International Journal of Environmental Health Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cije20

Histological investigation in aging male and female gerbil prostates after prenatal exposure to pequi (*Caryocar brasiliense Cambess*) oil and 17α-ethinylestradiol

Patrícia Matias Carvalho e Silva, Jéssica Adriane Zuffo, Maria Eduarda Hellou de Souza Mendes, Lucas Tavares Silva, João Victor Oliveira de Souza, Tracy Martina Marques Martins & Ana Paula da Silva Perez

To cite this article: Patrícia Matias Carvalho e Silva, Jéssica Adriane Zuffo, Maria Eduarda Hellou de Souza Mendes, Lucas Tavares Silva, João Victor Oliveira de Souza, Tracy Martina Marques Martins & Ana Paula da Silva Perez (15 Dec 2023): Histological investigation in aging male and female gerbil prostates after prenatal exposure to pequi (*Caryocar brasiliense Cambess*) oil and 17α-ethinylestradiol, International Journal of Environmental Health Research, DOI: 10.1080/09603123.2023.2294067

To link to this article: https://doi.org/10.1080/09603123.2023.2294067

Published online: 15 Dec 2023.

INTERNATIONAL JOURNAL OF ENVIRONMENTAL HEALTH RESEARCH
https://doi.org/10.1080/09603123.2023.2294067

Histological investigation in aging male and female gerbil prostates after prenatal exposure to pequi (*Caryocar brasiliense Cambess*) oil and 17α-ethinylestradiol

Patrícia Matias Carvalho e Silva[a], Jéssica Adriane Zuffo[b], Maria Eduarda Hellou de Souza Mendes[b], Lucas Tavares Silva[c], João Victor Oliveira de Souza[c], Tracy Martina Marques Martins[d] and Ana Paula da Silva Perez[e]

[a]Institute of Agricultural Sciences, Postgraduate Program in Animal Bioscience, Federal University of Jataí, Jataí, Goiás, Brazil; [b]Institute of Agricultural Sciences, Veterinary Course, Federal University of Jataí, Jataí, Brazil; [c]Institute of Health Sciences, Medicine Course, Federal University of Jataí, Jataí, Brazil; [d]Faculty of Medicine, Federal University of Pará, Altamira, Brazil; [e]Institute of Health Sciences, Medicine Course and Postgraduate Program in Animal Bioscience, Federal University of Jataí, Jataí, Brazil

ABSTRACT

The female prostate, also known as Skene's gland, is present in both humans and rodents. Prenatal exposure to ethinylestradiol (EE2), a synthetic estrogen found in oral contraceptives, induces pormotes neoplasic prostate lesions in gerbils (*Meriones unguiculatus*). Conversely, pequi oil (Pe), extracted from the Brazilian Cerrado fruit, has antioxidant, anti-inflammatory, and anticancer properties, mitigates risks associated with chronic diseases related to lifestyle and aging. This study evaluates the impact of prenatal exposure to Pe (300 mg/kg) on senile gerbil offspring's male and female prostates under normal conditions and EE2 exposure (15 µg/kg/day). Histological and morphometric analyses revealed that Pe reduced male body weight and prostate epithelial height, along with a thinner muscle layer. In females, EE2 exposure reduced prostatic weight, while Pe exposure lowered epithelial height and the relative stromal compartment volume, increasing the muscle layer. Pequi oil holds potential in mitigating alterations induced by exposure to the endocrine disruptor EE2.

ARTICLE HISTORY
Received 11 August 2023
Accepted 7 December 2023

KEYWORDS
Pequi oil; female prostate; male prostate; ethinylestradiol; morphometry

Introduction

The prostate gland, typically associated with the male reproductive system, is also found in females and is known as the Skene's gland (Zaviacic and Ablin 1998). The female prostate has been observed in various rodent species, including the Mongolian gerbil (*Meriones unguiculatus*), which we use as an experimental model in our research (Biancardi et al. 2017). In gerbils, the female prostate plays a crucial role in nourishing sperm within the female body through prostatic fluid, exhibiting characteristics similar to those found in males (Biancardi et al. 2017). Morphologically, the female gerbil prostate closely resembles the ventral male prostate (Santos and Taboga 2006).

In parallel to men, the female prostate can develop various pathological disorders. Notably, female prostatitis is the most prevalent cause within the spectrum of urethral syndromes (Gittes 2002). Additionally, iatrogenic lesions may occur in the periurethral region, including cases of cysts

CONTACT Ana Paula da Silva Perez paulabio_perez@ufj.edu.br Institute of Health Sciences, Federal University of Jataí (UFJ), Medicine Curse, Jatobá campus, BR 364, 3800, Jataí, 75801-615, Brazil

(Martín et al. 2010). Instances of adenocarcinoma formation within Skene's gland have also been documented (Saeed and Osunkoya 2021).

It's worth noting that the female prostate comprises approximately 15% to 25% of the size of the ventral male prostate (Santos and Taboga 2006). Both prostatic glands originate from the urogenital sinus epithelium, the precursor of the urethra (Staack et al. 2003; Sanches et al. 2014). In the case of the gerbil's female prostate, the epithelial buds make contact with the paraurethral mesenchyme on the 24th day of the prenatal period, traversing a narrow passage within the smooth muscle layer between the periurethral and paraurethral mesenchyme (Dos Santos et al. 2022). Conversely, in male gerbils, prostate budding occurs between the 20th and 21st prenatal days (Sanches et al. 2014).

At birth, male buds exhibit branching within the ventral mesenchymal pad, while female buds undergo this process only on the 5th postnatal day. The stage of canalization occurs on the 3rd postnatal day for females and on the 7th day for males. In both sexes, the presence of acini becomes evident after the 30th postnatal day (Sanches et al. 2016). Hence, it is evident that the development of the female gerbil prostate diverges markedly in its budding pattern from that of the male prostate (Sanches et al. 2016; Dos Santos et al. 2022).

Based on these structural similarities and distinct developmental processes, studies have undertaken a comparison of the impacts of exposure to endocrine-disrupting chemicals on both the ventral male prostate and the female prostate of gerbils (Perez et al. 2016; Costa et al. 2017; Gomes et al. 2023). For example, the synthetic estrogen 17α-ethinylestradiol (EE2) is a component of oral contraceptives and has been used by women for over 50 years (Stanczyk et al. 2013).

