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Effects of heat stress and long photoperiod on the prostate of rats

Hamid Reza Ghaffari¹, Javad Poursamimi²✉¹Department of Basic Sciences, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran²Department of Immunology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran

ABSTRACT

Objective: To examine light and heat effects on the morphological, histological, and micrometric structure of the prostate of rats.

Methods: Thirty adult male rats were divided into three groups. The control group was kept under 20°C–22°C and an artificial 12 h/12 h day/night cycle; the temperature group was under normal light and at (42±1)°C heat for 4 to 5 h daily, and the light group was exposed to 8 h/16 h day/night cycle with 20°C–22°C. Rats were weighed five times (at the beginning of the study and every seven days). Five milliliters (mL) of their peripheral blood were taken. The tissue staining was performed using the hematoxylin-eosin (H & E) stain and periodic acid–Schiff (PAS). In the following, tissue and cellular reactions to the PAS were examined.

Results: Folds were located entirely on the surface of the anterior lobe and periphery of the other lobes. The secretory units in the anterior lobe were more than the lateral lobe. A strong reaction of the secretory cells to the PAS was observed. Testosterone serum levels of the light group also significantly increased compared to the control group ($P<0.05$). The most histometric changes of the lobes were established in the lateral lobes. Heat stress resulted in a significant decrease in testosterone levels and transformed prostate tissue. The epithelium and parenchyma to scaffold ratio in the temperature group decreased.

Conclusions: Maximum and minimum changes in the ventral lobe happened under the ascent of temperature and light, respectively. The ventral lobe in the study of prostatic hyperplasia should be more considered.

KEYWORDS: Prostate; Heat stress; Photoperiod; Histology; Testosterone; Rat

1. Introduction

The prostate, which is the largest gland in the male reproductive system, is crucial for fertility and male reproductive physiology[1]. This gland is under the regulation of the pineal gland (part of the

thalamus) across the messages of nerve, light, and melatonin[2].

The pineal gland of animals generally functions as a neuroendocrine transducer, and the neuronal signals caused by environmental light are converted into endocrine messages[3]. The melatonin secreted from it increases in the dark and decreases in light conditions, leading to sleep-wake cycle patterns in the central nervous system[4,5]. Neurons with high sensitivity to light in the retina act as the reporter of light intensity to the hypothalamus. This is the reason the processed neuronal messages transferred to the pineal gland[6]. The pineal gland has also inhibitory effects on the reproductive glands (such as the prostate) and maturation. Moreover, its functional deficits is led to a decrease in the melatonin level and an incidence of untimely maturation[7]. The pineal gland in rodents regulates the gonadal secretion levels (the neuroendocrine-gonadal axis), so the removal of the pineal gland will highly develop the secretion of the follicle-stimulating hormone (FSH), luteinizing

Significance

Health encompasses not only the absence of disease but also complete physical, mental, and social well-being. It is important to consider the proper functioning of the reproductive system and the impact of environmental factors on overall health. The study explored the effects of temperature and light factors on prostate tissue in rats and revealed that balancing these factors can improve reproductive health indices.

✉To whom correspondence may be addressed. E-mail: javadpoursamimi@gmail.com; poursj1357@zbmu.ac.ir

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hormone (LH) and prolactin (PRL)[8,9]. The prostate gland is divided into 4 distinct lobes (the dorsal, ventral, and two laterals) biochemically and physiologically, which have different sensitivities to the androgenic hormones[10]. Androgenic hormones that indirectly influence the pineal gland, cause the growth of some aspects of the prostate, including the weight and the epithelial cell height of the lobes[9,11]. Moreover, they can stimulate proliferation and inhibit the apoptosis pathway in the lining epithelium of the prostatic ducts[12]. Some studies have manifested a rise in the secretion of androgenic hormones when rats are exposed to prolonged light (12 h) conditions[9]. Changes in exposure to light occur naturally. As the seasons replace, temperature and light also change, so the serum level of androgens and the activity rate of the prostate will change[13]. In addition to the effects of temperature and light on reproduction, it is manifested that photoperiod had more influence on reproduction and caused more changes in the serum levels of testosterone and dihydrotestosterone (a testosterone derivative)[14,15]. Dihydrotestosterone has the most stimulatory effect on the function of the prostate[16]. A temperature of 4 °C to 25 °C is normal for the majority of animals[17]. However, over 25 °C causes heat stress and eventually results in the loss of testicular weight and destructive effects on sperm morphology, motility, and life span[9,18]. Since the hypoxia effect and cell death in the rat's testicles have been studied previously[19] and it has been manifested that the rats do not reproduce seasonally and mate at every time, our attention was drawn to the prostate tissue changes in response to the temperature and light.

2. Materials and methods

2.1. Experimental design

Thirty healthy adult male rats of Wistar strain [weight (180±20) g, and age (3.5±0.4) months] were obtained from the animal house of Zabol University of Medical Sciences. After the seven days of acclimatization period (a healthy condition, a suitable cage with enough food, filtered tap water, and heat in the normal range 20 °C–22 °C), they were randomly divided into three groups (the control, temperature, and light groups, $n=10$ in each group) based on a lottery. There were two options for each group. Numbers (No.) 1 and 2 were assigned to the control groups, 3 and 4 for the temperature groups, and 5 and 6 for the light groups. In sum, the allocated rats in triple groups were the same. A person in charge of feeding the mice, gave them a certain amount of water and food daily. He did not know the identity of the groups.

