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ORIGINAL PAPER

Prenatal and pubertal exposure to ethinylestradiol induces Long-Term stromal and epithelial changes in the gerbil dorsal prostate

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Abstract

17α-Ethinylestradiol (EE2) is a synthetic estrogen derived from 17β-estradiol and widely used in oral contraceptives. It interferes with the endocrine system and disrupts hormonal balance. This study investigated the long-term effects of prenatal and pubertal EE2 exposure on the dorsal prostate of aging gerbils. Adult female gerbils (90–120 days old) received EE2 (15 µg/kg/day) and were assigned to three groups (n = 5): Control (untreated), EE2/PRE (exposed during gestational days 18–22), and EE2/PUB (exposed during postnatal days 42–49). After 12 months, the animals were euthanized, and dorsal prostates were collected for biometric, histopathological (including quantification of prostatic acini/section and lesion multiplicity), and Morphometric analyses (epithelial height and muscle thickness), with stereological evaluation of the epithelium, lumen, muscle, stroma, blood vessels, and collagen fibers. Tissue sections were stained with Hematoxylin–Eosin, Gömori's Trichrome, and Picrosirius Red. Results showed increased muscular thickness and decreased vascular volume in the EE2/PRE group, while the EE2/PUB group exhibited reduced volumes of the epithelium, lumen, and collagen fibers. Lesion analysis revealed a reduction in prostatic intraepithelial neoplasia (PIN) and an increase in luminal inflammation in the EE2/PUB group. These findings indicate that the biological effects of EE2 vary according to the timing of exposure, with both prenatal and pubertal periods representing critical developmental windows. EE2 exposure during these stages can induce alterations in epithelial-stromal interactions in a lobe-specific manner. Hormonal imbalance triggered ERα/ERβ signaling, influencing cellular differentiation and promoting inflammation. These distinct outcomes highlight how endocrine-disrupting chemicals (EDCs) compromise prostate homeostasis through hormone reprogramming and receptor-mediated pathways.

Keywords Ethinylestradiol · Puberty · Prenatal · Dorsal prostate

Introduction

The Mongolian gerbil (*Meriones unguiculatus*) is a polyestrous rodent with spontaneous ovulation and well-defined reproductive cycles, making it a suitable model for developmental studies. Females reach sexual maturity around 50–60 days and exhibit estrous cycles lasting approximately 4–6 days (Nishino and Totsukawa 1996). Males mature slightly later, reaching full reproductive capacity by ~ 90 days, as shown by histological and hormonal data indicating the onset of spermatogenesis and epididymal sperm reserves from day 60 onward (Pinto-Fochi et al. 2016). Gestation typically lasts 24–26 days, although delayed implantation may extend this period when postpartum lactation is present (Nowak 1991). With high reproductive efficiency and well-characterized developmental stages,

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the species offers a reliable framework for assessing endocrine-disrupting chemical effects during critical windows such as late gestation and peripuberty.

Within this developmental context, the prostate of the Mongolian gerbil undergoes critical organogenesis during specific prenatal and postnatal periods. Prostate development begins between the 18th and 22nd days of gestation and continues through the first postnatal day, corresponding to the budding phase. Postnatally, high androgen receptor (AR) expression in the periductal region drives branching and differentiation of prostate buds (Sanches et al. 2014; 2016). The final growth and glandular maturation occur during puberty, between postnatal days 42 and 49, marking this as a key window for prostate morphogenesis and potential vulnerability to endocrine disruptors (Sanches et al. 2014, 2016; Pinto-Fochi et al. 2016; Prins 2021).

Exposure to 17α-ethinylestradiol (EE2), a chemical endocrine-disrupting compound (EDC), can compromise this development and prostate function, posing risks to the health of the genital and endocrine systems (Perez et al. 2011; 2012, 2016; Barreiros et al. 2016; Fleury et al. 2021; Stukenborg et al. 2021). EDCs are exogenous chemical substances that interfere with or block hormonal functions (You and Song 2021; Bertram et al. 2022; Pan et al. 2024).

Studies using the Mongolian gerbil have demonstrated that prenatal and pubertal exposure to ethinylestradiol (EE2) induces long-lasting alterations in reproductive organs such as the prostate, ovary, and the Skene's gland (female prostate) (Perez et al. 2016; 2017; De Souza et al. 2022). In females, exposure to low doses of EE2 (15 µg/kg/day) between gestational days 18–22 significantly alters follicular development, leading to epithelial modifications in the ovary during aging. In both sexes, prenatal exposure promotes atypical morphological patterns and the development of prostatic intraepithelial neoplasia throughout life (Perez et al. 2016). These findings support the relevance of using the gerbil as a translational model to investigate the environmental effects of EE2 during critical developmental periods.

EE2 is a synthetic estrogen commonly used in oral contraceptives and increasingly recognized as a persistent environmental contaminant with endocrine-disrupting potential. A recent study by Nobre et al. (2024) demonstrated that EE2 adsorbed onto microplastics significantly increased toxic effects in tropical estuarine invertebrates—including alterations in phase I and II detoxification enzymes, oxidative stress in gills, lysosomal membrane destabilization, and cellular damage in the hepatopancreas and gills—after only 3 to 7 days of exposure. This highlights the capacity of EE2 to potentiate toxicity when combined with other environmental pollutants. Moreover, EE2 is frequently detected in surface and wastewater at concentrations ranging from sub-ng/L to tens of ng/L (Desbiolles et al. 2018). Its degradation half-life in aerobic aquatic environments can exceed 81 days, depending on specific physicochemical conditions. Studies show that its environmental persistence can be mitigated by photodegradation and microbial activity under certain conditions—such as the presence of humic substances, dissolved iron, and light exposure (Wang et al. 2015; Soares-Filho et al. 2018, He et al. 2022). In contrast, in humans, EE2 has a plasma half-life ranging from 13 to 27 h, owing to its resistance to hepatic metabolism (Stanczyk et al. 2013).

At the molecular level, EE2 ($C_{20}H_{24}O_2$) is a synthetic derivative of the natural estrogen hormone estradiol, sharing structural similarities with E2, the primary female sex hormone. However, due to the addition of the ethinyl group, EE2 is more stable and bioavailable, with a slightly higher molecular weight compared to natural estradiol. This molecular modification confers high estrogenic potency and resistance to metabolic degradation, explaining its extensive use not only in oral contraceptives but also in hormone replacement therapy and the treatment of menstrual disorders such as dysmenorrhea and menorrhagia (Barreiros et al. 2016; Rowen 2017).

