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Bisphenol A exposure alters hormonal modulation and responsivity in the prostate of aged female gerbils

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ABSTRACT

The female prostate is a gland regulated by steroid hormones for homeostasis. Bisphenol A (BPA) is an endocrine disruptor related to the progression of malignant lesions in prostate. The aim of this study was to analyze the hormonal modulation and responsiveness of the prostate of aged female gerbils previously exposed to BPA. Females were exposed to 50 μg/kg/day during pregnancy and lactation, and the prostate was analyzed at 18 months of age. Control groups were included to normalize the analysis. The samples were analyzed using histological and immunohistochemical techniques for the expression of androgen (AR), estrogen (ERα and ERβ), prolactin (PRL), and progesterone (PR) receptors, as well as steroidogenic enzymes (5α-reductase and aromatase) and the proliferation (PHH3) and epigenetic (EZH2) markers. Protein quantification was also performed for the receptors and enzymes described, as well as morphological and morphometric analyses of the gland. The results showed an increase in the epithelial expression of AR and ERα and a decrease in the expression of ERβ in this compartment. In addition, a decrease in epithelial expression was observed for the 5α-reductase and aromatase in the prostate epithelium and an increase in the expression of PHH3 and EZH2. By Western blot analyses, significant differences were observed in the protein quantification of the receptors and enzymes described. Thus, the current study showed the role of BPA in intraprostatic hormonal modulation, through alterations in the expression of hormone receptors and the conversion of enzymes in the female prostate, which can lead to the progression of malignant lesions in this tissue.

1. Introduction

The female prostate (Fig. 1) is an exocrine gland associated with the female urogenital system, that has become the subject of experimental studies since its description in some mammals (Santos and Taboga, 2006; UNTERGASSER et al., 2005). This gland is regulated by the action of steroid hormones, including androgens and estrogens (Biancardi et al., 2017; Fochi et al., 2008). Predominantly, androgens are responsible for the differentiation of this gland and estrogens regulate its development before puberty and its differentiation during adulthood (Dehm and Tindall, 2007; McPherson et al., 2008). In addition, progesterone and prolactin are responsible for the sexual activities and homeostasis of this gland (Grindstad et al., 2018; Zanatelli et al., 2021). These hormones play a relevant role, regulating development and homeostatic support during female cycles (Ruiz et al., 2023).

The cellular activity of hormones is mediated by nuclear or cytoplasmic receptors, which activate different pathways (Prins and Putz, 2008; Rochel-Maia et al., 2013). Androgen receptors (AR) are a nuclear

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receptor responsible for the activation of proliferative pathways (Balk and Knudsen, 2008). Estrogens act on the prostate by two members of the nuclear receptor family (ERα and ERβ). These receptors present a relevant role in the prostate development, mediating opposite activities, by activating proliferative and antiproliferative pathways in this prostatic tissue (McPherson et al., 2008; Rochel-Maia et al., 2013). During the development of the female prostate, steroidogenic enzymes, 5α-reductase (SRD5A1 and SRD5A2) and aromatase (CYP19A1) have an important function. These enzymes are responsible for the conversion of testosterone in dihydrotestosterone (DHT) or 17β-estradiol, respectively (Blakemore and Naftolin, 2016; Ellem and Risbridger, 2010; Traish, 2012). DHT is an androgenic hormone, which is involved with prostate growth and development, and differentiation of its secretory cells. This hormone acts on the prostate by the AR receptor. 17β-estradiol, on the other hand, is an estrogen hormone and is also related to the normal development of this gland and to the progression of pro-proliferative lesions in this tissue. Estrogens use the ERα and ERβ receptors to act on the female prostate (Ellem and Risbridger, 2010). The conversion by enzymes 5-alpha reductase and aromatase is then responsible for the maintenance of prostatic homeostasis, since disorders in this hormone

