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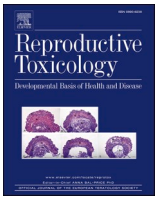

The impacts of exposure to bisphenol A in the adult female prostate *Meriones unguiculatus*

Carolina Marques Bedolo [a], Thalles Fernando Rocha Ruiz [b], Gustavo Matheus Amaro [a], Patricia Simone Leite Vilamaior [a], Ellen Cristina Rivas Leonel [c], Sebastião Roberto Taboga [a,b,*], Silvana Gisele Pegorin de Campos [a]

[a] *Department of Biological Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), Rua Cristóvão Colombo, 2265, Jardim Nazareth, 15054-000 São José do Rio Preto, São Paulo, Brazil*

[b] *Department of Structural and Functional Biology, Institute of Biology, State University of Campinas (UNICAMP), Bertrand Russell, s/n, 13083-865 Campinas, São Paulo, Brazil*

[c] *Department of Histology, Embryology and Cell Biology, Institute of Biological Sciences, Federal University of Goiás (UFG), Avenida Esperança, s/n, Câmpus Samambaia, 74690-900 Goiânia, Goiás, Brazil*

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ABSTRACT

The female prostate is associated with the urogenital system and presents homology in morphological terms with the male prostate. Due to its responsiveness to endogenous hormones, this gland is under a constant risk of developing prostatic pathologies and neoplasia when exposed to certain exogenous compounds. Bisphenol A (BPA) is an endocrine disruptor found in different plastic and resin products. Studies have emphasized the effects of perinatal exposure to this compound on different hormone-responsive organs. However, there have been few studies highlighting the influence on female prostate morphology of perinatal exposure to BPA. The objective of this study was to describe the histopathological alterations caused by perinatal exposure to BPA (50 μg/kg) and 17-β estradiol (E2) (35 μg/kg) in the prostate of adult female gerbils. The results showed that E2 and BPA induced proliferative lesions in the female prostate and acted along similar pathways by modulating steroid receptors in the epithelium. BPA was also found to be a pro-inflammatory and pro-angiogenic agent. The impacts of both agents were marked in the prostatic stroma. An increase in the thickness of the smooth muscle layer and a decrease in AR expression were observed, but no alterations in the expression of ERα and ERβ, leading to estrogenic sensitivity of the prostate. However, a peculiar response of the female prostate was to diminish the collagen frequency under BPA exposure correlated to smooth muscle layer. These data therefore indicate the development of features related to estrogenic and non-estrogenic tissue repercussions by BPA perinatally exposure in gerbil female prostate.

1. Introduction

The prostate, despite being generally described and studied in males, presents embryological and postnatal development in females [1–3]. This is due to androgen signaling, which may increase during the perinatal period in some species [4]. In humans, the existence of a female prostate is controversial, although histological and immunohistochemical evidence has confirmed the presence of an analogous glandular tissue surrounding the urethra [5,6]. Additionally, some believe the function of this tissue is associated with the production of secretions during ejaculation and orgasm after sexual arousal [7].

Among rodent models, *Meriones unguiculatus* (Mongolian gerbil), has a high incidence of functional prostates in females and has been an excellent model for the characterization and evaluation under experimental conditions of this gland [4,8]. Its features are homologous those of the male prostate [9,10], and is described as Skene's paraurethral gland in humans [11,12]. It is also responsive to hormones that regulate prostate development and homeostasis [13]. Studies indicate that the

Abbreviation: AR, androgen; BPA, Bisphenol A; ED, endocrine disruptor; ERα, estrogen receptor α; ERβ, estrogen receptor β.

* Correspondence to: São Paulo State University (Unesp), Department of Biological Sciences, Institute of Biosciences, Humanities and Exact Sciences, Cristóvão Colombo St, 2265, Jardim Nazareth, 15054-000 São José do Rio Preto, São Paulo, Brazil.

E-mail address: sebastiao.taboga@unesp.br (S.R. Taboga).

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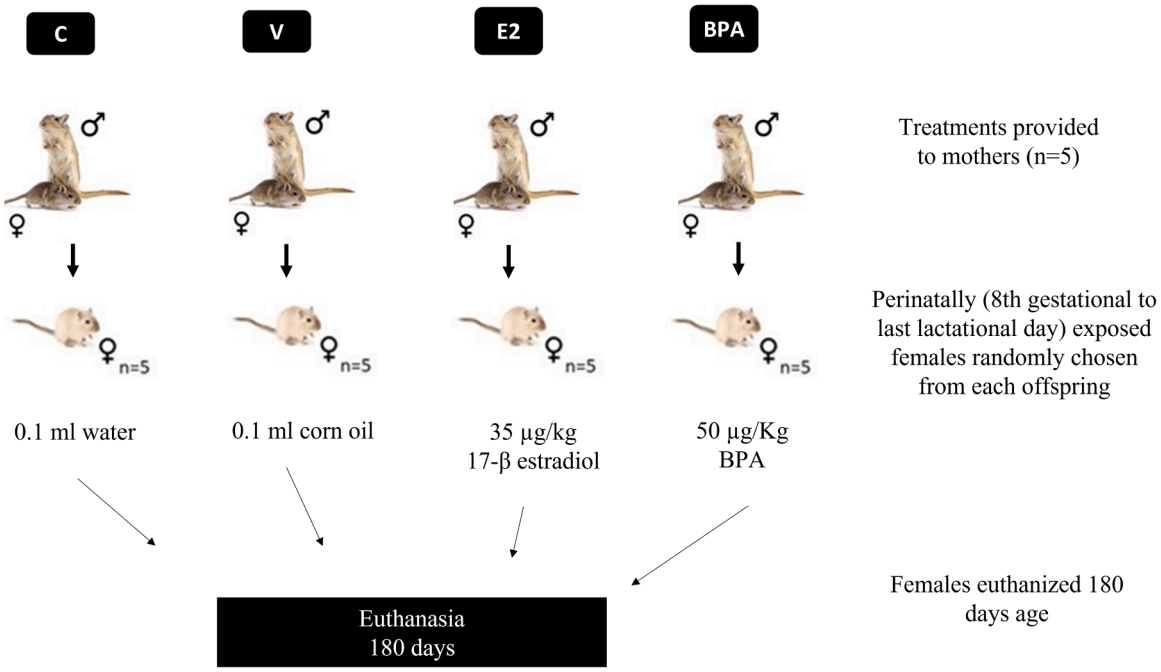

Fig. 1. Experimental design. C: control group; V: vehicle group; E2: estradiol group; BPA: Bisphenol A group.

