Advances in goat artificial ovary

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Background

The majority of thousands of oocytes in the ovaries are small, non-growing and reside in preantral follicles (PFs). The development of a culture system for preantral follicle may be very useful for understanding the complex mechanism in folliculogenesis at early stages of development as wells as could offer a significant way for the propagation of livestock, including goats. The *in vitro* follicular culture aims to mimic what happens with a few pre-ovulatory follicles, which escapes from atresia and ovulate. For this reason, this technique is also known as "artificial ovary". This abstract describes the results of a number of studies aimed to evaluate the effects of several substances on *in vitro* culture of caprine preantral follicles highlighting the many advances, limitations and prospects.

Review

Caprine PFs are usually cultured either in ovarian cortical slices or after isolation. Although IVC (*in vitro* culture) of PFs enclosed in cortical slices is practical, non-time-consuming, maintains three dimensional follicle architecture and preserves interactions between follicles and surrounding stroma cells, the cortical tissue may act as a barrier to IVC medium perfusion. Conversely, IVC of isolated PFs allows monitoring of individual follicles throughout the growing period, but is time-consuming, may be affected by the isolation procedure, demands a more sophisticated IVC system and is often applied to secondary and not to primordial and primary follicles. In general, several studies with farm animals and primates have successfully shown the activation and transition of primordial follicles to primary stages during *in vitro* culture of ovarian cortical slices. However, using these mammalian models primary follicles do not grow to secondary stages. Despite that, we succeeded to overcome this problem using an appropriate concentration of growth differentiation factor-9 (GDF- 9). Indeed, Martins *et al*. (2008) demonstrated that GDF-9 (200 ng/ml) maintained the survival of PF and promoted activation of primordial follicles. Furthermore, GDF-9 stimulated the transition from primary to secondary follicles, maintaining ultrastructural integrity of the follicles. Despite a small number of publications on IVC of isolated caprine PFs, promising results such as maintenance of follicular survival, follicle and oocyte growth, antrum formation, meiosis resumption and development of embryos after IVC of oocytes enclosed in caprine PFs were achieved (Saraiva *et al*., 2010). The main results obtained by our research group (for review see Figueiredo *et al*., 2010) after 18-day *in vitro* culture of isolated goat preantral follicles are as follows: follicular survival (89.7%), antrum formation (100%), growth rate (25.5 μm/day), recovery rate of oocyte ≥110 μm (77.1%), meiosis resumption (78.1%), nuclear maturation-MII (29.4%) and embryo production (n = 4).

Conclusions

The basic culture system for the *in vitro* culture of caprine PF which maintains follicular survival is well established. Primordial follicle activation and their further growth up to secondary stage *in vitro* were achieved. Isolated primary follicles can growth up to antral stage although this rate is still low. Antrum formation and fully grown oocytes were obtained from *in vitro* culture of large secondary follicles even yielding a few mature oocytes and embryos. At present, the key challenge for researchers is to increase the rates of maturation and *in vitro* production of embryos from goat oocytes enclosed in PF grown *in vitro*, specially from very early stages, in order to produce, in the future, large number of offsprings.

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Equine preantral follicles: ultrasound-guided Biopsy Pick-Up method to harvest ovarian tissue for *in vitro* processing, evaluation and culture

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Since preantral follicles are of great abundance in mammalian ovaries and the vast majority (>99.9%) will never become ovulatory, the ability to rescue these otherwise wasted follicles seems very appealing. Considering there are striking similarities in antral follicle dynamics between women and mares (Ginther *et al.*, 2004, 2005; Baerwald, 2009; Gastal, 2009, 2011; Ginther, 2012), the mare might become a good model to study early folliculogenesis in women, with several advantages related to using an animal model. Cryopreservation and/or *in vitro* culture of preantral follicles could potentially serve many purposes, such as large-scale embryo production from individuals with high genetic merit, conservation of rare or endangered species and preservation of fertility in humans whose preantral follicle population has been jeopardized. The success of these technologies is completely dependent upon understanding the specific mechanisms that regulate follicle and oocyte growth and development. The amazing benefits that could potentially be provided through *in vitro* culture of preantral follicles make the understanding of early folliculogenesis a top priority research area.

During the past 10 yr, the field of ovarian folliculogenesis has seen a large amount of focus placed on the study of preantral follicles. Although there is limited knowledge of the mechanisms that control preantral follicle dynamics, researchers are beginning to understand how preantral follicles undergo activation and growth through studies on *in vitro* culture. Obtaining material to be used for *in vitro* culture can be difficult, mainly because for several species, the only sources of preantral follicles are slaughterhouse or ovariectomized ovaries. This problem is even more amplified in mares, especially in the United States where accessible ovarian tissue is very scarce due to the closing of all equine abattoirs in 2007. Hence, a transvaginal, ultrasound-guided ovarian biopsy procedure that would allow for the repeated collection of preantral follicles *in vivo* could be very beneficial in providing material for the study of *in vitro* ovarian follicular dynamics (Lass *et al.*, 1997; Aerts *et al.*, 2005).