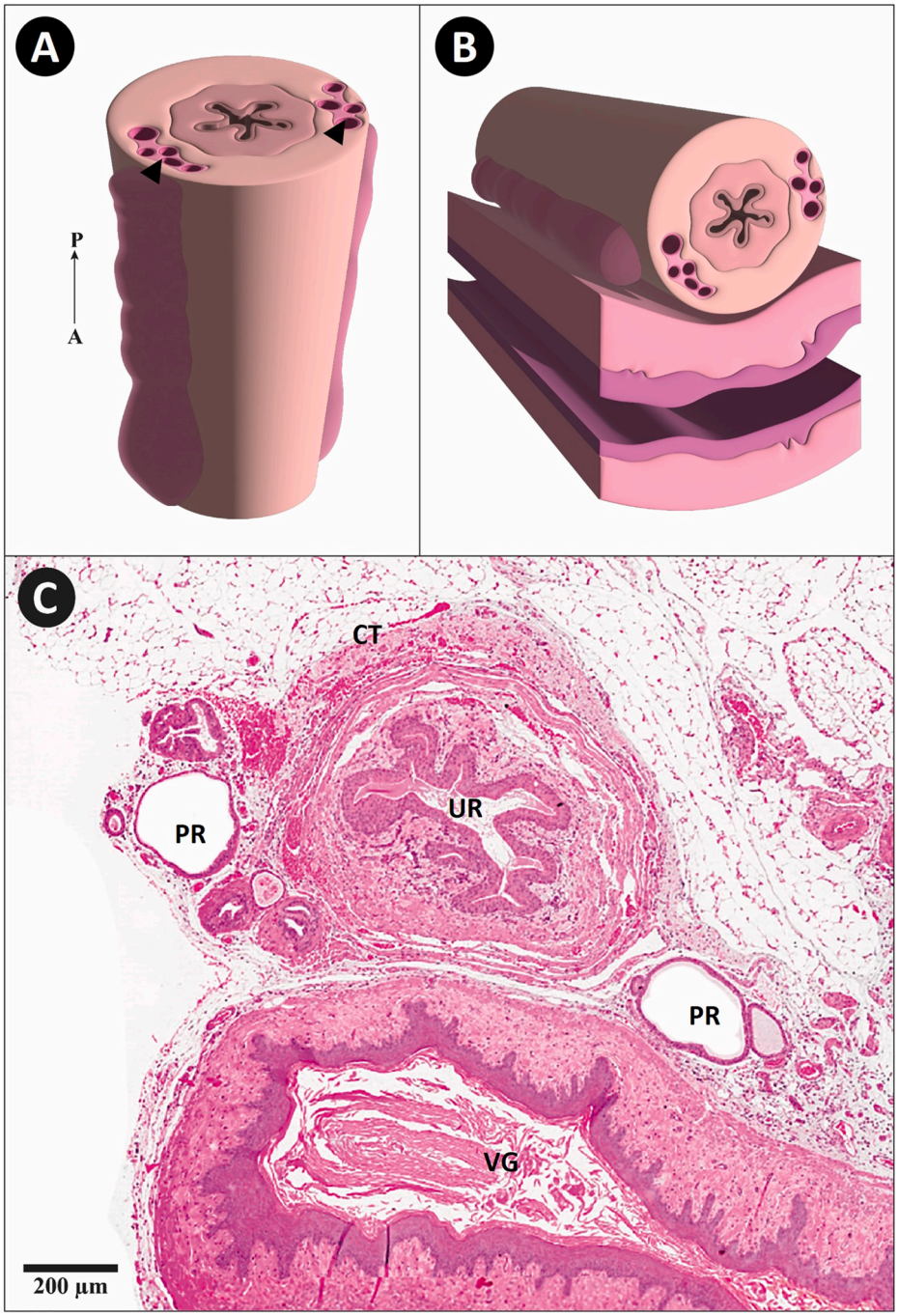


Fig. 1. Three-dimensional reconstruction of the prostate of the adult female gerbil (*Meriones unguiculatus*) in longitudinal **(A)** and transverse **(B)** views. The prostatic urethra (beige), acini (pink), and vagina are shown. The arrow indicates the orientation of the gland in relation to the anterior **(A)** and posterior **(P)** urethra. The acini (arrowhead) which insert into the connective tissue are also shown. **C**: Histological sections of the female prostate are shown, highlighting the urethra (UR), vagina (VG), connective tissue (CT), and female prostate (PR). **Scale bar:** 200 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modulation can lead to lesion development (Castro et al., 2013, 2018; Sánchez et al., 2022).

Over the years, the prostate gland has been studied in females using different models, such as the Mongolian gerbil (*Meriones unguiculatus*) (Dos Santos et al., 2003; Ruiz et al., 2021b; Ruiz et al., 2023c; Santos and Taboga, 2006). The females of these rodents have a well-developed prostate and its morphology and distribution of tissue components converge with those found in humans (Dos Santos et al., 2003). In gerbils, the prostate has a structure similar to the ventral lobe of the male prostate, located around the urethra and presenting an epithelium composed mostly of secretory cells (Dos Santos et al., 2003). In addition, the female prostate of this species shows spontaneous development of prostatic disorders, such as preneoplastic lesions, being an efficient model for studying endocrine dysregulation (Ruiz et al., 2023).

Studies have shown that the consequent lesions and alterations in the gland can occur based on the phase of estrous or gestational cycle (Fochi et al., 2008). There is also a relationship between these alterations and the aging process, in which there is a decrease in steroid hormone levels, along with the appearance of pathological disorders associated with this process (Jakiel and Baran, 2005). However, it was reported that endogenous and exogenous agents can play this role, leading to the development of pre-malignant lesions, such as hyperplasia and carcinomas (Cannarella et al., 2021; Wang et al., 2021). These agents include aging and the action of environmental endocrine disruptors, such as bisphenol A (BPA) (Costa et al., 2016; Custodio et al., 2010; Wang et al., 2021), leading to a different scenario for pathological development. BPA is an endocrine disruptor (ED) of the xenoestrogen class, which is widespread in the environment (Bleak and Calaf, 2021; Timms et al., 2005; Vom Saal and Vandenberg, 2021). According to previous studies, this ED interferes in the reproductive cycle, and can lead to epigenetic modifications, including modifications in histones (Acevedo et al., 2018; Bae and Hennighausen, 2014; Manikkam et al., 2013), promoting the expression of EZH2 (Enhance of Zeste Homolog 2), for example. This marker is a histone lysine found in the mammary gland and prostate of rodents. In addition, it is related to the repression of several genes and the consequent progression of pre-neoplastic lesions in these glands (Bae and Hennighausen, 2014; Doherty et al., 2010). Studies have linked exposure to this ED with morphological and physiological alterations, not only in the prostate, but also in the mammary and adrenal glands, which can even lead to the development of diseases in these glands (Ruiz et al., 2021; Tarafdar et al., 2022).

Thus, hormones and their target actions are crucial to the maintenance of the homeostasis of the female prostate, and imbalances in these hormones and their receptors interfere in this homeostasis. These imbalances can be caused by the action of some EDs, like BPA. Therefore, the aim of the current study was to analyze the expression of hormone receptors and hormonal modulation via converting enzymes in the prostate of aged female gerbils after exposure to BPA during gestation and lactation.

