Female prostate: a review about the biological repercussions of this gland in humans and rodents

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Abstract

The prostate is not a gland exclusive to the male reproductive system since it is also found in females of several mammals, including humans and rodents. In males, prostatic morphogenesis is an event controlled by androgens, which act indirectly via paracrine factors secreted by the mesenchyme. In females, prostatic embryological development occurs in an environment without steroid hormones, but the presence of these hormones in an adult organism induces the differentiation and secretory activity of prostatic cells. The increasing interest in female prostate studies comes from its biological role in the production of prostatic fluid, which participates in the nutrition and maintenance processes of spermatozoa that are introduced into the female reproductive system and has the potential to cause benign and malignant lesions. In recent decades, the occurrences of prostatitis (Skeneitis), benign prostatic hyperplasia, and urethral adenocarcinoma in post-menopausal women have been common. The installation of these disorders in aged women seems to be associated with the hormonal imbalance caused by the failure of the ovaries to produce steroids. Experiments involving testosterone and anti-estrogen administration in female rodents have shown that the morphology and physiology of the female prostate are regulated by androgens and estrogens. While androgens induce the differentiation, development, and secretory activity of the gland, the estrogens appear to modulate the androgenic effects, maintaining the normal physiology and growth of the prostate. Long-term exposure to synthetic hormones (contraceptives and hormonal replacement drugs), which interferes with women's hormonal balance, can cause important changes in the female prostate morphophysiology. Thus, it is necessary to frequently monitor the female prostate in order to prevent prostatic disorders that can endanger the quality of life of women.

Keywords: female prostate, prostate morphogenesis, rodents, androgens, estrogens.

Introduction

Development of the female prostate in mammals has been reported since the seventeenth century (de Graaf, 1672). Studies in humans have shown that the female prostate plays an important role in the reproductive process and in sexual behavior (Zaviačič, 1999). Besides, frequent reports of urogenital system disorders, whose origin is the prostate, have increased the care and attention directed to this gland (Sharifi-Aghdas and Ghaderian, 2004; Kato *et al.*, 2005; McCluggage *et al.*, 2006). Although there are several works that describe the histological structure of the female human (Zaviačič, 1999) and rodent (Shehata, 1974; 1975; 1980; Gross and Didio, 1987; Flamini *et al.*, 2002) prostate, issues related to the function and physiology of this gland have been discussed only recently (Zaviačič *et al.*, 2000a; b; Santos *et al.*, 2003a; Custódio *et al.*, 2004; Santos *et al.*, 2006). The objective of this work is to review the main studies on the female prostate, emphasizing the events that cause its embryological development as well as factors that control its function, physiology, and pathology during adult life.

Embryological development of the urogenital system

The developing urogenital tract contains epithelial structures of mesodermal (mesonephric or Wolffian duct and paramesonephric or Müllerian duct) and endodermal origin (urogenital sinus- UGS) that are associated with undifferentiated embryonic connective tissue known as the mesenchyme (Cunha *et al.*, 2002; Marker *et al.*, 2003; Staack *et al.*, 2003). The initial stages of gonadal development are identical in male and female embryos, and this phase is called the indifferent or ambisexual stage of sex differentiation. In mice, the male and female gonads become morphologically distinguishable only on Day 13 of gestation (Staack *et al.*, 2003). In male mice, after Day 13, the mesenchymal cells of the gonadal rudiment aggregate and condense into epithelial cords that become seminiferous tubules. After Day 14 of gestation, the Leydig cells of fetal testes differentiate and start to secrete testosterone (Pointis *et al.*, 1980). Testosterone prevents programmed cell death of mesonephric ducts and stimulates their development to form the epididymis, vas deferens, seminal vesicles, and efferent ducts. Meanwhile, the Sertoli cells initiate production of Müllerian inhibiting substance (MIS), which elicits regression of the paramesonephric ducts. In females, the fetal ovaries are relatively inactive endocrinologically

and are not required for the development of the female urogenital system. When there is no androgenic stimulus, the mesonephric duct recedes, and in the absence of MIS, the paramesophrenic duct develops giving rise to the oviducts, uterine horns, cervical canal, and the upper portion of the vagina (Staack et al., 2003).

The prostate develops from the UGS, which is an endodermal tube derived from the hindgut and ends in the cloaca. The urorectal septum subdivides the cloaca into the UGS ventrally and the rectum and anal canal dorsally. The cloaca subdivides in such a manner that the mesonephric and paramesonephric ducts terminate in the UGS. The UGS is further subdivided into the bladder and the definitive UGS. In mice, the two elements derived from the UGS are clearly distinguished on Day 13-14 of gestation (Staack et al., 2003).

The UGS is composed of an epithelial layer (urogenital sinus epithelium; UGE), which is derived from the endoderm and surrounded by a mesenchymal layer (urogenital sinus mesenchyme; UGM) originated from the mesoderm. This structure is found in the neck of the developing bladder and it arises in both male and female mice and rats 13 days post conception and after 7 weeks of gestation in humans. The urogenital sinus is morphologically indistinguishable between males and females until about Day 17-18 of gestation in mice and not until Week 10-12 in humans. After that, prostatic morphogenesis, a process that is both initiated by and dependent on circulating androgens produced by the fetal testes, begins (Marker et al., 2003).

In males, the initial event in prostatic morphogenesis is the outgrowth of solid epithelial buds from the UGE into the surrounding UGM. In rodents, most of the prostatic ducts are unbranched at birth. However in the neonate, as these ducts elongate within the UGM, they begin to bifurcate and emit lateral branches and give rise to three different prostatic lobes: the ventral prostate, dorsolateral prostate, and anterior prostate or coagulating gland (Marker et al., 2003). The process of ductal branching morphogenesis occurs simultaneously with ductal canalization (lumen formation) and epithelial and stromal cytodifferentiation (Wang et al., 2001).

Prostatic morphogenesis is dependent on steroid hormones. Androgens are necessary to initiate prostatic development, to continue embryonic and neonatal growth, and then to start prostatic secretory activity during puberty (Isaacs et al., 1994). However, the androgenic action is not exerted directly on the epithelial cells. Under the influence of androgens, the mesenchymal cells produce and secrete specific paracrine factors that control growth and prostatic gland differentiation. Hence, with epithelial cell differentiation, the levels of androgenic receptors (AR) increase, and the expression of epithelial estrogenic receptors (ERβ) and stromal estrogenic receptors (ERα) is induced.

Mesenchymal-epithelial interactions play a key role in directing the growth and development of the prostate because the mesenchymal paracrine signaling to the epithelium is essential to prostatic embryogenesis.

Thus, androgen action on the mesenchymal cells results in specific paracrine factors that act on the epithelial cells inducing their proliferation (Thomson et al., 2001). On the other hand, there is also a paracrine signal from the epithelium to the mesenchyme. This signal regulates the differentiation of the mesenchyme that surrounds the growing buds in a stroma composed of smooth muscle cells and fibroblasts (Hayward et al., 1996). Hence, during prostatic morphogenesis, the AR is necessary to the mesenchyme but not to the epithelium since its expression precedes prostatic bud development. In the epithelial cells, AR function is limited to the regulation of secretory proteins and perhaps cellular differentiation (Donjacour and Cunha, 1993).

