lineage were divided in eight experimental groups (n=5/group): control (CT) (saline solution) and treated with DD, DT, DD + DT, LC, DD+LC, DT+LC, DD+DT+LC. Each androgen was administered weekly, in a single dose of 7.5 mg/Kg of body weight, intraperitoneal, during eight weeks. The vitamin supplement was administered weekly, in a single dose of 1 ml, for gavage, in the eight weeks of treatment. The testicular sections of 5 µm- thick, included in Paraplast, were stained by hematoxylin and eosin for morphometric analyses in light microscopy. Ten transversal sections/animal/groups were measured by computational system Image Pro-Plus version 4.0. The results were evaluated by Kruskal-Wallis variance analysis, complemented with Dunn test at 5% significance level. The values of tubular area and major tubular diameter were significantly decreased in the groups treated with steroids, combined or not to vitamin supplement, in comparison to control groups. The minor tubular diameter was decreased in the androgenized groups, with exception of the DT group. The treatment with LC decreased significantly the minor tubular diameter (Table 1). The androgenic treatments associated or not to vitamin supplement were capable of to promote seminiferous tubular atrophy.

Table 1. Morphometrical results in rat testis in different experimental groups.

Experimental Groups	Major tubular diameter (µm)	Minor tubular diameter (µm)	TUBULAR ÁREA (µm ²)
CT	333.87 ± 29.44 a	233.10 ± 21.85 a	64349.11 ± 8420.68 a
DD	249.60 ± 21.86 b	212.41 ± 25.91 b	41781.27 ± 7137.86 b
DT	251.27 ± 37.54 b	226.56 ± 34.04 ab	44130.22 ± 13699.32 bd
DD+DT	296.22 ± 32.58 c	202.15 ± 23.75 bef	50801.49 ± 11838.66 fd
LC	259.08 ± 27.72 b	167.49 ± 39.68 c	32729.29 ± 11543.95 ce
DD+LC	256.27 ± 39.45 b	213.74 ± 44.60 bd	40287.54 ± 13692.11 b
DT+LC	261.20 ± 33.09 b	187.63 ± 31.70 cde	40472.44 ± 6299.98 be
DD+DT+LC	284.97 ± 40.01 c	186.61 ± 35.95 cf	40304.87 ± 11275.24 b

Medians (± interquartile deviation) with at least a same letter in the same column are statistically equal to each other (p<0.05).

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Testicular Damage Induced by Carbamazepine in Different Sexual Development Phases*

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Abstract

Oliva SU, Miraglia SM. Testicular Damage Induced by Carbamazepine in Different Sexual Development Phases. ARBS Annu Rev Biomed Sci 2008;10:A66. Carbamazepine (CBZ) is a first-line antiepileptic drug (AED), although it is also utilized for treatment of psychiatric disorders and neuropathic pain. The CBZ utilization has been associated with male reproductive tract damage, including hormonal alterations, sexual dysfunction and reduction of sperm quality. The wide and long use of CBZ is a common schedule in children and adolescents and alters the testosterone level in adult rats and humans. In addition, hypothalamic-pituitary-gonadal (HPG) axis during the pre-puberal and puberal period is more susceptible to toxic agents than in adult phase. The aim of this work was to evaluate the CBZ side effects on the spermatogenic process of rats from the pre-puberty until sexual maturation. Sixty 23-day-old male albino Wistar rats were distributed into three controls groups (n=10; C43, C63 and C93) and three CBZ-treated groups (n=10; CBZ43, CBZ63 and CBZ93). CBZ-treated rats received CBZ diluted in propylene glycol (20 mg/Kg/i.p). Daily treatment occurred during 20, 40 and 70 days, according to the different phases of rat sexual maturation. Control animals only received propylene glycol. At the end of each different treatment periods, left testes were removed and fixed by immersion in Bouin's liquid, for 48 hours. Testicular fragments were included in Paraplast Plus. The 5 µm sections were stained with HE or submitted to the Periodic Acid-Schiff (PAS) and counterstained with Hematoxylin. Histopathological, morphological and stereological analyses were achieved using image analysis system (Leica Qwin, Cambridge, England). Plasmatic levels of LH, testosterone and estradiol were also measured. Increase of volume density and volume of lymphatic space indicative of the interstitial edema, reductions of the volume density and volume of the seminiferous epithelium, Sertoli cell and germinal lineage cell alterations, large quantity of cellular debris and sloughed germ cells at various phases of maturation filled the seminiferous tubule lumen and intraepithelial vacuolization, reductions of testosterone levels and increase of estradiol levels were observed in the rats that were CBZ-treated since the weaning. Lower testosterone levels observed in puberty and adult CBZ-treated rats maybe have happened due to the CBZ negative effect on Leydig cell function. Although alterations of plasmatic testosterone and estradiol level may be partially responsible by the testis damage, the possible direct effects of CBZ on germinal lineage cells and Sertoli cells should be also considered because this drug are highly lipid soluble and presumed to cross the blood-testis barrier into the seminiferous tubules. The preliminary results obtained suggest that CBZ administered since pre-puberty provokes gradative and specific side effects in rat testes, resulting in more intensive damage in the adult phase.