Research by Perez et al. (2016) further highlights that the male ventral prostate responds more sensitively to prenatal EE2 exposure compared to the female prostate in gerbils. This difference in sensitivity between the male and female prostate glands serves as a basis for comparing the effects of EDCs. This information contributes to a better understanding of how EE2 and similar compounds can impact physiological processes and potentially lead to prostatic issues and changes in hormonal regulation (Sanches et al. 2020).

In addition to EDCs impacting the prostate, various plant-based products have been extensively studied for their potential to combat prostate cancer. Clinical trials have examined the effects of beverages, extracts, and dietary preparations on prostate cancer risk. Notably, *Camellia sinensis* (green or black tea) and *Solanum lycopersicum* (common tomato) have received more thorough investigation (Cicero et al. 2019).

In our study, we utilized pequi (*Caryocar Brasiliense Cambess*), a native fruit of the Brazilian Cerrado widely used in culinary preparations and as a key ingredient in various food products. Pequi oil is rich in nutritional value, featuring abundant antioxidants like carotenoids, phenolic compounds, fatty acids, and vitamins, making it a candidate for medicinal food (Nascimento-Silva and Naves 2019).

Significantly, a study conducted by Palmeira et al. (2016) demonstrated that oral administration of pequi oil to mice at doses of 100 or 400 mg/kg resulted in a reduction in the development of liver preneoplastic lesions and adenomas, suggesting its potential as a chemopreventive agent. Moreover, research by Vale et al. (2019) reported a protective effect of pequi oil supplementation in male adult rats, guarding liver cells against damage induced by oxygen free radicals during strenuous exercise. Additionally, pequi oil exhibits antimicrobial properties and has the potential for analgesic and anti-inflammatory effects (Miranda-Vilela et al. 2009; Baptista et al. 2018; Junior et al. 2020).

However, there are no existing studies on the effects of pequi on prostate morphophysiology. Therefore, our research aimed to assess and compare the impact of prenatal exposure to pequi oil and ethinylestradiol on the morphology and morphometry of the ventral male prostate and female prostate in gerbils throughout the aging process.

Material and methods

Animal experimentation

Our study involved 25 adult female gerbils (*Meriones unguiculatus*) (90 and 120 days). Each female gerbil was paired with one male gerbil of the same age to form distinct families. The gerbils used in this experiment were obtained from the Bioterium Center of Paulist State University (UNESP) (São José do Rio Preto, SP, Brazil) and housed in polyethylene boxes at the Bioterium of the Federal University of Jataí (UFJ) under controlled light conditions and an average temperature of 23°C. They had access to filtered water provided in glass bottles and were given food ad *libitum*, with the following composition: 23% protein, 12% minerals, 5% fiber, and 4% total lipids.

All animal experiments were conducted in compliance with the approved guidelines of the Ethics Committee on the Use of Animals (CEUA), protocol No. 004/2018 of UFJ. The study adhered to the ethical standards outlined in Law No. 11.974/2008 and Resolution Normative No. 01 of 2010/07/09 by the National Council for the Control of Animal Experimentation (CONCEA).

The pregnant females ($n = 5$) were separated into five groups: *Control*, *EE*, *EE/Ve*, *EE/Pe* and *Pe*. *Control group*: The pregnant females in this group did not receive any treatment. *EE group*: Pregnant females in this group were received via gavage from the 18th to the 22th day of gestation, 15 µg/kg/day of 17α-ethinylestradiol (EE2, Sigma, St. Louis, MO, USA) diluted in 100 µl of mineral oil Nujol® (CAS 8020-83-5; Sigma-Aldrich, St Louis, MO). The dosage administered in this study (Perez et al. 2016) was comparable to that of oral contraceptives used by women (Stanczyk et al. 2013). This exposure period occurs during the prostatic morphogenesis in gerbils (Sanches et al. 2014). *EE/Ve group*: The females in this group received only 1 ml/day of mineral oil during the same gestation period mentioned in the previous methodology. *EE/Pe group*: The females in this group received 15 µg/kg/day of 17α-ethinylestradiol (EE2) using the same methodology as the EE group. Additionally, they received 300 mg/kg of pequi oil, adapted from Vale et al. (2019) via gavage from the 18th to the 26th day of gestation. *Pe group*: In this group, female subjects received a gavage of 300 mg/kg of pequi oil, administered from the 18th to the 26th day of gestation (Figure 1).

The male and female pups in all experimental groups were euthanized upon reaching completed 12 months of age. Before euthanasia, the female pups were cycled, and they were all in the proestrus phase. This step was taken as the female prostate undergoes changes in its morphometry and morphology during different phases of the estrous cycle (Fochi et al. 2008).

Pequi oil

The pequi (*Caryocar Brasiliense Cambess*) was obtained in the Jatai, Goiás. After removing its peel, the pulp was subjected to maceration to extract the oil, using ethanol as a solvent. Subsequently, the oil obtained was exposed to a temperature of 40°C to allow the alcohol to evaporate. Finally, the oil was stored in a freezer for preservation (Vale et al. 2019).

Histology and morphometry

After euthanasia, the biometric analyses involved measuring absolute and relative body weights, as well as the weights of the male ventral prostate and the combined female prostate and urethra.

The male ventral prostate and female prostate from the experimental groups were fixed in 4% tamponed paraformaldehyde and then subjected to histologic processing before being embedded in paraffin. Subsequently, the glands were sectioned into 5 µm thick slices and underwent specific staining protocols, including Hematoxylin-Eosin (HE) for morphometric analysis, Gömori's Trichrome (GT) for stereology, relative volume (%), and Periodic Acid-Schiff (PAS) for histochemical analysis (Tolosa et al. 2003).

For the morphometric analysis, 200 measurements were collected from each male and female ventral prostate in the experimental groups ($n = 5$). The images of the histological sections stain with HE was captured using a Leica photomicroscope. The collected measurements (μm) included the epithelial height and the thickness of the muscle layer in the analyzed prostates. The Image Software Pro-Plus version 4.5 for Windows was used for data analysis (Perez et al. 2016). The secretory activity of the male ventral and female prostate was marked by examining histologic sections stained with PAS stain under an optical microscope, facilitating the realization of this analysis.