Table 1
Biometric parameters of female gerbils. Data expressed as mean±SEM. The superscript letters (a,b,c) correspond to the significant statistical differences between experimental groups. ANOVA followed by Turkey's Multiple Comparison test, $p < 0.05$. *Relation urethra+prostate/body weight.

	Experimental groups			
	C	**V**	**E2**	**BPA**
Body weight (g)	66 ± 1.673	68.8 ± 2.059	70.8 ± 1.356	74 ± 3.847
Urethra+prostate (g)	0.008 ± 0.001^{bc}	0.020 ± 0.004^{a}	0.028 ± 0.002^{a}	0.013 ± 0.002^{b}
Relative weight*	0.012 ± 0.002^{bc}	0.029 ± 0.005^{a}	0.039 ± 0.003^{a}	0.018 ± 0.003^{b}

female prostate can develop neoplastic alterations and respond to exogenous factors that cause injury in mammals [14].

Remarkably, several natural or synthetic exogenous chemicals bind to hormone receptors and interfere with their signals and intracellular functions in hormone-responsive organs [15]. Among the endocrine disruptors (EDs), Bisphenol A (BPA) is a xenoestrogen that acts through estrogen receptors to modulate events associated with an increased incidence of prostate cancer [16,17]. BPA is a synthetic phenol used in the manufacture of polycarbonate plastics, which are utilized in food and beverage containers, medical devices and epoxy resins [18]. Exposure to BPA should be reduced because the negative impacts of this environmental pollutant affect health profoundly. In addition to prostate alterations, BPA is associated with numerous other health outcomes, such as breast cancer, menstrual irregularities, genital abnormalities in male infants, infertility in men and women, early puberty, and metabolic disorders such as diabetes and obesity [19].

In the female prostate, peripubertal exposure to doses of BPA considered safe increases the development of proliferative lesions mediated by the estrogen receptor (ERα) in adulthood in the long term [20]. On the other hand, neonatal exposure to BPA, in both high and low dosages, decreases cell proliferation rates during the branching process of prostate development. BPA also promotes antiproliferative effects mediated primarily by the estrogen receptor (ERβ) [21]. In this context, estrogen receptors are the main mediators of the endocrine disruption caused by BPA. However, some authors have demonstrated that BPA can act through different receptor pathways [22,23], act in a nonselective manner, and act at various levels in biological systems [24]. The multiple binding capabilities of BPA amplify its impact on physiological processes in humans and animals and can promote carcinogenesis along many signaling pathways [25]. Regarding the association with ER, although BPA has been found to have a lower affinity for nuclear ERs relative to 17β-estradiol (E2), its estrogenic potency is equal to E2 for responses mediated by non-nuclear ERs [26]. As a positive estrogen control the estrogenic effects of BPA in laboratory studies are routinely compared with E2.

Hormone-responsive organs are highly susceptible to neoplastic programming during *in utero* exposure to EDs. Such exposure is able to mimic or disrupt steroid hormone actions, which impair the development of reproductive organs and adversely impact reproductive health, both at birth and during puberty or adulthood [27,28]. Currently, many pathologies arising during adulthood are being related to events linked to disturbances during pregnancy [28]. When considering the systems that could be affected by an adverse gestational environment, the developing female reproductive system may be particularly important [29]. In this sense, the female prostate stands out as a model system due it undergoes intense remodeling during postnatal life according to hormonal oscillations [8,30]. As BPA disrupts the modulation of estrogenic pathways, to which females are more sensitive, more pronounced effects are observed in females in comparison with males [4]. For this reason, in the present study, the deleterious effects of BPA on the prostate of female adult gerbils exposed to BPA during the perinatal period of development were described, with particular attention to the modulation of steroid receptors in the prostatic epithelium and stroma. Our data add to increase the evidence indicating the negative impact of BPA on the female reproductive system.

2. Material and methods

2.1. Experimental design

The animals were kept in the Animal Maintenance Facility of the Department of Biology from the Institute of Biosciences, Humanities and Exact Sciences (IBILCE) of the São Paulo State University (UNESP). The