2. Material and methods

Twenty-five female Mongolian gerbils (*Meriones unguiculatus*) were kept in polysulfone isolators with drinking and feeding troughs and feed and water *ad libitum*. The females were allocated with fertile males. The first day of gestation was defined after the birth of the first litter, which was discarded. The female gerbil exhibits postpartum estrus, and the day of parturition is thus Day 1 of the next gestation, that will only be normal length if the first litter is removed. To access the BPA exposure outcomes, the females were divided into three experimental groups: control group (gavage with water) (n = 8); vehicle group (gavage with oil) (n = 8); and BPA group - daily safe exposure (50 μg/kg) (n = 9). The dosage of BPA chosen for daily administration was considered safe according to the US EPA (United States Environmental Protection Agency) and was shown to be a carcinogenic inducer in hormone-responsive organs (Ruiz et al., 2021; Ruiz et al., 2022, 2023; Ruiz et al., 2023a; Ruiz et al., 2021). Furthermore, according to the EFSA (European Food Safety Authority), a new TDI dose was established for BPA (0.2 μg/kg/day) (Vom Saal et al., 2024).

The females were treated using a protocol whereby the exposure period lasted for the second pregnancy (24–26 days) and lactation (21 days). At 18 months of age, all the animals were anesthetized with isoflurane (3% in O2), weighed, and euthanized by decapitation. The female prostate was removed along with the bladder, the prostatic complex was dissected from the base of the bladder to the vaginal insertion, and the adjacent adipose tissue was removed. The female prostates were collected and fixed in paraformaldehyde 4% for 24 h. After fixation, the samples were dehydrated in ethanol, clarified in xylol, infiltrated, and included in paraplast on the automatic processor Leica TP 1020. The female prostates were sectioned (5 μm thickness) and the slides were mounted on a Leica RM 2255 automatic rotary microtome. For the western blotting analyses, the samples were frozen at −80 °C.

2.1. Morphological analyses

For general morphological analyses, serial sections of the female prostate were obtained, to provide a cranial-caudal overview, and stained with Hematoxylin-Eosin and immunohistochemistry for the α-SMA (alpha smooth muscle actin). Five animals per group and five sections per animal, with equivalent distances, were analyzed. The percentage of the tissue alterations was based on the ratio of the number of normal, atrophic, and hyperplastic acini to the total number of acini per animal.

Morphometry was also performed. Five sections per animal (n = 5) with equivalent distances were analyzed and marked with α-SMA immunohistochemistry. These sections were submitted to measurement of the height (μm) of epithelial cells and smooth muscle layer under the epithelium. For each analysis, 50 measurements were obtained, being 10 measurements per animal from each experimental group (n = 5). The evaluations were performed under light microscopy and the histological images were digitized with a BX61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120® Virtual Microscopy Slide Scanning System (VS120-S5), using QuPath® software.

2.2. Immunohistochemistry

This technique was used to analyze the expression of androgen (AR; Santa Cruz Biotechnology, Inc., sc-816, rabbit polyclonal, 1:50) and estrogen (ERα; Elabscience, Inc., EAB-31381, rabbit polyclonal, 1:50; and ERβ; Santa Cruz Biotechnology, Inc., sc-8974, rabbit polyclonal, 1:75), prolactin (PRLR; Cell Signaling, Inc., CST#13552, rabbit, 1:50) and progesterone (PR; Cell Signaling, Inc., CST#8757, rabbit) receptors, as well as the expression of hormone-converting enzymes, 5α-reductase (5-α-reductase 2; Santa Cruz Biotechnology, Inc., sc-20659, rabbit polyclonal, 1:50), and aromatase (CYP19; Elabscience, Inc., EAB64300, rabbit, 1:50). In addition, the expression of histone lysine methyltransferase (EZH2; Cell Signaling, Inc., CST#5246, rabbit, 1:75), smooth muscle fiber protein α- SMA (α-SMA; Santa Cruz Biotechnology; Inc., sc-53142, mouse monoclonal, 1:75**),** and the proliferation marker PHH3 (PHH3; Santa Cruz Biotechnology, Inc., sc-8656, rabbit, 1:100) in the female prostate were analyzed using this technique. For these analyses, the samples underwent the following steps: deparaffinization and rehydration of the sections with baths in xylol, alcohol, and water, respectively; antigenic recovery, in 10 mM citrate buffer or Tris EDTA, in a water bath at 92 °C or 98 °C; blocking of $^{\text{endogenous peroxidases, with 10\%}}_{\text{H}2\circ2}$ $^{\text{in methanol; inhibition of non-specific proteins}}$ with bovine serum albumin or using a solution of 5% skimmed milk; incubation of the primary antibody (varies for each receptor and enzyme described) overnight; incubation of the secondary antibody and polymer link (polymer detection system Novolink™ 1, Leica Biosystems Newcastle 118 Ltd., Newcastle, United Kingdom); development of the bonds with 3–30 diaminobenzidine tetrahydrochloride (DAB; Novolink™ DAB, RE7270-CE,

Leica Biosystems, Buffalo 121 Grove, USA); and counterstaining with hematoxylin. The previous steps were interspersed with washes in TBS or PBS and the slides were analyzed under a light microscope.

To quantify the positive cells for the receptors and markers described, the total area of the prostate was analyzed, both in the epithelium and in the stroma, using QuPath® software. The percentage of positive cells was defined by the quantification of immunoreactive cells divided by the total number of cells counted. This procedure was performed on five samples/animals per group (three sections per animal, n = 5), which were equally spaced (~100 μm) to make a craniocaudal comparison, more representative of whole female prostate. On the other hand, for the quantification of cells positive for the enzymes described and prolactin receptor, the relative frequency of cells immunoreactive for the technique was used. The relative frequency (%) was estimated using the M130 multipoint test system applied to the prostate according to the procedure described by (Huttunent et al., 1981). In this technique, 10 fields were photographed per animal at 400× magnification, in which 130 points were placed, classified according to the analysis as positive or negative for the marker, using Image-Pro® Plus software (v. 6.0).