Among the paracrine factors that interfere with prostatic morphogenesis, it is relevant to mention the family of homeobox transcription factors NKx3.1, Hoxa-13, Hoxb-13 and Hoxd-13, fibroblast growth factors FGF-7 and -10 (Huang et al., 2004), the glycoprotein Sonic hedgehog (Shh), the Shh receptor, patch (ptc), transcription factors of the family Gli, and BMP-4 (Pu et al., 2004). The NKx3.1 gene is expressed in the urogenital sinus epithelium (UGE) before the beginning of prostatic budding and it seems to play a role in epithelial differentiation and in prostatic development, mainly during the epithelial cell determination process in this gland (Bieberich et al., 1996). Hoxa-13, Hoxb-13, and Hoxd-13 are expressed in the urogenital sinus mesenchyme (UGM), and they are involved in prostatic development. These factors are expressed at high levels during fetal life, but their levels decline postnatally. FGF-10 and FGF-7 are produced by the UGM cells and interact with a unique receptor, FGFR2iiib, which is expressed by the UGE cells. Together, these factors play a critical role in the expansion and branching of the prostatic buds (Huang et al., 2004). Shh glycoprotein is secreted by the UGE cells in the mesenchymal interface of the developing prostate, activating its receptor patch (ptc), which is expressed in the mesenchymal cells. The Shh-ptc complex triggers a cascade of molecular signals that increase the levels of the gli transcription factors, which mediate the Shh effects. In vertebrates, there are three types of gli transcripts (gli-1, -2, -3) that are redundant and share one function. It is believed that the Shh gene participates in budding initiation and prostatic expansion. Furthermore, Shh seems to regulate many other genes involved in prostatic development, including Hoxa-13, Hoxd-13, and NKx3.1 (Pu et al., 2004). The bone morphogenetic proteins (BMP-4) are members of the transforming growth factor-β (TGF-β) family, and in general, act as inhibitors of proliferation during prostate development. In the mouse prostate, BMP-4 mRNA is localized in the mesenchyme, and its levels decline postnatally. In Fig. 1, a model is proposed in which these paracrine factors act to direct rodent prostatic morphogenesis during the postnatal period (Huang et al., 2004).
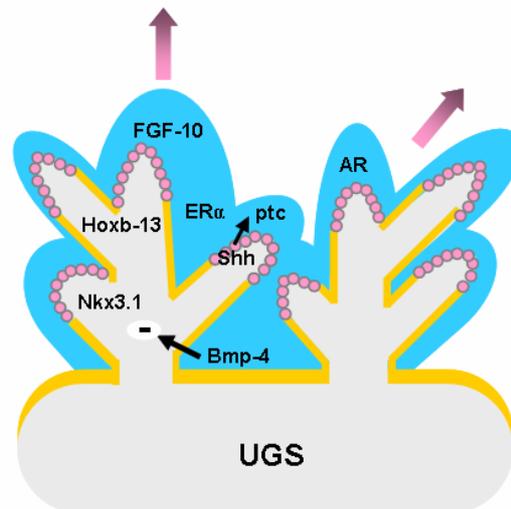
Figure 1. In a normal male prostate, androgen receptor AR and estrogen alpha receptor ERα expression occurs in the stromal cells (blue) located near the outgrowing prostatic buds (grey). Bmp-4 expression, which is high before prostate budding, declines rapidly after birth, releases its inhibitory effects on ductal outgrowth. Shh is secreted by distal tip epithelial cells (pink), activates ptc receptors on adjacent stromal cells, and participates in ductal outgrowth and prostatic differentiation. Fibroblast growth factor (FGF)-10 is expressed by distal mesenchymal cells and stimulates ductal outgrowth and branching. NKx3.1 is expressed early by undifferentiated epithelial cells and is involved in initiation of prostate development and epithelial differentiation. Hoxb-13 is expressed in epithelial cells and is involved in maintaining a differentiated phenotype. The smooth muscle layer is shown in yellow (adapted from Huang *et al.*, 2004).

In female human and rat embryos, the absence of testosterone induces the UGS to give rise to the lower vagina and the urethra (Shapiro *et al.*, 2004). The UGM starts to surround the urethral epithelium and is subdivided into three areas: the periurethral mesenchyme, the mesenchymal zone that undergoes smooth muscle differentiation, and the mesenchymal zone that contains the ventral mesenchymal pad (VMP). The latter structure has a localization analogous to the male ventral prostate and represents the UGM without the invasion of the UGE (Thomson *et al.*, 2002). The differentiation of a part of the UGM in smooth muscle is crucial to prostatic morphogenesis in both males and females since this event regulates paracrine signaling between the epithelium and the mesenchyme (Thomson *et al.*, 2002).

During mesenchyme differentiation, androgens regulate the thickness and continuity of the smooth muscle layer in such a way that the absence of androgens in female embryos causes the formation of a continuous, thick muscle layer. This layer separates the VMP from the urethral epithelium, preventing the prostatic epithelial buds that are arising in the urethra from making direct contact with the VMP. Hence, the dense layer of smooth muscle prevents the interaction of VMP with the growing prostatic buds, blocking the paracrine communication between the epithelium and mesenchyme (Thomson *et al.*, 2002). In males, with the presence of androgens, the formation of smooth muscles is inhibited or delayed, and the prostatic buds emerge out of the urethra and can penetrate the VMP. Henceforth, the mesenchyme-epithelial paracrine interaction is established and causes branching and outgrowth of the ventral prostate. The illustrative model of prostatic induction in males and females was proposed by Thomson and co-workers and is shown in Fig. 2.

The formation of prostatic buds is a constitutive process in males and females, but the branching and outgrowth of these buds is regulated by androgens. In females, the separation between the VMP and the urethral epithelium, which is caused by the smooth muscle layer, prevents the formation of a fully-developed and lobulated prostatic gland. Then, the reduced prostatic tissue observed in females of several species is formed from the UGS, which has not suffered any androgenic stimulus. Although the adult female prostate is smaller than the male prostate (about 15% - 25% of the size of a ventral male prostate), it has a differentiated and functional secretory epithelium (Zaviačič *et al.*, 2000a; Santos *et al.*, 2003a; Custódio *et al.*, 2004). As the female prostate grows and develops in an environment with low levels of androgens (only 5% of the total androgenic precursors produced in the male organism), it is believed that factors other than these hormones can act in the development and maintenance of this gland in adults (Timms *et al.*, 1999).
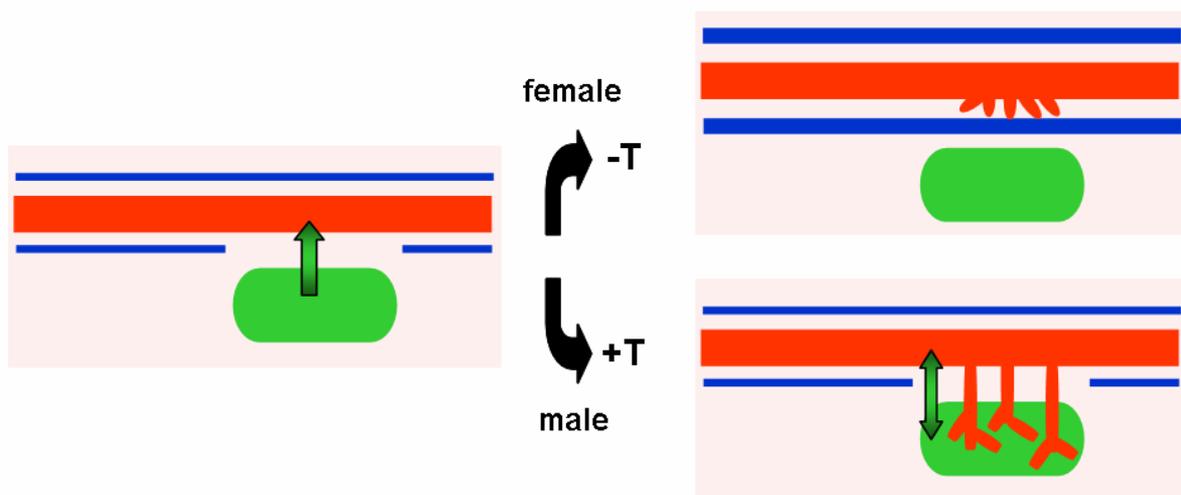
Figure 2. Descriptive model of ventral prostatic development in female and male rat embryos. On the left side, the ventral mesenchymal pad (VMP; green) is shown during the initial phases of prostatic induction (Days 17-18 of gestation). Observe that the smooth muscle layer (blue) is discontinuous and it allows signaling between the VMP and the urethral epithelium (red). On the right side, prostatic morphogenesis is shown in males and females (after Day 21.5 of gestation). In females, the absence of testosterone allows the smooth muscle to form a thick and continuous layer, preventing paracrine signaling between the outgrowing prostatic buds (red) and the VMP. In males, testosterone induces the formation of a thin and discontinuous muscle layer that allows the epithelial-mesenchymal interaction. Emerging urethral-epithelial buds (red) penetrate the VMP. In the neonate, these buds undergo side branching inside the VMP, giving rise to the ventral prostate (adapted from Thomson *et al*., 2002).

