Effectiveness of selenium in maintaining morphological architecture of prostatic compartments in mongolian gerbil

Eficácia do selênio na manutenção da arquitetura morfológica dos compartimentos prostáticos em gerbil da Mongólia

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ABSTRACT

Male prostate is a reproductive gland that is under steroid hormones influence, which is object of several studies related to proliferative lesions that may arise during the aging process. Selenium is a micro-nutrient that has been described as a toxic mineral if administered at high concentrations but can also be beneficial mineral exerting a protective action on various tissues used properly. This mineral can be found in some foods such as Brazil nuts, grains, cereals and some vegetables. Mongolian gerbil prostate have morphophysiological similarities between the human prostate and has been used for comparative studies. This work aims to analyze the morphology and stereology of Mongolian gerbil prostate after selenium supplementation, followed by testosterone injection. Animals were divided into 3 groups (5 per group): GC intact animals; TG testosterone injection (1 mg/kg) for 21 days; TSG, selenium administration and testosterone injection (1 mg/kg) for 21 days. After experimental phase, animals were euthanized and prostate was dissected, fixed in metacarn, embedded, and sections were stained with hematoxylin-eosin (HE) for morphological and morphometric/stereological analyses. Analyzes showed that prostate epithelium of TG showed points of stratification and higher columnar cells when compared to CG and TSG. Already stereological analysis showed that the volume of epithelium in TG and TSG was higher than in CG. Besides, non-muscular volume of TG stroma showed higher than in TSG and CG. In conclusion, selenium is, in this concentration, an

important factor that acts in maintenance of prostate morphology, even under the influence of exogenous testosterone.

Keywords: prostate, proliferative lesion, sodium selenite, testosterone.

RESUMO

A próstata masculina é uma glândula reprodutiva que está sob influência de hormônios esteróides, sendo objeto de diversos estudos relacionados às lesões proliferativas que podem surgir durante o processo de envelhecimento. O selênio é um micronutriente que tem sido descrito como um mineral tóxico se administrado em altas concentrações, mas também pode ser um mineral benéfico exercendo ação protetora em vários tecidos utilizados adequadamente. Esse mineral pode ser encontrado em alguns alimentos como castanha-do-pará, grãos, cereais e alguns vegetais. A próstata do gerbil da Mongólia tem semelhanças morfofisiológicas com a próstata humana e tem sido usada para estudos comparativos. Este trabalho tem como objetivo analisar a morfologia e estereologia da próstata do gerbilo da Mongólia após suplementação de selênio, seguida de injeção de testosterona. Os animais foram divididos em 3 grupos (5 por grupo): animais GC intactos; Injeção de testosterona TG (1 mg/kg) por 21 dias; ETG, administração de selênio e injeção de testosterona (1 mg/kg) por 21 dias. Após a fase experimental, os animais foram eutanasiados e a próstata foi dissecada, fixada em metacarno, embebida e os cortes foram corados com hematoxilina-eosina (HE) para análises morfológicas e morfométricas/estereológicas. As análises mostraram que o epitélio prostático do TG apresentou pontos de estratificação e células colunares superiores quando comparado ao GC e ao TSG. Já a análise estereológica mostrou que o volume do epitélio no GT e no GET foi maior que no GC. Além disso, o volume não muscular do estroma do TG mostrou-se maior que no ET e no GC. Concluindo, o selênio é, nesta concentração, um importante fator que atua na manutenção da morfologia da próstata, mesmo sob influência da testosterona exógena.

Palavras-chave: próstata, lesão proliferativa, selenito de sódio, testosterona.

1 INTRODUCTION

Prostate is a reproductive accessory gland that secretes the seminal fluid contributing to sperm motility and nutrition. Its development and growth start in the fetal period and extends to sexual maturity (CUNHA; DONJACOUR; SUGIMURA, 1986; MARKER et al., 2003). This gland is dependent on steroid hormones that are important for development and differentiation during embryogenesis and for maintenance during adult life (CUNHA; CHUNG, 1981). In addition, the prostate consists of a secretory epithelium and a stroma, which are controlled by androgens (GRADELA et al., 2013), being the testosterone, which is converted to dihydrotestosterone, one of the most important hormones to maintenance of prostate (CARVALHO-SALLES; TAJARA, 1999).