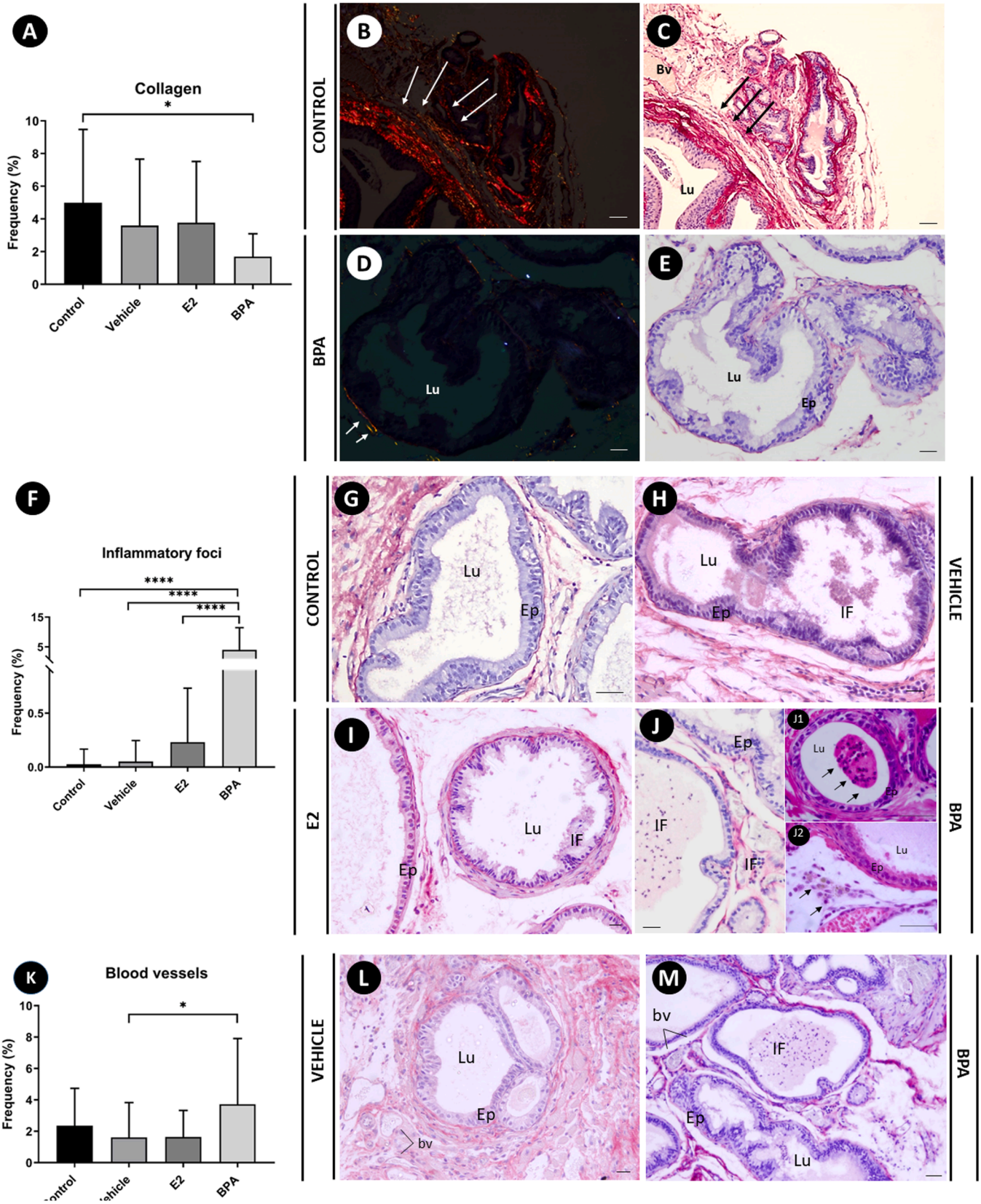

Fig. 2. Percentages (mean±SEM) and pictures of collagen deposition, inflammatory foci, and blood vessel incidence in female prostate tissue from different groups (n = 5). Arrows in (B-D): collagen fibers; Ep: epithelium; Lu: lumen; IF: inflammatory foci; bv: blood vessels; arrows in (J1-J2): inflammatory cells. ANOVA followed by Turkey's Multiple Comparison test, $p < 0.05$. Scale bars: 50 μm. Stains: Picrosirius red counterstained with hematoxylin.

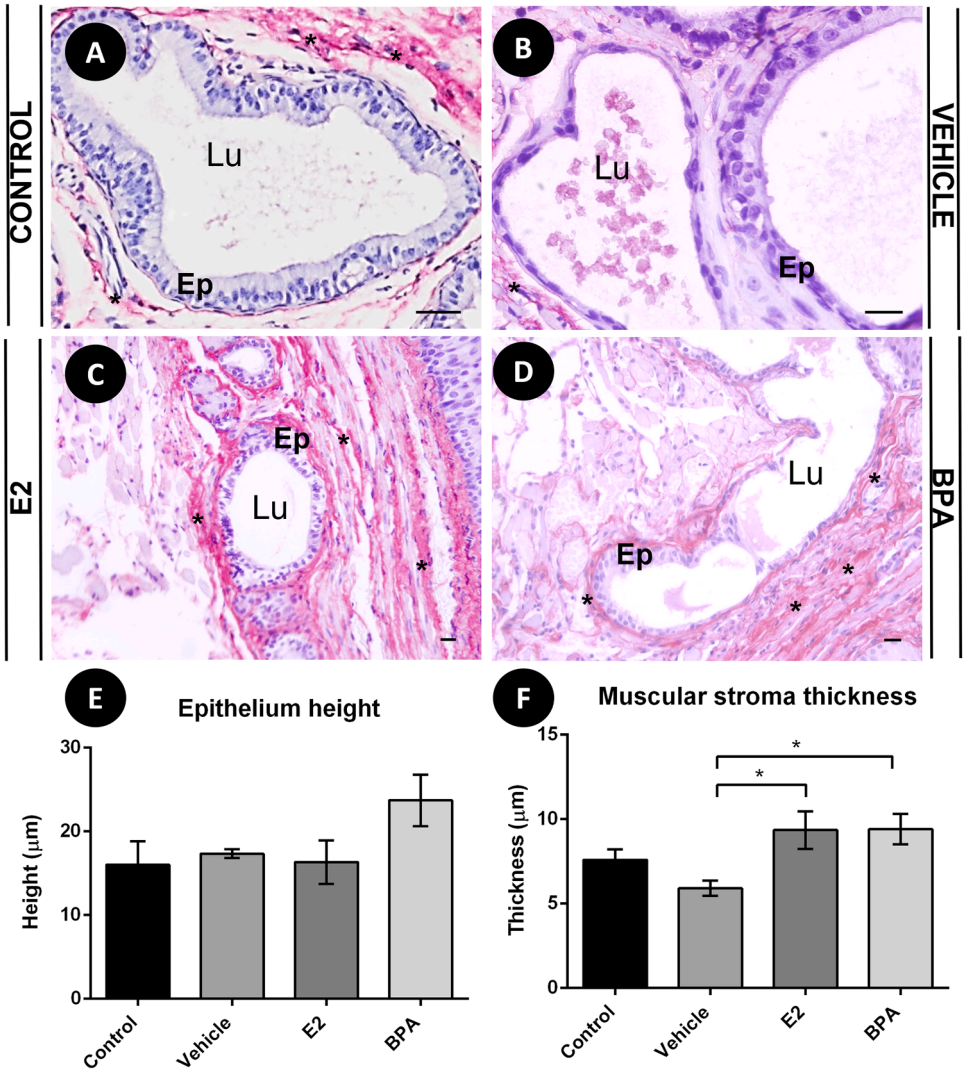

Fig. 3. Measurement of muscular stroma and epithelial thickness in the female prostate of animals evaluated in different groups (n = 5). Ep: epithelium. Lu: lumen; Asterisks: collagen fibers. ANOVA followed by Turkey's Multiple Comparison test, $p < 0.05$. Asterisks indicate significant differences between the groups analyzed (*$p < 0.05$). Scale bars: 20 µm. Stains: Picrosirius red counterstained with hematoxylin.