The developmental frequency of a functional prostate gland in females is very high. In humans, about 90% of women develop mature prostatic tissue that is active in secretory processes (Zaviačič *et al.*, 2000b). In rodent females, prostatic morphogenesis seems to be influenced by the intrauterine position of the animals during gestation (Clark *et al.*, 1991; Timms *et al.*, 1999). Thus, in the female rat fetus, the development of prostatic buds is more frequent when gestation occurs with twin female fetuses (67%) but rarely occurs between two males (29%). This occurs because animals bearing two female or two male fetuses have natural differences in testosterone (T) and estrogen (E) levels. Females bearing two female fetuses have higher levels of E, which is the hormone responsible for modulating the effects of androgens on the developing prostate and induces larger growth of prostatic buds during glandular morphogenesis (Timms *et al.*, 1999).

Female prostate: new concepts and change of paradigms

The first report of the "female prostate" occurred in 1672 when Reinier de Graaf used this term to describe a set of glands located around the urethra, which according to him, had considerable homology with the male prostate (de Graaf, 1672). Two centuries later, Alexander Skene described the female prostate as being formed by two paraurethral ducts that open into orifices on both sides of the urethra and as having limited underprovided secretory function (Skene, 1880). Since then, this set of glands has been called "Skene's paraurethral glands." For a long time, this gland was considered a vestigial organ without any biological importance to the female organism (Zaviačič and Ablin, 2000). However, since 1950, new studies have discussed the female prostate, mainly in relation to the biological role that this organ could have in woman (Huffman, 1948; 1951; McCrea, 1952; Tepper *et al.*, 1984; Wernet et *al.*, 1992; Zaviačič, 1993; Zaviačič *et al.*, 1993; 1997a; b; 2000a; b). One of the main works about the female prostate, which describes the human female prostate and investigates its structural and functional aspects as well as its sexological implications, is the research developed by Zaviačič *et al.* (1999).

Zaviačič presents the human female prostate as a paraurethral set of numerous glands and ducts that are inserted into a fibromuscular stroma (Zaviačič *et al.*, 2000a). The glands are lined by differentiated and mature epithelium that has two main cell types: the basal cells, which are stem cells responsible for the maintenance of the prostatic cell population; and the secretory or luminal cells that continually produce the prostatic fluid (Zaviačič *et al.*, 2000b). The luminal cells are the most numerous ones and express the prostate-specific antigen (PSA) and the prostate specific acid phosphatase (PSAP), two important prostate markers (Zaviačič *et al.*, 1993).

Together with the studies of the human female

prostate, many studies have demonstrated the occurrence of a prostate in females of several rodents species such as *Praomys natalensis* (Smith *et al.*, 1978; Gross and Didio, 1987), *Rattus novergicus* (Shehata, 1980; Vilamaior *et al.*, 2005), *Lagostomus maximus maximus* (Flamini *et al.*, 2002), *Meriones libycus* (Shehata, 1974; 1975), and *Meriones unguiculatus* (Santos *et al.*, 2003a; Custódio *et al.*, 2004). Furthermore, the morphological characteristics presented by these works indicate that the prostate of the females of these species is equivalent to the male ventral prostate and that its epithelial cells show a functional phenotype.

Until now, the biological role of prostate development in the female organism has not been clear. Biochemical studies demonstrated that prostatic fluid released during female ejaculation has the same chemical composition as male prostatic fluid. The more abundant components of the human female ejaculate are PSA, PSAP, zinc, and fructose (Zaviačič, 1993; 1999). Evidence indicates that the fructose produced by this gland flows in a small amount from the urethra to the vagina and plays an important role in reproduction. Thus, since fructose is the main source of energy for spermatozoa, female prostatic fluid also contributes to the success of fertilization. Recent studies have detected levels of PSA in the serum and in the urine of women (Zaviačič and Ablin, 2000; Schmidt *et al.*, 2001). These works have indicated that the prostate is the main producer of PSA in females although there are other extraprostatic sources (Diamandis and Yu, 1997; Yu and Berkel, 1999; Galadari *et al.*, 2004; Kocak, 2004; Sauter *et al.*, 2004).

Another area of exploration for the possible functions of the female prostate is its importance to female sexual sensitization (Zaviačič, 1993). A few reports have associated the female prostate with the Gräfenberg spot (G-spot), emphasizing the relevance of stimulus of this spot in order to produce the phenomenon of female ejaculation (Schubach, 2002). According to Gräfenberg, the G-spot refers to an "area" or "zone" richly innervated in the upper-anterior wall of the vagina through which the female prostate can be accessed (Gräfenberg, 1950). However, controversial articles have maintained that the female prostate and the G-spot are the same structure (Addiego *et al.*, 1981; Hines, 2001).

In addition to the biological and behavioral implications mentioned above, the main focus of interest about the female prostate comes from its capacity of developing severe lesions during aging. Recent works have described the occurrence of cancers of the urethra, whose origin is the female prostate (Dodson *et al.*, 1994, 1995; Ali *et al.*, 1995; Ebisuno *et al.*, 1995; Sloboda *et al.*, 1998; Islam *et al.*, 2001; Sharifi-Aghdas and Ghaderian, 2004; Kato *et al.*, 2005; McCluggage *et al.*, 2006). Furthermore, there is evidence that other prostatic pathologies, such as

prostatitis and benign prostatic hyperplasia, can occur in the female prostate with the same level of severity observed in the male prostate (Zaviačič, 1999). Acute cystitis is the most common form of infection of the female urinary system, and the incidence of this lesion in young women in the USA is from 0.5-0.7 episodes per person per year. Currently, it is known that the origin of this cystitis is the female prostate. When the inflammation of the prostate occurs (prostatitis or Skeneitis) it disseminates throughout the entire female reproductive system resulting in urethro-prostato-cystitis (Zaviačič, 1999). This phenomenon occurs due to communication of the system of prostatic glands with the urethra and the anterior wall of the vagina.

Based on the information stated above, it is impossible to consider the female prostate an insignificant vestigial organ, and thus, the definition of Skene must be reassessed. In spite of the fact that the female prostate has smaller dimensions compared to the male prostate, it is active in secretory processes and requires the same attention as any other organ in the female organism because alterations in its physiology can endanger the health and quality of life of women. Hence, studies that elucidate the processes that maintain homeostasis of this gland are necessary because little is known about the physiological events that preserve the functionality of the female prostate in normal as well as in pathological conditions.