The control group (20 °C–22 °C) was kept on an artificial day/night cycle (12 h/12 h) for four successive weeks. The temperature group was kept daily at (42±1) °C for 4 to 5 h and day/night cycle (12 h/12 h) for four weeks. The light group (20 °C–22 °C)

was exposed under a day/night cycle (16 h/8 h) for four weeks. Mice were weighed at the beginning of the study, and every seven days. In sum, they were weighed 5 times. The exclusion criteria of mice were loss of weight <160 g, and not eating or drinking food and water, respectively.

2.2. Sampling and histological analysis

Triple mice groups were assessed four times during 28 days. The study volume was set on base our previous study[20,21]. At the end of the period (28 days), rats were anesthetized with ketamine and xylazine. Peripheral blood (5 mL) was collected in tubes without anticoagulant and was centrifuged (10000× g for 10 min at 4 °C). Sera were stored at -20 °C until analysis (4th week). The enzyme-linked immunosorbent assay (ELISA), and histological analysis by the hematoxylin-eosin (H & E) assay[22] and periodic acid–Schiff (PAS)[23] were also performed for all samples obtained from the rats. In brief, 30 prostate tissues were separately identified by an anatomist. They were initially fixed in the buffered formalin (10%), buffered formalin, embedded in paraffin, and cut at 5–6 μ m thicknesses using a rotary microtome. For dehydration, clarification, and kerosene saturation were performed using the Histokinette Set Model 2000. The H & E staining was adequate for high-quality analysis nuclear and its details. Slides of 0.5 cm thickness in each lobe were obtained using the microtome and were placed in Bouin's fixative solution for 10 h and then examined using PAS staining. It was adequate for analyzing cytoplasm and its details (glycoproteins).

2.3. Quantitative histometric analysis

For analysis of the epithelium thickness, cell number, alveolar diameters, and parenchyma-to-scaffold ratio, the Dino-Lite digital microscope was used. Five fields of the objective microscope (Olympus) were inspected randomly using the magnification factor of 40 (×40), and a magnification of 100 (×100) was used for the examination of the alveolar cavity diameter.

2.4. Serum testosterone level

Serum testosterone levels of samples were measured using the ELISA kit (Cat. No. ab108666) from Abcam, Inc. Optical density (OD) was measured at a wavelength of 450 nm.

2.5. Statistical analysis

The data of experimental groups (control, temperature, and light) were obtained and analyzed using IBM SPSS Statistics version 26. To check the distribution of data (normal *vs.* non-normal), the Kolmogorov-Smirnov (K-S) test was used. In addition, parametric and nonparametric tests were applied for variables with normal and non-normal distribution, respectively. One-way ANOVA and Tukey's

post hoc tests were used to compare the mean between groups. The data were presented as mean $\pm$ SD. *P* value <0.05 was considered significant.

2.6. Ethics statement

The study was approved by the Vice-Chancellor's Office for Research at Zabol University of Medical Sciences and achieved the code: IR.ZBMU.REC.1399.137, through the university's research committee on date: 2021-02-08. All experiments also were approved by the Institutional Animal Ethics and Care Committee of the Faculty of Medicine, Zabol University of Medical Sciences.

3. Results

3.1. Macroscopic findings and body weight variations

Four lobes (dorsal, ventral, anterior, and lateral) were identified and confirmed. The ventral lobe was the largest. The lateral lobe also was composed of sub-sections 1 and 2. The mean body weight of rats in all groups did not show any change compared to the beginning of the study, except in the light group [(204.0 $\pm$ 1.7) g *vs.* (198.0 $\pm$ 1.3) g], which nonsignificantly decreased (*P*>0.05) (Table 1). However, statistical examination of the data using paired-samples *t*-test discovered a significant difference between the temperature group and the control group (*P*=0.023).

3.2. Histologic observations

3.2.1. Macroscopic view of prostate gland

The macroscopic results showed that this gland consists of 4 anterior, ventral, dorsal, and lateral lobes, and the lateral lobe includes two types 1 and 2 lateral lobes. Transverse section from the dorsal lobe of the rat prostate gland to the anterior part of the bladder was showed (Figure 1). The location of the type 1 lateral lobe and dorsal lobe has been shown. The seminal vesicles and ducts, anterior lobe ducts, vas deferens, and ampulla region are seen.

3.2.2. Ventral lobe

The cellular connective tissue in the center region of the ventral lobe in the control group was more (Figure 2A and 2B), and the parenchyma also was more extended than the scaffold. The folded secretory units were observed in the peripheral area, and the tubular secretory units in the central area (Figure 2A). A row of myoepithelial cells was observed on top of the alveolar basement membrane (Figure 2B). The scaffold area changes in the temperature group were apparently more than control, and light groups (Figure 2C). The folded units in the light group also were remarkable, and its secretory ducts also appeared denser than the control and temperature groups.

Table 1. Body weight (g) of rats in the study.

Days	Control group	Temperature group	Light group
Day 7	205.0 $\pm$ 1.1	203.0 $\pm$ 1.6 ^a	204.0 $\pm$ 1.7
Day 14	203.0 $\pm$ 1.4	202.0 $\pm$ 1.4 ^a	201.0 $\pm$ 1.5
Day 21	206.0 $\pm$ 1.6	200.0 $\pm$ 1.7 ^a	199.0 $\pm$ 1.2
Day 28	207.0 $\pm$ 1.5	203.0 $\pm$ 1.6 ^a	198.0 $\pm$ 1.3

Data are expressed as mean $\pm$ SD. ^a*P*<0.05: compared to the control group.