animals were kept in polysulfone BPA-free isolators (Alesco®), in racks equipped with air exhaustion at a temperature of 22 + /- 2ºC, with food (LABIMIX – Ratos e Camundongos; Agromix Industria e Comercio de Alimentos) and water ad libitum. The protocol was approved by the local Ethics Commission on Animal Use (113/2015).

Fertile females and males were kept in a polysulfone isolator. The first litter was discarded to ensure the pregnancy onset. The second litter was used for this experiment. Pregnant 4-month-old females were treated from 8th day of pregnancy (implantation day) until the 21st day post-birth (end of lactation), totalizing 38 days of treatment. Exposure was administered by gavage: Group 1 (C), 0.1 ml of water (daily), with no other associated compounds; Group 2 (V), 0.1 ml of corn oil (daily doses - dilution vehicle); Group 3 (E2), 35 µg/kg of 17β-estradiol [31] 3 times a week; and Group 4 (BPA), 50 µg/kg of BPA in daily doses, considering a safe dosage according to EFSA and US EPA [32–34] (Fig. 1). In this experiment, only group E2 received the treatment 3 times a week due to females aborted their litters when receive daily dosage the 17β-estradiol treatment. Also, group 3 (E2) was a positive bioidentical estrogen control [35].

After weaning, litters were separated from the mothers, and one female was randomly chosen from each offspring group (n = 5) and used for analysis (Fig. 1). Litter effects were avoided since each animal analyzed was from a different family. The females obtained from each litter were kept and euthanized 180 days after birth; the animals were euthanized only in the estrous phase of the reproductive cycle to avoid possible alterations. A vaginal smear was performed on the morning of the euthanasia to confirm the estrous cycle phase. All females were submitted to this protocol to standardize the estrous phase of the cycle, avoiding errors associated with hormonal oscillations. For euthanasia of the animals, a mixture of 3 mg/kg Xylazine hydrochloride (Anasedan, Ceva, Paulinia, SP, Brazil) and 10 mg/kg Ketamine hydrochloride (Cetamin, Syntec, Barueri, SP, Brazil) was injected subcutaneously. The females were then weighed and decapitated. The prostate tissue was collected for morphological and immunohistochemical analysis. The prostate was removed along with the bladder and the prostatic complex was dissected from the base of the bladder to the vaginal insertion and the adipose tissue adjacent was removed. The prostate was removed and immediately fixed in paraformaldehyde 4% for 24 h. After fixation, the samples were transferred to ethanol 70% and histologically processed in a tissue processor (TP1020; Leica Biosystems). The paraffin blocks were sliced (5 µm thick) and the sections placed on glass slides.

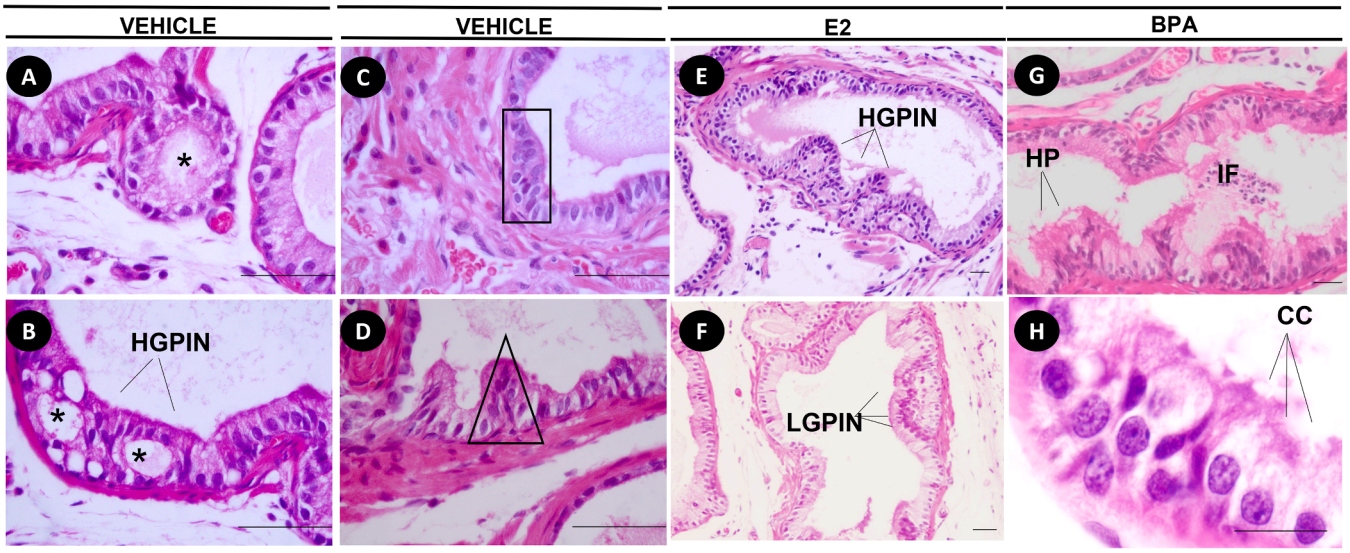

Fig. 4. Morphological alterations in the female prostate gland. (A-D) Vehicle; (E-F) E2; (G-H) BPA. The morphological alterations: microacini (asterisks), flat epithelial atypia (rectangle), papillary epithelial atypia (triangle), low-grade (LGPIN) and high-grade (HGPIN) prostatic intraepithelial neoplasia, hyperplasia (HP), inflammatory foci (IF), and clear cells (CC). Scale bars: (A-G) 20 μm; (H) 10 μm. Stains: HE.