Female prostate: experiments with rodents

The morphology and function of the human female prostate has been described by many researchers in collaboration with Dr. Milan Zaviačič (Zaviačič, 1993; Zaviačič *et al.*, 1993; Zaviačič *et al.*, 1997a; b Zaviačič, 1999; Zaviačič and Ablin, 2000; Zaviačič *et al.*, 2000a; b). In the rodent, the female prostate has been described by many researchers (Shehata, 1974; 1975; 1980; Gross and Didio, 1987; Flamini *et al.*, 2002); however, none of these works has investigated the mechanisms that control the function of the female prostate gland. The experiments with human female prostatic tissue are very limited because the female prostate can only be obtained through necropsies of women who suffered cerebral death (Zaviačič, 2000a). Hence, it is necessary to adopt experimental models that use a prostatic gland similar to the human female prostate in order to extrapolate the data to the human species.

Our research group has adopted the Mongolian gerbil (*Meriones unguiculatus*, Gerbilinae, Muridae) as an experimental model because the female prostate of this rodent has great homology with the human female prostate and the male gerbil ventral prostate (Taboga *et al.*, 2001). In addition, the occurrence of prostates in females of these animals is very common since we can find a fully developed gland in 80% of the adult females studied.

Anatomically, the female gerbil prostate is composed of a cluster of glands and ducts that are

concentrated in both sides of the median urethra (Fig. 3A-C). The ducts insert into the urethra musculature and open into various areas of the urethral lumen. The secretory portion of the adult female prostate of the gerbil is lined with an epithelium that varies from simple cubic to columnar pseudostratified (Fig. 4A). The epithelial cells consist of two types: the basal cells, which form a discontinuous layer of stem cells for the maintenance of prostatic growth (Fig. 4A); and the secretory cells, which have a cytoplasm rich in organelles involved in the process of synthesis and secretion of glycoproteins (Fig. 4A, D, E). The secretory cells express proteins reactive to human anti-PSA antibodies in their apical portion (Fig. 4B) and strong immunostaining to PSAP througout the cytoplasm (Fig. 4C). Levels of PSA-like protein can also be detected in the serum of these animals (levels of PSA serum in adult females: 0.1-1.3 ng/ml; Santos *et al.*, 2006) and have values close to the ones observed in adult women (0.1-0.9 ng/ml; Zaviačič and Ablin, 2000; Schmidt *et al.*, 2001).

Immunocytochemical studies have shown that the female gerbil prostate expresses receptors for two important steroid hormones, estrogen and testosterone. The secretory epithelial cells (Fig. 5A, B) and fibroblasts (Fig. 5A, C) test positive for androgen receptors (AR). The estrogen alpha receptor (ERα), which is often expressed in stromal cells of human and rodent males (Prins *et al.*, 2001; Rovuele *et al.*, 2001; Omoto *et al.*, 2005), has also been detected in the epithelial secretory cells (Fig. 5D-F). The occurrence of ER α-positive epithelial cells seems to be associated with the development of prostatic hyperplasia and metaplasia. It has also been described in the human prostatic epithelium (Härkönen and Mäkelä, 2004).

The prostatic glands and ducts are associated with a fibromuscular stroma, which is rich in cells, fibers, and blood vessels (Santos *et al.*, 2001). Reticular fibers and collagen fibers are observed in the basal epithelium, always in association with the smooth muscle cells (Fig. 6A, B). Ultrastructurally, smooth muscle cells have a large nucleus, around which there is a considerable cluster of organelles (Fig. 6C). Bundles of collagen fibers are abundant and observed near the smooth muscle cells while elastic fibers are very scarce (Fig. 6C).
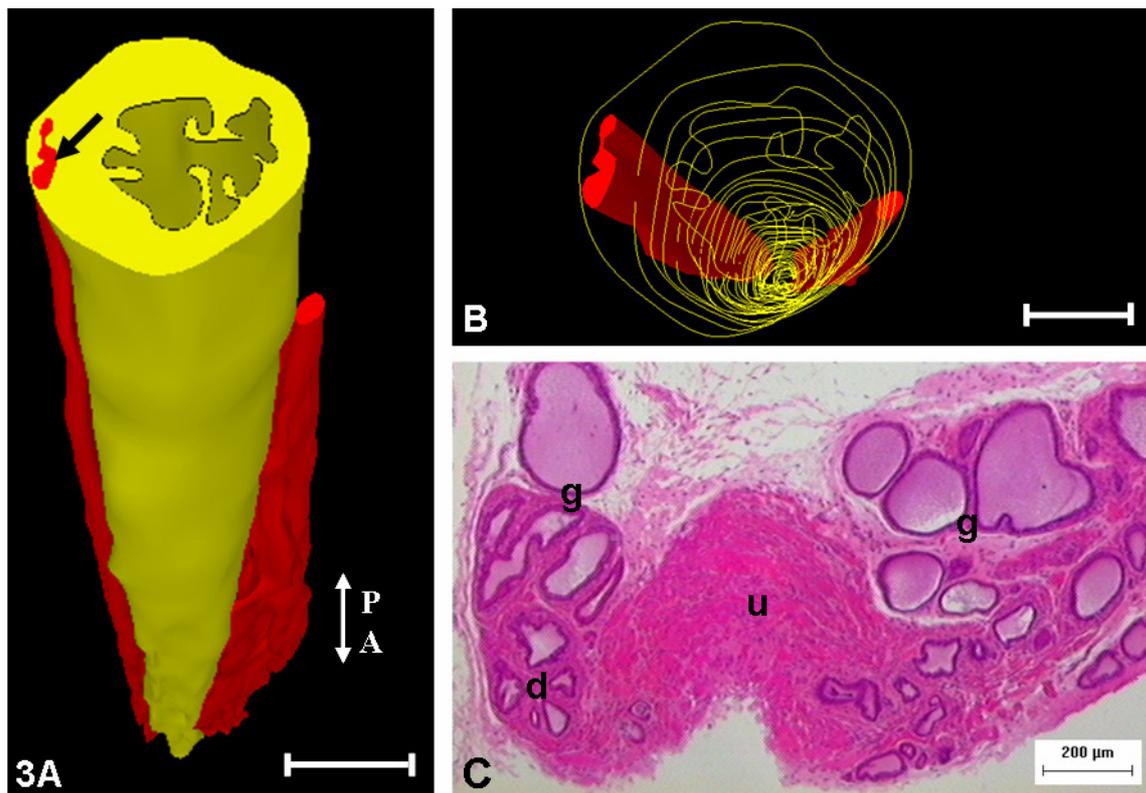
Figura 3. Three-dimensional reconstruction of the adult female gerbil prostate (*Meriones unguiculatus*) in longitudinal (**A**) and transversal (**B**) views. The prostatic urethra (yellow), prostate ducts, and alveoli (red) are shown. The double arrow indicates the gland orientation relative to the anterior (A) and posterior (P) urethra. Ducts that insert into the urethral musculature (arrows) are also shown. **C**: histological sections of the female prostate are shown in addition to the ducts (d) and prostatic alveoli (g) orientation relative to the prostatic urethra (u). (Bar = a: 25 µm, b: 20 µm).
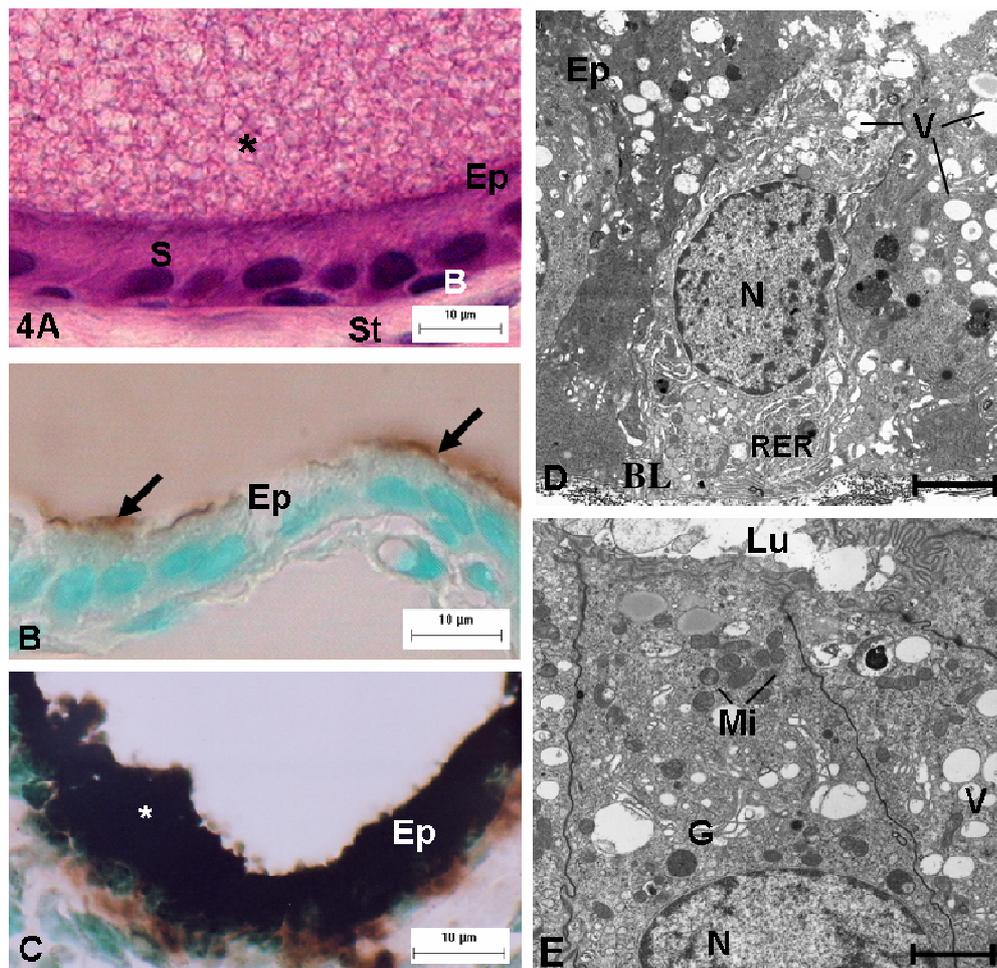
Figure 4. **A**: detail of the secretory epithelium (Ep) stained using the hematoxylin-eosin method. Secretory cells (S), basal cells (B), stroma (St), and secretion (*) are shown. **B**: prostate specific antigen (PSA) expression in apical portion (arrows) of secretory epithelium (Ep) counterstained with Methyl-Green. **C**: cryosection of the adult female prostate showing a positive reaction for prostate specific acid phosphatase (PSAP) counterstained with Methyl-Green (*). **D**: epithelial ultrastructure (Ep) showing the secretory cells with large nuclei (N) and cytoplasm with a fully-developed rough endoplasmatic reticulum (RER) and secretory vesicles (V). (basement lamina, BL; Bar = 2 µm) **E**: detail of the apical portion of a secretory cell where it is possible to observe mitochondria (Mi), the Golgi complex (G), and secretory vesicles (V). (lumen, Lu; nuclei, N; Bar = 1.4 µm).