It is known that EE2, upon binding to androgen receptors (AR) in the endodermal structures of the urogenital sinus (UGS), can compete with 5α-dihydrotestosterone (DHT) and estradiol (E2), both of which are derivatives. EE2 mimics E2 and interferes with the binding of DHT to AR, compromising the essential hormonal interaction for prostate development. Communication between the urogenital epithelium (UGE) and urogenital mesenchyme (UGM) cells, mediated by these hormones, is fundamental for organogenesis and the maintenance of prostate functions (Prins and Putz 2008; Wilson 2011; Buskin et al. 2021).

The interaction of dihydrotestosterone (DHT) with androgen receptors (AR) in the prostate lobes—initially composed of solid, unbranched epithelial buds during the prenatal phase—activates androgen signaling and stimulates the production of paracrine factors such as fibroblast growth factors (FGFs), Wnts, NKX3.1, HOXB13, BMP4/7, FOXA1/2, and Sonic Hedgehog (SHH) signaling (Buskin et al. 2021; Olson et al. 2021). These factors regulate epithelial differentiation and cellular patterning, promoting the branching morphogenesis of prostate buds during development (Buskin et al. 2021). In gerbils, prostate development continues during puberty, driven by AR-mediated processes. However, with aging, the prostatic gland may respond to the androgen declines, being observed an epithelial proliferation and stromal remodeling (Pegorin de Campos et al. 2006).

As an exocrine accessory sex gland dependent on hormones, the prostate's development is regulated by multiple

signaling pathways, including endocrine, paracrine, and autocrine mechanisms, as well as transcription factors (Meeks and Schaeffer 2011; Montano and Bushman 2017). In gerbils, the prostate consists of four distinct lobes: anterior (coagulating gland), dorsal, ventral, and dorsolateral, organized in pairs on the right and left sides (Rochel et al. 2007). In humans, the prostate has a zonal morphology, divided into central, peripheral, and transitional zones (Ittmann 2018).

Morphologically, the dorsal lobe is more developed and exhibits an acinar tubular organization, with greater secretory epithelium height and thicker smooth muscle layers compared to the ventral lobe. Rochel et al. (2007) demonstrated that despite these lobes having distinct characteristics, they share physiological similarities: both possess a stratified epithelium and fibromuscular stroma, have a secretory function, undergo branched morphogenesis during development, indicating the possibility of shared developmental pathways, and respond similarly to hormonal stimulation (mainly androgenic). These shared characteristics are substantial for the analysis of the present study.

Recent single-cell transcriptomic studies comparing rats and humans have demonstrated that the dorsal prostate lobe, particularly when considered alongside the lateral lobe (forming the dorsolateral prostate, DLP), exhibits molecular and cellular signatures closely resembling those of the human peripheral zone (PZ)—a region critically implicated in prostate cancer development and inflammation (Crowley and Shen 2022; Aparicio et al 2025). Moreover, the peripheral zone constitutes approximately 70% of the glandular tissue in the human prostate and serves as the primary site for prostatic adenocarcinomas (Yu et al 2023). Consequently, selecting the dorsal prostate lobe for our experimental model not only provides strong translational relevance but also ensures sensitivity to aging-related changes and dietary interventions.

This clinical relevance, combined with its well-characterized anatomy and consistent experimental responsiveness, facilitates management in animal facilities, making it a widely used model in aging and reproductive biology studies that exhibit significant responses under various experimental conditions (Ruiz et al 2023; Zucão et al 2024). Thus, the present study aims to investigate how prenatal exposure to the endocrine disruptor EE2 affects the morphometric and stereological characteristics of the dorsal prostate gland in gerbils during aging.

Material and Methods

Animals and Experimental Design

A total of 15 adult nulliparous female gerbils, aged 90 to 120 days, were used in this study, with 5 animals allocated to each experimental group. Each female was housed in a cage with a male of the same age range, forming different family groups.

The animals were housed in polypropylene cages at the Animal Facility Center of UNESP in São José do Rio Preto, SP, in the Laboratory of Microscopy and Microanalysis building, under a controlled temperature of 23 °C and fluorescent lighting with a 12-h light/dark cycle. Food was provided ad libitum, with a rodent-specific diet and filtered water in glass drinking bottles.

All experimental procedures were conducted in accordance with ethical guidelines for animal research (Fig. 1). The study was approved by the Ethics Committee on Animal Use (CEUA) of UNESP under protocol No. 020/2009 and by the CEUA of UFJ under protocol No. MB 01/2023, regulating the use of biological material in this research.

EE2/PRE Group: Five pregnant females received a daily dose of 15 µg/kg of EE2 (Sigma, St. Louis, MO, USA) by gavage, diluted in 100 µl of Nujol® mineral oil (CAS 8020–83-5; Sigma-Aldrich, St. Louis, MO), following a methodology previously used by our research group (Fleury et al. 2021; Perez et al. 2016, 2017). Male offspring (n = 5) were exposed to EE2 exclusively during prenatal period, specifically from gestational day 18 to 22, a period corresponding to the onset of prostate morphogenesis in gerbils (Sanches et al. 2014).

EE2/PUB Group: EE2 was administered to five male gerbils at a dose of 15 µg/kg/day by oral gavage, restricted to the period between postnatal days 42 and 49, a period corresponding to puberty in gerbils (Pinto-Fochi et al. 2016).

Control Group: The male gerbil did not undergo any treatment at any stage of its life. Male offspring were weaned at 30 days of age. To ensure heterogeneity, one male from each litter was selected, totaling five animals per experimental group. Figure 1 illustrates the experimental design.

All male gerbils from the experimental groups, originating from different families, were weighed and subsequently euthanized at 12 months of age at the Animal Facility Center of UNESP, in São José do Rio Preto, SP, in accordance with CEUA-UNESP guidelines. Euthanasia was performed by deep anesthesia with xylazine (3 mg/kg) and ketamine (10 mg/kg), followed by decapitation.