Table 2
Analyses incidence and multiplicity of prostatic lesions in female prostate of Mongolian gerbils. Control (C), Vehicle (V), 17-β estradiol (E2) and Bisphenol A (BPA) groups. Superscript letters a and b indicate significant differences between the groups. Two-way ANOVA was followed by Tukey or Duns. Incidence analysis was performed in percentage. Values expressed as mean.

		Incidence (%)				Multiplicity			
		C	V	E2	BPA	C	V	E2	BPA
Clear cells		40	100	100	80	0–27(5.5)[b]	13–56(34.67)[a]	3–6(22.2)[a,b]	0–40 (21.6)[a,b]
PIN total		-	60	60	-	0 (0)	0–17 (5.34)	0–10 (3)	0 (0)
Inflammatory foci	Intraluminal	-	40	20	60	0 (0)	0–2 (0.5)	0–2 (0.4)	0–12 (3.8)
	Periglandular	-	40	-	40	0 (0)	0–11 (2.34)	0 (0)	0–8 (2.8)
	Total	-	80	20	100	0 (0)	0–13 (2.83)	0–2 (0.4)	0–20 (6.6)
Epithelial atypia	Flat	40	60	40	-	0–2 (0.34)	0–3 (1.7)	0–2 (0.6)	0 (0)
	Papillary	40	40	60	40	0–1(0.34)	0–6 (1.5)	0–2 (1.2)	0–6 (1.8)
Hyperplasia		40	20	40	80	0–3 (0.67)[a,b]	0–9 (1.5)[a,b]	0–2 (0.6)[a,b]	0–6 (2.8)[a]

2.2. Analysis of biometric parameters

The prostates were removed in association with the urethra due to the proximity of the structures; the complex was then weighed and standardized. The relative weight was calculated from the urethra + prostate weight divided by the body weight (ratio) of each animal.

2.3. Morphological analysis

For the general morphological analysis, the histological sections were stained with Hematoxylin-eosin (HE). The HE staining was performed to analyze morphological alterations in the epithelium and stroma, including stereology, incidence and multiplicity. The picrosirius technique was utilized to analyze the collagen fibers in the stroma. To compare the relative volumes of different prostatic compartments (epithelium, lumen, muscular stroma, non-muscular stroma, blood vessels, inflammatory foci and collagen fibers) between experimental groups, stereological analysis was performed on slides stained with picrosirius. In total, 30 prostate fields (6 images of different sections per animal, n = 5) were randomly recorded for each experimental group with a BX61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120® Virtual Microscopy Slide Scanning System (VS120-S5), using Image Pro-Plus 6.0 software (Media Cybernetics; MD; USA). The relative frequency was estimated using the M130 multipoint test [36] applied to the prostate according to the procedure described previously [37]. Morphometry was also performed. Histological sections stained with picrosirius were submitted to measurement of the height (μm) of secretory epithelial cells and thickness (μm) of the smooth muscle layer of the epithelium. For each analysis, 200 measurements were obtained from each experimental group. The evaluations were performed under light microscopy (Olympus BX60) coupled to a polarized light, which enabled the birefringence of collagen fibers to be identified due to their molecular composition.

2.4. Analysis of prostatic lesions

The analyses of prostatic lesions were based on a previously published description [38]. Microacinar budding had previously been described as high-grade prostatic intraepithelial neoplasia (HGPIN) [39]. Hyperplasia and prostatic intraepithelial neoplasia (PIN) were considered as lesions. Moreover, the number of clear cells, the presence of flat or papillary epithelial atypia, and intraluminal and periglandular inflammatory foci were considered to be morphological alterations and were quantified. Clear cells are a subtype of merocrine secretory cells, which differ from the typical merocrine by presenting pale cytoplasm and less electron-dense cytoplasmic contents, of low-density and variable size of secretory vesicles, evaluated ultrastructurally. These cells, which accumulate neutral carbohydrates in their cytoplasm [40]. Guerra et al. [41], have been described as having a flat epithelial atypia (FEA), corresponding to epithelial stratification, where cells are grouped along the acini, forming a stratified strip by the acini, not driving the stratification into the lumen. Papillary epithelial atypia (PEA),