It is known that many histological changes occur in the prostate caused by hormonal decompensation and that is why there are a large number of studies on its morphology and physiology, as it has frequently been affected by some proliferative lesions such as Benign

Prostatic Hyperplasia (BPH), inflammations and neoplasms (GRADELA et al., 2013). These lesions may occur due to the aging process, which promotes imbalance in prostatic hormone levels, leading to changes in the interaction between stroma and epithelium, thus resulting in uncontrolled cell proliferation, causing some of the proliferative lesions (GRADELA et al., 2013).

The identification of proliferative lesions that affect the prostate has justified studies using rodents as experimental animals, with some of them trying to establish a relationship between rodent prostate lobes and human prostate areas (GRADELA et al., 2013; ROCHEL et al., 2007). Among rodents, the Mongolian Gerbil has been the target of studies related to the prostate, as it is a docile animal, easy to handle and has a low incidence of natural diseases (PINHEIRO et al., 2003), in addition to having similarity with the prostate of (Fig. 3) (ROCHEL et al., 2007) and to be an excellent experimental model related to aging (PEGORIN DE CAMPOS et al., 2006).

Due to the importance of studies on morphofunctional changes in the prostatic compartments, research seeks to identify possible treatments that can promote the reversal and/or prevention of these alterations, focusing on investigations about molecules found in human food, such as Selenium (Sel). This micro-nutrient with antioxidant action can be found in some foods such as Brazil nuts, grains, cereals and some vegetables (FERGUSON; PHILPOTT; KARUNASINGHE, 2004; RAYMAN, 2000; TAPIERO; TOWNSEND; TEW, 2003) and it has antioxidant properties, reduces the risk of non-transmissible chronic diseases, increases the resistance of the immune system, protects against the action of heavy metals and xenobiotics and also inhibits the proliferation of abnormal cells in both rodents and humans (BJORKHEM-BERGMAN, 2004).

This work aimed to evaluate the prostate compartments through morphological, morphometric and stereological methods to verify the influence of Selenium on the maintenance of prostate architecture after administration of exogenous testosterone

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

Adult male gerbils were used, 90 days old, divided into 3 groups (five animals each): CG, intact adult animals; TG, animals that received subcutaneously 0.10 mL of testosterone cypionate for 21 days, at intervals of 48 hours, according to (POLLARD; LUCKERT, 1987); TSG who received weekly Sodium Selenite in concentrations of 10 mg/kg by gavage (in 0.5 mL of saline solution), in addition to the subcutaneous application of testosterone cypionate

similar to the TG group. Selenium doses were administered two weeks before the testosterone application. All procedures were approved by the ethics committee (CEUA-UFMT 23108.076606/2015-16).

2.2 HISTOLOGICAL ANALYSIS

Prostatic samples were fixed by immersion in Metecarn's solution (5% parformaldehyde, 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2) for 4h. After fixation, tissue fragments was washed with running tap water, dehydrated in an ethanol series, embedded in paraffin and sectioned at 3m on a rotatory microtome. Histological sections were subjected to hematoxylin–eosin (H&E) staining for general studies and microscopic analyses were performed on light photomicroscope (Olympus, Hamburg, Germany). Microscopic fields were digitized using the software Image-Pro Plus, Version 4.5 for Windows.

2.3 MORPHOMETRIC AND STEREOLOGICAL ANALYSIS

Random fields of hematoxylin–eosin (H&E) histological sections of prostate from each experimental group were analyzed by Image-Pro® Plus version 6.0 for Windows to morphometric evaluation of epithelium height. Stereological measurements were obtained by the M120 multipoint test system and 60-line test proposed by (WEIBEL, 1963) to compare the relative proportion (relative volume in %) of each component of prostatic tissue (epithelium, lumen, muscular stroma and non-muscular stroma). 30 random microscopic fields were captured from each experimental group. The relative values were determined by counting the points coincident with the test grade divided by the total number of points.