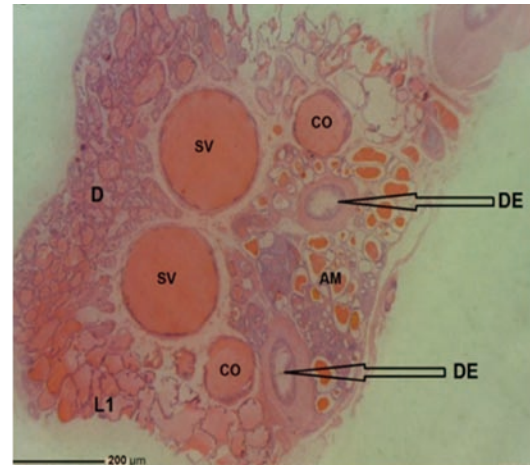


Figure 1. Transverse section from the dorsal lobe of the rat prostate gland to the anterior part of the bladder. The position of lateral lobe 1 (L1) and dorsal lobe relative to each other, as well as seminal ducts of vesicles, ducts of anterior lobes, vas deferens and ampullary gland are shown ($\times 40$). Ampullary gland (AM), anterior lobe ducts (CO), dorsal lobe (D), vas deferens (DE), and seminal vesicle ducts (SV) are seen.

3.2.3. Dorsal lobe

A filamentous structure with a layer of smooth muscle surrounding the ducts in the control group was observed (Figure 2D). The alveolar secretory units were more than the tubular units, and their cytoplasm was more acidophilus than the ventral lobe. However, they were extended throughout the dorsal lobe (Figure 2D, 2E, 2F). Analysis of these findings did not show significant differences in the temperature group compared with the control group (*P*>0.05). In addition, the light group did not have any significant difference with the control and temperature groups (*P*>0.05).

3.2.4. Anterior lobe

Alveolar and secretory units were more than the tubular units. The secretory units were located on the periphery of the gland (Figure 2G and 2H). The epithelial folds in the control group had extended from the secretory units to the beginning of the tubular units (Figure 2G). Several smooth muscle layers surrounded the tubular secretory units (Figures 2H). The cell number of the scaffold remarkably was low. The nucleus of secretory cells usually was located in the cell center. The anterior lobe was different in terms of foamy secretory cells and apical acidophilic cells from others. The tubular units also contained a deep cavity with linear folds and eosinophilic secretions. The intensity of folds of the secretory units in the temperature group