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The Effects of Different Temperatures on the Duration of Spermatogenesis and the Investigation of Preferential Location of Early Spermatogonia in Zebrafish*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Nóbrega RH, Schulz RW, França LR. *The Effects of Different Temperatures on the Duration of Spermatogenesis and the Investigation of Preferential Location of Early Spermatogonia in Zebrafish*. *ARBS Annu Rev Biomed Sci* 2008;10:A67. The zebrafish (*Danio rerio*) is a small tropical freshwater teleost native to India. During the last decade, it has become an important vertebrate model in scientific research due to several advantageous characteristics such as: external fertilization with high number of progeny, easy handling, genomic sequence available and easily traceable development of transparent embryos. In this context, this specie, that reproduces at the physiological temperature of 27-28°C, is a suitable model for studies involving biology of reproduction. However, there are still few data in the literature related to testis function and spermatogenesis for this teleost. Investigate the effects of different temperatures on the duration of spermatogenesis and also to evaluate the distribution of early spermatogonia in the seminiferous tubules at the physiological temperature. For this purpose, sexually mature males were kept at the following temperatures: 20°C (n=15), 27°C (n=12), 30°C (n=7), and 35°C (n=12) for at least one week. After this period, they received one single intracoelomatic injection of ³H-thymidine (2μCi/g), and were sacrificed at approximately 2h, 12h, 1, 2, 3, 4, 5, 6 and 7 days after thymidine injection. The testes were removed, weighed, and routinely prepared for histological, morphometric and autoradiographic evaluations. The position of early spermatogonia was recorded as being: 1) in contact to the interstitium or 2) in contact to other tubules. The position of 500 early spermatogonia were evaluated per each animal (n=5) and expressed as percentage. To normalize this value and determine if the distribution of early spermatogonia is random or not, we stipulated a ratio between the length of each tubular region (contacting interstitium or other tubule) and the total tubular perimeter using Image J (NIH). The combined duration of meiotic and spermiogenic phases of spermatogenesis was 2, 4 and 6 days at 30°C, 27°C and 20°C, respectively. At 35°C, the spermatogenic process apparently did not progress beyond the first meiotic division and more apoptotic germ cells and seminiferous epithelium composed only by Sertoli cells were observed. The topographical distribution of early spermatogonia was found to be preferential and 76% of these cells were localized near to the interstitial area of the seminiferous tubules, which comprises only 1/3 of the total tubular perimeter. Considering the few teleosts investigated up to date, the results found showed that the duration of spermatogenesis in zebrafish is comparatively very short in all temperatures evaluated. Similar to recent studies in mammals, early spermatogonia in zebrafish has a preferential location in the seminiferous tubules area facing the interstitial compartment.