Recent studies in our laboratory validated the use of a transvaginal, ultrasound-guided ovarian Biopsy Pick-Up (BPU) method to harvest preantral follicles using the mare as a model to study early folliculogenesis. The following general findings were obtained: 1) the BPU method provided sufficient material for the study of early folliculogenesis in mares; 2) preantral follicle quantity, morphology and viability did not differ according to phase of the estrous cycle; 3) number of follicles, but not follicle morphology or viability, was greater for younger *versus* older mares; 4) rate of atresia generally increased with follicle class; 5) proportion of primordial to primary follicles was higher for histological *versus* tissue chopper analysis, even though overall follicle morphology rates were similar; 6) rate of morphologically normal follicles, follicle viability and follicle class proportions were similar between follicles from *in vitro* BPU and scalpel blade dissected fragments; 7) number of follicles per mg of tissue was similar within animals between tissue chopper and histological analyses; 8) preantral follicles submitted to an *in vitro* culture system appeared to respond positively by undergoing activation and growth during a 7 day culture period, with some follicles remaining morphologically normal; and 9) α-MEM$^+$ seemed to be superior to TCM-199$^+$ as a base culture medium for equine preantral follicles.

In conclusion, the results from our recent studies validated the transvaginal, ultrasound-guided BPU method as a way to harvest ovarian fragments from mares containing large numbers of morphologically normal and viable preantral follicles for the study of early folliculogenesis in the equine species. The ovarian fragments can be submitted to histology or processed using a tissue chopper for further analysis of the preantral follicles. The fragments can also be submitted directly to *in vitro* culture, where follicle growth and development can be achieved after 7 days of culture in α-MEM$^+$ medium. Finally, the BPU method can be used to repeatedly harvest large numbers of primordial and primary follicles from equine ovaries without jeopardizing short-term reproductive function. Successful *in vitro* culture and cryopreservation systems for this species might make the BPU method a feasible technique to provide material to enable the utilization of oocytes within the abundant preantral follicles present in the equine ovary, many of which are essentially wasted *in vivo* through inactivation or atresia during development. In the future these technologies could potentially provide a means for the preservation of genetic material from valuable individuals or for large-scale embryo production.

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Morphology shines a bright light on mechanisms of testicular toxicity: a tribute to Lonnie Russell

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Every generation invents new technologies and pursues science in ways previously not available. Lonnie Russell (Fig. 1) lived during the zenith of the 'Ultrastructure' period, at a time when the transmission electron microscope (TEM) was just becoming mainline technology in the research laboratory. Of course, TEM is now just another tool of the biological sciences and one for which fewer students receive training. However, during Dr. Russell's life, TEM was one of the most significant inventions for which Ernst Ruska received the 1986 Nobel Prize in Physics. Lonnie's first manuscript in 1973 (Russell and Gardner, 1973) was titled, "Ultrastructure of the pseudohermaphrodite rat testis." More than half of his 167 publications contained data obtained using the TEM. Electron micrographs were one of Lonnie's scientific passions, as he collected many thousands of TEM negatives, which he carefully catalogued during his career, starting with the heavy glass plates and then later the thin photographic films. Lonnie also enjoyed the techniques involved in TEM: fixation, embedding in plastic resins, ultramicrotomy, sitting at the TEM in the dark and taking the photographs, and even the darkroom development of the negatives and printing of images, which he also catalogued in numerous file cabinets by research project. In collaboration with John Bozzola, they wrote one of the finest technique books on the subject (Bozzola and Russell, 1998).

Morphology obtained by brightfield microscopy and immunohistochemistry, as well as TEM, provides an essential starting point for understanding the mechanisms of testicular pathogenesis observed after exposure to various toxicants and in cases of gene deletion that result in testicular anomalies. It is interesting that the second manuscript to be published by Lonnie was an investigation of testicular ultrastructure in a case of genetic mutation (Russell and Gardner, 1974). Therefore, he was among the first group of scientists to use morphology, especially TEM, to help explain testicular failure associated with male infertility.

Figure 1. Dr. Lonnie Russell in 2000 at the Boston meeting of the American Society of Andrology. Lonnie Russell was Professor in the Department of Physiology at Southern Illinois University School of Medicine in Carbondale. He received a B.S. in zoology from the University of Tulsa in 1967, an M.S. in Medical Sciences from the University of Oklahoma in 1969, and a Ph.D. in Anatomy from the University of Nebraska in 1974.