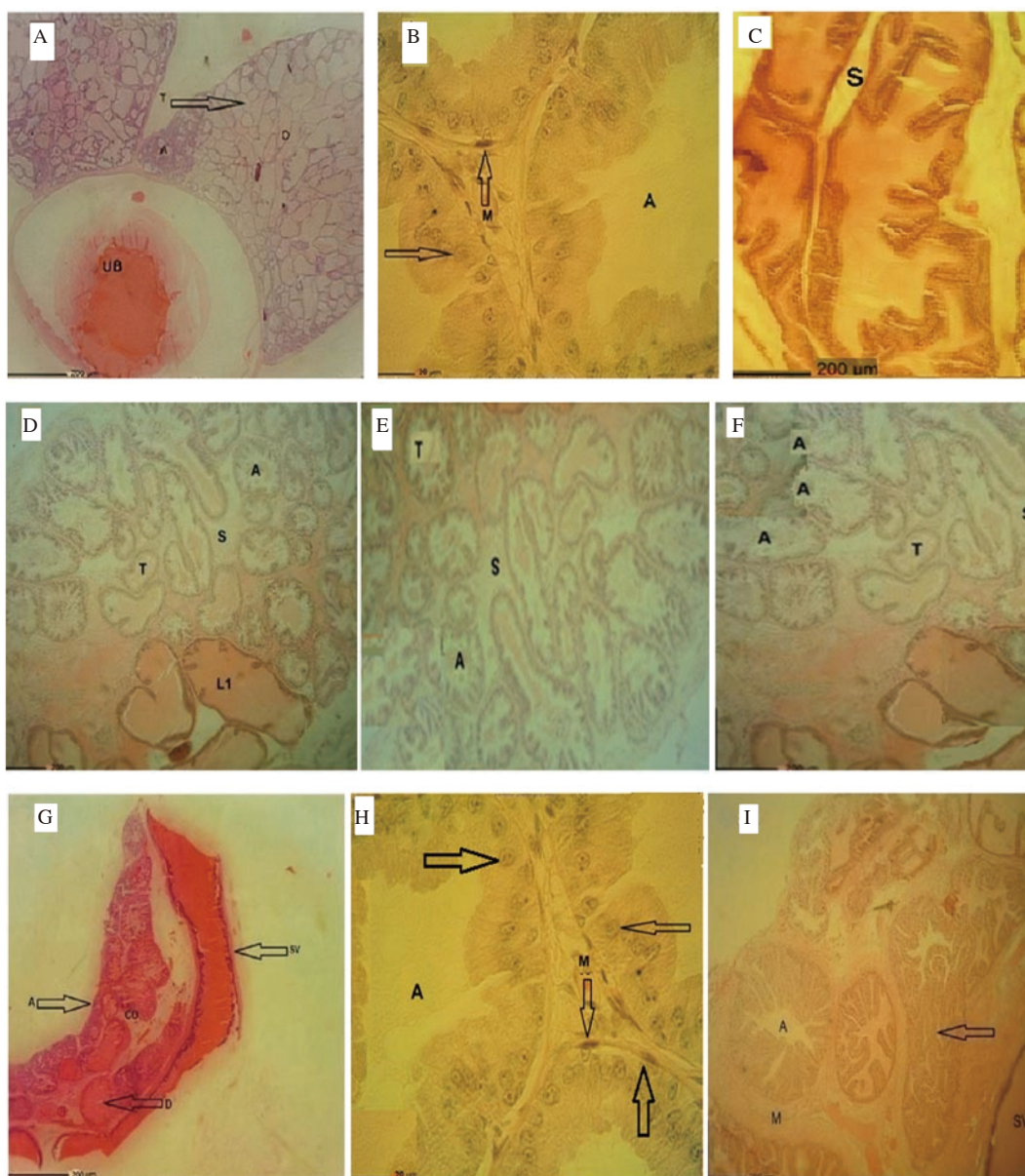


Figure 2. A–C: Transverse section of the prostate ventral lobe of rats in different groups. A: In the control group ($\times 4$, H & E), the lobe location relative to the bladder is shown (arrow). Dispersion of secretory tubules is seen. Alveolar secretory units (A), the urinary bladder (UB), tubular secretory unit (T), secretory tubule (D). B: In the light group ($\times 40$), myoepithelial cells (M) (arrow) have emerged in the wall of the alveolar secretory units (A). C: In the temperature group ($\times 40$), an increase in scaffolding (S) between secretory units and tubules is seen. D–F: Transverse section of the prostate dorsal lobe of rats in different groups. D: In the control group ($\times 40$, H & E), the secretory tubule distribution and scaffold (S) and the location of the lobe relative to the lateral 1 (L1) lobe are shown. Alveolar secretory unit (A), tubular secretory unit (T). E: In the light group ($\times 40$), alveolar secretory units have emerged (A). F: In the temperature group ($\times 40$), an increase in folded alveolar secretory units (A) is seen. G–I: Transverse section of the prostate anterior lobe of rats in different groups. G: In the control group ($\times 40$, H & E), folding of the majority of the alveolar secretory units is clear (arrows). Alveolar secretory units (A), muscle cells (M), seminal vesicles (SV), anterior lobe (CO), large folded secretory ducts (D). H: In the light group ($\times 40$), the folded units (A) are located in the periphery (opposite pole of the seminal vesicle). The decrease in the height of secretory cell and the number of myoepithelial cells (M) is observed (arrows). I: In the temperature group ($\times 40$), an increase in folded alveolar secretory units (A) is seen (arrows).

was lower than in the light group (Figure 2I). These findings in the light group had more changes than the other groups (Figure 2H).

3.2.5. Lateral lobes

The most incremental approach was observed in the scaffold of the temperature group, which was higher than the control and light groups (Figures 3).

3.2.6. PAS staining

The most and least color intensity were seen in anterior (Figure 4A, B, C), and the type 1 and type 2 lateral lobes (Figure 4D and E and F) and the ventral lobe (Figure 4G), respectively. Moreover, high-intensity PAS staining was observed in the tubular secretory units. Secretory cells in the light groups, which were under the photoperiod cycle, were PAS-positive.

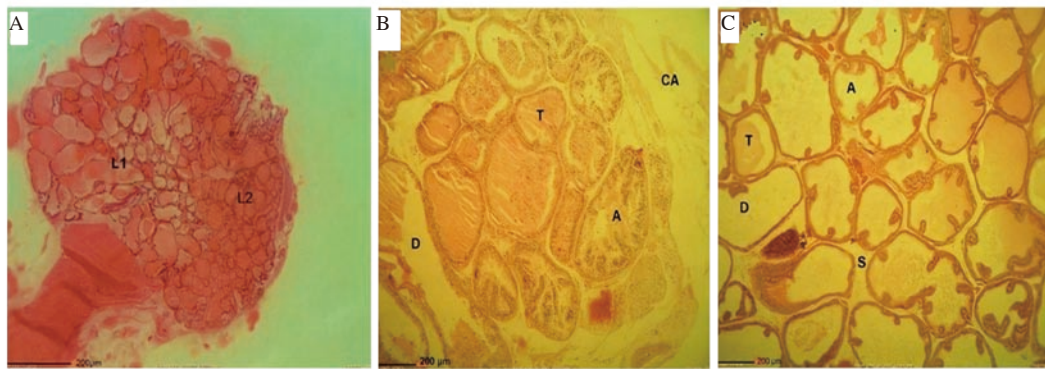


Figure 3. Histologic observations of the prostate lateral lobes of rats in different groups. A: Transverse longitudinal sections in the prostate lateral lobes 1 and 2 in the control group ($\times 40$, H & E). Note the enlarged and folded excretory tubules in the secretory units of lateral lobe 1 (L1) and the filling of the secretory excretory tubules with secretory material in lateral lobe 2 (L2). B: Longitudinal section of the prostate lateral lobe type 2 in the light group. The excretory tubules are not folded and the secretory units are more folded compared to lateral type 1. Alveolar secretory units (A), capsule (CA), tubular secretory units (T), secretory ducts (D). C: Longitudinal section of the prostate lateral lobe type 1 in temperature group ($\times 40$, H & E). Note the presence of folds in the walls of tubules and secretory units and the intensity of staining of the majority of the secretory cells of the lateral lobe type 1. Alveolar secretory units (A), tubular secretory units (T), secretory ducts (D), loose scaffold connective tissue (S).