To determine the relative volume (stereology) of the different prostatic compartments (epithelium, muscle, lumen, and stroma) in the studied experimental groups, we captured 30 random fields from each group using slides stained with GT. The measurements followed the 130-point, 10-line system proposed by Weibel et al. (1966). Using this approach, we collected data for each analyzed field, enabling the calculation of the relative frequency of the epithelial, muscular, stromal, and luminal compartments within the prostate gland. For morphological and morphometric analyses of the prostates, images were taken using a Leica photomicroscope (DM750).

Statistical analysis

Statistical analyses of morphometric data were performed using spreadsheets and graphs in GraphPad Prism 5.00 software. Firstly, the data obtained in this study were assessed for normality using the Kolmogorov-Smirnov test. For data with non-parametric distribution, the Kruskal-Wallis test was applied, while data with parametric distribution underwent analysis of variance (ANOVA), followed by multiple comparisons between groups using the Tukey test. The significance level adopted was 5% ($p < 0.05$), and the results were expressed as mean ± standard deviation (SD).

Results

Morphometry analysis of the male prostate

Table 1 presents the body weights of male gerbils, indicating a lower body weight in the Pe group compared to the EE/Ve group. However, there were no significant changes observed in the absolute and relative weight of the ventral prostate.

Histologically, the ventral male prostate exhibits acini coated by a columnar to cuboid simple epithelium and is surrounded by fibromuscular stroma (Figure 2(a-c)). Prenatal exposure to ethinylestradiol and pequi oil altered the morphological standard of this gland during aging. Microscopically, the presence of prostatic intraepithelial neoplasia (PIN) and changes in the glandular morphometry were observed (Figure 2(g,h)).

Morphometric data revealed an increase in the epithelial height of the ventral male prostate in the EE group compared to the other groups. However, the prostatic epithelium in the male EE/Ve,

Table 1. Biometric data of male and female gerbils of the experimental groups. The data are expressed as mean ± SD ($n = 5$).

PARAMETERS	CONTROL	EE/Ve	EE	EE/Pe	Pe
MALE					
Body weight (g)	$83.63 \pm 9.70^{a,b}$	92.65 ± 9.14^{a}	$85.60 \pm 10.82^{a,b}$	$9.18 \pm 12.48^{a,b}$	7.12 ± 13.53^{b}
Ventral prostate (g)	0.03 ± 0.02^{a}	0.03 ± 0.01^{a}	0.026 ± 0.01^{a}	$.04 \pm 0.02^{a}$	$.03 \pm 0.01^{a}$
Relative of the ventral prostate (mg)	0.34 ± 0.21^{a}	0.32 ± 0.14^{a}	0.38 ± 0.18^{a}	$.48 \pm 0.26^{a}$	$.54 \pm 0.25^{a}$
FEMALE					
Body weight (g)	68.89 ± 9.51^{a}	65.00 ± 8.70^{a}	69.71 ± 5.47^{a}	69.84 ± 7.85^{a}	69.75 ± 6.30^{a}
Urethra and prostate (g)	0.06 ± 0.01^{a}	$0.03 \pm 0.005^{a,b}$	0.02 ± 0.01^{b}	$.05 \pm 0.03^{a,b}$	$.04 \pm 0.03^{a,b}$
Relative of the urethra and prostate (mg)	0.93 ± 0.23^{a}	$0.66 \pm 0.06^{a,b}$	0.34 ± 0.08^{b}	$.83 \pm 0.46^{a}$	1.01 ± 0.57^{a}

Significantly difference groups are denoted by superscript letters (a,b) with $p < 0.05$.

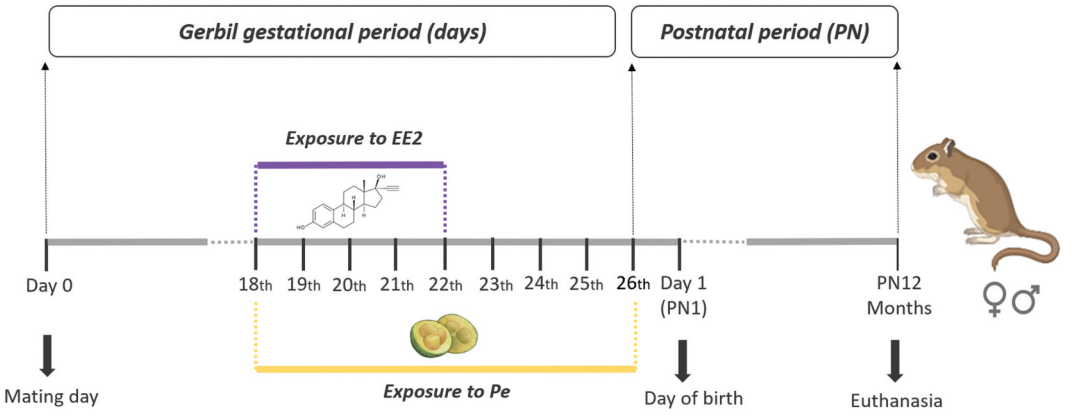

Figure 1. Experimental design utilized in the study. Pregnant females in the control group received no treatment. The EE group received a treatment of 15 µg/kg/day of EE2, while the EE/Ve group received 1 ml/day of the vehicle containing EE2. Both treatments were administered from the 18th to the 22nd day of pregnancy. From the 18th to the 26th day of pregnancy, the Pe group was treated with 300 mg/kg of pequi oil, and the EE/Pe group received 300 mg/kg of pequi oil along with 15 µg/kg/day of EE2, but only between the 18th and 22nd days of pregnancy. Both male and female pups were euthanized upon reaching 12 months of age.

EE/Pe, and Pe groups decreased significantly compared to the control group, especially in males exposed to ethinylestradiol (Figure 2(p)). Other alterations observed were in the thickness of the muscle layer surrounding the ventral male prostate. Compared to the control group, the vehicle group exhibited a decrease in the muscle layer, and this decrease was less pronounced in the other groups (Figure 2(q)).

Morphometry analysis of the female prostate

In the experimental group of female gerbils, there were no significant alterations in body weight. However, the weight of the urethra and female prostate in the EE group was smaller compared to the control group. Furthermore, within the EE group, the relative weight of these structures decreased when compared to the EE/Pe and Pe groups (Table 1).