Of particular interest to me was the early work of Lonnie on microtubules of the seminiferous epithelium. In 1977, he reported microtubule linkages to an important Sertoli cell organelle that he named 'ectoplasmic specialization' (ES; Russell, 1977). In 1981, he published a detailed study showing that microtubule disrupting agents caused sloughing of germ cells along with portions of apical cytoplasm from the Sertoli cells (Fig. 2). After reading this paper and others on the cytoskeletal elements involved in spermiation (Russell *et al*., 1981, 1989), my understanding of the Sertoli cell-spermatid relationship and the importance of microtubules allowed me to begin a 15 yr study of Benomyl, a microtubule poison, which not only induces testicular atrophy, but also results in the obstruction of efferent ductules (Hess and Nakai, 2000). Benomyl is a common fungicide that disrupts microtubule

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formation and at first it was thought that its effects on male reproduction would be an inhibition of spermatogonial mitosis, which is dependent upon microtubules for the organization of chromosomes. However, it was not established that this mechanism would cause testicular atrophy. Subsequent studies then revealed that the first event following exposure to Benomyl was germ cell sloughing (Fig. 3) with attached Sertoli cell cytoplasm (Hess *et al*., 1991; Nakai and Hess, 1994; Nakai *et al*., 2002), similar to that seen with colchicine (Russell *et al*., 1981). Sloughing was found to be stage-dependent, with the most susceptible stages being those with elongated spermatid heads residing closer to the tubule lumen (Fig. 4). The mechanism proposed for such selective sloughing was the involvement of vimentin intermediate filaments that could help hold the ES and attached spermatid heads near the Sertoli cell nucleus (Vogl *et al*., 2008; Upadhyay *et al*., 2011) but would be absent as the elongated spermatids moved toward the lumen (Fig. 4). The sloughed conglomerate of cells then became occluded in the small efferent ductules (Fig. 5) that carry sperm from the rete testis to the epididymis (Hess, 2002).

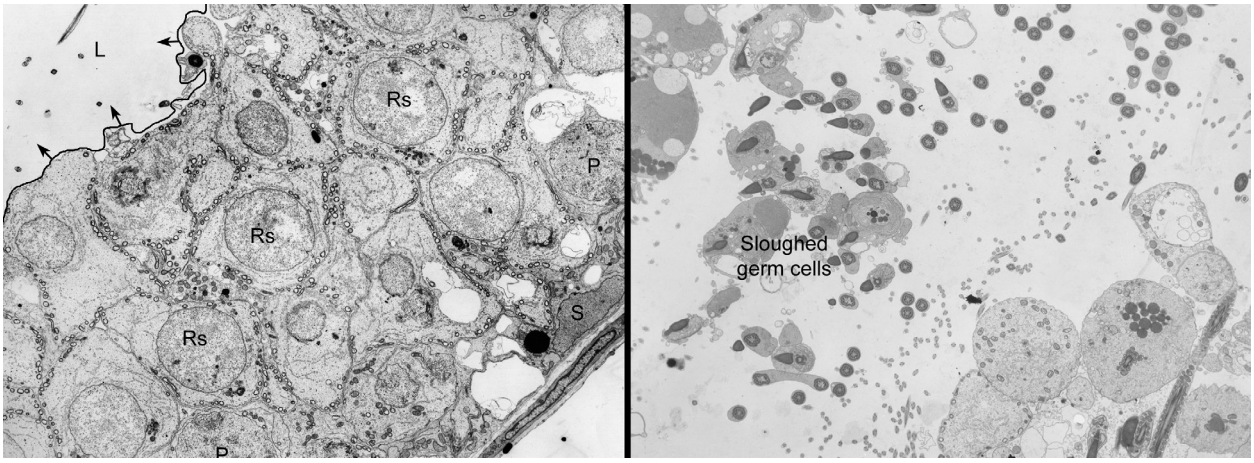

Figure 2. Transmisson electron micrographs from the Lonnie Russell collection. a) Stage VII tubule showing area where elongated spermatids have sloughed (black line with arrows) into the lumen (L) after exposure to colchicine. Rs, round spermatid; S, Sertoli cell; P, pachytene spermatocyte. b) Sloughed germ cells are found in the lumen along with normal cross sections of sperm.