3.3. Histometric analysis

3.3.1. Ventral lobe

The epithelial thickness of secretory units in the light group nonsignificantly increased compared to the control group and the temperature group ($P > 0.05$). The alveolar diameter of the secretory units in the temperature group slightly increased compared to the control group ($P > 0.05$) but significantly increased compared to the light group ($P < 0.05$). The cavity diameter of the secretory units in the temperature group decreased compared to the control group and the light group ($P > 0.05$).

The count of cylindrical cells in 50 μm length of epithelial tissue of secretory units in the temperature and light groups increased compared to the control group ($P > 0.05$).

The percentage of the ratio (parenchyma to scaffold) in the temperature group was significantly lower than the control group ($P < 0.05$). However, the light group was slightly lower than the control group ($P > 0.05$) (Table 2).

3.3.2. Dorsal lobe

The epithelial thickness of secretory units in the light and temperature groups increased compared to the control group ($P > 0.05$). The diameter of alveoli of secretory units in the temperature and light groups decreased compared to the control group ($P > 0.05$). The cavities diameter of the tubular secretory units in the light and temperature groups increased compared to the control group ($P > 0.05$). The cylindrical cell count in 50 μm length of epithelial tissue in the temperature group significantly increased compared to the control group ($P < 0.05$). Moreover, the light group significantly decreased compared to the control group ($P < 0.05$). The percentage of the ratio (parenchyma to scaffold) in the temperature and light groups was significantly lower than the control group ($P < 0.05$) (Table 2).

3.3.3. Anterior lobe

The epithelial thickness of secretory units in the light group increased compared to the control group. However, the temperature groups significantly decreased compared to the control group ($P < 0.05$). The diameter of alveoli of the secretory units in the temperature and light groups increased compared to the control group ($P > 0.05$). The cavity diameter of the secretory units in the light and temperature groups significantly increased compared to the control group ($P < 0.05$). The light group also significantly increased compared to the temperature group ($P < 0.05$). The cylindrical cell count in 50 μm length of epithelial tissue in the light and temperature groups increased compared to the control group ($P > 0.05$). The percentage of the ratio (parenchyma to scaffold) in the temperature and light groups was lower than the control group ($P > 0.05$) (Table 3).

3.3.4. The type 1 lateral lobe

The epithelial thickness of the light group significantly increased compared to the control group ($P < 0.05$). However, the temperature group significantly decreased compared to the control group ($P < 0.05$). The diameter of alveoli of the secretory units in the temperature group significantly increased compared to the light and control groups ($P < 0.05$). The cavity diameter of the secretory units of the light group significantly increased compared to the temperature and control groups ($P < 0.05$). The cylindrical cell count of the temperature group increased compared to the control and light groups ($P > 0.05$). The percentage of the ratio (parenchyma to scaffold) in the temperature group was lower than the control and light groups ($P > 0.05$) (Table 3).

3.3.5. The type 2 lateral lobe

The epithelial thickness in the temperature and light groups decreased compared to the control group ($P > 0.05$). The diameter of