2.3. Western blotting

To quantify the proteins, the samples were subjected to Western Blotting analysis. In summary, protein extracts were obtained from the prostate complexes frozen at −80 °C using the tissue digestion method and an extraction buffer. From these extracts, total protein concentrations were quantified by absorbance of the samples using the BCA Protein Assay kit (Pierce BCA Protein Assay Kit, Thermo Fischer Scientific, Rockford, IL, USA) in a microplate reader. After this, the proteins were subjected to SDS page gel (12% acrylamide) electrophoresis (90V/120 min) and then transferred to nitrocellulose membranes. These membranes were subjected to the immunoblotting protocol: blocking of non-specific proteins with 5% skimmed milk in TBSt; incubation in the primary antibodies overnight at 4 °C; washing in TBSt buffer; and incubation in the corresponding secondary antibodies (HRP; Cell Signaling; Inc., CST#7074, rabbit, 1:1000; and HRP; Cell Signaling; Inc., CST#7076, mouse, 1:1000) and in a solution of the ECL Pierce detection substrate (32.109, ThermoFischer, Rockford, IL, USA). This technique was performed to analyze the androgen (AR; Cell Signaling; Inc., CST#5153, rabbit; 1:500), estrogen alpha (ERα; Invitrogen; Inc., MA5-14502, rabbit, 1:1000), and estrogen beta receptors (ERβ; Santa Cruz, Inc., sc- 373853, rabbit, 1:1000) and the enzymes aromatase (CYP19; Elabscience; Inc., E-AB-68290, rabbit, 1:500) and 5α-reductase (5α-reductase 2; Santa Cruz; Inc., sc-20659, rabbit, 1:1500), and the GAPDH protein (GAPDH; Cell Signaling; Inc., CST#2118, rabbit, 1:1000). Finally, these membranes were revealed in the ChemiDoc® image system (BioRad, Hercules, CA, USA). The density of the bands was quantified using ImageJ software (version 1.52a, Wayne Rasband, NIH, USA) and molecular weight was estimated based on ladder bands in Image Lab Software (Version 6.1) (Bio-Rad Laboratories, 2020).

2.4. Statistical analysis

The data from the Western Blotting analyses are shown in boxplots (median) and the other analyses are expressed in bar graphs (error of the mean) and were statistically analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc., USA). Firstly, the normality of the data obtained was checked using the Kolmogorov-Smirnov test. Data with parametric distribution were then subjected to the ANOVA test and data that did not show parametric distribution were subjected to the Kruskal-Wallis test. The analyses were also followed by post-tests (Tukey and Dunn's), according to the first distribution analyses.

3. Results

3.1. Histopathological analyses

The quantity of normal, atrophic, hyperplastic acini, and acinus with PIN (Fig. 2E–I) did not show significant differences among the different groups. However, the height of the different compartments of the tissue showed statistical significance, with a decrease in the BPA group compared to control and vehicle groups (Fig. 2J and K). In addition, the height of the muscle layer did not show statistical differences between the groups (Fig. 2 K). The expression of the proliferation marker (PHH3) was analyzed using immunohistochemical techniques (Fig. 2 H). An increase in PHH3 expression was observed in the BPA and vehicle groups, compared to the control group, with significant differences.

3.2. Hormone receptor expression

The expression of AR and ERα in the prostate of females exposed to BPA showed a significant increase compared to the other experimental groups (Fig. 3A and B). In the epithelial cells, AR and ERα expression (Fig. 3C and D) increased significantly in the BPA and vehicle groups compared to the control group. Furthermore, stromal AR expression (Fig. 3 C) showed an increase in the BPA group, and epithelial ERβ expression (Fig. 3 E) showed a decrease in the BPA group compared to the control and vehicle groups. In addition, the western blotting analyses showed an increase in ERα in the BPA and vehicle groups compared to the control group (Fig. 3 H), while there were no significant differences in the quantities of AR and ERβ between the groups (Fig. 3G–I). Finally, there were no significant differences in the PR and PRL expression between the groups (Fig. 3F–J).

3.3. EZH2 expression

The expression of histone lysine methyltransferase (EZH2) was analyzed using immunohistochemical techniques (Fig. 4). As seen in Fig. 4-B, there was an increase in epithelial expression in the BPA group compared to the control and vehicle groups.

3.4. Steroidogenic enzyme expression

The expression of steroidogenic enzymes was evaluated by immunohistochemistry and Western blotting (Fig. 5A and B). Epithelial aromatase enzyme decreased significantly in the BPA group compared to the control and vehicle groups (Fig. 5 C). Furthermore, the expression of aromatase in the stroma demonstrated an increase in the BPA group compared to the control and vehicle groups (Fig. 5 C). Furthermore, the present study showed an epithelial decrease in the expression of the 5α-reductase enzyme in the BPA group compared to the control group (Fig. 5 D). However, there were no significant differences between the vehicle and BPA. Finally, in the western blotting analyses (Fig. 5E and F), a significant decrease in the enzyme 5α-reductase was observed in the BPA group compared to the control and vehicle groups.