Following euthanasia, the 12-month-old male gerbils were weighed, and the prostate complex along with the dorsal prostate lobe were excised and weighed to assess

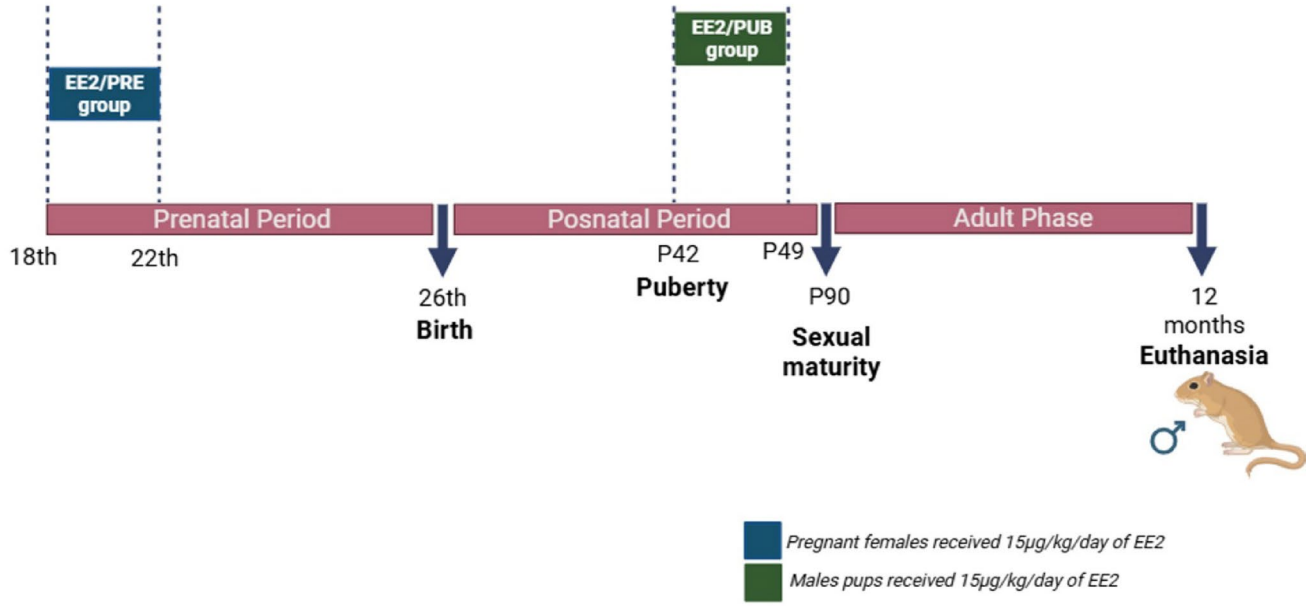

Fig. 1 Experimental Design Scheme, representing the treatment periods. In the EE2/PRÉ group (red), pregnant females (n = 5) received 15 µg/kg/day of EE2 by gavage from the 18th to the 22nd day of gestation. In the EE2/PUB group (blue), male offspring (n = 5) from different litters received the same dosage of 15 µg/kg/day of EE2 by gavage from the 42nd to the 49th postnatal day. In the control group, the offspring were not exposed to any compound. Male offspring from the experimental groups were euthanized at 12 months of age

absolute and relative prostate weights (expressed as gland weight/body weight).

Histological processing

Dorsal prostates from the experimental groups were fixed in either 4% buffered paraformaldehyde or Metacarn solution (acetic acid, chloroform, and methanol in a 1:3:6 ratio) and processed for histology. After fixation, tissues were embedded in paraffin (Histosec, Merck). At the Histopathology Laboratory of the Medical School at the Federal University of Jataí (UFJ), paraffin blocks were sectioned at 5 µm using a semi-automatic microtome (Leica RM2235). Sections were floated in a 46 °C water bath, oven-dried at 65 °C for 90 min to ensure complete drying and paraffin removal, and then subjected to two xylene baths.

Following deparaffinization, sections were rehydrated through a graded ethanol series (100%, 90%, 70%, and 50%) and immersed in distilled water for 10 min. Staining was performed using Hematoxylin–Eosin (HE), Gomori's Trichrome, and Picrosirius Red, following the methodology described by Dapson et al. (2011). After staining, sections were dehydrated and mounted using ERU-Mount-Easy medium (Path/Diagnósticos, Indaiatuba/SP, Brazil, Grupo Erviegas) for subsequent morphometric, histopathological and stereological analyses.

Histological images for morphometric, stereological, and histopathological analyses were acquired using an Olympus slide scanner and analyzed with OlyVIA software (version 2.9.1).

Morphometry

Histological sections were stained with HE, which allowed clear delineation of the borders of the muscle layer as well as the apical and basal regions of the epithelium. Transverse sections were prepared, with five slides per animal, and four digital images were captured per animal at 40 × magnification. This resulted in 100 measurements of epithelial height and 100 measurements of muscle layer thickness (in µm) per group. Considering a sample size of n = 5 animals per group, 20 measurements were performed per animal (5 measurements per slide), following the methodology adapted from Perez et al. (2017). All measurements were carried out using Image-Pro Plus software, version 6.0 (Windows 11).

Stereology of prostatic compartments

Stereological analysis was conducted to determine the relative volume of different prostatic compartments in the experimental groups. To achieve this, five slides were prepared for each subgroup. On each slide, six digital images were captured at 40 × magnification, and 30 random fields per subgroup were analyzed from Gömori's Trichrome-stained slides. The analysis focused on prostatic compartments, including the epithelium, lumen, muscle, stroma, and blood vessels.

The relative volume of collagen fibers in the stromal compartment of the dorsal prostate was quantified using histological sections stained with Picrosirius Red. Sections from five animals per experimental group were analyzed at 40× magnification, totaling 250 measurements per group. The measurements were conducted using 130 points and 10 lines, following an adaptation of the Weibel (1963) system and its modifications for the prostate (Perez et al. 2011).

Stereological analysis was performed using Image-Pro Plus software (version 6.0, for Windows 11). Based on the collected data, the relative frequencies of various prostatic compartments were calculated, including epithelial, muscular, non-muscular stromal, luminal, blood vessel, and collagen fiber compartments. The stereological grid configuration used the following parameters: grid shape – linear segments; length (%) – 20 (horizontal), -50 (vertical); spacing – 57 (horizontal), 54 (vertical); layout – orthogonal.