The female prostate is primarily located paraurethrally and consists of acini. These acini are surrounded by a similar epithelium, akin to the one found in the ventral male prostate. This epithelium exhibits significant secretory activity and is encircled by fibromuscular tissue (Figure 3 (a-c)). Prenatal exposure to EE2 resulted in the development of microinvasive carcinoma (Figure 3(g)) and prostatic intraepithelial neoplasia (PIN) during the aging process (Figure 3(h)). It's study noting that distinct morphometric alterations were observed in the glands of the experimental groups (Figure 3(p,q)).

The acinar epithelial height was notably reduced in the female prostate for the EE/Ve, EE/Pe, and Pe groups when contrasted with both the control and EE groups (Figure 3(p)). Regarding the muscular layer, a significant augmentation was evident in the EE group when compared to the EE/Ve group. This increase was similarly observed in the EE/Pe and Pe groups in comparison to the control, EE/Ve, and EE groups (Figure 3(q)).

Stereology and histochemical analysis

Table 2 presents the relative volume (stereology) (%) of prostatic compartments in male and female gerbils from the experimental groups. The Pe group showed a significant increase in the luminal compartment of the female gerbil prostate compared to the EE/Ve and EE groups. The muscular compartment of the prostate gland in the Pe group showed a reduction compared to

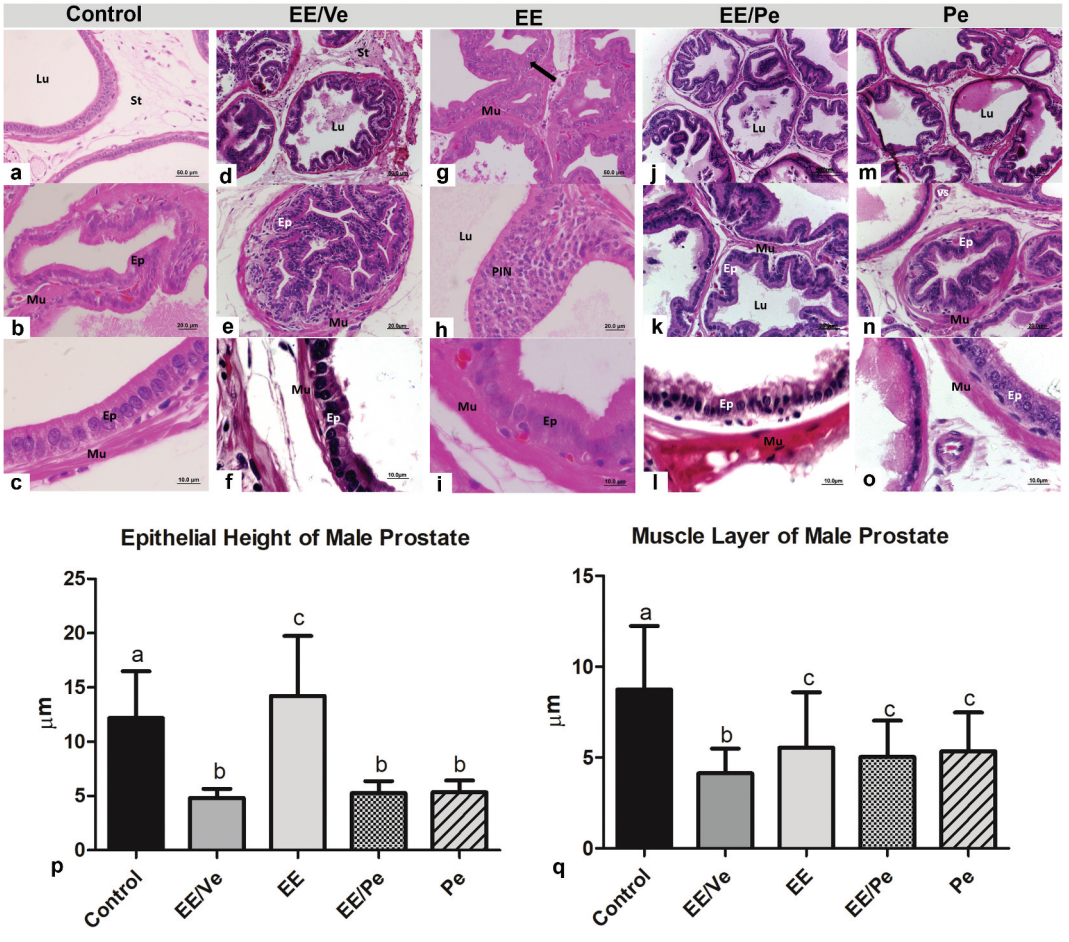

Figure 2. Histological sections of the ventral male gerbil prostate from the experimental groups were subjected to Hematoxylin-Eosin (HE) staining (A to O) for morphological analysis. The glands were characterized by different components including epithelium (ep), muscular layer (mu), luminal region (Lu), and stromal compartment (St). Notably, a specific detail highlighting the presence of prostatic neoplasia intraepithelial (PIN) was observed (arrow) in the EE group's gland (C and H). A graphical representation depicted epithelial height (P) and muscle layer thickness (Q). The results were presented as mean ± SD. Significant differences among the groups were indicated by superscript letters (a,b, c) with $p < 0.05$.

the group. The stromal compartment of the prostatic gland in females that received EE2 and pequi oil during the prenatal period was smaller in comparison to the EE/Ve group. In relation to relative volume of the epithelium observed a reduction in the ventral male prostate of EE group when compared to the control group. These morphological findings are depicted in Figure 4(a-g).

When examining male stereology, a significant increase in the prostatic lumen was observed in the ventral prostate of gerbils exposed to EE2, setting it apart from the other experimental groups. Conversely, a substantial reduction in prostatic lumen size was noted in the EE/Pe group when compared to the EE group. Additionally, a decrease in lumen size was evident in animals exclusively exposed to pequi oil, in contrast to the EE and EE/Pe groups.

The relative volume of the muscular compartment within the ventral male prostate exhibited a significant increase in the EE/Pe group as compared to the EE group. This elevation in relative volume was also evident in the Pe group when compared to the EE/Ve and EE groups. Regarding the stromal compartment, a noteworthy and statistically significant increase was observed solely in the ventral male prostate of the Pe group, distinguishing it from the control group (Figure 4(h-n)).