4. Discussion

The key findings presented in the current study demonstrated that BPA exposure during windows of susceptibility (pregnancy and lactation) affected morphological and western blotting expression of receptors, and modulated hormonal signaling in the female prostate. The results showed an increase in AR expression in the stromal cells and a decrease in ERβ expression in the epithelial cells. In addition, in the BPA group, aromatase and 5α-reductase were changed. In the epithelium, BPA caused a decrease in aromatase and an increase in this enzyme in the stroma. In another way, 5α-reductase decreased with the action of BPA. Furthermore, BPA exposure led to increased EZH2 expression, a remarkable epigenetic marker, in the epithelium, and a decrease in this

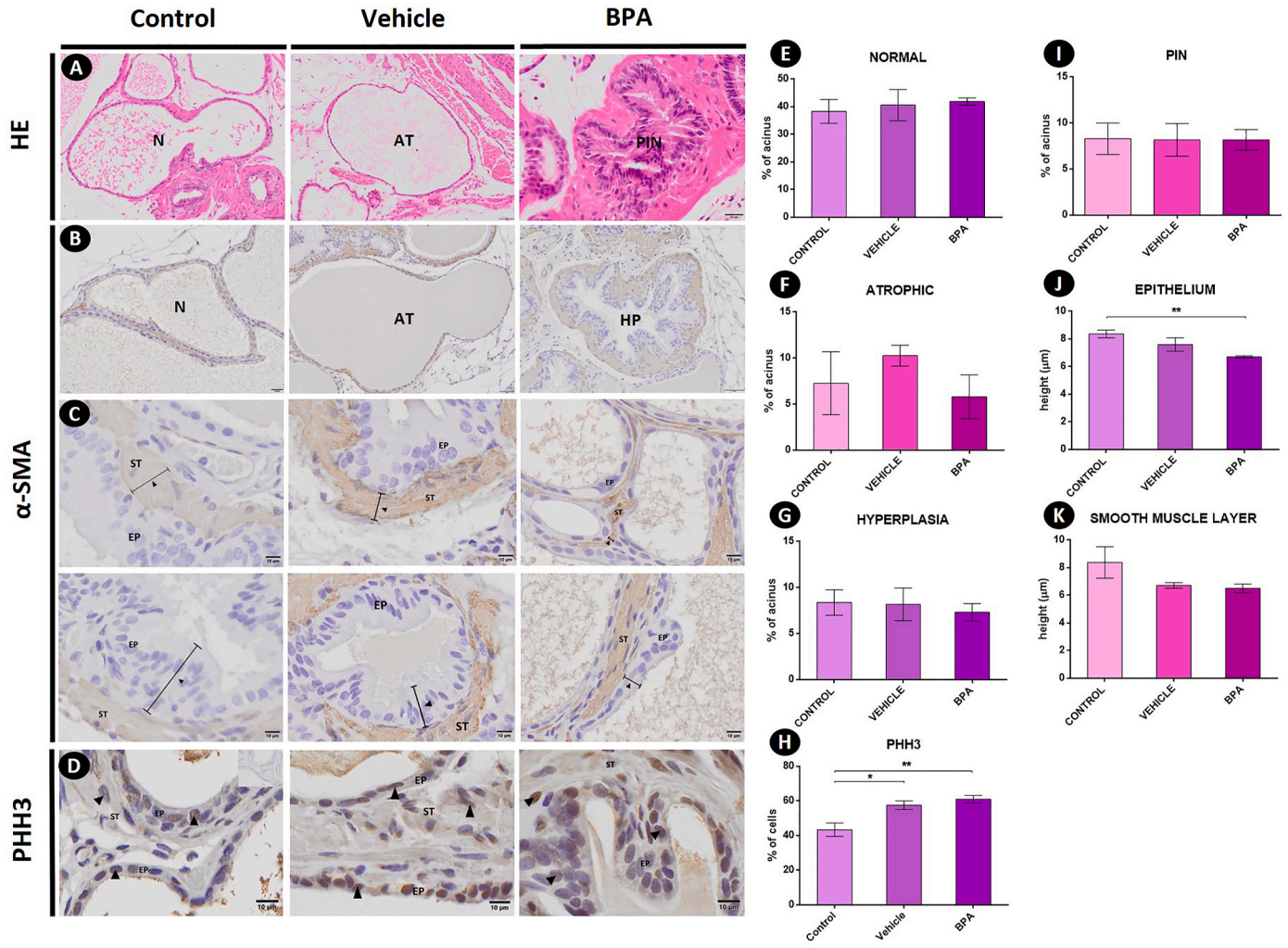


Fig. 2. Morphological **(A, B)** and morphometric **(C)** analyses, and the expression of the proliferation marker (PHH3) **(D)** in the female prostate. Representation of the different types of acini in HE **(A)** and with α- SMA expression **(B)**. **(C)** Representation of the height (arrowhead) of the epithelium and the smooth muscle layer**. (D)** Total expression (arrow) in the experimental groups for the marker described. Percentage of normal **(E)**, atrophic **(F)**, and hyperplastic acini **(G),** and acinus with PIN **(I)** on the different groups (control, vehicle, and BPA). Height of epithelium **(J)** and smooth muscle layer **(K)** in the different groups. **(H)** Percentage of total cells positive for the marker described (PHH3). The asterisks show the statistical difference between the experimental groups ((*$p < 0.05$; **$p < 0.01$). **Groups:** control, vehicle, and BPA. **Abbreviations:** N: normal acinus; AT: atrophic acinus; HP: hyperplastic acinus. EP: epithelium; ST: stroma**. Scale bars:** (A–D) 10 µm and (A3, B3) 20 µm. *Inset*: Negative Control.

compartment in the female prostate. In addition, exposure to corn oil (vehicle group) also showed some significant differences. This has already been demonstrated in the male prostate, in which corn and mineral oil interfere in homeostasis, revealing their potential to act as endocrine disruptors (Guerra et al., 2019). However, the present work and the following discussion focus on the action of BPA exposure.