Histopathological analysis

Sections of the dorsal prostate from the experimental groups, stained with hematoxylin and eosin (H&E), were subjected to histopathological analysis. To quantify the number of prostatic acini profiles per sectional area, 30 randomly selected fields were analyzed for each group (n = 5), as described by Fochi et al. (2008). Lesion multiplicity was also assessed, including the presence of prostatic intraepithelial neoplasia (PIN) and luminal inflammation (LI). Lesions were classified based on the criteria established by Shappell et al. (2004) and further supported by findings in gerbils (Falleiros-Junior et al. 2016; Quintar et al. 2017). Multiplicity was evaluated by counting the number of lesions across entire sections in five animals per group, analyzing 25 previously scanned sections (Perez et al. 2016).

Statistical analysis

Statistical analyses for morphometric, histopathological, stereological, and collagen fiber quantification data were conducted using GraphPad Prism 5.00. Initially, data normality was assessed using the Kolmogorov–Smirnov test. Non-parametric data were analyzed using the Kruskal–Wallis tests. Parametric data were analyzed using Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test. The significance level was set at 5% ($p < 0.05$), and results were expressed as mean ± standard deviation.

Results

Biometry

Exposure to EE2 during the prenatal and pubertal periods did not result in significant changes in the body weight of the animals during aging, nor in the absolute and relative weight of the dorsal prostate, when compared to the control group (Fig. 2a-c).

Morphometry

Morphometric analysis revealed a significant increase in the epithelial height lining the acini of the dorsal prostate in the EE2/PUB group ($p < 0.0001$) compared to the control and EE2/PRE groups (Fig. 3a). In contrast, the muscle thickness surrounding the prostatic acini did not show a significant difference (Fig. 3b). These findings were further supported by histological analysis of HE-stained sections (Fig. 5a–c).

Stereology of the prostatic compartments

Stereological analysis showed a decrease in the relative volumes of the epithelial and luminal compartments, along with an increase in the muscle compartment of the dorsal prostate in the EE2/PUB group ($p < 0.0001$), compared to the other groups (Fig. 4a–c). Although the total stromal compartment was not significantly altered between groups (Fig. 4d), changes were observed in its components. Specifically, a significant decrease in the volume of blood vessels was found in the EE2/PRE group compared to the control and EE2/PUB groups ($p < 0.0001$; Fig. 4e). The histological features of the prostatic compartments are illustrated in Figs. 5d–f. The reduction in collagen fibers ($p < 0.0001$; Fig. 4f) was observed in the prostatic stroma of the EE2/PUB group compared to the other groups (Fig. 5g-i).

Histopathological analysis

The number of prostatic acini per section did not show significant differences among the experimental groups, as shown in Fig. 6a, c, e, and g. However, lesion multiplicity revealed a lower incidence of prostatic intraepithelial neoplasia (PIN) in the dorsal prostate of the EE2/PUB group ($p < 0.0001$) compared to the control and EE2/PRE groups (Fig. 6b, d, h). Another notable histopathological alteration was the increased inflammation observed in the luminal compartment of the EE2/PUB group ($p < 0.0001$) compared to the other groups (Fig. 6f, i).

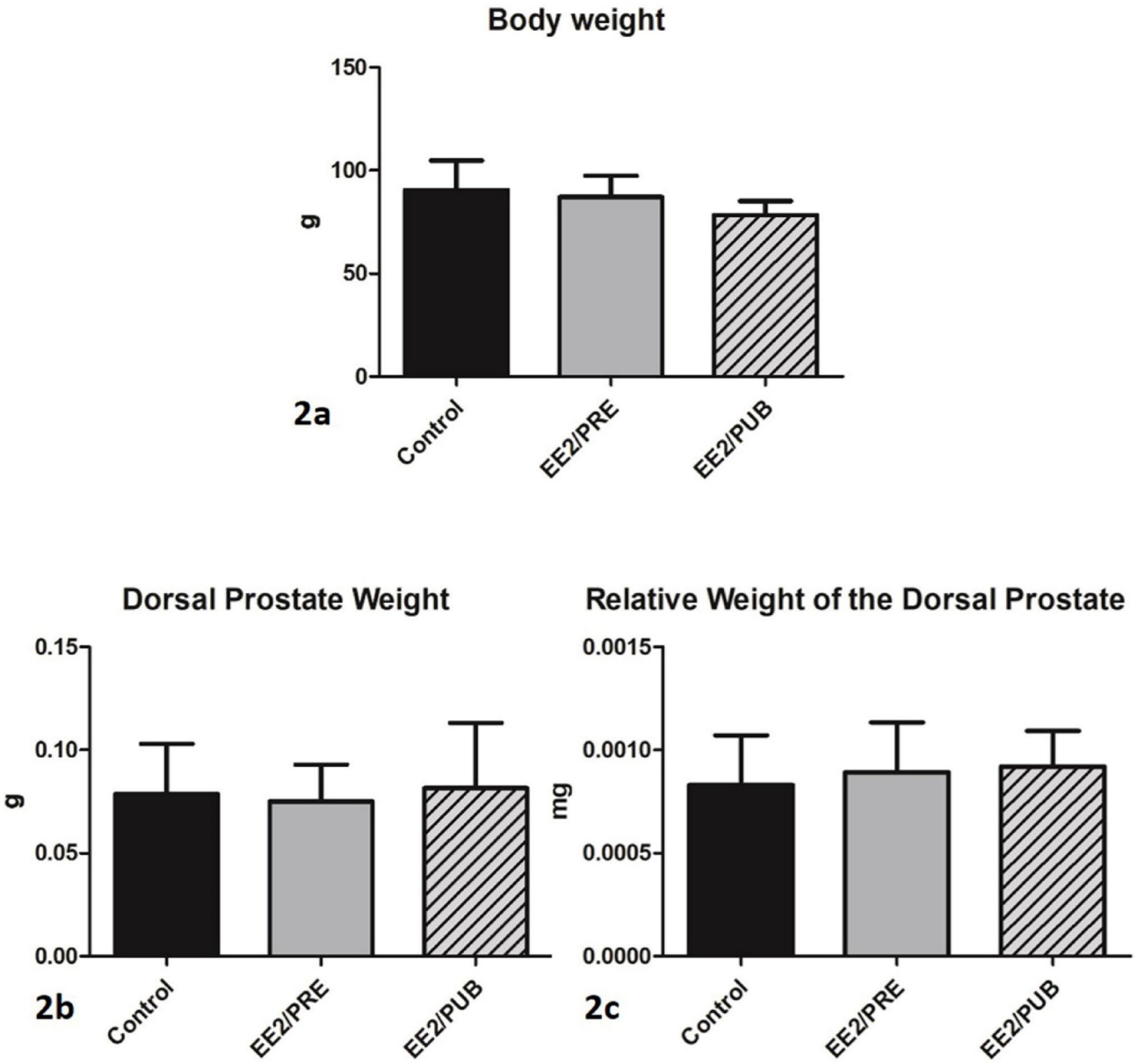

Fig. 2 Biometry of the experimental groups. **2a** Body weight of male gerbils (12 months). **2b** Absolute weight of the dorsal prostate. **2c** Relative weight of the dorsal prostate. Data are expressed as mean ± standard deviation ($p < 0.05$)