2.4 STATISTICAL ANALYSIS

All statistical analyses were performed with GraphPad Prims 7.00. The ANOVA and Tukey HSDs test were employed, and $p \leq 0.05$ was considered statistically significant.

3 RESULTS AND DISCUSSION

Analysis of histological sections demonstrated that the morphology of the prostatic epithelium of the CG group showed cubic columnar cells tending to be prismatic, with a central nucleus and with little dense secretory activity in the lumen. Furthermore, smooth muscle cells were found in the muscular stroma involving the prostatic acini with presence of extracellular matrix. (Fig. 1a-d). Therefore, in this group, no significant changes/modifications were identified in their morphology during the experiment period.

After 21 days of treatment, morphological and morphometric analyzes of the prostate of animals in the TG experimental group demonstrated the presence of some histological changes. The epithelium showed many stratification points and taller columnar cells (Fig. 2a-f) when compared to the CG (Fig. 1a-d) and TSG (Fig. 3a-f) groups. Morphometric analysis (Fig. 05) indicated significantly higher epithelial cell in TG when compared with CG ($p < 0,001$) and TSG ($p < 0,01$). Furthermore, the presence of high secretory activity of epithelial cells in the TG group was identified, with several secretion vesicles being visible in their apical region (Fig. 2b-f). According to MOSTOFI et al. (1992), these histological changes have proliferative characteristics and occur due to disturbances in the homeostasis of the population of secretory cells.

In this case, these disturbances are due to the administration of exogenous testosterone, used in this experiment, which corroborates the experiment carried out by CAMPOS et al. (2007), who describe in their studies that in the long term, testosterone can cause histological changes such as the presence of inflammatory cells in acini, cells undergoing apoptosis and intraepithelial neoplasias. These intraepithelial neoplasias have been described by BOSTWICK et al. (2000), as precursor lesions of prostate cancer.

Stereological analyzes (Fig. 5) revealed that epithelium and non-muscle stroma of the TG group showed a greater volume when compared to animals in the CG. In contrast, these compartments presented a smaller volume in TSG when compared to TG animals. These epithelial and stromal changes in the TG group may be a factor indicative of an imbalance in prostate homeostasis, as the results of SUGIMURA et al. (1986) indicated the stromal compartment as being the first target of androgen action, since the epithelial reaction is mediated by stromal factors. Results from POLLARD and LUCKERT (1987), CAMPOS et al. (2007) as well as LEE et al. (2017), describe lesions of a proliferative genesis in prostate epithelial cells, associated to presence of T. In this sense our results also show that testosterone appears to act by promoting the modification of stromal composition, which, as suggested by the aforementioned authors, may be a preponderant factor in the induction of proliferative lesions in the prostate gland.

In the prostate of animals in the TSG group, the presence of taller cubic/columnar cells (Fig. 3b-d) was found in the epithelium when compared to the CG group, but statically smaller when compared to the TG group (Fig. 4), with visible secretory activity and a lower amount of stratification points (Fig. 3e-f). Together with the lower epithelial and stromal volume, these results lead us to propose a protective effect of Sel, even under the influence of some type of factor promoting/inducing proliferative activity (in this case, testosterone), as several studies,

in different organs and tissues, has suggested this protective action. TUJI et al. (2005), evaluated the radioprotective effect of Sel on the submandibular glands of Wistar rats, and found a lesser intensity in the alteration of the acinar cells of this gland. Furthermore, BJÖRKHEM-BERGMAN et al. (BJORKHEM-BERGMAN, 2004) described the protective effect of Sodium Selenite in rats that had chemically induced hepatocarcinogenesis.

ROTRUCK et al. (1972) and CHOW and TAPPEL (1974)also described this effect of Sel on the tissue repair process in erythrocyte cells against hemolysis. This protective effect of Sel was also described in the study carried out by CEKAN et al.(1985), who demonstrated a significant decrease in the number of fetal malformations after intraperitoneal injection of sodium selenite.