The exposure to BPA has a relevant impact on the female prostate, leading to alterations in the proliferative pathways and promoting the development of pre-neoplastic lesions in this organ, as well as histo-pathological alterations (Chen et al., 2016; Rochel-Maia et al., 2013; Wang et al., 2021). Contributing to this scenario, studies in males demonstrate that aging plays a significant role in the alterations related to endocrine disruptors, since it promotes an increase in atrophy in the prostatic epithelium (Huang et al., 2018; Kido et al., 2014). Furthermore, with aging, the levels of testosterone decrease, and this hormone appears to be a repressor to ERα expression and necessary to ERβ expression in females (Attard et al., 2009; Jakiel and Baran, 2005; Preventicon and African, 1984). Both estrogen receptors act in synergy, regulating the activation of pro- and antiproliferative pathways. Under prostatic homeostatic conditions, the ERα expression is predominantly observed in stroma cells, while ERβ is mainly expressed in epithelial cells (Imamov et al., 2004).

BPA exposure led to unbalance of expression of both receptors. In addition, the AR expression was founded on the basal secretory epithelial cells, since the activity of the prostate is regulated by the cross-regulation between androgens and the responsivity of epithelial cells (Cordeiro et al., 2008). In females, this androgen receptor is also responsible for activating pro-proliferative pathways and regulating the tissue homeostasis with the estrogen receptors (Dehm and Tindall, 2007; Rochel-Maia et al., 2013). Thus, with the high expression of ERα and AR receptors and the low expression of the ERβ receptor in the epithelium, the dynamic of responsivity could develop pre-neoplastic lesions (Rochel-Maia et al., 2013; Wilson, 2011). It has been shown that ERα has a proliferative function, since it appears to be greatly increased in cases of prostate cancer (Lafront et al., 2024). On the other hand, most studies attribute a protective function to ERβ, since it is responsible for activating antiproliferative pathways, acting as a tumor suppressor when increased, and inactivating the proliferative pathway of AR (Risbridger et al., 2001; Rochel-Maia et al., 2013). However, the opposite action of this receptor has also been demonstrated, which could facilitate the