Discussion

The data from this study demonstrate that early exposure to EE2, an EDC, induces distinct morphological changes in the dorsal prostate tissue of gerbils during aging. These alterations vary depending on whether the exposure occurred during the prenatal or pubertal period.

In our study, gerbils aged 12 months were classified as ageing animals based on previous literature indicating that this age represents a transitional phase between adulthood and senescence in this species. According to Cheal (1986), significant age-related alterations in plasma hormone levels are already evident in male gerbils at this stage, indicating early reproductive ageing. As a result, several studies have used 12-month-old gerbils to investigate early age-related changes in reproductive organs, highlighting early hormonal exposure and morphophysiological alterations in prostate tissue during ageing (Perez et al. 2016; 2017; Fleury et al. 2021). Together, these findings support the use of 12-month-old gerbils in our study as a suitable model for examining age-dependent morphological changes in the prostate.

Initially, no significant changes were observed in body weight or in the absolute and relative weights of the dorsal prostate in gerbils exposed to EE2 during either

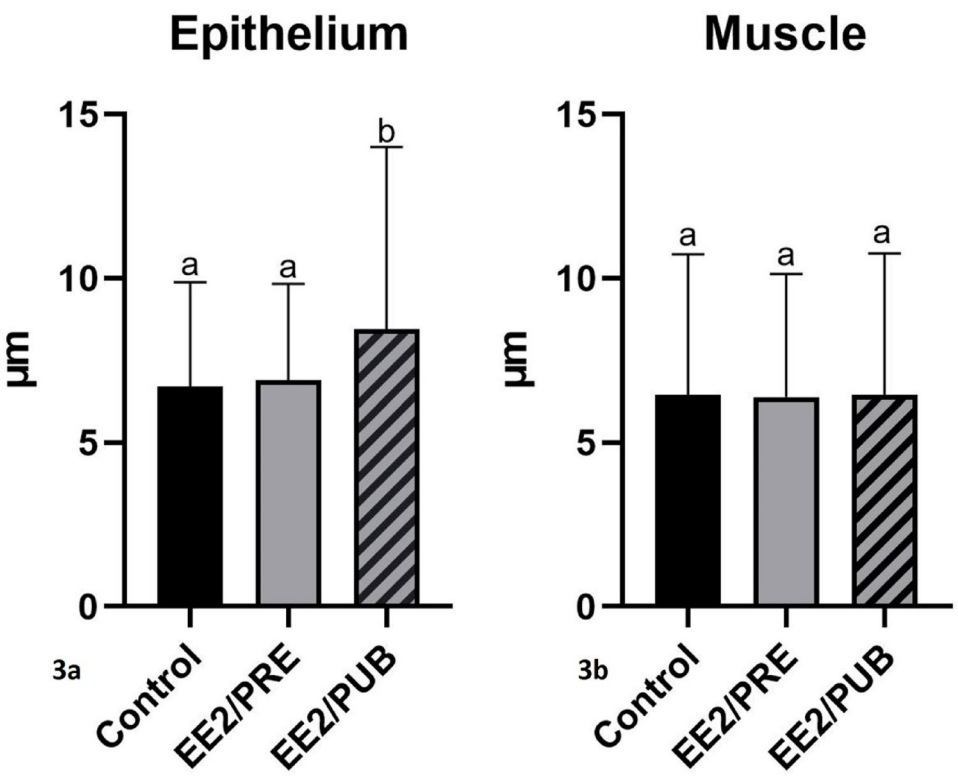

Fig. 3 Data from the dorsal prostate morphometry of the experimental groups. **3a** Epithelial height of the prostatic acini. **3b** Muscle thickness surrounding the prostatic acini. Data are expressed as mean ± standard deviation. Superscript letters (a, b) indicate statistically significant differences between the experimental groups ($p < 0.05$)

developmental window. These findings contrast with previous studies reporting that early EE2 exposure is associated with metabolic alterations and increased prostate growth, particularly in the ventral lobe and in later stages of life (Perez et al. 2011, 2016, 2017; Fleury et al. 2021; Sanches et al. 2021).

However, an increase in the relative volume of the muscular compartment was observed in the dorsal prostate of aged gerbils exposed to EE2 during the pubertal period. These findings may be attributed to early hormonal reprogramming mechanisms. Exposure to synthetic estrogens during critical windows of prostate development—such as the fetal period—can induce permanent alterations in stromal differentiation, particularly in smooth muscle cells (Prins et al. 2001; Ricke et al. 2006). This process, known as estrogenic imprinting, leads to altered hormone sensitivity in adulthood and predisposes the tissue to hyperplasia and stromal remodeling (Prins et al. 2021).

Furthermore, studies have shown that prenatal exposure to EE2 can disrupt the balance of testosterone and estradiol levels, promoting proliferative responses in the prostatic muscular compartment. This effect may be mediated through activation of estrogen receptors ERα and ERβ (Perez et al. 2016). Such structural changes, including increased muscular thickness, are commonly observed in conditions like benign prostatic hyperplasia (BPH), reinforcing the role of endocrine disruptors in age-related prostatic pathologies.