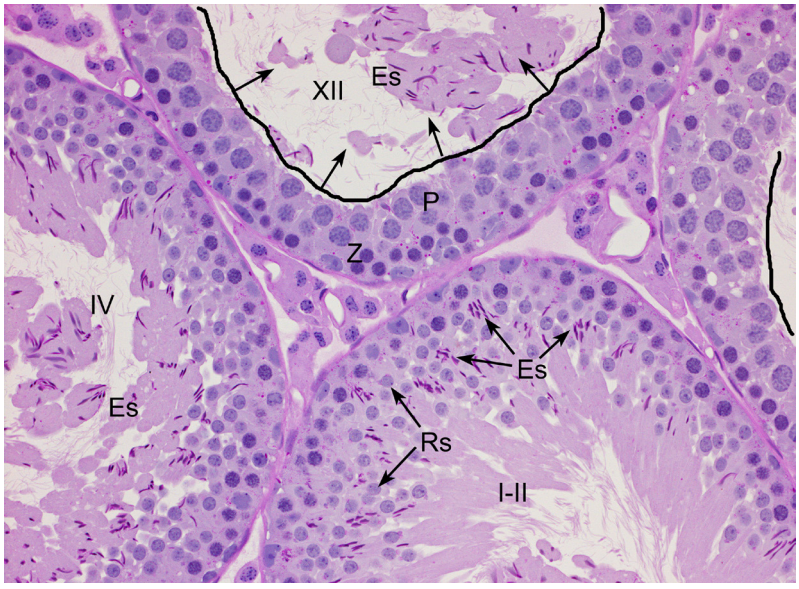

Figure 3. Rat testis exposed to Benomyl for 4 h (Nakai *et al*., 1992). Note sloughing (line with arrows) of elongated spermatids (Es) in Stage XII but not in Stages I-II. Elongated spermatids in Stage IV are starting to move toward the lumen and appear to be starting to slough, whereas those in Stages I-II are intact. Rs, round spermatids; P, pachytene spermatocytes; Z, zygotene spermatocytes.

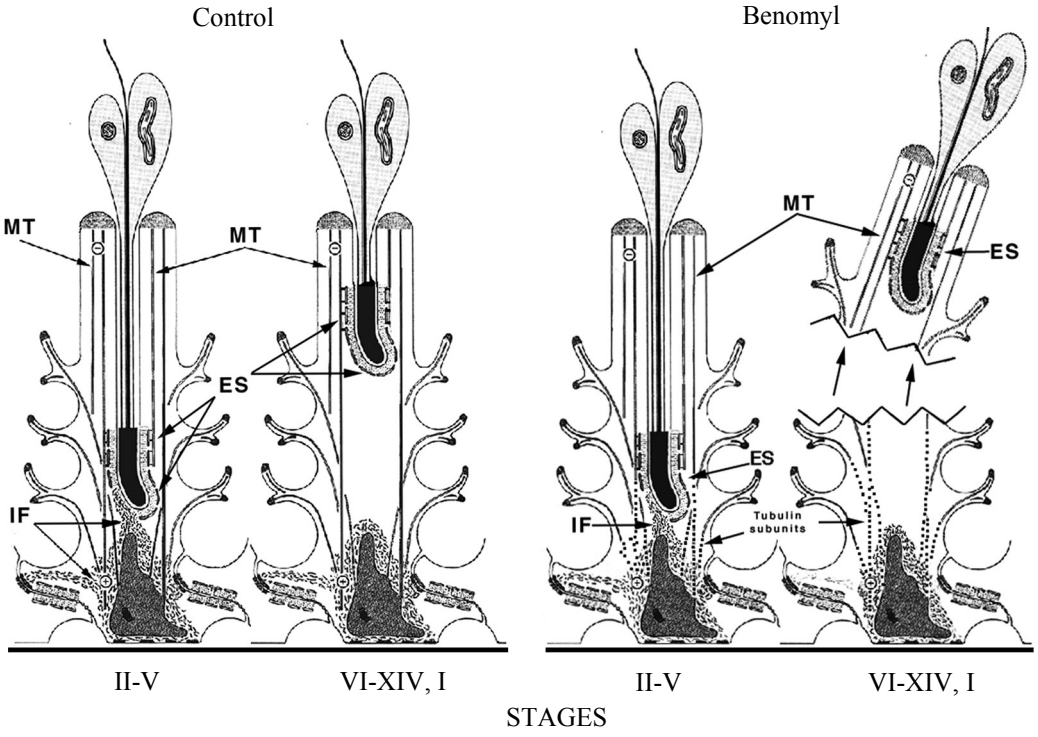

Figure 4. Drawing to depict the relationship between elongated spermatid/ectoplasmic specialization (ES) and microtubules (MT) and intermediate filaments (IF; vimentin) in the Sertoli cells of the rat. In Stages II-V, where elongated spermatid heads penetrate deeper within the epithelium along MT tracts, the IF surrounding the Sertoli cell nucleus attach to the ES. In Stages VI-XIV and I, the elongated spermatids are extended near the lumen. It is hypothesized that following Benomyl exposure, which prevents microtubule formation, Stages lacking IF attachments to the ES will be more susceptible to sloughing of the elongated spermatids.