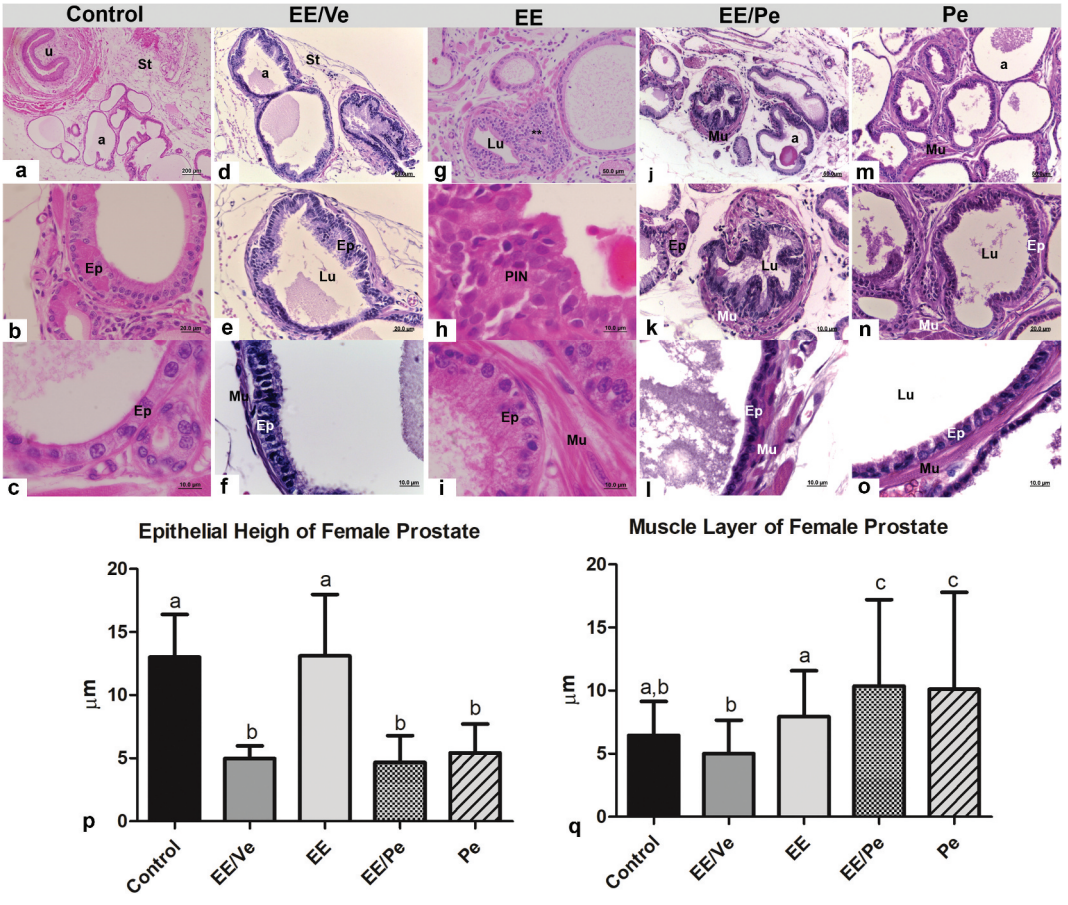

Figure 3. Histological sections of the female gerbil prostate from the experimental groups were subjected to Hematoxylin-Eosin (HE) staining (A to O) for morphological analysis. The gland's morphological features were characterized, including prostatic acinus (a), epithelium (ep), muscular layer (mu), luminal region (Lu), and stromal compartment (St). Notably, the presence of prostatic neoplasia intraepithelial (PIN) was observed in the EE group's gland (C and H), indicated by asterisks. A graphical representation illustrated epithelial height (P) and muscle layer thickness (Q). The results were presented as mean ± SD. Significant differences among the groups were denoted by superscript letters (a,b,c) with $p < 0.05$.

Table 2. Relative volume (%) of prostatic compartments in male and female gerbils of experimental groups. The data are expressed as mean ± SD ($n = 5$).

PARAMETERS	CONTROL	EE/Ve	EE	EE/Pe	Pe
MALE					
Stereology of the Epithelium (%)	33.92 ± 12.03^{a}	$30.42 \pm 12.69^{a,b}$	21.46 ± 8.71^{b}	$26.49 \pm 10.60^{a,b}$	$28.95 \pm 13.70^{a,b}$
Stereology of the Lumen (%)	$26.15 \pm 13.23^{a,c}$	$30.26 \pm 13.59^{a,c}$	45.58 ± 17.85^{b}	33.87 ± 14.76^{a}	2.36 ± 13.12^{c}
Stereology of the Muscle (%)	$12.46 \pm 8.02^{a,b,c}$	$12.28 \pm 9.82^{a,b}$	9.42 ± 5.66^{a}	$14.18 \pm 3.94^{b,c}$	16.62 ± 8.04^{c}
Stereology of the Stroma (%)	27.72 ± 17.41^{a}	$27.29 \pm 13.34^{a,b}$	$19.27 \pm 8.07^{a,b}$	$25.46 \pm 16.78^{a,b}$	34.08 ± 17.33^{b}
FEMALE					
Stereology of the Epithelium (%)	17.05 ± 9.16^{a}	14.10 ± 4.12^{a}	18.69 ± 10.01^{a}	14.82 ± 6.85^{a}	17.40 ± 11.22^{a}
Stereology of the Lumen (%)	$29.83 \pm 18.00^{a,b}$	16.41 ± 3.25^{a}	21.77 ± 12.76^{a}	$39.42 \pm 23.07^{a,b}$	36.92 ± 14.80^{b}
Stereology of the Muscle (%)	$8.36 \pm 5.88^{a,b}$	$9.10 \pm 3.55^{a,b}$	12.87 ± 14.58^{a}	$9.25 \pm 7.80^{a,b}$	5.10 ± 2.58^{b}
Stereology of the Stroma (%)	$41.97 \pm 18.33^{a,b}$	60.38 ± 8.22^{a}	$46.67 \pm 14.18^{a,b}$	37.08 ± 20.41^{b}	$4.61 \pm 21.59^{a,b}$

Significantly difference groups are denoted by superscript letters (a,b,c) with $p < 0.05$.