It is worth noting that the experimental design and the animals used in the present study were the same as those used in Perez et al. (2017), with the only difference being the specific prostatic lobe analyzed. Based on this, and according to Perez et al. (2017), exposure to EE2 at a dose of 15 µg/kg/day during puberty led to a reduction in testosterone levels, which resulted in both structural and ultrastructural alterations in the ventral prostate of male gerbils. Similarly, rats subjected to prolonged exposure to a mixture of EDCs during critical developmental periods exhibited a reduction in the volume of the epithelial compartment of the ventral prostate, accompanied by decreased glandular dilatation (Sousa et al. 2023). This reduction likely reflects a decrease in cellular volume, which may impair glandular homeostasis. In our study, we also observed a decrease in the epithelial and luminal compartments of the prostate in gerbils exposed to EE2 during puberty, which may be linked to reduced testosterone levels and consequent morphophysiological changes in the gland.

It is well established that the prostatic epithelium is highly sensitive to hormonal regulation and responds to the estrogenic activity of compounds such as EE2. EE2 binds to estrogen receptors (ERα and ERβ), activating intracellular pathways that regulate cell proliferation, apoptosis, and tissue microenvironment. This leads to increased mitotic activity and epithelial hyperplasia, measurable as epithelial thickening (Falleiros-Júnior et al. 2016). Previous studies have shown that early or prolonged estrogen exposure

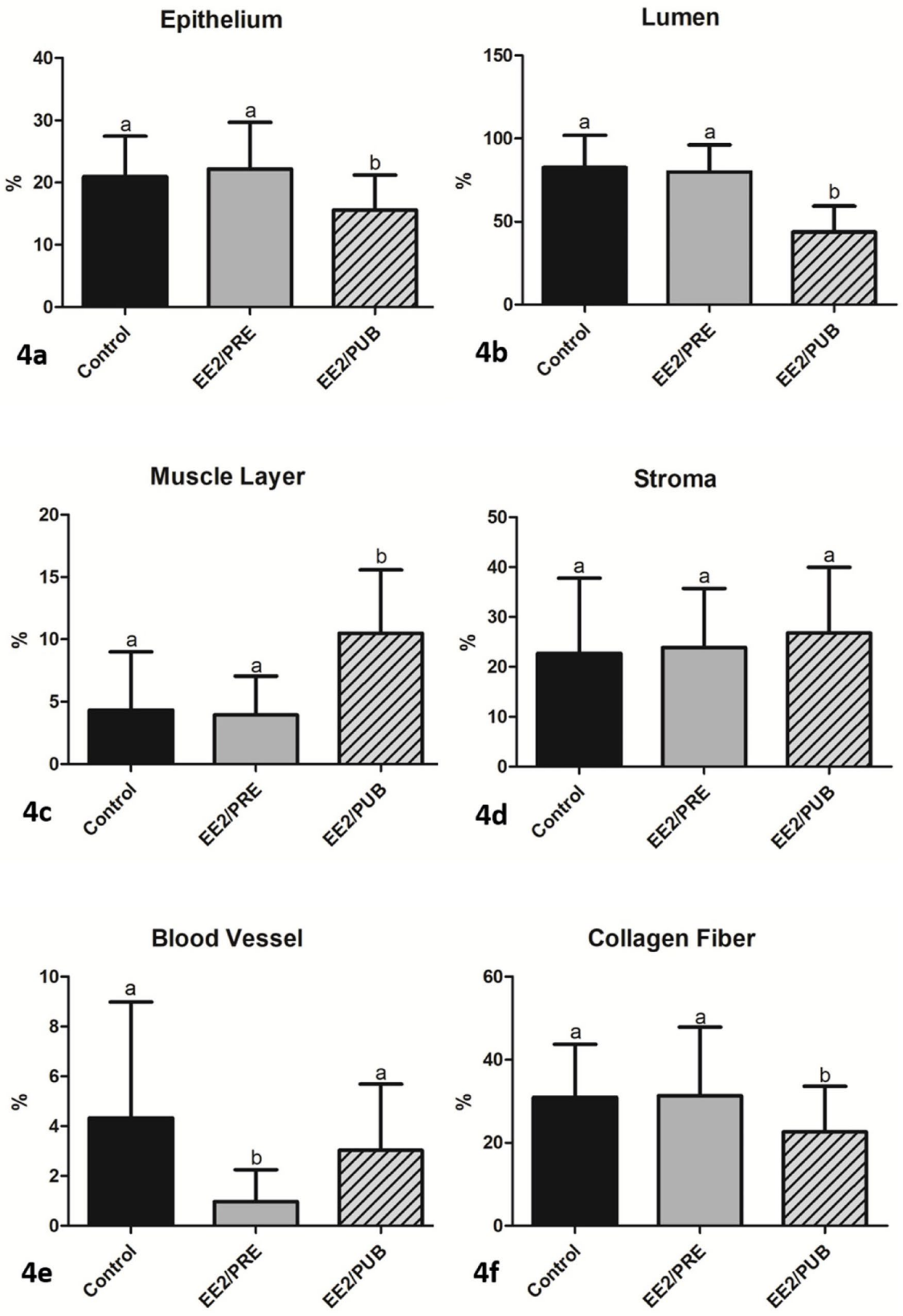

Fig. 4 Stereology (relative volume %) of the compartments in the dorsal prostate of the experimental groups. **a** Epithelium. **b** Lumen. **c** Muscle. **d** Stroma. **e** Collagen fibers. **f** Epithelium. **b** Lumen. **c** Muscle. **d** Stroma. **e** Blood vessels. **f** Collagen fibers. The superscript letters (a,b) indicate a statistically significant difference between the experimental groups ($p<0.05$)