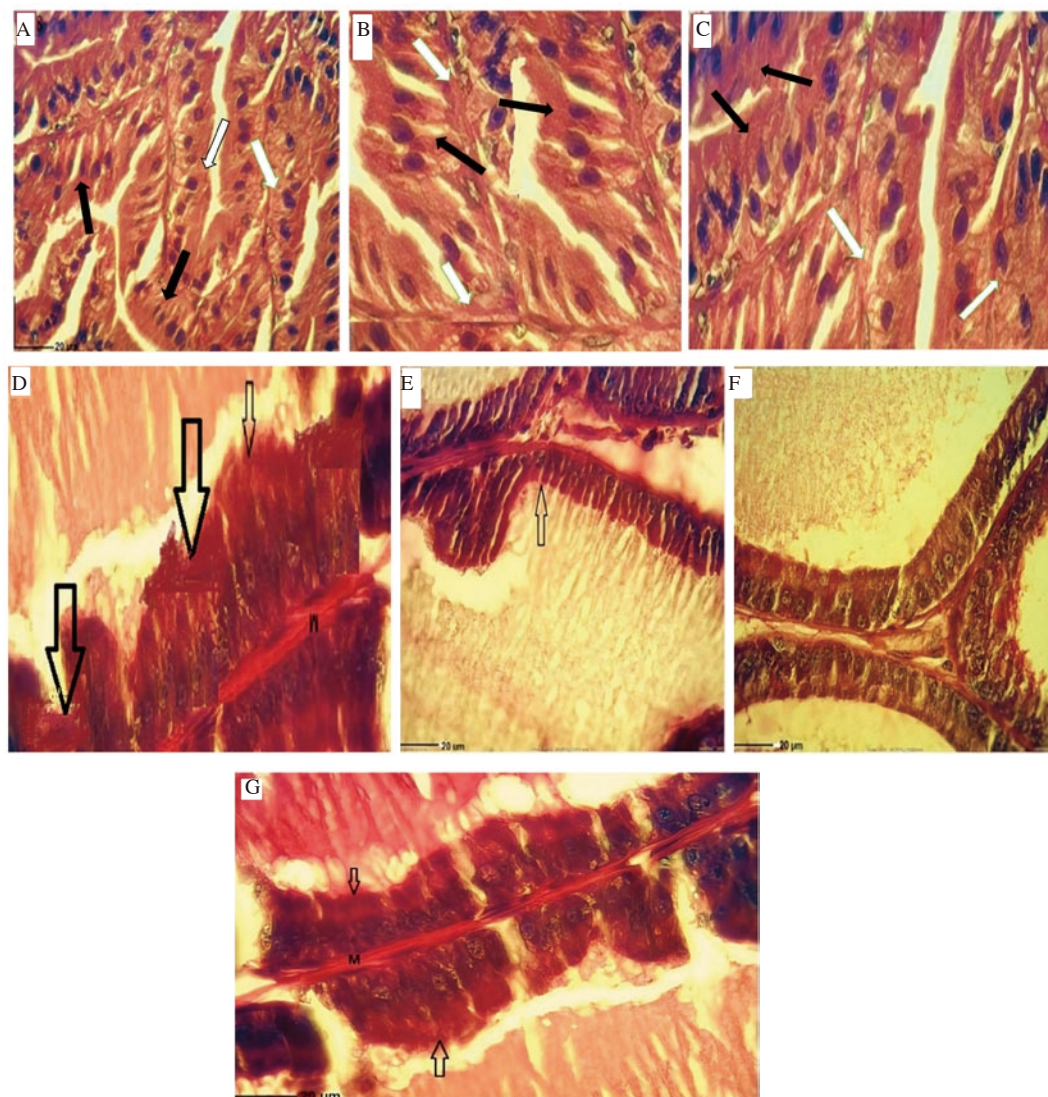


Figure 4. A–C: The prostate anterior lobe of rats in different groups. A. Cross-section of the prostate anterior lobe in the control group ($\times 40$). The PAS-positive of the apical cytoplasm of the secretory cells is significant (arrows). Dark arrows indicate the apical cytoplasm of the secretory cells, while white arrows indicate myoepithelial cells. B. Longitudinal section of the prostate anterior lobe in the light group. Similar to the previous image, the dark arrows indicate the apical cytoplasm of the secretory cells, while the white arrows indicate myoepithelial cells. C. Longitudinal section of the prostate anterior lobe in temperature group ($\times 40$). In this image, the dark arrows indicate the secretory cells, while the white arrows indicate myoepithelial cells. **D–F:** The prostate lateral lobe of rats in different groups. D. Cross-section of the prostate lateral lobe in the control group ($\times 40$). The PAS-positive of the apical cytoplasm of the secretory cells is significant (arrows), and the myoepithelial cells (M) between the secretory units are also PAS positive. E. Transverse section of the prostate lateral lobe type 1 in the light group ($\times 40$), the apical cytoplasm of the secretory cells is seen (arrow). F. Transverse section of the prostate lateral lobe type 1 in the temperature group ($\times 40$). **G:** Cross-section of the prostate ventral lobe in the control group ($\times 40$). The PAS-positive of the apical cytoplasm of the secretory cells is significant (arrows), and the myoepithelial cells (M) between the secretory units are also PAS positive. However, the data of the light and temperature groups are not significantly different compared to the control group which is not shown.

alveoli of the secretory units in the temperature group significantly increased compared to the light and control group ($P < 0.05$). The cavity diameter of the secretory units in the temperature group decreased compared to the control group ($P > 0.05$) and significantly decreased compared to the light group ($P < 0.05$). The cylindrical

cell count in the temperature group increased compared to the control group ($P > 0.05$) and significantly increased compared to the light group ($P < 0.05$). The percentage of the ratio (parenchyma to scaffold) in the temperature group was significantly lower than the control and light groups ($P < 0.05$) (Table 3).

Table 2. Histometric analysis of ventral and dorsal lobes of rats.

Parameters	Control group	Temperature group	Light group
The epithelial thickness, μm			
Ventral lobe	24.00 $\pm$ 1.07	23.00 $\pm$ 3.20	26.00 $\pm$ 2.97
Dorsal lobe	18.00 $\pm$ 2.40	20.00 $\pm$ 3.71	21.00 $\pm$ 3.52
The alveolar diameter, μm			
Ventral lobe	351.00 $\pm$ 42.20	404.00 $\pm$ 29.86 ^b	310.00 $\pm$ 35.93
Dorsal lobe	296.00 $\pm$ 59.03	247.00 $\pm$ 60.22	252.00 $\pm$ 23.71
The cavity diameter, μm			
Ventral lobe	298.00 $\pm$ 60.80	236.00 $\pm$ 59.47	299.00 $\pm$ 61.86
Dorsal lobe	175.00 $\pm$ 71.21	184.00 $\pm$ 78.02	195.00 $\pm$ 14.49
The count of cylindrical cells in 50 μm length of epithelial tissue, n			
Ventral lobe	9.00 $\pm$ 1.30	10.00 $\pm$ 0.83	10.00 $\pm$ 1.22
Dorsal lobe	9.00 $\pm$ 1.64	10.00 $\pm$ 1.30 ^a	8.00 $\pm$ 0.54 ^a
The percentage of the ratio (parenchyma to scaffold), %			
Ventral lobe	79.00 $\pm$ 1.92	68.00 $\pm$ 6.85 ^a	75.00 $\pm$ 1.86
Dorsal lobe	72.00 $\pm$ 2.80	59.00 $\pm$ 9.50 ^a	55.00 $\pm$ 7.61 ^a

Data are expressed as mean $\pm$ SD. a: Compared to the control group, $P<0.05$; b: Compared to the light group, $P<0.05$.