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Vitamins C and E Reduce Oxidative Stress and Incidence of Morphological Alterations in Sperm of Hyperglycemic Adult Male Rats*

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Abstract

Fernandes GSA, Assumpção TA, Silva CF, Campos KE, Damasceno DC, Kempinas WG. Vitamins C and E Reduce Oxidative Stress and Incidence of Morphological Alterations in Sperm of Hyperglycemic Adult Male Rats. ARBS Annu Rev Biomed Sci 2008;10:A68. Hyperglycemia is associated with impairment of the reproductive system function, eventually leading to reduced male fertility. Its etiology may involve oxidative damage by reactive oxygen substances, and protection against this damage can be offered by antioxidant supplementation. The aim of this study was to investigate the antioxidant effects of oral administration of vitamin C and/or E on the reproductive system of hyperglycemic adult male rats. Adult male rats (90 days old; n=10/ experimental group) received a single dose of streptozotocin (40mg/kg BW) and were divided in four hyperglycemic experimental groups: hyperglycemic control group, hyperglycemic group treated with 150mg of vitamin C diluted in 0.5mL of water; hyperglycemic group treated with 100mg of vitamin E diluted in 0.5mL of corn oil, and hyperglycemic group treated with 150mg of vitamin C plus 100mg of vitamin E for 30 consecutive days. The normoglycemic group received only the vehicles (0.5mL of water plus 0.5mL of oil). At the end of this period the rats were killed and the following were analyzed: body and reproductive-organs weights, germ-cell counts in the testis, sperm morphology classified as to head (without curvature or isolated form) and tail abnormalities (broken, isolated or rolled into a spiral), and blood biomarkers of oxidative stress (malondialdehyde – MDA, superoxide dismutase – SOD, glutathione peroxidase – GSH-Px and reduced glutathione – GSH). Differences were considered significant when $p < 0.05$. There was a significant reduction ($p < 0.05$) of the biomarkers of oxidative stress in the hyperglycemic groups treated with vitamin C and/or E in comparison to the hyperglycemic group, being equal to the normoglycemic group. There was a significant reduction ($p < 0.05$) in the body weight and weights (absolute and relative to body weight) of the testis, seminal vesicle (full and empty), prostate and epididymis absolute weight of hyperglycemic animals in comparison to the normoglycemic. Vitamin C and/or E reduced ($p < 0.05$) the number of morphological alterations in sperm, when compared to the hyperglycemic group, being equal to the normoglycemic group. There were no significant differences in the daily sperm production and in the sperm counts in the testis. In these experimental conditions, vitamins C and E worked as antioxidants reducing the oxidative stress and, as a result of that, diminished the number of morphological alterations found in sperm of hyperglycemic adult male rats.

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The Calcium Influx Induced by Progesterone is Stimulated by PKA Activation in Human Sperm Exposed to the Papaverine, a Phosphodiesterase Inhibitor*

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I Workshop on Male Reproductive Biology, 05-07 November 2007