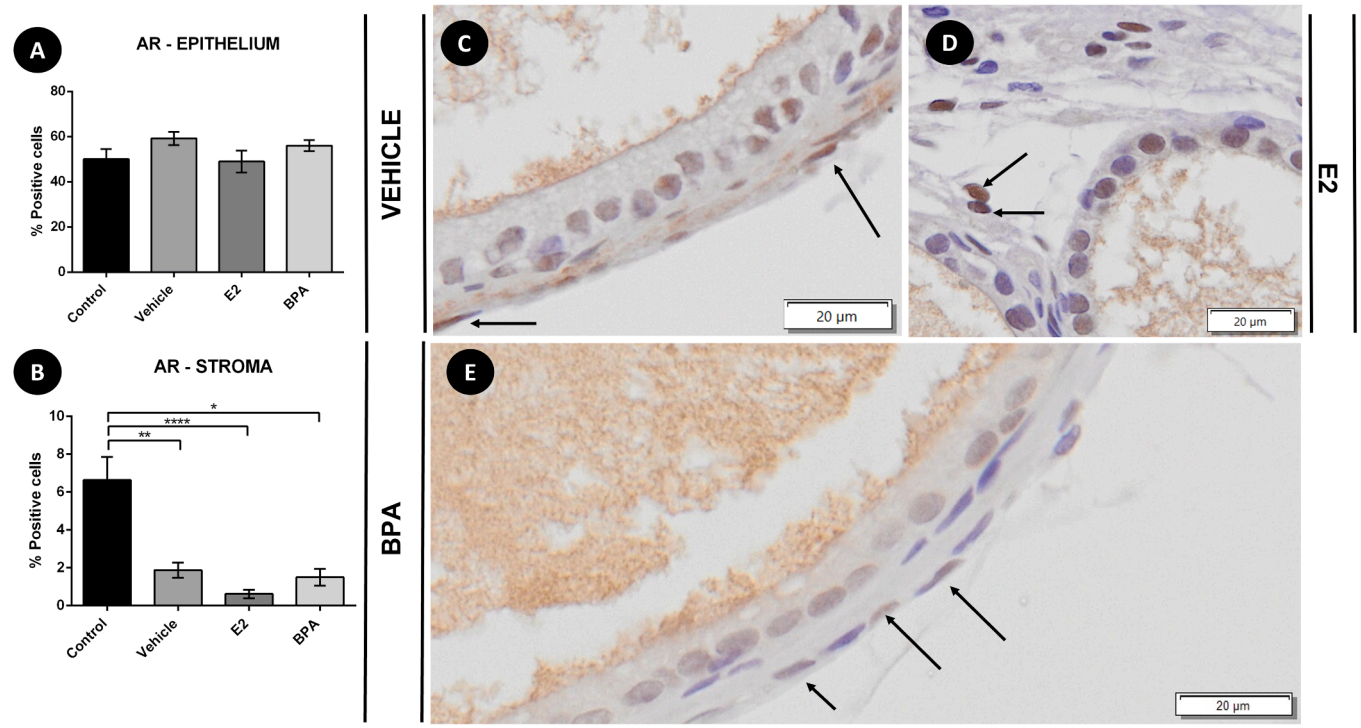

Fig. 5. Percentages of AR expression in epithelial and stromal cells of female prostate tissue in different groups (n = 5). Arrows indicate immunoreactive stromal cells. ANOVA followed by Turkey's Multiple Comparison test. Asterisks indicate significant differences between the groups analyzed (*p < 0.05; **p < 0.01; ***p < 0.001). Scale bars: 20 µm.

corresponding to the foci of epithelial cells grouped in a short region of acini, drive the stratification into the lumen. Inflammatory foci are inflammatory cell infiltrates located in the lumen (intraluminal) or periglandular region the prostate acini.

The multiplicity of lesions corresponds to the average number of altered foci present in the prostate of each animal group. Additionally, the incidence of prostate lesions (number of animals affected by a class of injury) was quantified for each prostate in the different groups. For both incidence and multiplicity quantification, the following morphological alterations were considered: clear cells, intraluminal and periglandular inflammatory foci, flat and papillary epithelial atypia, and lesions such as hyperplasia and PIN.

2.5. Immunohistochemistry

Immunohistochemistry analyses were performed to identify the immunoexpression of androgen (AR), estrogen alpha (ERα), and beta (ERβ) receptors. The primary antibodies used were AR (polyclonal rabbit, anti-AR, 1:75, sc-816, Santa Cruz Biotechnology, CA, USA), ERα (polyclonal rabbit, anti-ERα, 1:50, sc-542, Santa Cruz Biotechnology, CA, USA), and ERβ (polyclonal rabbit, anti-ERβ, 1:50, sc-8974, Santa Cruz Biotechnology, CA, USA). The protocol applied followed an order of procedure. Antigen retrieval was performed using citrate buffer (pH: 6.0) for 40 min at 98 °C. Then, the slides were then placed in 3% H2O2, twice, for 15 min, for endogenous peroxidase blocking. Next, nonspecific proteins were blocked for 30 min in 5% skimmed milk. The sections were then incubated with the different types of primary antibodies in a humidified environment overnight at 4 °C. For identification of the antibodies in the tissue, the sections were incubated with specific polymers (EnVisionTM, Dual Link System - HRP, Dako, CA, USA) for one hour at room temperature. Revelation of the binding sites was then performed with 3, 3`-diaminobenzidine tetrahydrochloride dihydrate (DAB - NovolinkMaxDAB, RE7270-CE, Leica Biosystems, Buffalo Grove IL, USA) for up to 2 min and counterstaining was performed with Harry's hematoxylin.

For the quantification of AR, Erα and ERβ, the frequency of positive cells from 30 fields per group (n = 5) was analyzed. For this, the percentage of AR, ERα, and ERβ positive cells was calculated as the number of positive nuclei divided by the total number of nuclei in each field. Positive cells were considered in the epithelium and stroma of the prostate.

2.6. Statistical analysis

Data were analyzed for normality (Kolmogorov-Smirnov) and then compared by one-way ANOVA (parametric data) or the Kruskal-Wallis test (nonparametric data). Results were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Biometric parameters

The biometric parameters were presented in Table 1. Prostatic complex weight showed significant differences between the BPA (0.013 g ± 0.002) and E2 (0.028 g ± 0.002) groups. Relative weight was also statistically different between the BPA (0.018 g ± 0.003) and E2 (0.039 g ± 0.003) groups. There were no significant changes in body weight between the groups.

3.2. Stereological and morphometric analysis

The frequencies of collagen, inflammatory foci and blood vessels of the prostrate of female gerbils showed significant alterations, as demonstrated by the stereological analysis (Fig. 2). Collagen fibers decreased in the BPA group in comparison to the control group (Fig. 2A-E). Inflammatory foci were enhanced in the BPA group in comparison to the other groups (Fig. 2F-J). In addition, the incidence of blood vessels was higher in the BPA group compared to the vehicle group (Fig. 2K-M). Morphometric analysis of compartments showed significant differences