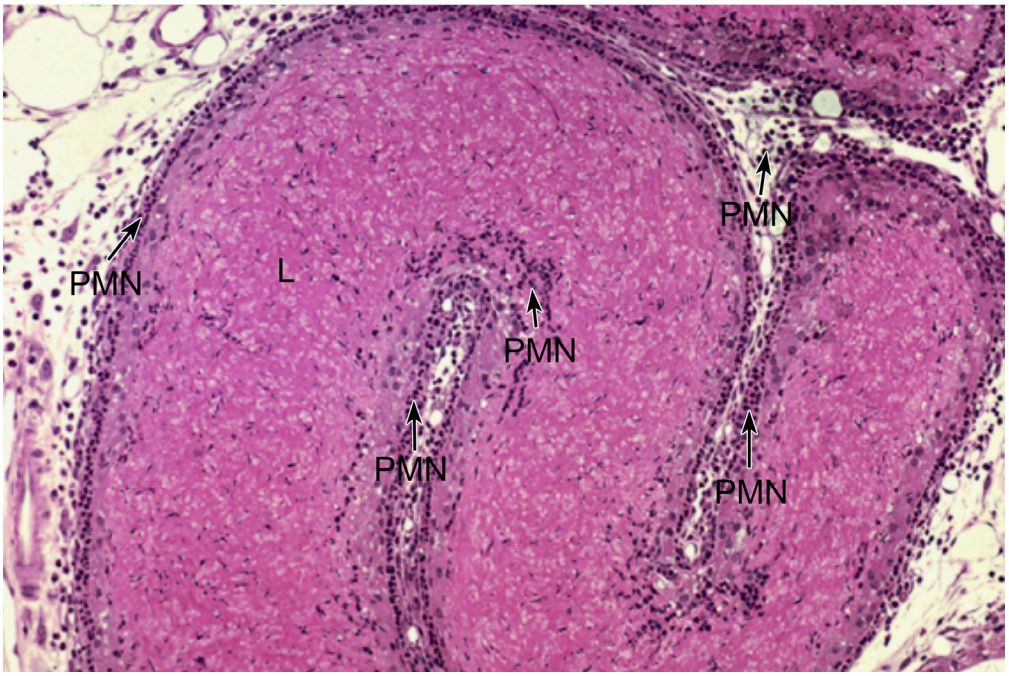

Figure 5. Rat efferent ductule 24 h after exposure to Benomyl (Nakai *et al*., 1992). The lumen (L) is completely obstructed with sloughed germ cells and dilated. Neutrophilic polymorphonuclear leukocytes (PMN) surround and penetrate the epithelium.

Atrophy of the testis after exposures to the fungicide Benomyl was determined to be caused not by microtubule effects in the testis, but rather back pressure of fluid accumulation after occlusions in the efferent ductules (Nakai *et al*., 1992). The seminiferous epithelium continues to release sperm and fluid for several hours after obstruction or impairment of fluid reabsorption in the efferent ductules, resulting in the buildup of luminal fluid volume and increased intratesticular pressure (Hess *et al*., 1997; Hess and Nakai, 2000). We have never discovered the biochemical mechanism by which Benomyl interferes with efferent ductule function. However, it appears to induce more rapid fluid reabsorption, thereby compacting the sloughed germ cells within the lumen (Hess and Nakai, 2000). We speculate that the disruption of apical microtubules in the ductal epithelium may interfere with apical membrane recycling and maintenance of the microvillus structure, where the fluid and ion transport systems are localized (Hess, 2002). Regardless of the mechanism of ductal occlusion, the significance of this study was the discovery that testicular atrophy was ultimately caused by the accumulation of fluid and back pressure within the testis, which adds to our understanding of testicular pathogenesis, because there are important differences in efferent ductule anatomy between rodents and larger mammals, including humans (La *et al*., 2012).