Abstract

Jiménez-Trejo F, Torres-Flores V, Neri-Vidaurre PC, Calderón-Salinas V, Molina-Guarneros J, González-Martínez MT. The Calcium Influx Induced by Progesterone is Stimulated by PKA Activation in Human Sperm Exposed to the Papaverine a Phosphodiesterase Inhibitor. ARBS Annu Rev Biomed Sci 2008;10:A69. Progesterone induces a fast transient calcium influx in human sperm through the activation of non genomic receptors. During sperm capacitation, a complex process required for sperm to be able to fertilize the egg, the calcium influx induced by progesterone is enhanced. Sperm capacitation is mediated by an increase in cAMP content and the subsequent PKA activation. In this work we examined the effect of increasing intracellular cAMP on the calcium influx induced by progesterone in non capacitated human sperm. Sperm was exposed with the phosphodiesterase inhibitor papaverine for 5 minutes, a treatment that increased both the cAMP content and the PKA activity several-fold. Human sperm was obtained from a panel of 12 healthy volunteers with 3-6 days of sexual abstinence. Sperm cells ($100-200 \times 10^6$) were separated from the seminal plasma (isotonic percoll gradients (75/50 % percoll)). Ejaculates were normal according to the World Health Organization protocol. The cells were loaded with $5 \mu\text{M}$ fura ff in 2 ml HHSM for 40 minutes at 36°C . The fluorescence was detected at 488 nm with an optical filter (Andover Corp., Salem, NH), alternately exciting at 340 nm and 380 nm, at 0.83 Hz. The 340/380 fluorescence ratios were converted to intracellular calcium. The cAMP was measured with the Cyclic AMP Competitive EIA kit from Zymed Laboratories (South San Francisco, Ca) The activity of de PKA in sperm extracts was measured as the incorporation of ^{32}P from $\text{ATP-}^{32}\text{P}$ to a kemptide substrate, provided by an up-state kit (Lake Placid, NY). Detection of tyrosine phosphorylated proteins by western blot. The calcium influx induced by progesterone was increased by papaverine to levels close to those found in capacitated sperm. This effect was partially inhibited by H89 (45 %) and by genistein (42 %) and the sum of both inhibitors reduced the stimulating effect of papaverine by 89 %. The inhibitory effect of gensitein on the progesterone-induced calcium influx could be related to its capability to inhibit the papaverine-stimulated increase in cAMP content and PKA activity. The calcium transport mechanism activated by progesterone is directly stimulated by PKA activation and indirectly, by the activity of tyrosine kinases.

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The Human Sperm, a Neuroendocrine Cell: Presence of Serotonergic Components*

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Abstract

Jiménez-Trejo F, Méndez-Martínez B, Gutiérrez-Ospina G, González-Martínez MT. The Human Sperm, a Neuroendocrine Cell: Presence of Serotonergic Components. ARBS Annu Rev Biomed Sci 2008;10:A70. Neuroendocrine cells (NE) are neuroectoderm-derived cells that have the ability of taking up, storing, and releasing both indolamines and catecholamines. Diverse neural markers (neuronal proteins and neurotransmitters), have been described in peripheral organs and in the human spermatozoa. Exocytosis in NE and sperm is essential to the functions of these cells and is strongly influenced by similar receptors. 'Neuronal' receptor types in sperm may also play a role in the control of sperm motility in the acrosomal reaction. One important molecule for neural systems is serotonin (5-HT). Synthesized through a metabolic pathway in which the limiting step is catalyzed by enzyme tryptophan hydroxylase. 5-HT are involved in males reproduction and in invertebrates 5-HT participates in sperm motility and fertilization capacities. With this in mind, the aim of our work was evaluate the presence of some components of the serotonergic system in human sperm, using both HPLC and immunodetection techniques. Human sperm was obtained from a panel of 6 healthy volunteers with 3-6 days of sexual abstinence. Sperm cells ($100-200 \times 10^6$) were separated from the seminal plasma. Ejaculates were normal according to the World Health Organization protocol. We detecting tryptophan hydroxylase, and serotonin receptors $5HT_{1B}$, $5HT_{2A}$, $5HT_3$ and tyrosine phosphorylated proteins too by immunocytochemistry and western blot. Chromatographic analyses attempt documented the presence of the activity of tryptophan hydroxylase in human sperm. Human sperm displayed immunoreactivity to $5HT_{1B}$, $5HT_{2A}$, and $5HT_3$ serotonin receptors, labeled positive for enzyme AA-NAT. Western blot analyses sperm homogenates documented the presence of some immune markers. HPLC analyses documented the presence of the activity of tryptophan hydroxylase in the human sperm ($n=10$) $0.2715 \pm 0.052 \text{ nM/mgprotein/hr}$. In the other hand, sperm treated with different drugs relation with serotonergic system change to expression by tyrosine phosphorylated proteins ($\sim 106 \text{ kDa}$). The presence of markers of serotonergic system could be modified the sperm motility by molecular and biochemical alteration of the physiology in human sperm. The activity of tyrosine kinases (directly stimulated by PKA activation), may be dependent to different pharmacological agents related with serotonin system. These results suggest a functional relationship between serotonergic components and the human sperm. We concluded with a series of questions that remain unanswered and that may be a guide for future research.

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