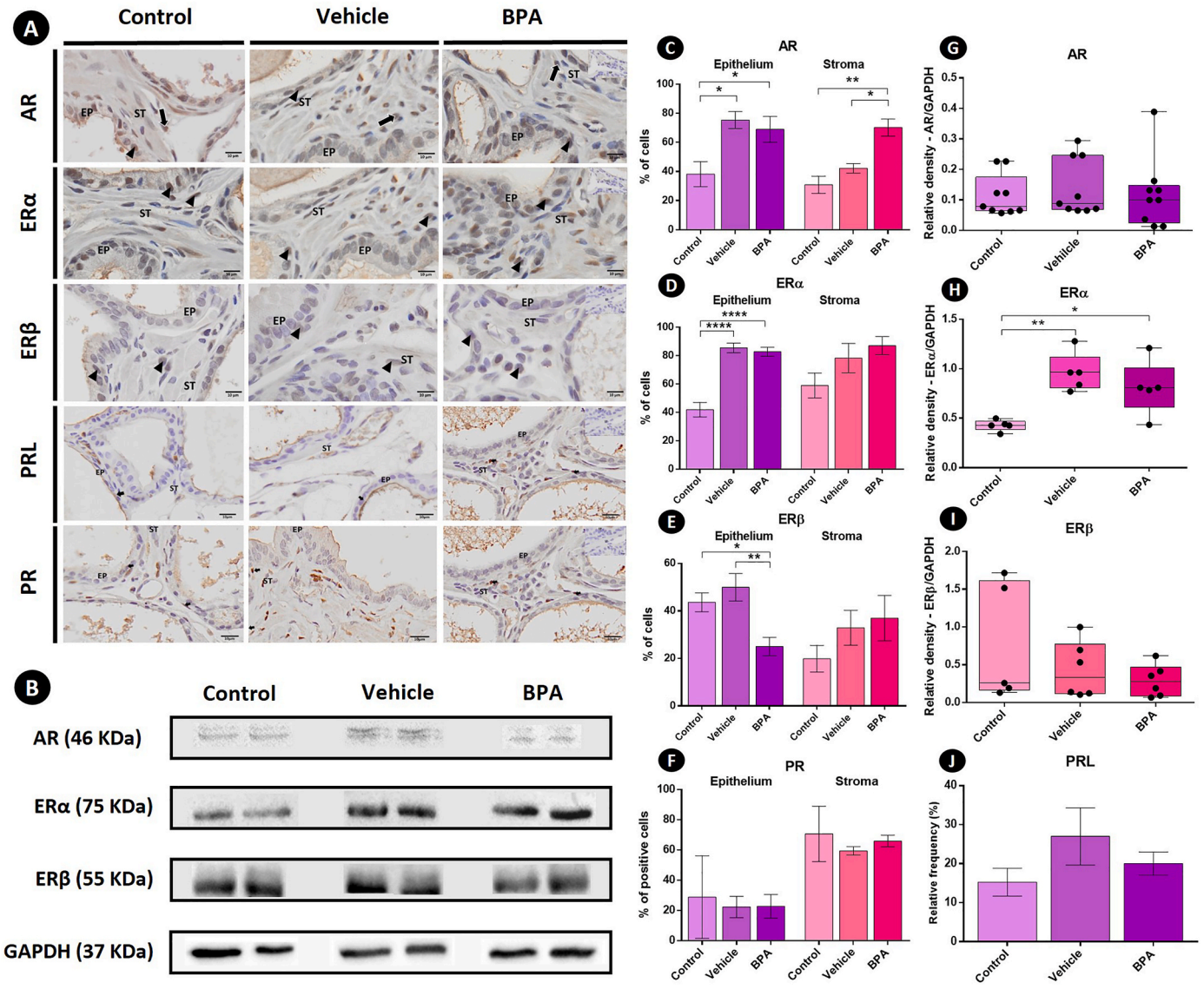


Fig. 3. Expression of AR, ERα, ERβ, PR, and PRL in the female prostate. **(A)** Epithelial (arrowhead) and stromal (arrow) expression in the experimental groups for the receptors described. **(C-F, J)** Percentage of epithelial and stromal cells positive for the receptors described. Protein quantification in Western Blotting for the receptors described **(B)**. **(G–I)** Relative density for the receptors described. The asterisks show statistical differences between the experimental groups (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$). **Groups:** control, vehicle, and BPA. **Abbreviations:** EP: epithelium; ST: stroma. **Scale bars:** (A, D, G) 10 μm. *Inset:* negative control.

progression of these lesions (Li et al., 2022). This can occur in cases where ERα expression is greatly increased. Furthermore, under specific conditions, this receptor may be responsible for the progression of cancer cells, for example. This happens through ERβ interfering with other ligands, promoting the amplification of cancer cell growth or the activation of cascade responses, accelerating the differentiation of these cells. However, although this action has been documented, many studies are still needed to really understand how ERβ behaves in these cases (Li et al., 2022).

Through the hormone receptors, the hormones act in tissue alterations, mainly for the female prostate. The regulation of this gland is mediated by androgens and estrogens signaling for the development and differentiation of this gland during the life cycle (Biancardi et al., 2017; Dehm and Tindall, 2007; Fochi et al., 2008). These hormones act together at the prostate and, according to previous studies, alterations in their serum levels could be responsible for the progression of some diseases in this tissue, such as preneoplastic lesions and prostate cancer (Bosland, 2000; Leav et al., 1988; Ricke et al., 2007). The reduction in testosterone can lead to a significant change in the expression of the hormone receptors, being responsible for the decrease in ERβ expression and the increase in ERα expression in females, favoring the appearance of pre-malignant lesions in the prostatic tissue (Jakiel and Baran, 2005; Rochel-Maia et al., 2013). However, unlike some previous results (Ruiz et al., 2023b), other studies show an increase in the plasma levels of estradiol with BPA exposure, explaining the increase in the expression of their receptors in the female prostate (Castro et al., 2018; Ricke et al., 2007). It is probable that this observed decrease is being supported in the gland, by the increase in the aromatase enzyme in the tissue and the estrogen levels on the prostate are also being increased, increasing the expression of their receptors.

The testosterone is converted into 17β-estradiol or Dihydrotestosterone (DHT) to act in the female prostate by their own hormone receptors (Sánchez et al., 2013, 2022). This conversion is mediated by two main enzymes: aromatase and 5α-reductase. Aromatase converts testosterone into 17β-estradiol (Ellem and Risbridger, 2010). This enzyme is important to the local maintenance of the testosterone-estrogen ratio and when its expression changes, there is a large chance of the development of some pathologies in this gland. This

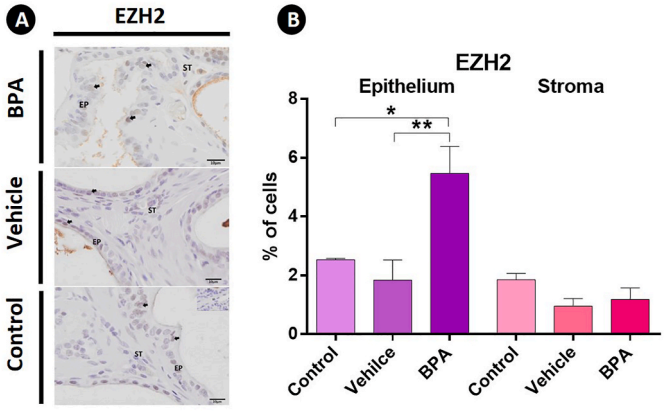


Fig. 4. Expression of histone lysine methyltransferase (EZH2) **(A)** in the female prostate. **(A)** Epithelial and stromal expression (arrow) in the experimental groups for the receptors described. **(B)** Percentage of epithelial and stromal cells positive for the receptor described. The asterisks show statistical differences between the experimental groups (*p < 0.05; **p < 0.01). **Groups:** Control, vehicle, and BPA. **Abbreviations:** EP: epithelium; ST: stroma. **Scale bars:** (A) 10 μm. *Inset*: Negative Control.

occurs because of the proliferative actions of estrogen (Castro et al., 2013, 2018; Ellem and Risbridger, 2010; Sánchez et al., 2022). On the other hand, 5α-reductase is responsible for converting testosterone to DHT and this androgenic hormone acts by the AR in the female prostate, activating pro-proliferative pathways and regulating tissue development (Castro et al., 2013, 2018; Sánchez et al., 2022).

BPA has been described as having an antiandrogenic action, since it decreases the quantity of circulating testosterone (Sánchez et al., 2013). Previous studies also show decreases in 5α-reductase, as well as serum testosterone and DHT intraprostatic levels (Ruiz et al., 2023b; Sánchez et al., 2013). The regulation of 5α-reductase is testosterone-dependent, since there was a decrease in the serum levels of this hormone and, consequently, a decrease in this isoenzyme and intraprostatic androgens levels. However, the expression of AR increased in this tissue, associated with the antagonist action of BPA (Castro et al., 2013, 2018; Sánchez et al., 2013, 2022). Therefore, these enzymes are significant to hormonal availability *in situ*, and alterations in their levels, allied with the high expression observed in the estrogen alpha and androgen receptors, can cause the imbalance in tissue homeostasis.