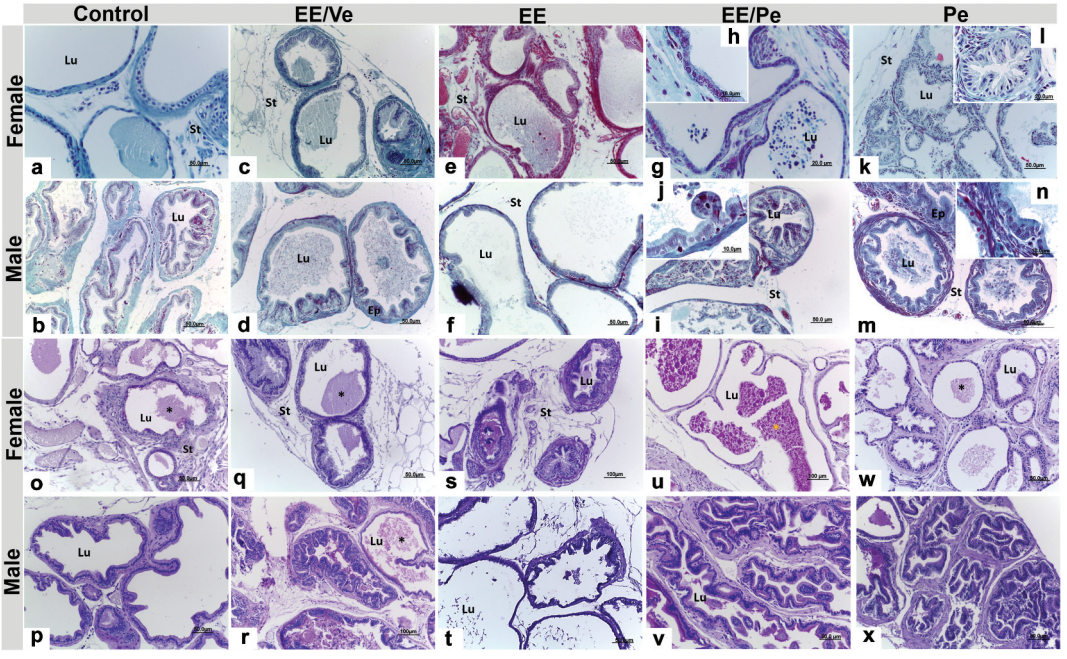

Figure 4. Histological sections of the female prostate and ventral male prostate from the experimental groups were subjected to Gömori's trichrome (GT) staining (A to N) to characterize the prostatic compartments. The luminal region (Lu) and stroma (St) were specifically examined. Additionally, sections were stained with Periodic Acid-Schiff (PAS) (O to X) to provide insights into glandular secretory activity. The presence of prostatic secretion within the luminal compartment was marked by asterisks. A detailed examination revealed an increase in this secretion's appearance in the female prostate (yellow asterisk).

The Figure 4(o-x) illustrates the secretory activity observed in the glandular prostatic tissue of both female and male gerbils belonging to the experimental groups. The histological sections, positively stained with PAS, accentuate this activity, particularly noticeable in the luminal area. Notably, the female prostate of the EE/Pe group exhibited an abundance of secretions within the lumen when juxtaposed with the treated group of females (Figure 4(o-s)). Conversely, in the male ventral prostate, this disparity was not observed (Figure 4(t-x)).

Discussion

Our study delved into the consequences of prenatal exposure to ethinylestradiol and pequi oil on the morphometry and morphology of prostatic tissue in both aging male and female gerbils. Notably, our research revealed distinct outcomes of this exposure on the glands. These observations align with previous research where singular exposure to EE2 was the focal point (Perez et al. 2016). Importantly, these findings emphasize the gerbil as a valuable experimental model for advancing research in the field of reproductive biology (Ruiz et al. 2023). This work holds the distinction of being the pioneering study in investigating the effects of pequi oil on both male and female prostates.

Male and female rats aged 8–10 weeks were administered doses of pequi oil via gavage over a 24-day period. Notably, a reduction in body weight was observed in males receiving 1000 mg/kg, while females did not exhibit this reduction (Traesel et al. 2016). A separate study involving humans indicated that the consumption of pequi oil capsules (at 400 mg/kg) over 14 days led to a decrease in total cholesterol and LDL levels in men above 45 years of age, in comparison to women (Miranda-Vilela et al. 2009). In our research, male gerbils received an oral dose of 300 mg/kg of the same oil during the prenatal period, resulting in a decrease in their body weight

during aging. These findings collectively emphasize that irrespective of the concentration or timing of pequi oil consumption, which contains a rich composition of monounsaturated and saturated fatty acids (Nascimento-Silva and Naves 2019), it consistently influences body weight in male rodents and men.

As noted by Cunha et al. (2009), changes in the relative weight of organs are indicative of potential toxicity. In the case of female subjects exposed to EE2, a decline in both the relative and absolute weight of the female prostate was observed. However, animals that received pequi oil did not exhibit alterations in these measurements. Pequi oil exhibited no indications of maternal toxicity, embryofetotoxicity, or developmental toxicity at any dosage level, even at 1000 mg/kg/day. This suggests its potential safe use during pregnancy (Traesel et al. 2017). Our study reveals that prenatal exposure to EE2 May lead to potential toxicity in the female prostate during aging, whereas exposure to pequi oil does not seem to have this effect on the gland.

In gerbils, a higher incidence of prostatic lesions occurs during the aging process. These disorders are linked to an increase in epithelial and stromal components, as evidenced by acinar expansion and increased glandular presence. These alterations have been observed in both gland types (Campos et al. 2008; Custodio et al. 2010). According to Perez et al. (2016), prenatal exposure to EE2 (15 µg/kg/day) leads to an increased proliferation of epithelial cells and the development of neoplastic lesions in both male and female gerbils during aging. However, both isolated exposure to pequi oil and concurrent exposure to EE2 and exposure to vehicle (EE/Ve) led to a reduction in epithelium height in the male and female prostate. Additionally, in male prostates, there was a noticeable decrease in the thickness of the muscle layer.

According to Palmeira et al. (2016), the administration of pequi oil reduced the development of liver preneoplastic lesions and induced remodeling of these lesions in mice. The chemopreventive properties of the oil are likely attributed to its antioxidant content. In our study, we observed the development of prostatic lesions in both males and females during aging, particularly following prenatal exposure to EE2. This fact was not observed in the prostatic gland of animals exposed prenatally to pequi oil, providing one of the initial pieces of evidence for the protective effect of pequi oil on prostate tissue.