Table 3. Histometric analysis of anterior, type 1 and 2 lateral lobes of rats.

Parameters	Control group	Temperature group	Light group
The epithelial thickness, μm			
Anterior lobe	22.00 $\pm$ 2.56	18.00 $\pm$ 2.35 ^a	24.00 $\pm$ 2.43
Type 1 lateral lobe	19.00 $\pm$ 1.26	16.00 $\pm$ 1.26 ^a	21.00 $\pm$ 1.43 ^a
Type 2 lateral lobe	21.00 $\pm$ 2.30	17.00 $\pm$ 2.37	17.00 $\pm$ 1.59
The alveolar diameter, μm			
Anterior lobe	387.00 $\pm$ 34.01	522.00 $\pm$ 109.10	696.00 $\pm$ 82.45
Type 1 lateral lobe	380.00 $\pm$ 37.82	417.00 $\pm$ 92.91 ^{ab}	300.00 $\pm$ 22.65
Type 2 lateral lobe	212.00 $\pm$ 79.18	444.00 $\pm$ 57.95 ^{ab}	290.00 $\pm$ 44.55
The cavity diameter, μm			
Anterior lobe	202.00 $\pm$ 40.00	281.00 $\pm$ 44.46 ^a	405.00 $\pm$ 79.38 ^{ac}
Type 1 lateral lobe	280.00 $\pm$ 68.92	250.00 $\pm$ 23.73	525.00 $\pm$ 156.70 ^{ac}
Type 2 lateral lobe	211.00 $\pm$ 46.06	166.00 $\pm$ 37.41 ^b	247.00 $\pm$ 53.28
The count of cylindrical cells in 50 μm length of epithelial tissue, n			
Anterior lobe	8.00 $\pm$ 1.58	9.00 $\pm$ 1.92	9.00 $\pm$ 1.41
Type 1 lateral lobe	8.00 $\pm$ 0.83	9.00 $\pm$ 0.54	8.00 $\pm$ 0.79
Type 2 lateral lobe	8.00 $\pm$ 1.00	9.00 $\pm$ 0.83 ^b	8.00 $\pm$ 0.23
The percentage of the ratio (parenchyma to scaffold), %			
Anterior lobe	62.00 $\pm$ 5.11	57.00 $\pm$ 9.50	58.00 $\pm$ 3.87
Type 1 lateral lobe	69.00 $\pm$ 12.65	56.00 $\pm$ 6.68	68.00 $\pm$ 8.41
Type 2 lateral lobe	79.00 $\pm$ 8.22	44.00 $\pm$ 8.03 ^{ab}	61.00 $\pm$ 8.34

Data are expressed as mean $\pm$ SD. a: Compared to the control group, $P<0.05$; b: Compared to the light group, $P<0.05$; c: Compared to the temperature group, $P<0.05$.

Table 4. The mean percentage of folded units in all of lobes of rats.

The mean percentage of folded units, %	Control group	Temperature group	Light group
In the ventral lobe	49.30 $\pm$ 8.27	34.20 $\pm$ 1.64	74.81 $\pm$ 1.90 ^{ac}
In the dorsal lobe	53.20 $\pm$ 2.94	45.40 $\pm$ 2.70	72.80 $\pm$ 1.48 ^{ac}
In the type 1 lateral lobe	76.20 $\pm$ 2.38	55.60 $\pm$ 2.70	88.20 $\pm$ 1.30 ^{ac}
In the type 2 lateral lobe	61.40 $\pm$ 2.30	32.20 $\pm$ 2.38	70.80 $\pm$ 1.92 ^{ac}

Data are expressed as mean $\pm$ SD. a: Compared to the control group, $P<0.05$; c: Compared to the temperature group, $P<0.05$.

3.3.6. The mean percentage of the folded units in the ventral, dorsal lobes and lateral lobes

In ventral lobe, the percentage of folded units of the light group was significantly higher than the temperature and control groups ($P<0.05$). In dorsal lobe, the percentage of folded units of the light group also was significantly higher than the temperature and control

groups ($P<0.05$) (Table 4).

In type 1 lateral lobe, the percentage of folded units in the light group was significantly higher than the temperature and control groups ($P<0.05$). In type 2 lateral lobe, the percentage of folded units in the light group was significantly higher than the temperature and control groups ($P<0.05$) (Table 4).

3.3.7. Serum testosterone levels

The serum testosterone levels in the light group [(4.94±1.01) pg/mL] significantly increased compared to the control group [(2.70±0.91) pg/mL] ($P<0.05$). In addition, the temperature group [(1.08±0.52) pg/mL] significantly decreased compared to the control group ($P<0.05$).