Science is a continuum, one discovery built upon another. I had long admired the manuscripts published by Lonnie Russell, which gave me the focus for understanding why germ cells slough in testes of animals exposed to Benomyl, which lead to the discovery that testicular atrophy was caused by efferent ductule obstruction. This last discovery also provided the foundation for an important breakthrough in our understanding of estrogen's function in the male reproductive system. After development of the estrogen receptor-α knockout mouse (*Esr1*KO), it was noted that there were dilations of the rete testis (Fig. 6) and seminiferous tubule lumens (Lubahn *et al*., 1993). Because my laboratory had observed the effects of Benomyl on efferent ductules and rete testis, we hypothesized that loss of *Esr1* would also induce efferent ductule occlusions. However, morphological examination of the *Esr1*KO mouse revealed dilation of efferent ductules and back pressure atrophy of the testis without ductal obstruction (Hess *et al*., 1997). Estrogen was found to be responsible for expression of several genes that maintain ion and fluid reabsorption by the ductal epithelium, the first reported function of *Esr1* in the male reproductive tract (Hess *et al*., 2002, 2011; Carreau and Hess, 2010).

Lonnie Russell saw science through the eyes of photographs. I too have follow in his footsteps because photography is also my passion and morphology seems to shine a bright light on the mechanisms associated with testicular pathogenesis. While Lonnie had an enormous enthusiasm for life outside of science, it is possible that the microscope gave him the most fun and enthusiasm within science.

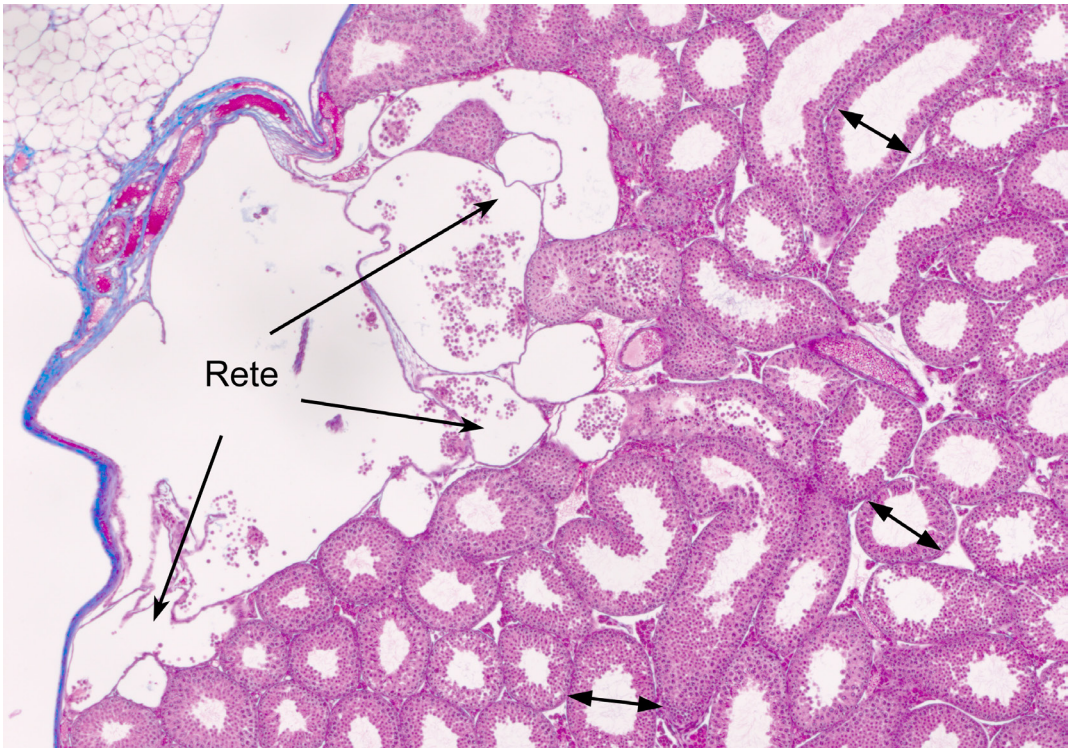

Figure 6. *Esr1* knockout mouse showing dilated rete testis and seminiferous tubule lumens.

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Searching the killer: new factors contributing to the deletion or prostate epithelial cells

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Androgen regulation of prostate physiology is complex. The absence of prostate in response to the complete androgen insensitivity and the regressive changes attained by androgen deprivation make it intuitive an all or nothing regulatory mechanism. This is reinforced by the urgent need of controlling prostate cancer progression.

Our laboratory is engaged in finding strategies to deal with prostate diseases and one major goal is to understand prostate development and physiology, trying to identify molecular targets that can become useful for treating both benign prostate hyperplasia (BPH) and prostate cancer (PCa).

PCa is a leading cause of death in males, second only to skin cancer. As a matter of fact, with reducing the number of deaths due to cardiovascular diseases and extended lifespan, cancer in general is currently becoming prevalent cause of death (64 and 186 per 100,000 deaths in 1900 and 2010, respectively; Jones *et al*., 2012) Nonetheless, BPH, a major cause of suffering in males, affects much younger men (24% of the prostates in men between 41 and 50 yr) and hits twice as many individual (86% of the prostates in men between 81-90 yr; Bostwick *et al*., 1992).