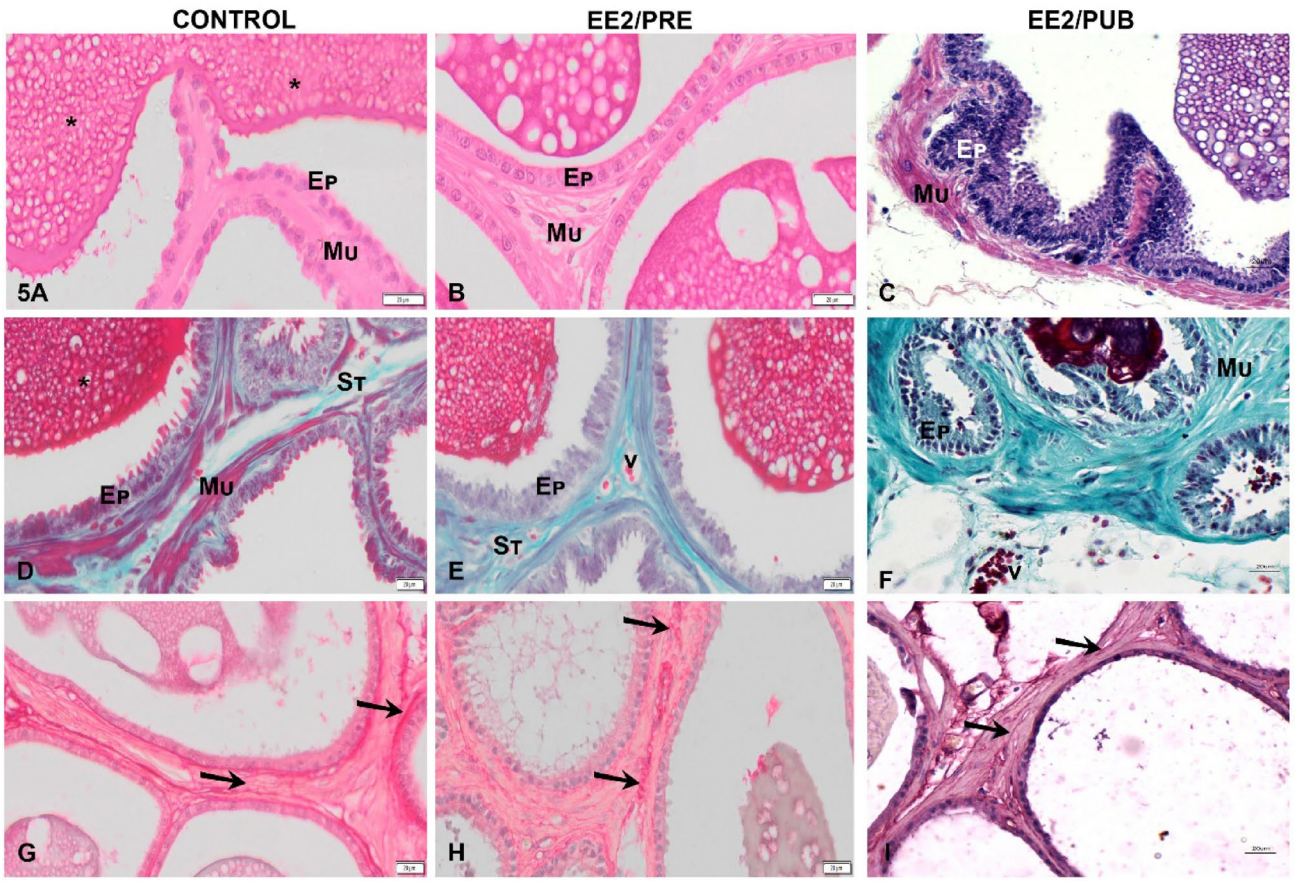

Fig. 5 Histological sections of the dorsal prostate of the experimental groups: Control, EE2/PRE and EE2/PUB. HE staining (**a**–**f**) highlighting the prostatic epithelium (Ep), smooth muscle layer (Mu) and secretion in the luminal region (**). Gömori Trichrome stain (**g**–**l**) non-muscular stroma (St), blood vessels (Vs), muscle (broad arrow) and luminal compartment (**). Picrosirius Red staining: collagen fibers (arrow) present in the stromal compartment

induces histoarchitectural changes in the rodent prostate, including increased epithelial thickness, reduced glandular lumen, and stromal alterations (Fleury et al. 2021; Carvalho and Silva et al. 2024). These findings align with the present study, which demonstrated a significant increase in epithelial height following EE2 exposure, supporting its role as an endocrine disruptor capable of altering prostate morphology.

A study in rats has shown that the balance between testosterone and estrogen is crucial for regulating microvessels in prostatic tissue, as hormonal imbalances directly influence the expression of vascular endothelial growth factor (VEGF) (Wang et al. 2022). Exposure to endocrine-disrupting chemicals (EDCs) during critical developmental windows alters the prostatic stroma in rats, affecting non-cellular stromal components such as collagen fibers and blood vessels (Sousa et al. 2023; Bueno et al. 2024). In gerbils prenatally exposed to EE2, a reduction in blood vessel volume within the prostatic stroma was observed, which may impair glandular angiogenesis and increase susceptibility to lesions.

The spatial organization of collagen fibers, key components of the extracellular matrix (ECM), plays a pivotal role in regulating the growth and progression of prostate neoplasms (Karamanos et al. 2021; Zadvornyi et al. 2023). In prostate cancer, collagen fibers are typically shorter, thicker, and less aligned compared to those in BPH, and increased expression of matrix metalloproteinases (MMPs) reflects active ECM remodeling. These ECM changes are being investigated as potential biomarkers for prostate cancer progression. In the dorsal prostate of the EE2/PUB group, a significant reduction in the relative volume of collagen types I and III was observed. This alteration in a critical ECM component highlights the stromal compartment's responsiveness to pubertal EE2 exposure and suggests a potential mechanism by which EDCs may contribute to prostate tissue remodeling and pathology.

Exposure to EE2 at the same dose used in our study (15 µg/kg/day) during the prenatal period has been shown to increase the predisposition to prostatic intraepithelial neoplasia (PIN) in the ventral prostate of aged gerbils (Perez et al. 2016). Similarly, when exposure occurs at the same dose