SHIBATA and collaborators (1996) described in their studies the existence of striking histological similarities between the process of emergence of neoplastic lesions in mice, their experimental model, with the same process seen in gerbils, which in turn is very similar to that seen in humans. Furthermore, experiments that promote the emergence of neoplastic processes induced by androgens have stood out as a good biological tool, allowing us to better monitor the processes involved in the evolution of histological changes that trigger prostate neoplasms in humans (BOILEAU, 2003).

4 CONCLUSION

Results indicate that testosterone promotes morphological changes in the epithelium and stroma of the Gerbil prostate, but the ingestion of Sel attenuates these changes, preventing the induction of proliferative lesions in this gland. Therefore, Selenium shows promise as a protective molecule in maintaining prostate morphophysiology, even under the influence of exogenous testosterone.

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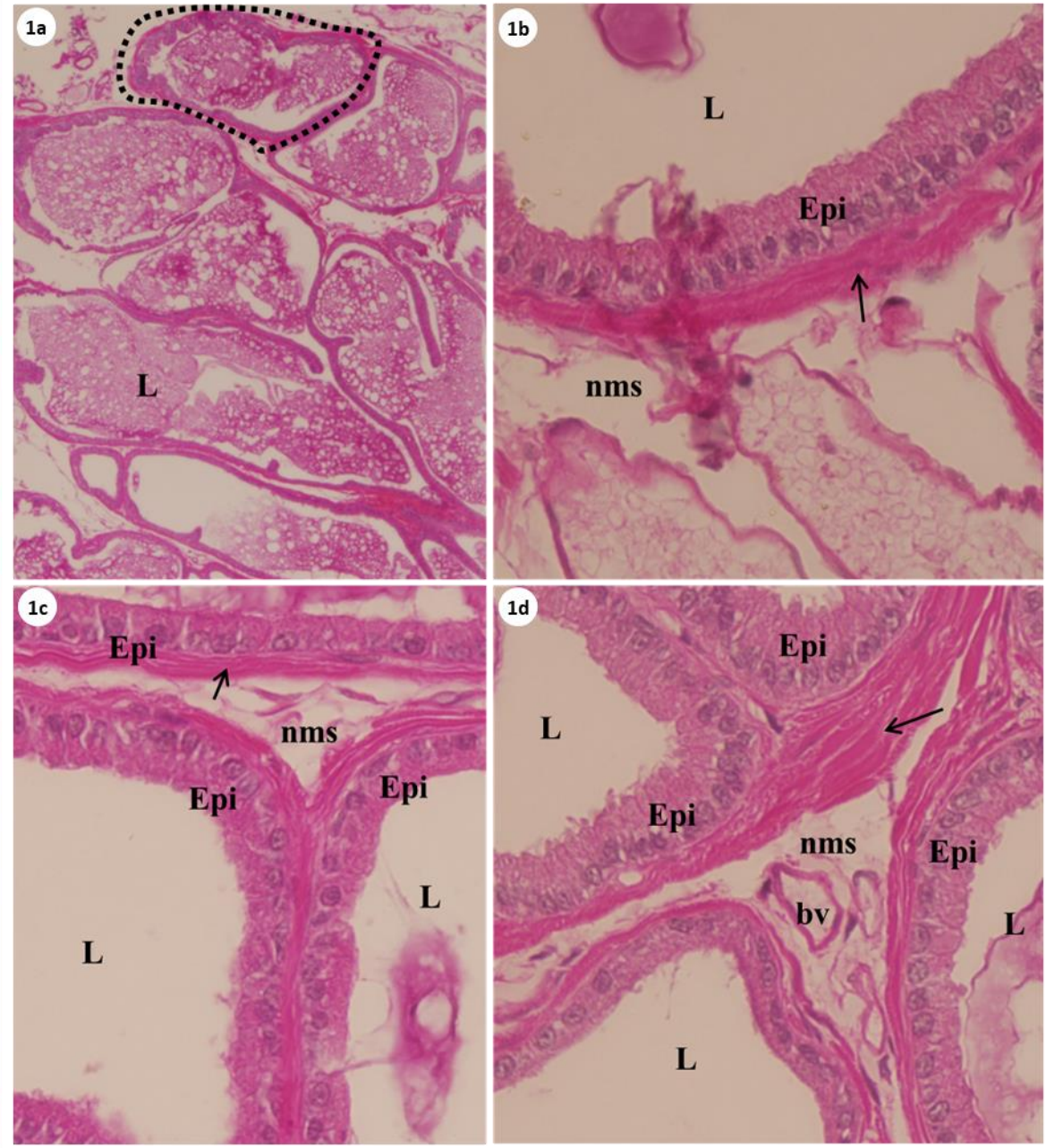
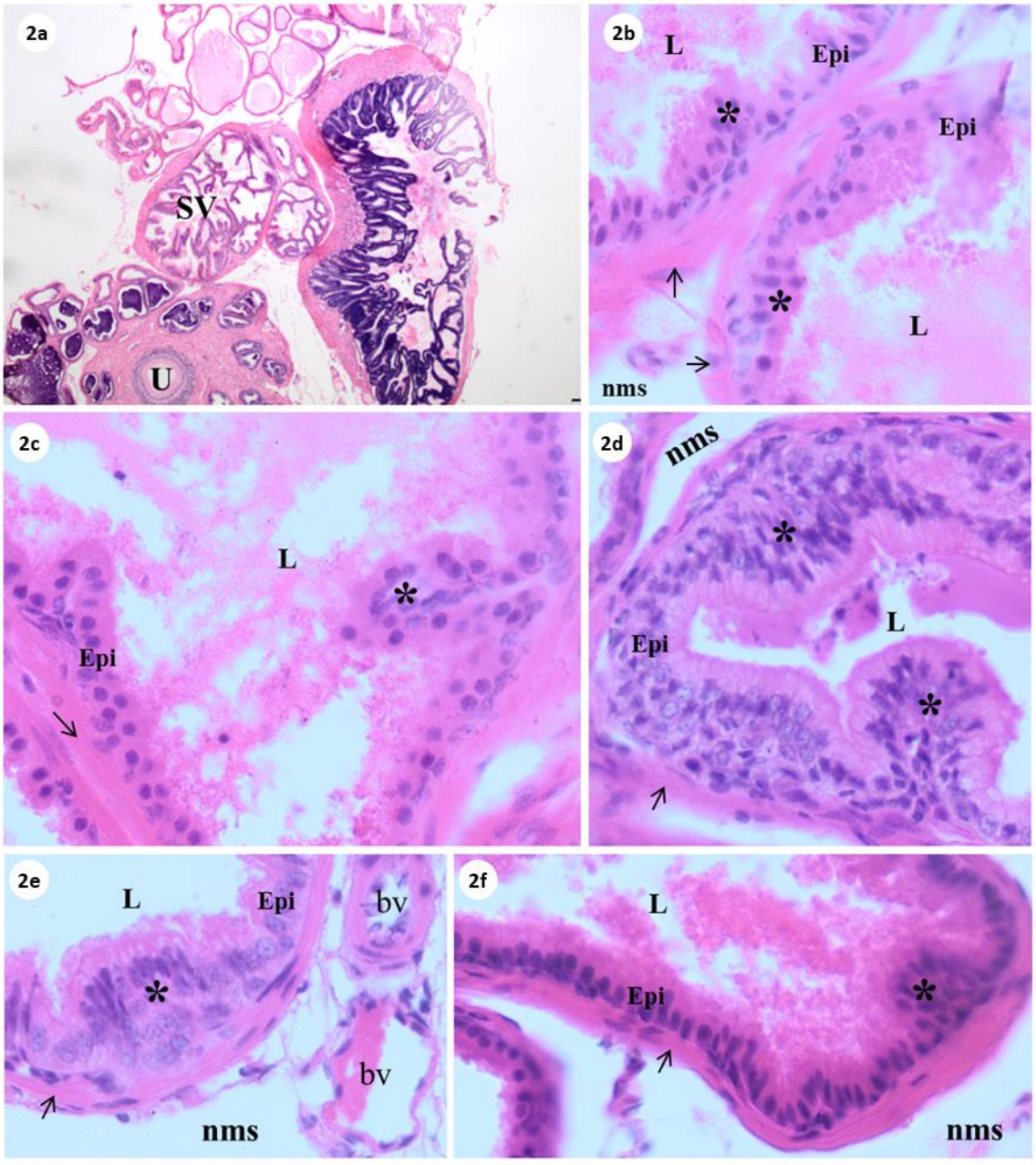
ANNEX