4. Discussion

Considering the macroscopic findings of this study uncovered 4 lobes (dorsal, ventral, anterior, and laterals) for the rat's prostate which the largest of that was the ventral lobe. Moreover, the lateral lobe was subdivided into types 1 and 2. The mean body weight of mice in the light group decreased. Fink *et al*, in accordance with our findings, reported that the mean weight of the animals (brush-tailed possum) did not change under light exposure[24]. The rat's prostate previously was subdivided into only a dorsal lobe and two ventral lobes by Hernandez *et al*[25], and two lobes by Jesik *et al*[26] and Price[27], and 4 lobes by Hayashi[28]. However, our findings manifested that the rat's prostate is composed of an anterior, a dorsal, a ventral, and two lateral lobes, which mainly contain the scaffold proteins and parenchyma. Hayashi *et al* reported that the anterior lobe contains the largest tubular secretory units[28]. An animal study by Hernandez *et al* also manifested that the secretory units of the parenchyma only consist of the acinar structure[25]. The nucleus of secretory cells is often located in the center of alveolar secretory cells of the ventral, dorsal, and type 2 lateral lobes. This finding was confirmed by Brandes and Jesik *et al* for the ventral and lateral lobes[29,26]. Our study manifested that the rat's prostate commonly contains a highly thick epithelium wall and folds in the alveolar wall like a hyperplasia. Folds were located entirely on the surface of the anterior lobe and periphery of the other lobes. Moradi *et al*, in accordance with our findings, expressed that the height, number, and folding of the secretory cells and wall of the prostate alveolar lobe is a sign of its high secretion and function[23]. Although the scientific report by Jesik *et al* revealed a high intensity of acidophilic stain of the dorsal lobe compared to the others[26], our study uncovered that a high intensity of acidophilic stain is in the type 1 lateral lobe compared to the other sections. In addition, the anterior lobe contained more secretory units than the lateral lobe which was a sign of its high function. On the opposite of this, Hayashi *et al* identified the anterior lobe as a non-active lobe in terms of secretion[28]. Our findings manifested this lobe is very active and contains a large excretory tubule. According to our study, rats' testosterone serum levels of the light group were higher than the control group. Carballada *et al* in the same direction reported that hamsters' testosterone serum levels are altered by photoperiod[15]. Therefore,

some of our findings include the growth of the prostate and its function, the epithelial thickness, the parenchyma-to-scaffold ratio, and low count of the cylindrical cell, likely related to the testosterone serum level, is regulated by photoperiod cycle. Concerning this, Banerjee *et al* also detected further changes in the ventral lobe than the dorsal lobe after testicular secession (the testosterone cutoff)[30]. Carballada's findings revealed that the dorsal lobe of the hamster prostate was more reactive than the ventral lobe under short-term photoperiod (16 h/8 h day/night cycle)[15]. The folds' expansion in the alveolar secretory units was considered a sign of further changes that caused it to differentiate from the dorsal lobe. In general, there are morphological and histological differences in the prostate lobes. However, the results of this study uncovered some differences in the photoperiodic reactions of the lobes and histometric changes in the type 1 and 2 lateral lobes. The high sensitivity of the prostate lateral lobe to the androgens (*e.g.*, prolactin) was uncovered by Wylot *et al*. Their previous study also revealed that the serum levels of prolactin in rats depend on the length of the day, so that the maximum of it was between day/night and night/day[31]. It may be why the alveolar area of the rat's prostate and the height of epithelial cells were high when they were treated with prolactin[25]. Some studies by Nevalainen *et al* also indicated high expression of prolactin receptors on the prostate membrane in the lateral, ventral, and dorsal lobes[32]. Liu *et al* also elucidated that prolactin increases the cellular changes in the lateral lobe and zinc (Zn) concentration in their mitochondria[33]. The linkage of the androgen hormones to their specific receptors and an increase in androgen secretion of the prostate epithelial cells also is indicated by Słuczanowska-Głabowska *et al*[34].

Our findings showed that the diameter of the tubular secretory units in the light group was much bigger than the other groups. For verification of this finding, there was the Fink *et al*'s finding that showed, if the diameter of the cavity increases their activity will also increase[24]. However, a short photoperiod will also activate apoptosis pathway[15]. In our study, the photoperiod without the induction of hyperplasia, improved rats' physiological function. Contrary to the hamster, the changes caused by photoperiod in the ventral and dorsal lobes of the rat's prostate were different compared to others. The lateral lobe was more sensitive to environmental factors and androgens.

Jara *et al* also revealed apoptosis after raising the temperature of the rat epididymis and the ventral lobe of the prostate[35]. Nakanoma *et al* also showed sensitivity to heat stress in prostate cancer cells. They reported that an increase to 43 °C triggers the apoptosis pathway[36]. In our study, heat stress resulted in a remarkable decrease in serum testosterone levels and their corresponding changes in prostate tissue. According to histometric analysis, the epithelial thickness and parenchyma-to-scaffold ratio were lower in the temperature

group compared to the other groups. Furthermore, the mean count of cylindrical cells in the secretory epithelial tissue was higher than in other groups. These findings elucidated a decrease in prostate function in the physiological range.

There are some limitations. Our study was an animal study of the effects of heat stress and long photoperiod on the prostate. To create hyperthermia only in the genital area of rats, without or less effect on the other parts of their body, was our limitation. In addition, having a free-sex relationship in male rats with females was another. However, these differences did not affect the study's findings.

In conclusion, in this study, it was found that the ventral lobe of the rat's prostate had the most changes in high temperature and low light. Secretory cells also were the most reactions to PAS staining in the light group and the least in the temperature group. Testosterone serum levels also significantly increased in the light group compared to the temperature group.

It is suggested that the ventral lobe should be considered in the study of prostatic hyperplasia. In addition, the apoptosis rate in the epithelium of the anterior lobe should be checked after light or temperature exposure.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Data availability statement

The data of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Hamid Reza Ghaffari and Javad Poursamimi contributed to project administration, study conceptualization and methodology. Javad Poursamimi performed the review, and editing of manuscript and analysis. Hamid Reza Ghaffari and Javad Poursamimi performed the experiment. Javad Poursamimi performed original manuscript writing, and submitted the data for analysis.

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