Along the years, we have been examining aspects of prostate development and regression after castration. Initial research was dedicated to understand the plasticity of smooth muscle cells and how extracellular matrix was remodeled in parallel to epithelial shrinkage (Carvalho and Line 1996; Carvalho *et al*., 1997a, b; Vilamaior *et al*., 2000, 2005; Antonioli *et al*., 2004, 2007; Corradi *et al*., 2004; Garcia-Florez *et al*., 2005; Felisbino *et al*., 2007; Augusto *et al*., 2008; Taboga *et al*., 2008).

Current interests range from the mechanisms of ductal branching and canalization in early development, extracellular matrix components, remodeling enzymes and control over cell behavior, smooth muscle cell differentiation and plasticity, androgen receptor expression and signaling, epithelial cell apoptosis, cancer cell homing to the bone marrow, prostate immunology and IgA transcytosis. Molecules of interest are the androgen receptor itself, matrix metalloproteinases, heparanase-1, heparan sulfate and heparan-sulfate binding growth factors and chemokynes, clusterin and ps20/WFDC1.

For the sake of conciseness, we have chosen to explore a series of results related to the identification of novel genes, the expression of the which is turned on in the androgen deprived environment achieved by orchyectomy and propose a mechanism of hierarchical expression of master regulatory genes which coordinate a series of events related to the altered physiology and gland shrinking, involving cell-cell communication and cell survival mechanisms besides apoptosis pathways.

Factors contributing to prostate weight loss are the elimination of the accumulated secretion in the lumen, a reduction in the synthetic machinery, reduced blood flow, smooth muscle cell atrophy, and epithelial cell death by apoptosis. More recently, we found that cell desquamation is an early an relevant event contributing to epithelial cell deletion (Rosa-Ribeiro and Carvalho, 2012; personal communication) We have reported that gland regression is continued for as long as 100 days after castration (Antonioli *et al*., 2007) and that affecting the epithelial apoptosis rate promoted similar reduction in the epithelial volume 7 days after castration (Garcia-Florez *et al*., 2005). We hypothesized that stromal changes would be necessary for allowing further reduction of the epithelium. A follow up on the apoptosis rate and the expression of matrix metalloproteinases (MMPs) led us to discover that a second peak of apoptosis takes place at day 11 after castration and is preceded by a remarkable increase in MMP-2, -7 and -9 expression and activity at day 10. Blocking of MMP activity using pharmacological inhibitors resulted in a 50% reduction in the rate of apoptosis at day 11, suggesting a causative relationship (Bruni-Cardoso *et al*., 2010). This mechanism was somewhat similar to that published for the post-weaning mammary gland involution (Lund *et al*., 1996).

This peak activity of MMPs at day 10 and the subsequent peak of apoptosis at day 11 suggested us that sequential events occur in the prostate following the sudden drop of circulating and tissue concentration of the androgens testosterone and dihydrotestosterone (Kashiwagi *et al*., 2005).

Interest on gene expression in response to castration is seen in a series of publications which led to the identification of androgen-repressed and androgen-stimulated genes. Genes were identified first by their relative abundance before and after castration and then by mass analyses. We became particularly interested on a study by Desai *et al*. (2004). They have examined the differences in gene expression by DNA microarray in the ventral and dorsolateral prostate and found 1456 genes and EST differentially expressed in the rat ventral prostate and 256 genes in the dorsolateral prostate. Their analyses demonstrated a significant variation in PTEN (phosphatase and

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tensin homologue) and an inflammatory/immune signature demonstrating the presence of different immune system cells in the prostate gland, particularly T-cell, macrophages and mast cells. In spite of the presence of these cells appearing in increasing amounts in the prostate after castration, it is perfectly clear that the regressive changes and the epithelial cell death is tolerogenic, at least under normal conditions, as no overt inflammation is observed in association with the organ shrinkage. Exploring this would however drive us in another direction.

We then decided to examine the genes in Desai's data for the presence of transient expression of genes involved with transcription regulation, including new genes annotated thereafter. Bioinformatics brought about 20 genes, which are under validation in laboratory. Immunohistochemistry showed that the product of one such gene was specific to stromal cells and another specific to scattered cells in the epithelium. The others showed broad expression in both compartments and in immune system cells. In particular, members of the NFκB family showed variable expression in cells in each compartment.