Recently in our laboratory, we conducted preliminary studies on the female prostate of *Wistar* rats (*Rattus novegicus;* Vilamaior *et al.*, 2005). The first results showed that the prostate of adult female rats have morphology similar to the ventral prostate lobe of young male rats (Fig. 7A-C). In these females, the gland is characterized by alveoli with an underdeveloped lumen and stroma full of smooth muscle cells, fibroblasts, and fibrous elements of that extracellular matrix. The epithelium is lined by cells that vary from cubic to columnar and sometimes have some buds. In the luminal compartment, a small development can be present with scarce secretion in its interior (Fig. 7B). These results refute several data from the current literature that suggest that in rat and mouse females, total involution of the mesenchymal and endodermic tissues, which would give rise to the gland during prostatic morphogenesis, occurs (Thomson, 2001; Thomson et al., 2002; Marker et al., 2003). These reports evidence that testosterone is a key component of prostatic morphogenesis, and its absence during female embryonic development results in the formation of vestiges insufficient for any functional role. However, according to our observations, one can infer that this gland possesses moderate secretory capacity although it does not show a level of development as accentuated as that of the female gerbil prostate (Santos *et al.*, 2005).
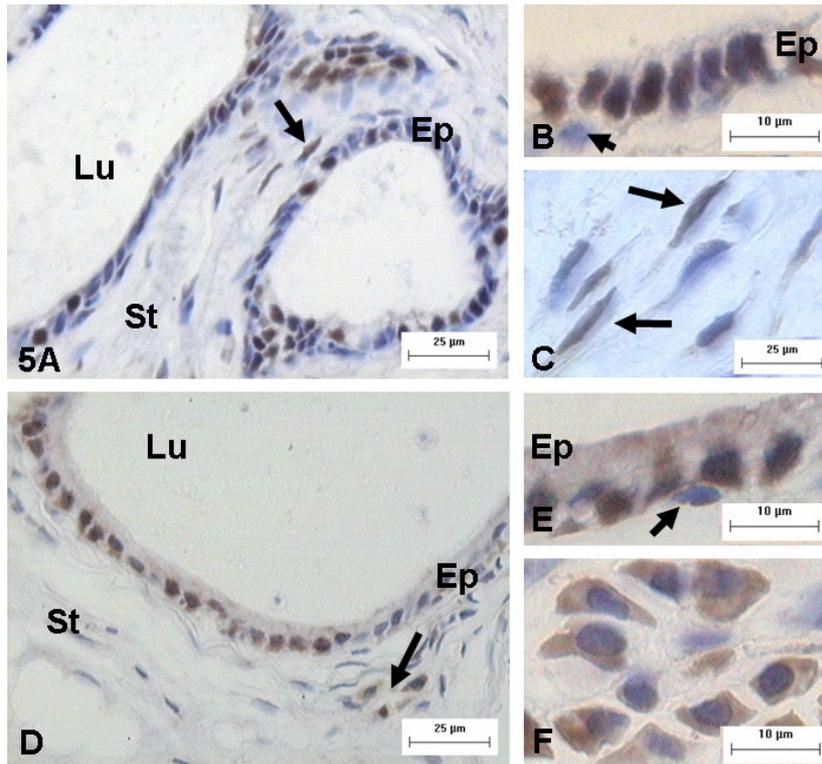
Figure 5. Immunocytochemical identification of the androgen receptor (AR; **A-C**) and estrogen receptor alpha (ERα; **D-F**) in a control female prostate counterstained with Harris's hematoxylin. **A**: strong staining is observed in the secretory epithelial cell nuclei (Ep) and in fibroblasts (arrows). (stroma, St; lumen, Lu). **B**: epithelial basal cells (Ep) are AR-negative (arrow). **C**: detail of fibroblasts with an AR-positive stain. **D**: ERα-positive stain is observed in epithelial secretory cells (Ep) and in the nucleus and cytoplasm of stromal cells (arrows). **E**: detail of the secretory epithelium (Ep) showing that basal cells (arrows) do not express ERα. **F**: a cluster of stromal cells showing intense cytoplasmatic and nuclear ERα staining.
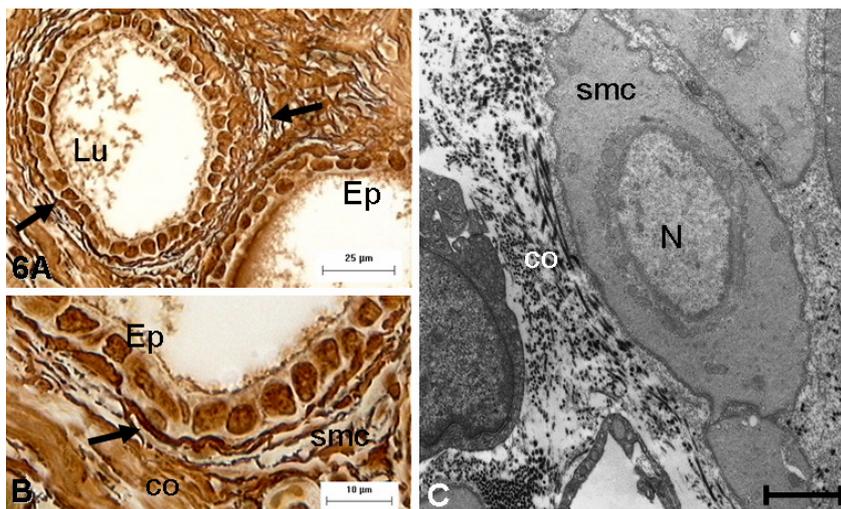
Figure 6. **A** and **B**: histological sections of the female gerbil prostate stained with Gömöri's reticulin. The reticular fiber network is stained in brown (arrows). Epithelium (Ep), lumen (Lu), collagen (co), and smooth muscle cells (smc). **C**: ultrastruture of the prostatic stroma showing smooth muscle cells (smc) and their association with collagen fibers (co). (nucleus, N; Bar = 1.4 µm).