Figure 1: Grupo CG. Cortes histológicos corados com HE de animais controle. 4a: Visão geral dos ácinos que estão envoltos pelo estroma. 4b-d: Componentes prostáticos. L- lúmen; Epi- epitélio; nms- estroma não muscular; bv- vaso sanguíne; setas-estroma muscular; (4a, 100x; 4b, 400x; 4c, 400x e 4f, 400x).



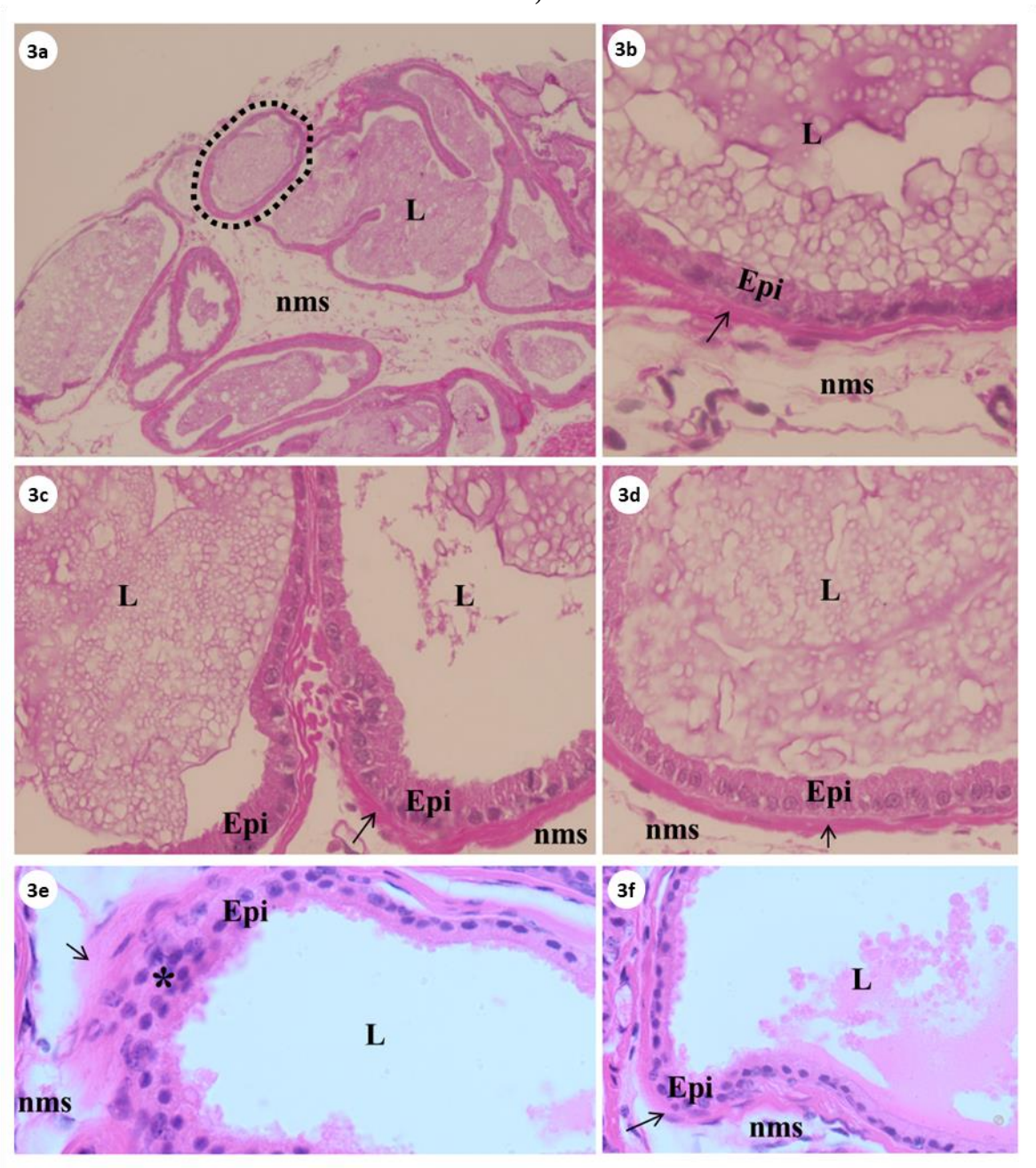
Source: Own authorship

Figure 2: Histological sections stained with HE from animals treated with Testosterone (TG). 2a: Overview of acini that are surrounded by stroma. 2b-f: Details showing the prostatic components. L, lumen; U, urethra; Epi, epithelium; nms, non-muscular stroma; bv, blood vessels; SV, seminal vesicle; * stratification points; arrow, muscular stroma. (2a- 40x; 2b- 400x; 2c- 400x; 2d- 400x e 2f- 400x).