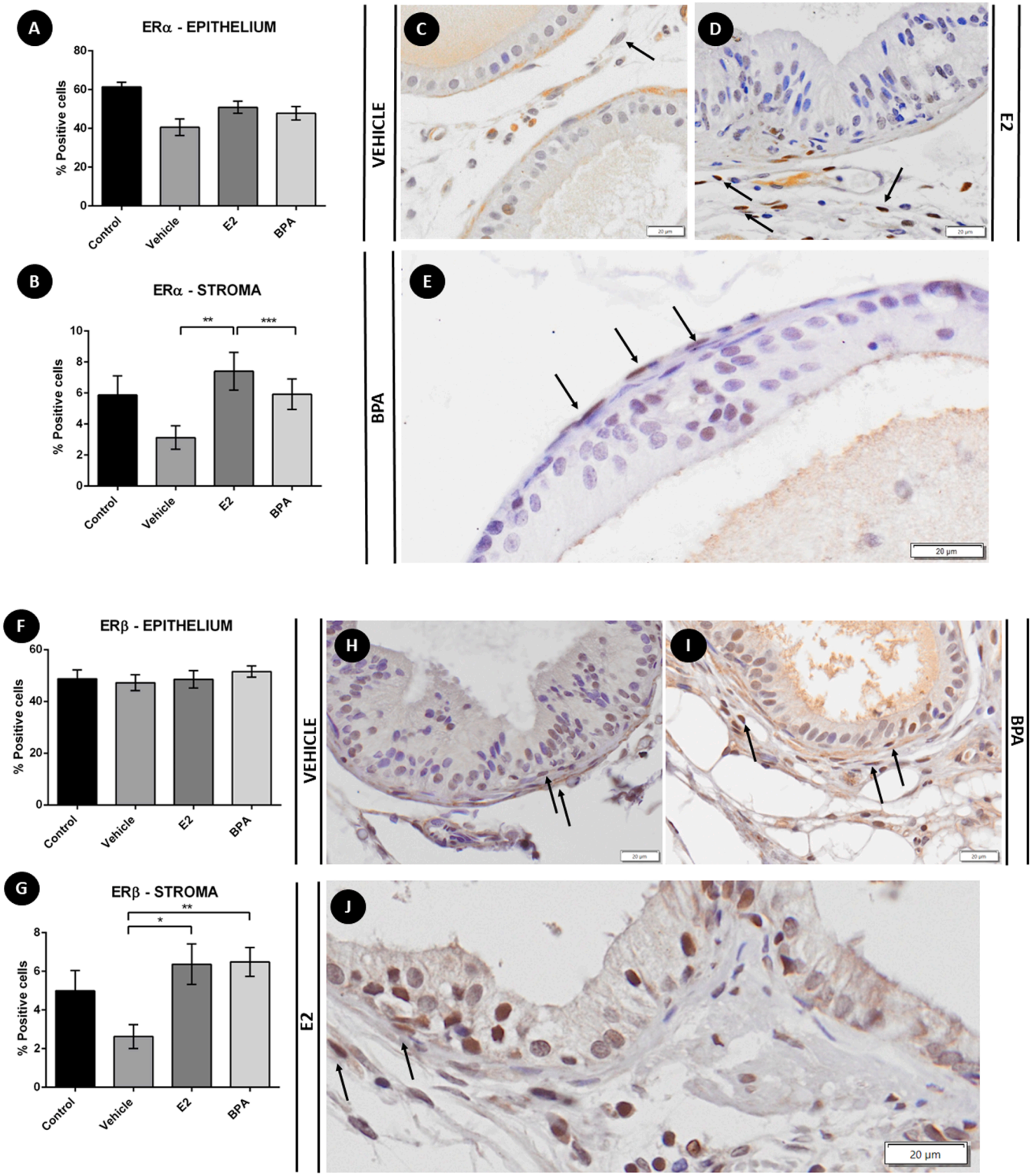

Fig. 6. Percentages of ERα and ERβ expression in epithelial and stromal cells of female prostate tissue in different groups (n = 5). Arrows indicate immunoreactive stromal cells. ANOVA followed by Turkey's Multiple Comparison test. Asterisks indicate significant differences between the groups analyzed (*p < 0.05; **p < 0.01; ***p < 0.001). Scale bars: 20 µm.

between experimental groups in relation to the thickness of the smooth muscle layer (Fig. 3). E2 (9.35 µm ± 1.11) and BPA (9.40 µm ± 0.89) groups presented a significant increase compared to the vehicle group (5.90 µm ± 0.58). There were no significant differences in epithelium height between the groups.

3.3. Incidence and multiplicity of prostatic lesions

Lesions, such as low-grade and high-grade prostatic intraepithelial neoplasia (LGPIN and HGPIN), benign hyperplasia, periglandular and luminal inflammatory foci, papillary epithelial atypia, flat epithelial atypia, clear cells and microacinar buddings in the prostate of female

gerbil were demonstrated in Fig. 4. PINs (Table 2) was present only in E2 (60%) and vehicle (60%) groups, without statistical significance for the multiplicity (Table 2). Foci of hyperplasia were present in all groups, being increased in the BPA group (80%) with statistical difference compared to vehicle group in the multiplicity of this lesion. Epithelial atypia was a common feature among the groups (Table 2). The prevailing subtype was papillary epithelial atypia. The presence of clear cells was observed in all experimental groups, but presented high multiplicity frequency in the vehicle group, without statistical difference with E2 and BPA. All animals from the BPA group presented intraluminal (60%) and periglandular (40%) inflammation (Table 2). The E2 group showed a lower percentage (20%) in relation to the other groups but did not demonstrate statistical significance among the experimental groups for the inflammation multiplicity.

3.4. Immunoexpression of hormonal receptors

The expression of AR and ER receptors in the epithelial and stromal compartments was quantified. Neither receptor showed any no significant difference between the groups in the epithelial compartment; however, there was some modulation in their expression in the stromal compartment. AR immunoexpression showed a decrease in stroma in vehicle, E2 and BPA groups compared to the control group (Fig. 5). Regarding ERs, there was an increase in stromal ERα expression in the E2 group compared to the vehicle and BPA groups (Fig. 6B). Stromal ERβ-positive cells were lower in the vehicle group in comparison to the E2 and BPA groups (Fig. 6G).