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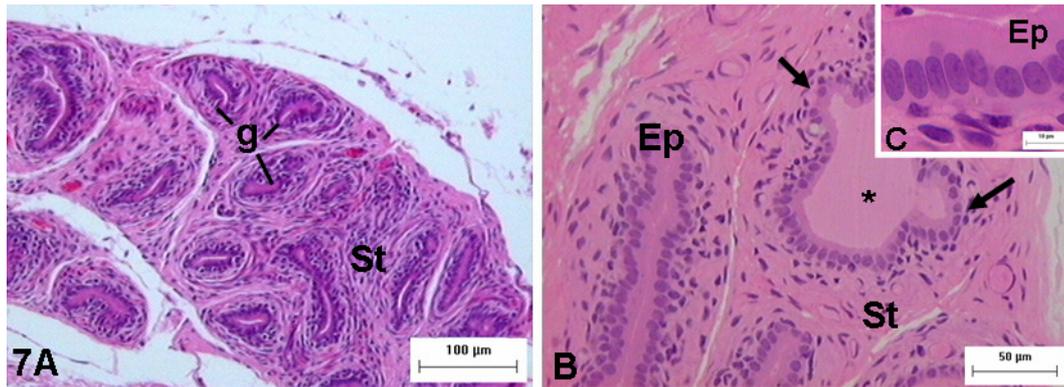
Figure 7. Histological sections of the adult female prostate of a *Wistar* rat (*Rattus novergicus*) stained with hematoxylin-eosin. **A**: the secretory portion of the gland is underdeveloped (g) and lines a reduced lumen. The stroma (St) is dense with a large amount of cells. **B**: prostatic alveoli with epithelial branching (arrows) and the reduced glandular lumen full of secretion (*). Stromal cells are displayed near the alveoli (St). **C**: simple columnar epithelium showing secretory cells with a large nucleus and cytoplasm.

The morphofunctional characteristics of the adult female prostate of gerbils and *Wistar* rats indicate that the growth and the activity of this gland are maintained in an environment with low androgen levels (serum testosterone levels in adult female gerbil: 0.4 – 2.7 ng/ml; Santos *et al.*, 2006). Thus, it is believed that other factors such as estrogen, for example, play an important role in physiological regulation of the prostate. Hence, it is fundamental to understand the elements that regulate the differentiation, growth, and secretion of female prostate because alterations in these events can cause lesions. That is the reason why we have developed additional experiments related to hormonal treatment of gerbil and rat female prostates, and the results obtained are outlined briefly in the following section.

Hormonal regulation of rodent female prostate

Androgenic effects on the gerbil and rat adult female prostate

Although the gerbil and *Wistar* rat adult female prostates present different levels of glandular development, both respond in a similar way to treatment with androgens. Experimental treatment of adult female gerbils with testosterone cypionate (T) for 21 days (Santos *et al.*, 2003b; Leite *et al.*, 2004) showed that this steroid hormone exercises a biphasic effect on prostatic regulation (Santos *et al.*, 2006). Initially, there was copious cellular proliferation that caused glandular growth (Fig. 8A-B). After the first 7 days of treatment, the prostatic gland showed great secretory activity that caused an increment in the luminal area. Neoplastic intraepithelial foci with pseudocribiform architecture were also observed, thus indicating that T administration can cause important tissue disruptions in the female gerbil prostate. Serologic data from this experiment showed that serum testosterone levels in the treated females increased in some cases to 12 times greater than the levels observed in untreated females (1.6 ng/ml in control females and up to 18.9 ng/ml in T-treated females); however, the serum estrogen levels were not altered significantly during the treatment (Santos *et al.*, 2006).

Initial results obtained in experiments with *Wistar* rat females (Vilamaior *et al.*, 2005) indicate that the pattern of prostatic response to treatment with T is similar to that in female gerbils. Nevertheless, because the prostate of untreated female rats is underdeveloped compared to female gerbils, the glandular alterations became evident during the first 3 days of hormonal treatment (Fig. 8C-D). The more noticeable alterations during this initial phase of treatment were intense cellular proliferation and enlargement of the luminal compartment, which had a large amount of PAS-positive secretion (Fig. 8C-D). These experiments showed that testosterone administration promotes growth and greater secretory activity in the female prostate of the rodents studied. These effects reproduce in detail the events that occur during prostatic development in male mice and Wistar rats (Vilamaior *et al.*, 2006). Thus, it is possible to conclude that the lack of androgens contributes to reduced female prostate development (Santos *et al.*, 2006) and that the secretory activity observed in the prostates of untreated females (Santos *et al.*, 2003a; b) is maintained by the small amount of testosterone present in the serum of these animals.