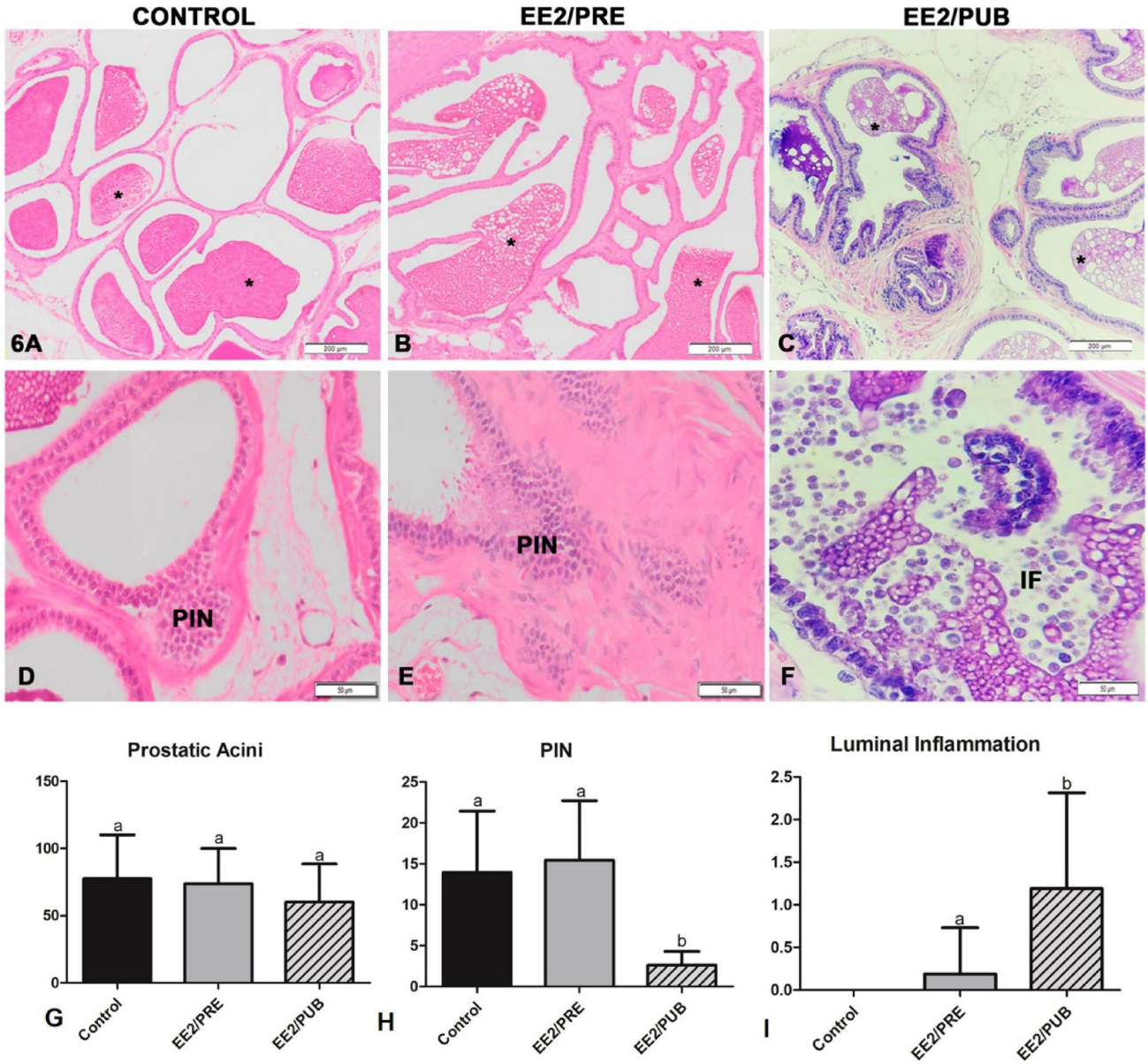

Fig. 6 Histopathological sections of the dorsal prostate from the experimental groups: Control (**a**, **b**), EE2/PRE (**c**, **d**), and EE2/PUB (**e**, **f**). Sections were stained with HE. Images show prostatic acini (**a**), prostatic intraepithelial neoplasia (PIN), and luminal inflammation (LI). Quantification of prostatic acini per section is shown in (**g**), while the multiplicity of PIN and LI are presented in (**h**) and (**i**), respectively. Superscript letters (a, b) denote statistically significant differences between experimental groups ($p < 0.05$)

and during the same postnatal period (days 42 to 49) as in our experiment, an increase in both PIN and prostatic inflammation is observed, along with decreased testosterone levels during aging (Perez et al. 2017). However, in our study, we noted a decrease in PIN development and an increase in luminal inflammation in the dorsal prostate of the EE2/PUB group. Rochel et al. (2007) reported histological differences between prostatic lobes in gerbils. Adult males castrated or treated with the anti-androgen flutamide exhibited greater androgen sensitivity in secretory activity in the ventral lobe compared to the dorsal lobe. This suggests that the epithelial response of the dorsal lobe may be slower or involve compensatory mechanisms related to androgenic regulation of secretory function (Goes et al. 2007). These findings support the idea that the prostate lobes of gerbils respond differently to hormonal changes—a pattern also observed in our study, although in response to synthetic estrogen.

In rats treated with exogenous estrogen, positive immunoreactivity for estrogen receptor beta (ERβ) was observed in dorsal prostate epithelial cells, suggesting upregulation of this pathway by estrogens (Cândido et al. 2012). ERβ is primarily expressed in epithelial cells and, unlike ERα,

plays a suppressive role in prostate cancer development and progression (Chen et al. 2022). Therefore, the action of ERβ may be associated with the reduced PIN incidence in the dorsal prostate of gerbils exposed to EE2 during puberty.

However, exposure to EE2 during puberty led to an increase in luminal inflammation in the dorsal prostate lobe. Silver et al. (2024) reported that, in mice, hormonal imbalance between estrogen and testosterone levels induced elevated expression of the chemokine CXCL17 in prostate epithelial cells, which attracted macrophages to the prostate tissue. These macrophages migrated into the glandular lumen and differentiated into foam cells, secreting inflammatory mediators such as VEGF and TGF-β1, thereby promoting inflammation. Similarly, EE2 exposure during puberty has been shown to reduce testosterone levels in gerbils (Perez et al. 2017), which may disrupt epithelial-stromal interactions and contribute to the establishment of an inflammatory microenvironment in the dorsal prostate.

Our study demonstrates that the response dynamics of the dorsal lobe of the gerbil prostate to EE2 exposure differ from those observed in the ventral lobe, as well as across different exposure periods. These findings reinforce that each prostatic lobe has distinct morphology and specific characteristics. Understanding these tissue-specific peculiarities is essential to elucidate the mechanisms of action of EDCs and their effects on prostate gland health.

In this context, even in the absence of molecular or functional assays, morphological analysis alone has proven valuable in identifying lobe-specific alterations and developmental susceptibilities. Although this study is limited to morphological analysis, the data provide meaningful and novel insights into the effects of endocrine-disrupting compounds on the dorsal prostate of Mongolian gerbils—an anatomically distinct and understudied region. These findings offer valuable baseline information and highlight sensitive developmental windows, supporting future investigations that may incorporate molecular and functional approaches to further explore underlying mechanisms.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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