The stroma components, including smooth muscle cells, play a crucial role in maintaining the normal physiological function of the gland, as well as in the development and progression of prostatic disorders (Sanches et al. 2021). In female gerbils exposed to EE2 during the prenatal period, an increase in the muscular layer thickness of the prostate has been observed, which is linked to a higher occurrence of prostatic intraepithelial neoplasia (Perez et al. 2016). In our study, we also noted an increase in the thickness of the prostatic muscle in female prostates of animals that received isolated pequi oil and were concurrently exposed to EE2. However, the relative volumes of the muscular and stromal compartments decreased in the Pe and EE/Pe groups, respectively. Morphologically, the dosage of pequi oil was not found to be associated with the development of prostatic disorders.

Studies have demonstrated that exposure to EE2 during developmental periods leads to distinct alterations in the morphophysiology of the ventral male prostate and female prostate in gerbils as they age (Perez et al. 2016; Fleury et al. 2021). Notably, the male prostate exhibited greater sensitivity to the effects of this synthetic estrogen compared to the female gland, resulting in the development of precancerous lesions in the ventral male prostate during the aging process (Perez et al. 2016). Regarding exposure to pequi oil and the vehicle (mineral oil), it was observed that this compound induced distinct morphological changes in these glands. However, pequi oil has the potential to mitigate the alterations induced by exposure to the endocrine disruptor EE2.

Acknowledgements

The authors acknowledge the Program of Initiation to Scientific, Technological Research and Innovation at UFJ (PIBIC/2019-2020).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the CNPq (National Council for Scientific and Technological Development) under Grant number 407761/2016-0.

ORCID

Tracy Martina Marques Martins http://orcid.org/0000-0003-0250-2234
Ana Paula da Silva Perez http://orcid.org/0000-0002-2195-2452

Data availability statement

The supporting data for the findings of this study can be obtained from the corresponding author upon making a reasonable request.

References

Baptista A, Gonçalves RV, Bressan J, Pelúzio MCG. 2018. Antioxidant and antimicrobial activities of crude extracts and fractions of cashew (*Anacardium occidentale L.*), cajui (*Anacardium microcarpum*), and pequi (*Caryocar brasiliense C.*): A systematic review. Oxid Med Cell Longev. 2018:1–13. doi: 10.1155/2018/3753562.

Biancardi MF, Dos Santos FCA, de Carvalho HF, Sanches BDA, Taboga SR. 2017. Female prostate: historical, developmental, and morphological perspectives. Cell Biol Int. 41(11):1174–1183. doi: 10.1002/cbin.10759.

Campos SG, Zanetoni C, Scarano WR, Vilamaior PS, Taboga SR. 2008. Age-related histopathological lesions in the Mongolian gerbil ventral prostate as a good model for studies of spontaneous hormone-related disorders. Int J Exp Pathol. 89(1):13–24. doi: 10.1111/j.1365-2613.2007.00550.x.

Cicero AFG, Allkanjari O, Busetto GM, Cai T, Larganà G, Magri V, Perletti G, Robustelli Della Cuna FS, Russo GI, Stamatiou K, et al. 2019. Nutraceutical treatment and prevention of benign prostatic hyperplasia and prostate cancer. Arch Ital Urol Androl. 91(3). doi:10.4081/aiua.2019.3.139.

Costa JR, Campos MS, Lima RF, Gomes LS, Marques MR, Taboga SR, Biancardi MF, Brito PVA, Santos FCA. 2017. Endocrine-disrupting effects of methylparaben on the adult gerbil prostate. Environ Toxicol. 32(6):1801–1812. doi: 10.1002/tox.22403.

Cunha LC, Azeredo FS, Mendonça ACV, Vieira MS, Pucci LL, Valadares MC, Freitas HOG, Sena AAS, Junior RSL. 2009. Avaliação da toxicidade aguda e subaguda, em ratos, do extrato etanólico das folhas e do látex de *Synadenium umbellatum Pax*. Rev Bras Farmacogn. 19:403–411. doi: 10.1590/S0102-695X2009000300012.

Custodio AM, Santos FC, Campos SG, Vilamaior PS, Oliveira SM, Góes RM, Taboga SR. 2010. Disorders related with ageing in the gerbil female prostate (Skene's paraurethral glands). Int J Exp Pathol. 91(2):132–143. doi: 10.1111/j.1365-2613.2009.00685.x.

Dos Santos FCA, Negre AFP, Rodríguez DAO, de Sousa GC, Rodrigues GA, Sanches BDA, Carvalho HF, Taboga SR, Biancardi MF. 2022. Female prostate development: morphological analysis of the budding dynamic. Microsc Microanal. 28(1):272–280. doi: 10.1017/S1431927621014008.

Fleury FG, Guimarães LRF, Rezende EB, Martins TMM, Caires CRS, Dos Santos FCA, Taboga SR, Perez APS. 2021. Prenatal and pubertal exposure to 17α-ethinylestradiol cause morphological changes in the prostate of old gerbils. Cell Biol Int. 45(10):2074–2085. doi: 10.1002/cbin.11656.

Fochi RA, Perez AP, Bianchi CV, Rochel SS, Góes RM, Vilamaior PS, Taboga SR, Santos FC. 2008. Hormonal oscillations during the estrous cycle influence the morphophysiology of the gerbil (*Meriones unguiculatus*) female prostate (skene paraurethral glands). Biol Reprod. 79(6):1084–1091. doi: 10.1095/biolreprod.108.070540.

Gittes RF. 2002. Female prostatitis. Urol Clin North Am. 29(3):613–616. doi: 10.1016/s0094-0143(02)00062-9.

Gomes LS, da Silva Lima D, Costa JR, Naves JS, Marques MR, Taboga SR, Ghedini PC, Biancardi MF, Alcantara Dos Santos FC. 2023. Neonatal exposure to aluminum chloride predisposes adult and senile gerbils to the prostatic hyperplasia. Cell Biol Int. 47(5):990–1003. doi: 10.1002/cbin.11995.