In the male organism, the androgens are related to the differentiation, growth, and maintenance of prostate secretory activity (Hayward *et al.*, 1996; Steers, 2001; Thomson, 2001). Thus, the data obtained by the testosterone treatment suggest that androgens play a similar role in the female prostate, which is related primarily to the differentiation of secretory epithelium and regulation of secretory activity.
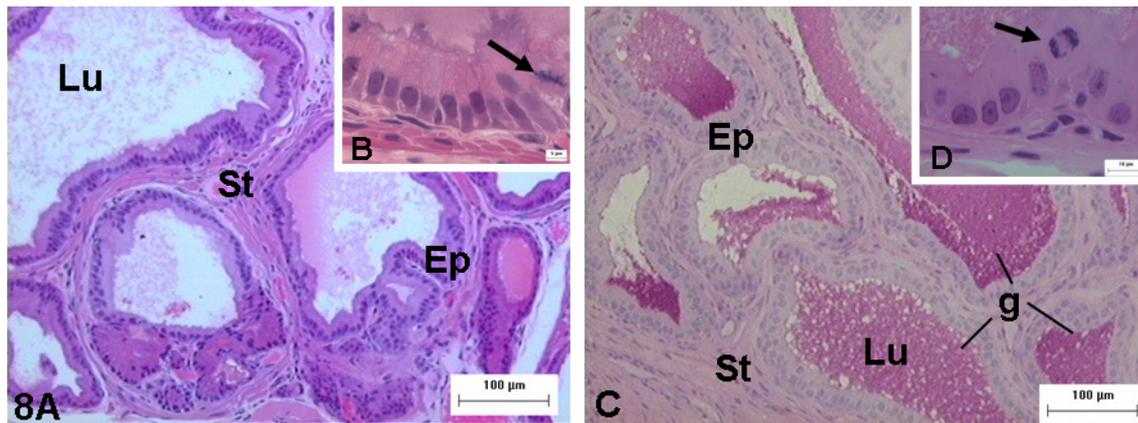
Figure 8. **A** and **B:** histological sections of adult female gerbil prostate after testosterone (T) treatment and stained with hematoxylin-eosin. Note the intense development that occurred in the epithelium (Ep), lumen (Lu), and stroma (St). Cellular proliferation is observed in the secretory epithelium (arrow). **C** and **D**: histological sections of a female rat prostate after 3 days of T treatment and stained using the periodic acid & Schiff method (PAS). The prostatic gland undergoes intense development, presenting many alveoli (g) and spreading of the luminal compartment (Lu) in which there is a large amount PAS-positive secretion. Note the presence of cell division (arrow) in the prostatic epithelium (Ep).

Anti-estrogenic effects on the adult gerbil female prostate

Although the tissue of the prostate is androgen dependent, its physiology and pathology are also influenced by estrogens (García-Flórez *et al.*, 2005). Besides modulating androgen effects, estrogens appear to increase tissue sensitivity to other hormones by increasing the number of their receptors (Timms *et al.*, 1999). As the estrogen serum levels are elevated in the female organism, it is believed that this hormone can act directly in the maintenance and physiology of the female prostate.

Studies involving the male rodent prostate indicate that control of hormonal development and prostatic function is complex and dependent on the balance among steroid hormones (Risbridger *et al.*, 2003; García-Flórez *et al.*, 2004). In male rats, the increasing of the estrogen:androgen ratio, which occurs with aging, precedes or coincides with an increase incidence of prostate cancer (Härkönen and Mäkela, 2004). In post-menopausal women, when the ovaries fail to produce estrogens, there is a greater probability of pre-malignant and malignant prostatic lesion development (Sloboda *et al.*, 1998).

Initial studies involving the senile gerbil prostate suggest that the occurrence of spontaneous prostatic lesions is more frequent and precocious in females than in males (unpublished data). These data indicate that estrogens play an important role in prostatic physiological maintenance and that the androgen:estrogen imbalance is the key factor in the installation of prostatic disorders. Based on this, our research group has developed preliminary evaluations of the effect of estrogen suppression on the female gerbil prostate. To evaluate the effects of estrogen suppression on the female prostate, two anti-estrogen drugs, letrozole and tamoxifen, were used. These two estrogenic inhibitors were developed for the treatment of estrogen-positive breast cancer as an attempt to interrupt breast cancer cell progression (Cabot *et al.*, 1996; Berstein *et al.*, 2002; Dixon *et al.*, 2003; Haynes *et al.*, 2003; Smith, 2003). Letrozole is a non-steroidal inhibitor of the aromatase enzyme capable of preventing 99% of peripheral estrogen conversion (Haynes *et al.*, 2003). Tamoxifen is a non-steroidal triphenylethylene that exercises an antagonistic action on estrogen by binding competitively to the estrogen receptor (Cabot *et al.*, 1996). The initial results obtained so far show that both drugs cause important alterations in prostate physiology of female gerbils (Santos *et al.*, 2004; 2005).

With letrozole treatment, it is possible to observe accentuated glandular hyperplasia, characterized by increases in epithelial and stromal cells as well as an increase in the total number of ducts and alveoli (Fig. 9A). Furthermore, intra-epithelial neoplastic foci in a pseudocribiform arrangement (Fig. 9B) were very frequent, evolving in some cases into more severe tissue disorders (Santos *et al.*, 2004). These morphological data observed using letrozole treatment reproduce, in part, the morphological alterations observed with androgen treatment. In fact, the hormonal alterations caused by the letrozole treatment are similar in some aspects to the serum hormonal modifications caused by the T treatment. Evaluation of serologic data showed that the letrozole treatment increases serum testosterone levels by six times without substantially altering estradiol levels. Immunocytochemical reactions to the AR (Fig. 9C) and ERα (Fig. 9D) show that the epithelial and stromal prostatic cells have greater androgen receptor expression but less frequent expression of the ER-positive cells.
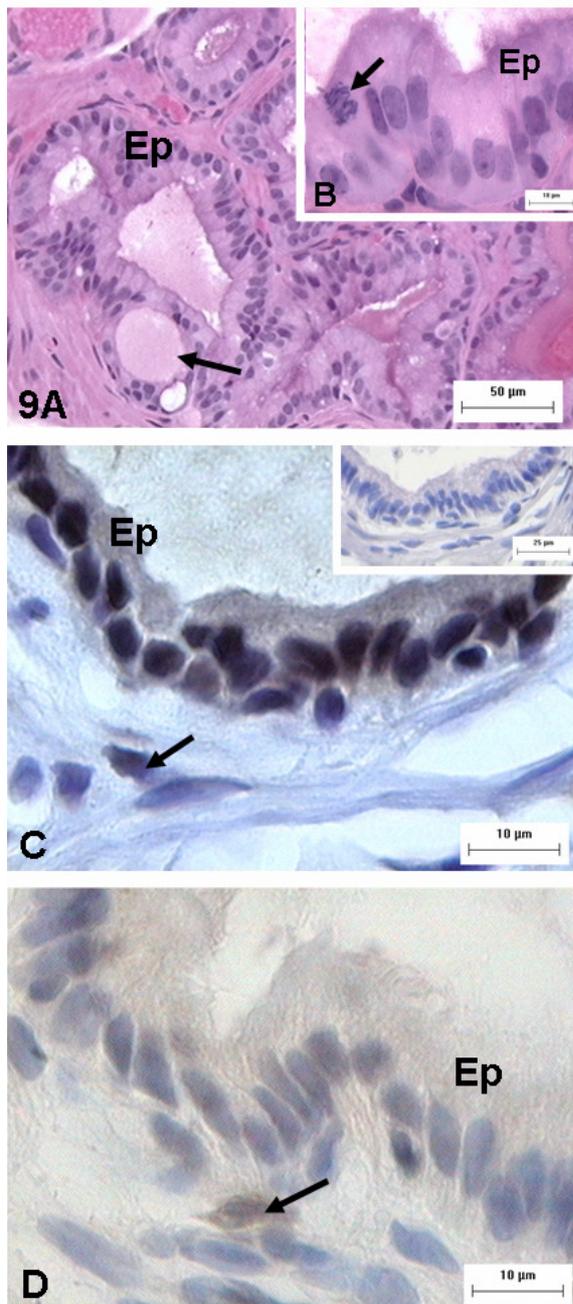
Figure 9. Histological sections of adult female gerbil prostate treated with letrozole. **A**: general view of the gland where an intense glandular hyperplasia and the occurrence of intraepithelial arcs are observed (arrow). **B**: detail of the prostatic epithelium (Ep) showing a great degree of development of epithelial cells and the occurrence of cell proliferation (arrow). **A** and **B** are stained with hematoxylin-eosin. **C**: immunocytochemical reaction to the androgen receptor. Intensely stained cells can be observed in the epithelium (Ep) and stroma (arrow). (inset: negative control). **D**: immunocytochemical reaction to the estrogen alpha receptor. Weak reactions are observed in the epithelial and stromal cells (arrow). **C** and **D** are counterstained with Harris's hematoxylin.