Furthermore, BPA exposure is related to epigenetic modifications (Bae and Hennighausen, 2014; Doherty et al., 2010). The present work shows some alterations in EZH2 expression. This marker is a histone methyltransferase and it is associated with the progression of premalignant lesions and some pathologies in this tissue (Bae and Hennighausen, 2014). The results suggest that the EZH2 can be regulated by estrogen, since there were also alterations in the expression of estrogen receptors present in the BPA group compared to the other groups (Nanjappa et al., 2019). This has already been demonstrated in other works, but in the uterine epithelium, where the high expression of EZH2 was linked to the high expression of estrogen receptors and the increase in E2 (Nanjappa et al., 2019).

5. Conclusions

The results of the current work demonstrated that BPA exposure during the included phases (gestation and lactation) was responsible for changing the homeostasis of the female prostate by different pathways. This involves the expression of hormone receptors and steroidogenic enzymes, and epigenetic modifications, which can lead to the progression of pre-malignant lesions in this tissue and some prostate pathologies, since these alterations activate proliferative pathways.

CRediT authorship contribution statement

Luara Jesus Ferrato: Writing – original draft, Methodology, Formal

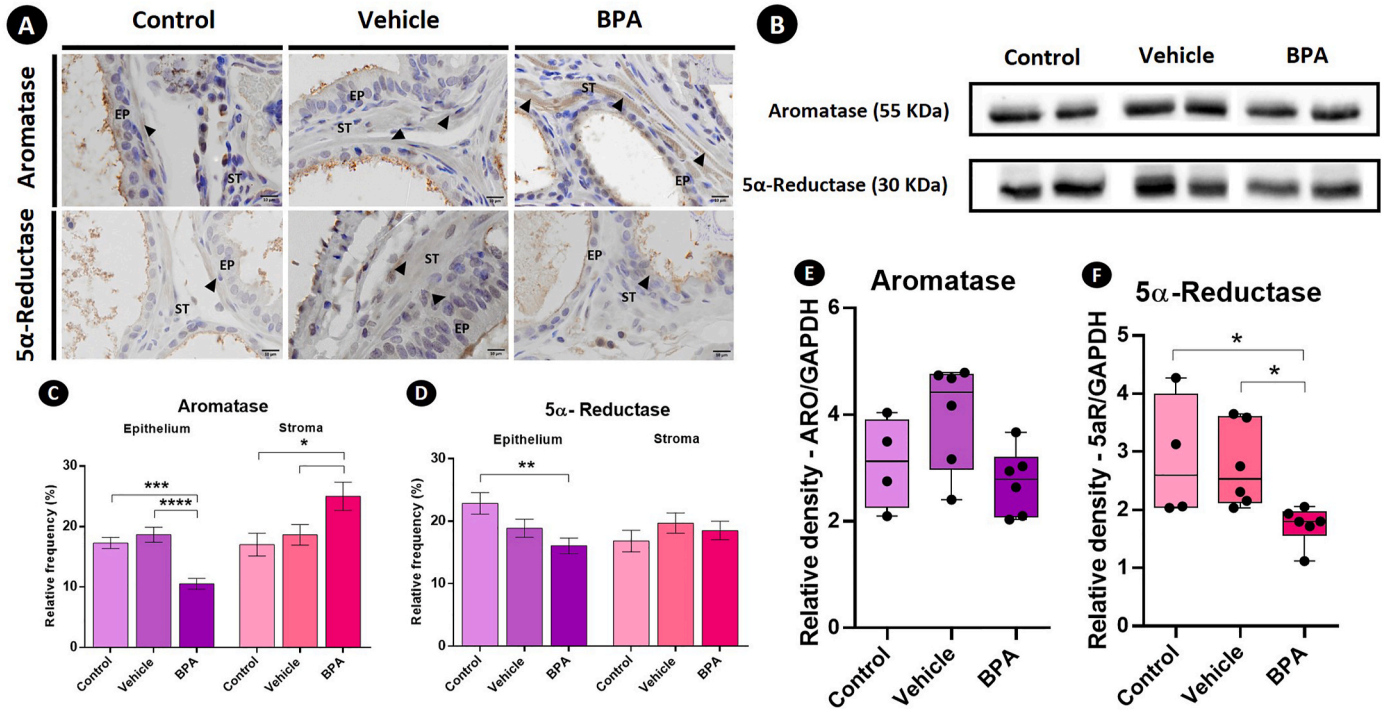


Fig. 5. Immunohistochemistry for the expression of the enzymes Aromatase and 5α-Reductase. **(A)** Epithelial and stromal expression (arrowhead) in the experimental groups for the enzymes described. **(C, D)** Percentage of epithelial and stromal cells positive for the enzymes described. Protein quantification in Western Blotting for the enzymes described **(B)**. **(E, F)** Relative density for the enzymes described. **Abbreviations:** EP: epithelium; ST: stroma. **Scale bars:** (A, B) 10 μm. The asterisks show the statistical difference between the experimental groups (*p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001). *Inset:* negative control.

analysis, Data curation, Conceptualization. **Thalles Fernando Rocha Ruiz:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lorena Gabriela de Souza:** Writing – original draft, Methodology, Formal analysis. **Gervásio Evangelista Brito-Filho:** Methodology. **Simone Jacovaci Colleta:** Methodology. **Ellen Cristina Rivas Leonel:** Writing – original draft, Supervision, Methodology. **Sebastião Roberto Taboga:** Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Ethics approval and consent to participate

This study has Ethic Committee in Animal Utilization (CEUA) homologation, under protocol nº 217/2019.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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