In line with our interest in finding new factors that could be involved with the regulation of gene expression in the androgen-deprived environment in the prostate gland, we have approached this experimentally by performing microarray analyses of the ventral prostate in different conditions previously shown to promote differential kinetics of cell death, i.e. castration, high dose estrogen and a combination of both (Garcia-Florez *et al*., 2005). The results revealed 2,693 genes differentially expressed and 580 that were common to the three experimental groups (Rosa-Ribeiro *et al*., 2012; UNICAMP, Brazil; personal communication). Lists of transcription factors and transcription regulators were filtered for the relative abundance of the corresponding binding sites in the up-most and down-most regulated genes and revealed at least 10 transcription factors which are also under validation in the laboratory.

The conclusions of these studies so far are that (i) a number of events take place in response to castration and occur at different periods after the drop in testosterone levels, (ii) sequential events occurring in the androgen deprived environment are not necessarily under androgen-regulation, (iii) early immediate genes are activated in response to the halt of androgen-stimulation, and (iv) some transcription factors retrieved from bioinformatics are restricted to individual cell types and would not be revealed by mass analysis of the entire organ, because they represent only a small fraction of the cell population. Finally, integrated approaches will be necessary for the identification of genes and their products that could be targeted for killing cells resisting to castration and to avoid the selection of cells contributing the progression for the androgen-independent stage of prostate cancer.

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Integrating science and animal management in conservation of endangered species

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Numerous species continue to decline rapidly throughout the world. The International Union for Conservation of Nature (IUCN) lists a host of mammals (20%), birds (13%), reptiles (21%) and amphibians (30%) as threatened with extinction. The best strategy for species conservation relies on mitigating the primary threats to their survival in their natural habitats and establishing protected areas to ensure their long term survival (*in situ* conservation). However, in reality, it is equally important to establish self-sustaining populations in captivity (*ex situ* conservation) to serve as insurance populations and provide animals for future reintroduction. This strategy has enabled the successful recovery and reintroduction of several charismatic species including the Black-footed ferret and the Przewalski's horse. These successes were made possible by integrating fundamental research and sound animal husbandry practices. Although most animals breed well naturally, assisted reproductive technologies also have played a significant role in circumventing behavioral incompatibility and maximizing genetic representation at least in the Black-footed ferret. Most *ex situ* management programs to date have focused on maintaining 90% of extant gene diversity over 100 years. Although this strategy is still considered the 'gold standard' for species management, recent surveys of wildlife species managed in zoological institutions both in the United States and Europe have revealed that only a small number of species are self-sustaining. While problems are occurring at multiple levels, they most often are related to small numbers of founder animals, lack of animal space and declining reproduction. If this trend continues, we will see 'extinction' of species in zoos worldwide. Captive breeding programs now have reached a critical juncture wherein alternative animal management strategies as well as reliable population control measures need to be explored. This lecture will review the successes achieved to date with species recovery and new strategies being evaluated for establishing self-sustaining populations of rare and endangered species in zoological institutions.

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The secret life of sperm from chromatin to expression

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Significant attention has been given to the somatic and egg genomes to address how large-scale changes in chromatin structure mediate the expression of our genome. While this has revealed some interesting trends, it has become clear that far less is known about the male gamete. In part, this reflects the unique packaging of the sperm genome by the small basic nuclear proteins into an even more condensed and transcriptionally inert state. The protamines essentially render the nucleus a tough nut to crack. The male haploid-specific protamine locus provides a platform upon which to build testable models that can dissect the underlying mechanistic framework of how large scale changes in chromatin structure modulate the expression of our genome. This has enabled a series of studies to begin to examine how structure both selects genes for expression yet compacts the genome to essentially an inert state in preparation for fertilization. Within the mature spermatozoan the PRM1, PRM2, TNP2 protamine locus presents as a single accessible chromatin loop domain. The boundaries of the domain are defined by nuclear anchors. Transgenic analysis has shown that these nuclear anchors function as boundary elements. Within mouse and man the extended open structure is formed by the pachytene spermatocyte stage. The formation of the open chromatin loop appears to be initiated through a series of defined nicks catalyzed by nuclear matrix associated Topoisomerase II. The PRM1, PRM2, TNP2 domain remains in this extended conformation even in the highly condensed transcriptionally silent mature spermatozoan nucleus. This appears independent of the extent of histone replacement by protamines. The majority of the histones that remain seem randomly distributed along the chromosome. Their positioning does not appear to be species specific, although a subset gives the impression of transcriptional ghosts that are echoed by spermatozoal RNAs. Interestingly, RNAs have been observed within the sperm nuclear matrix. The RNAs that are delivered at fertilization likely provide an essential component to early paternal genome reprogramming and may act as genetic and epigenetic effectors to the fetal onset of adult disease.

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