In the prostate, local estrogenic production occurs through testosterone aromatization into estrogen via the aromatase enzyme (Kaburagi *et al.*, 1986; Hiramatsu *et al.*, 1997; Negri-Cesi *et al.*, 1998; McPherson *et al.*, 2001; Risbridger *et al.*, 2003). Hence, it is believed that although serum estradiol levels do not change, letrozole administration causes suppression of estrogen activity in tissue. Therefore, it is suggested that increasing serum testosterone levels, caused by the inhibition of their aromatization into estrogen, is responsible for increasing AR expression and consequently for glandular development and a greater

secretory activity of the glands treated with letrozole. In a similar manner, the suppression of estrogen activity in tissue can be related to lower ERα expression in the epithelium and prostatic stroma and to the development of prostatic intra-epithelial neoplasia.

With tamoxifen treatment, the morphologic alterations observed were more severe and consisted of neoplastic disorders and glandular hypertrophy (Santos *et al.*, 2005). The prostatic alveoli presented luminal enlargement and epithelial structural changes (Fig. 10A). Intra-epithelial neoplasia with a pseudocribiform arrangement was found repeatedly in several prostatic

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alveoli. The epithelial cells became polymorphic, fully vacuolated, and without traces of a greater secretory activity (Fig. 10B). Low AR (Fig. 10C) and ERα (Fig. 10D) expression was observed in epithelial and stromal cells. Serologic data showed that tamoxifen did not cause significant alterations in serum testosterone or estrogen levels (Santos *et al.*, 2005). Although there were not any serum hormonal alterations with tamoxifen treatment, immunocytochemical data indicate that the tamoxifen action not only disrupted estrogenic activity but also interfered with the prostate gland's response to androgenic stimulus.

Estrogens indirectly participate in prostatic growth and differentiation by modulating AR signaling and regulating the prostatic response to this hormone (McPherson *et al.*, 2001). In male mice that have a deficiency in estrogen production, the absence of this hormone alters AR activity, causing these receptors to be responsible for the development of pre-malignant and malignant lesions (Risbridger *et al.*, 2003). Consequently, it is believed that the tissue changes observed in prostates of female mice treated with tamoxifen are due to alterations in the intraprostatic hormonal environment. By blocking the binding between estrogen and its receptor, the prostatic physiology as a whole can be altered because besides estrogenic suppression, less AR activity was also observed.
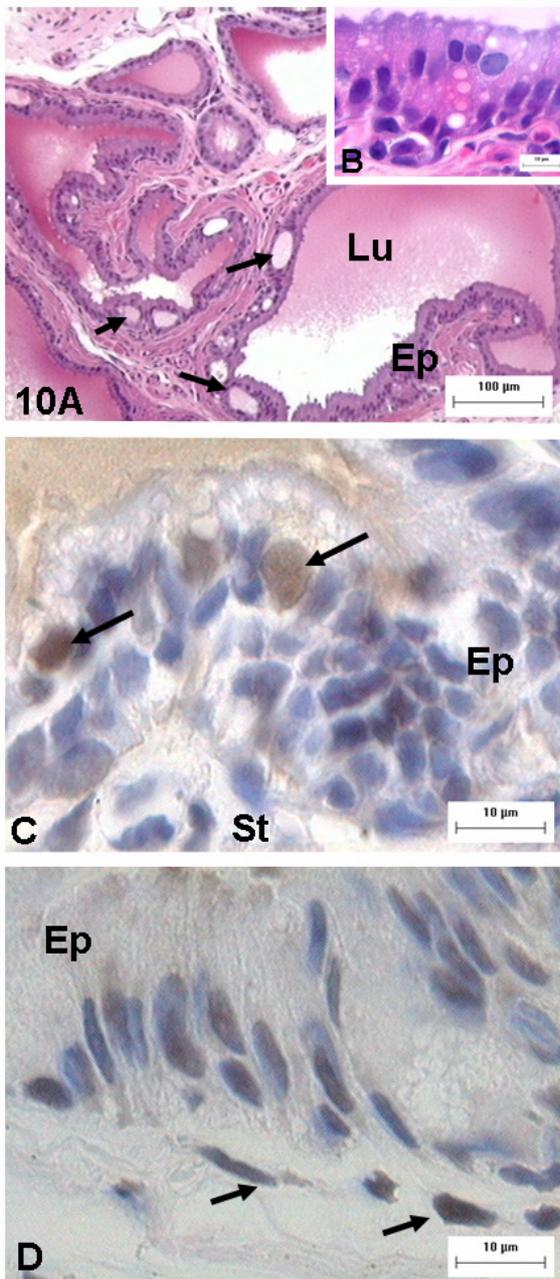
Figure 10. Histological sections of the adult female gerbil prostate treated with tamoxifen. **A**: prostatic alveoli with luminal hypertrophy (Lu) and intraepithelial neoplasia with pseudocribiform pattern (arrows) are shown. **B**: detail of polymorphic epithelial cells showing large amount of vacuoles. **A** and **B** are stained with hematoxylin-eosin. **C**: immunocytochemical reaction to the androgen receptor. Weak reaction are observed in the epithelium (arrows) and stroma (St). **D**: immunocytochemical reaction to the estrogen alpha receptor (ERα). Cells that stained positive for the ERα are observed in the epithelial (Ep) and stromal cells (arrow). **C** and **D** are counterstained with Harris's hematoxylin.

Concluding remarks

Androgens and estrogens participate in hormonal regulation of the female gerbil prostate. While androgens induce the differentiation, development, and secretory activity of the gland, estrogens appear to modulate the androgenic effects and maintain the normal physiology and growth of the prostate. Thus, the balance between these steroid hormones is crucial to prostatic homeostasis. Hormonal imbalance caused by anti-estrogen drugs (letrozole and tamoxifen) can trigger profound alterations in adult prostate morphophysiology. Some of these alterations are similar to those observed in the prostatic glands of post-menopausal women (Sloboda *et al.*, 1998; Islam *et al.*, 2001; Kato *et al.*, 2005) and in senile gerbil females (unpublished data). This corroborates the importance of internal hormonal balance to maintenance of prostatic health.

With the advent of contraceptive pills in recent decades, hormonal reposition drugs, hormonal therapies for breast cancer treatment, and even the exposure to environmental hormones known as *endocrine disruptors*, women are susceptible to severe hormonal alteration that can influence prostatic physiology. Because many health professionals have neglected the existence of a functional prostatic gland in females or even ignored its biological and pathological importance until now, little is known about the effects of long-term exposure to exogenous hormones on prostatic morphology. It is essential to monitor the female prostate frequently, mainly after menopause and during hormonal induction, in order to prevent development of prostatic disorders.

Acknowledgments

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