4. Discussion

The aim of the present study was to evaluate the effects of perinatal exposure to environmental BPA and 17-β estradiol doses on the female gerbil prostate. There are reports of some studies of BPA exposure and its consequences in adult life are partially presented in the literature [42], however data related to the female prostate are scarce. Biometric analyses showed alterations in the absolute and relative weight of the prostatic complex between the BPA and E2 groups, with the latter promoting a significant increase in the gland. These results are similar to those observed for adults and senile males exposed during the pubertal period to synthetic estrogen [43,44]. Additionally, Fochi et al. [45] demonstrated a peak of estradiol secretion increases the number of acini present in the prostate. The anabolic effect of estrogen on the female prostate is transferred during the gestational period and adds to the effects of circulating estrogens. BPA had no effect on prostatic weight, which may indicate an endocrine disruption not related to estrogenic canonical pathways [22,24].

As observed in other studies, exposure to BPA promoted inflammatory foci in the male prostate [16,42]. As previously described, BPA modulates the synthesis and secretion of some inflammatory cytokines, increasing focal inflammation [46]. These elements could contribute to the establishment of a pro-tumoral microenvironment [47–49]. Furthermore, angiogenesis has already been highlighted as an adverse effect caused by BPA [50], which may also contribute to a tumor microenvironment by an increase in inflammatory cells [51] or by the remodeling of stromal elements [52]. On the other hand, the E2 group shows a low frequency of inflammatory focus in the female prostate compared to BPA, which demonstrates the particular effect of this endocrine disruptor on inflammation progress [46].

Regarding epithelial alterations, hyperplasic foci were found in 80% the animals exposed to perinatal BPA. The increase in this proliferative lesion in the prostate has already been associated with the effect of BPA in male gerbils [16,53]. In the female prostate, the absence of more aggressive lesions may indicate that perinatal BPA exposure was not sufficient to promote neoplastic pathways during adulthood. The period of exposure and the window of susceptibility have some influence on the development of these disorders [54,55]. Furthermore, epithelial atypia and hyperplasia are frequently associated with the prostate of animals exposed to BPA [16]. The development of these lesions is due to changes in the cell format, which may or may not be associated with cell proliferation [42,56]. Also, the presence of "clear cells" in the epithelium of female prostate was observed, which appear to be a cell population of rapid stress response. Although little studied, it is known that such cells are responsive to estrogen and progesterone [57], which contributes to xenoestrogen activity of BPA during endocrine disruption.

The epithelial alterations observed after BPA exposure do not reflect the behavior of steroid receptors typically modulated by endocrine disruptors. The immunoexpression of AR, ERα and ERβ in the present study showed an interesting characteristic regarding its modulation in the compartments. The response of AR expression in the stroma was lower in the vehicle group, being similar to the result of the E2 and BPA groups, demonstrating that this response could not be associated with these compounds, but with their vehicle of administration. Guerra et al. [41] demonstrated that the use of corn oil promotes morphological alterations in male gerbil prostate, as well as AR-positive cells. The actions of E2 and BPA on steroidal receptors in the female prostate epithelium were similar and did not promote effective modulation of the same. A possible explanation might be that BPA exerts endocrine disruption due to its weak binding affinity for the estrogen receptors ERα and ERβ [58]. According to Shafei et al. [23], the actions of BPA are through ubiquitous bindings with different receptors leading to deleterious mechanisms via different molecular pathways. Our data also corroborate the findings that the action of BPA through multiple binding capabilities amplifies its impact on physiological processes [22–25], since there were differences in the expression of receptors between the groups.

Muscular stroma thickness was significantly increased in the E2 and BPA groups compared to the vehicle control. The increase in muscular stroma in the prostate was observed with finasteride treatment, a 5-alpha reductase inhibitor, of male and female gerbils, resulting from a less androgenic status [59]. Although the mechanisms of finasteride and BPA are different, their results for smooth muscle stroma are associated with anti-androgenic pathways. This result is probably associated with estrogenic pathways since the results for the E2 and BPA groups did not show any significant differences between them.

The stromal compartment presented a marked response under exposure to E2 and BPA, with a reduction in AR expression in both groups. Although, the response of AR expression in the stroma was lower in the vehicle group, being similar to the result of the E2 and BPA groups, demonstrating that this response could not be associated with these compounds, but with their vehicle of administration. Guerra et al. [41] demonstrated that the use of corn oil promotes morphological alterations in male gerbil prostate, as well as AR-positive cells. For ERα and ERβ expression, the groups exposed to E2 presented a significant increase in relation to the vehicle control group, while the BPA group showed an increase only in ERβ. In prepubertal rats prenatally exposed to BPA there was a decrease in AR-positive cells in the periductal stroma of the ventral prostate [60]. In cultures of human stroma fibroblasts, low-doses of BPA down-regulated AR levels of expression [61]. These data suggest that exposure to BPA altered the differentiation pattern of stromal cells of the prostate [60]. In this sense, among the fibrillar elements of the stroma, collagen fibers decreased in the BPA group, indicating a change in the secretory activity of stromal cells, such as fibroblasts and smooth muscle cells. Our results are in accordance with those of Yu et al. [62], in that the decrease in AR is associated with a decrease in collagen deposition by stromal cell elements. Collagen is one of the main components remodeled during the development of neoplasia [63,64]. Also, decreased AR in stroma may point to a decrease in the homeostatic control of this compartment [65], corroborating to muscular thickness increased due to low androgenic sensitivity. These findings are significant with regard to prostate cancer, where alterations in the stroma compartment may enhance the invasiveness and/or malignant potential of the nascent tumor [60].

5. Conclusion

In summary, exposure to BPA and E2 during pregnancy and lactation influences hormone-responsive organs, such as the prostate, in female gerbils. BPA and E2 showed important morphological repercussions in the prostate of females exposed perinatally to them. BPA is a pro-inflammatory agent in the female prostate, just as it is in the male. The stromal microenvironment is the most responsive and affected by the action of modulating endocrine agents. The decrease in AR expression and collagen fibers suggest a pro-tumor microenvironment.

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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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