Junior AJ, Leitão MM, Bernal LPT, Dos Santos E, Âm K-O, Justi P, Argandoña EJS, Kassuya CAL. 2020. Analgesic and anti-inflammatory effects of *Caryocar brasiliense*. Anti - inflamm Anti - allergy Agents Med Chem. 19 (3):313–322. doi: 10.2174/1871523018666190408144320.

Martín LB, Barguti I, Andraca AZ, Gómez IR, Castañón LB 2010. Cyst of the skene's gland: report of four cases and bibliographic review. Arch Esp Urol. 63(3):238–42.
Miranda-Vilela AL, Pereira LC, Gonçalves CA, Grisolia CK. 2009. Pequi fruit (*Caryocar brasiliense Camb.*) pulp oil reduces exercise-induced inflammatory markers and blood pressure of male and female runners. Nutr Res. 29 (12):850–8. doi: 10.1016/j.nutres.2009.10.022.
Nascimento-Silva NRRD, Naves MMV. 2019. Potential of whole pequi (*caryocar spp.*) fruit-pulp, almond, oil, and shell-as a medicinal food. J Med Food. 22(9):952–962. doi: 10.1089/jmf.2018.0149.
Palmeira SM, Silva PR, Ferrão JS, Ladd AA, Dagli ML, Grisolia CK, Hernandez-Blazquez FJ. 2016. Chemopreventive effects of pequi oil (*Caryocar brasiliense Camb.*) on preneoplastic lesions in a mouse model of hepatocarcinogenesis. Eur J Cancer Prev. 25(4):299–305. doi: 10.1097/CEJ.0000000000000187.
Perez APS, Biancardi MF, Caires CRS, Falleiros-Junior LR, Góes RM, Vilamaior PSL, Santos FCA, Taboga SR. 2016. Prenatal exposure to ethinylestradiol alters the morphologic patterns and increases the predisposition for prostatic lesions in male and female gerbils during ageing. Int J Exp Pathol. 97(1):5–17. doi: 10.1111/iep.12153.
Ruiz TFR, Vilamaior PSL, Grigio V, Colleta SJ, Zucão MI, de Campos SGP, Dos Santos FCA, Biancardi MF, Perez APS, Taboga SR, et al. 2023. The Mongolian Gerbil as a Useful Experimental Model in Reproductive Biology. Reprod Sci. 30(7):2092–2106. doi: 10.1007/s43032-023-01171-6.
Saeed F, Osunkoya AO. 2021. Skene gland adenocarcinoma: clinicopathologic features, comprehensive biomarker analysis, and review of the literature. Pathol Int. 71(10):712–714. doi: 10.1111/pin.13145.
Sanches BD, Biancardi MF, Santos FC, Góes RM, Vilamaior PS, Taboga SR. 2014. Budding process during the organogenesis of the ventral prostatic lobe in Mongolian gerbil. Microsc Res Tech. 77(6):458–466. doi: 10.1002/jemt.22370.
Sanches BDA, Carvalho HF, Maldarine JS, Biancardi MF, Santos FCA, Vilamaior PSL, Taboga SR. 2020. Differences between male and female prostates in terms of physiology, sensitivity to chemicals and pathogenesis—A review in a rodent model. Cell Biol Int. 44(1):27–35. doi: 10.1002/cbin.11214.
Sanches BDA, Maldarine JS, Vilamaior PSL, Felisbino SL, Carvalho HF, Taboga SR. 2021. Stromal cell interplay in prostate development, physiology, and pathological conditions. The Prostate. 81(13):926–937. doi: 10.1002/pros.24196.
Sanches BD, Maldarine JS, Zani BC, Biancardi MF, Santos FC, Góes RM, Vilamaior PS, Taboga SR. 2016. The Expression of the Androgen Receptor and Estrogen Receptor 1 is Related to Sex Dimorphism in the Gerbil Prostate Development. Anat Rec. 299(8):1130–9. doi: 10.1002/ar.23364.
Santos FCA, Taboga SR. 2006. Female prostate: a review about the biological repercussions of this gland in humans and rodents. Anim Reprod. 3(1):3–18.
Staack A, Donjacour AA, Brody J, Cunha GR, Carroll P. 2003. Mouse urogenital development: a practical approach. Differentiation. 71(7):402–413. doi: 10.1046/j.1432-0436.2003.7107004.x.
Stanczyk FZ, Archer DF, Bhavnani BR. 2013. Ethinyl estradiol and 17β-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. Contraception. 87(6):706–727. doi: 10.1016/j.contraception.2012.12.011.
Tolosa EMC, Rodrigues CJ, Behmer OA, Freitas Neto AG. 2003. Manual de técnicas para histologia normal e patológica. 2nd ed. São Paulo (SP): Manole; p. 331.
Traesel GK, de Lima FF, Dos Santos AC, Souza RIC, Cantadori DT, Kretschmer CR, Navarini VJ, Oesterreich SA, de Lima FF. 2017. Evaluation of embryotoxic and teratogenic effects of the oil extracted from *Caryocar brasiliense Cambess* pulp in rats. Food Chem Toxicol. 110:74–82. doi: 10.1016/j.fct.2017.10.018.
Traesel GK, Menegati SE, Dos Santos AC, Carvalho Souza RI, Villas Boas GR, Justi PN, Kassuya CA, Sanjinez Argandoña EJ, Oesterreich SA. 2016. Oral acute and subchronic toxicity studies of the oil extracted from pequi (*caryocar brasiliense, Camb.*) pulp in rats. Food Chem Toxicol. 97:224–231. doi: 10.1016/j.fct.2016.09.018.
Vale AF, Ferreira HH, Benetti EJ, Rebelo ACS, Figueiredo ACR, Barbosa EC, Simões K. 2019. Antioxidant effect of the pequi oil (*Caryocar brasiliense*) on the hepatic tissue of rats trained by exhaustive swimming exercises. Braz J Biol. 79(2):257–262. doi: 10.1590/1519-6984.180015.
Weibel ER, Kistler GS, Scherle WF. 1966. Practical stereological methods for morphometric cytology. J Cell Biol. 30 (1):23–38. doi: 10.1083/jcb.30.1.23.
Zaviacic M, Ablin RJ. 1998. The female prostate. J Natl Cancer Inst. 90(9):713–713. doi: 10.1093/jnci/90.9.713.