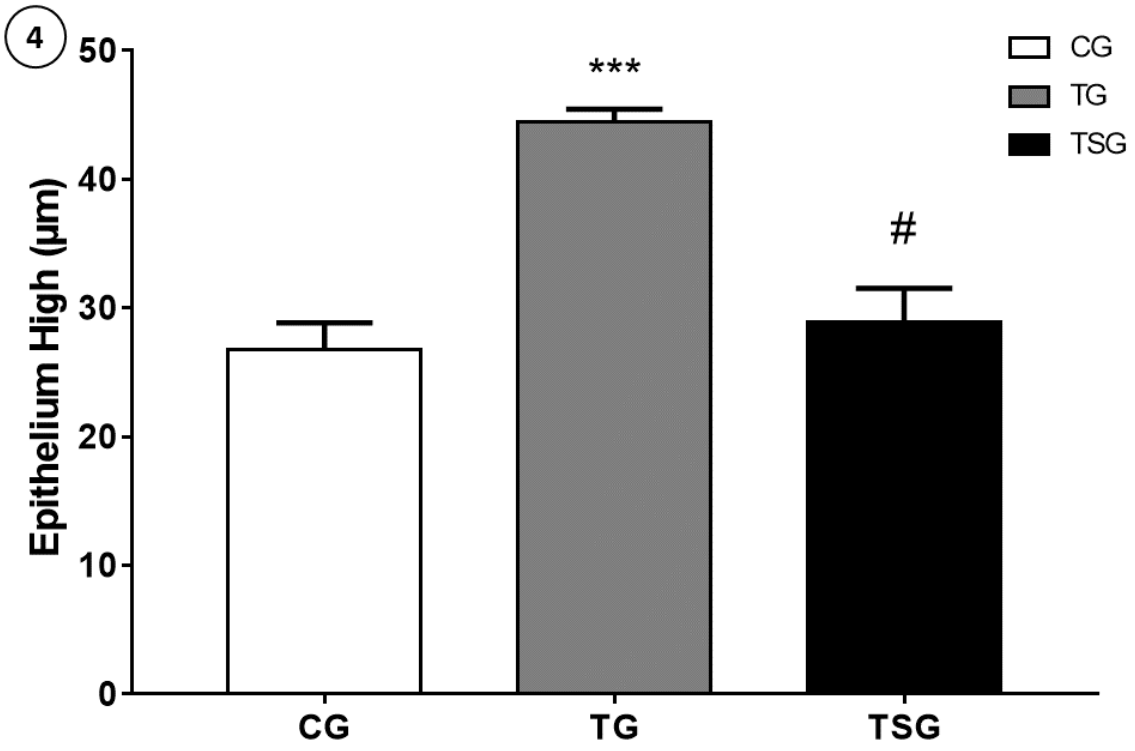
Source: Own authorship

Figure 3: Histological sections stained with HE from animals treated with Selenium and Testosterone (TSG). 3a: Overview of prostatic acini surrounded by stroma. 3b-f: Prostatic components. L, lumen; Epi, epithelium; nms- non-muscular stroma; * stratification focus; arrow, muscle stroma. (3a- 100x; 3b- 400x; 3c- 400x; 3d- 400x e 3f- 400x).



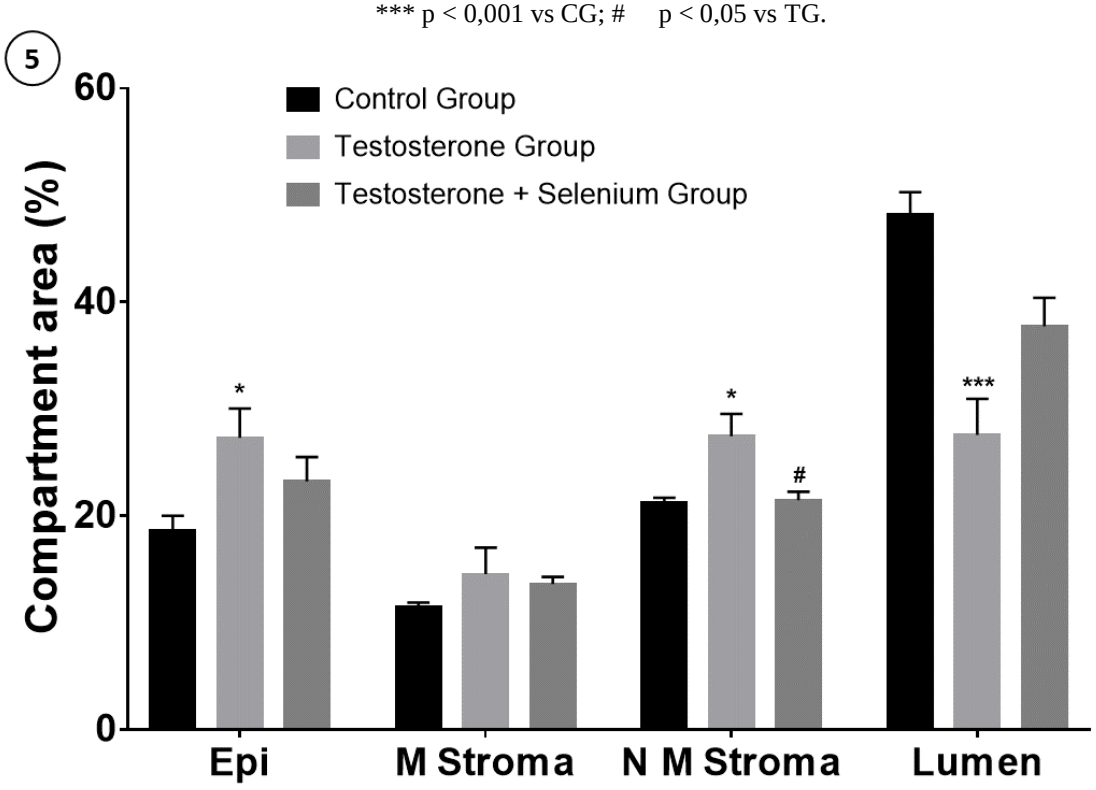
Source: Own authorship

Figure 4: Morphometric analysis on the epithelium of the different experimental groups. CG, Control Group; TG, Testosterone group; TSG, Testosterone and Selenium Group; *** p < 0,001 vs CG; # p < 0,01 vs TG.



Source: Own authorship

Figure 5: Prostatic compartments stereological analysis of the different experimental groups. * p < 0,05 vs CG; *** p < 0,001 vs CG; # p < 0,05 vs TG.



Source: Own authorship