The photoperiod and heat stress effects on histometrical structure of rat prostate gland

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Key words:
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Abstract:

BACKGROUND: There is not enough information about the effects of heat stress and photoperiod on different lobes structure. **OBJECTIVES:** The present study aims at determining the histological changes and the rate of changes in each lobes of rat prostate, affected by photoperiod changes and heat stress. **METHODS:** To this end, 15 adult male wistar rats were divided to three groups: 1. the control group in which the rats were kept in 12L: 12D and 25°C temperature condition, 2. the heat stress group in which the rats were kept in 12L: 12D and 42+1 °C temperature condition for 4 to 5 hours per day, and 3. the photoperiod group in which the rats were kept in 16L: 8D and 25°C temperature condition. After 30 days, samples were taken from different lobes and sections with 5 to 6μ thickness were made and stained by H&E and PAS. **RESULTS:** The microscopic results showed that histomorphometrical structure and histochemical reactions of the different lobs of normal prostate of the rats are different. The proportion of parenchyma to stroma decreased by heat stress; however, it increased by photoperiod. The maximum changes were seen in ventral lobe. The epithelial thickness, lumen diameter, and number of secretory units also increased by photoperiod (16L:8D), but it decreased by heat stress. The number of secretory cells were increased by heat stress because the cell size decreased; however, they decreased by long photoperiod regime. The number of folded secretory units increased by photoperiod, while heat stress has an adverse effect ($p<0.001$). The serum testosterone increased by long photoperiod and decreased by heat stress ($p<0.01$). **CONCLUSIONS:** This study shows that long photoperiod has important effects on increasing the rat prostate parenchyma and its activity.

Introduction

The prostate gland is the largest accessory sex gland in mammals. This gland surrounds the urinary bladder neck and urethra opening. Its secretion is important for sperm fertility (Guyton and Hall, 2010). Thomson (2001) reported that the earliest signs of prostate formation were observed in 17 or 18 days and approximately 9 to 10 weeks of embryonic development in mice, rats, and human, respectively. Androgens are essential factors for the survival of prostate epithelial cells. Although there are other androgen formation in ducts, channels, and having to be involved in the differentiation of epithelial tissue, the main androgen is testosterone (Donjacour and Cunha, 1998).

In the male, there is a period of growth in which the prostate is fully functional and it will be alternate with a period of regression in which the prostate

parenchyma is changed. During this regression period, a dramatic decreasing will occur in the weight and function of the prostate. These changes are regulated by environmental cues; the major ones are photoperiod and heat stress (Bronson and Heideman, 1994; Bronson, 1985). It has been shown that the photoperiod length has a major effect on the morphology and function of the mature testes of male hamsters (Breckon and Cawood, 1985; Darrow et al., 1980) and juvenile ones (Gunduz and Stetson, 1994). Moreover, the epididymis luminal diameter decreases in a short-day light regime. The aim of this study was to identify some details of the changes induced by long-day light regime (16:8h. light: dark) and induced heat stress on the different lobes of rat prostate.

Materials and Methods

Animals were prepared from laboratory animal center of Jondy Shapour university of medical sciences of Ahwaz. For this study, 15 adult male wistar rats were divided into three groups (5 rats in each group): 1. The control group (G1) in which the rats were kept in 12L: 12D and 25°C temperature condition, 2. The heat stress group (G3) in which the rats were kept in 12L:12D and 42 +1°C temperature condition for 4 to 5 hours per day, 3. The photoperiod group (G2) in which the rats were kept in 16L:8D and 25°C temperature condition. The rats were fed with standard diet. After 30 days, the rats were easy drawing with chloroform, and blood samples were taken from heart; in addition, then the level of testosterone was measured by the Elisa (Power work Biotek X52). Abdominal cavity was explored and samples were taken from different lobes of rat's prostate gland. Sections with 5 to 6µ thickness were made by paraffin embedding method and were stained by H&E and PAS (Bancroft and Gamble, 2003). The PAS staining was used to show the glycoprotein secretion in each lobe. The histomorphotrical studies were done using digital Dino-Lite lens and Dino-capture1 software. Secretory cells were counted in 50 micrometer length of the alveolus wall in magnification of 40.

Statistical Analyses: Data are expressed as mean ± standard variation. One-way analysis of variance (ANOVA) was performed on the data. Differences between groups were considered to be significant at $p< 0.05$.

Table 1. Epithelial thickness of secretory units in different lobes of rat prostate (µm). Letters in superscript means significant difference ($p<0.05$).

Groups / Lobes	Control (G1)	Photoperiod (G2)	Heat stress (G3)
Ventral	24±1.07	26±2.97	23±3.2
Dorsal	18±2.40	21±3.52	20±3.71
Anterior	22±2.56	24±2/43 ^{C}	18±2.35 ^{B}
Lateral 1	19±1.26	21±1.34	16±1.26 ^{AB}
Lateral 2	21±2.30	17±1.59	17±2.37

Table 2. Lumen diameter of secretory units in different rat prostate lobes (µm).

Groups / Lobes	Control (G1)	Photoperiod (G2)	Heat stress (G3)
Ventral	298±60.80	299±61.86	226±59.47
Dorsal	175±71.21	195±14.49	184±78.02
Anterior	202±40	405±79.38 ^{AC}	281±44.46
Lateral 1	280±68.92	525±156.7 ^{AC}	250±24.73
Lateral 2	211±46.06	247±53.28 ^{C}	166±37.41 ^{B}

Table 3. The number of secretory cells in 50 µ length of secretary unit wall in different rat prostate lobes. Letters in superscript means significant difference ($p<0.05$).

Groups / Lobes	Control (G1)	Photoperiod (G2)	Heat stress (G3)
Ventral	9±1.30	10±1.22	10±0.83
Dorsal	9±1.4 B ^{C}	8±0.54 ^{AC}	10±1.3 ^{AB}
Anterior	8±1.58	9±1.41	9±1.92
Lateral 1	8±0.83	8±0.79	9±0.54 ^{AB}
Lateral 2	8±1	9±0.83 ^{C}	10±1 ^{B}

Table 4. The proportion of parenchyma to stroma in different lobes of rat prostate. Letters in superscript means significant difference ($p<0.05$).

Groups / Lobes	Control (G1)	Photoperiod (G2)	Heat stress (G3)
Ventral	79±1.92 ^{C}	75±1.86	68±6.85 ^{A}
Dorsal	72±2.80	55±7.61	59±9.50
Anterior	62±5.11	58±3.87	57±9.5
Lateral 1	69±12.56	68±8.41	56±6.68 ^{AB}
Lateral 2	79±8.22 ^{BC}	61±8.34 ^{AC}	44±8.03 ^{AB}

Results

Microscopic results revealed that histomorpho-

Figure 1. The ventral lobe of rat prostate gland in control group (X4, H&E). The folded secretory units in peripheral area (A), tubular units (T), and ducts (D).

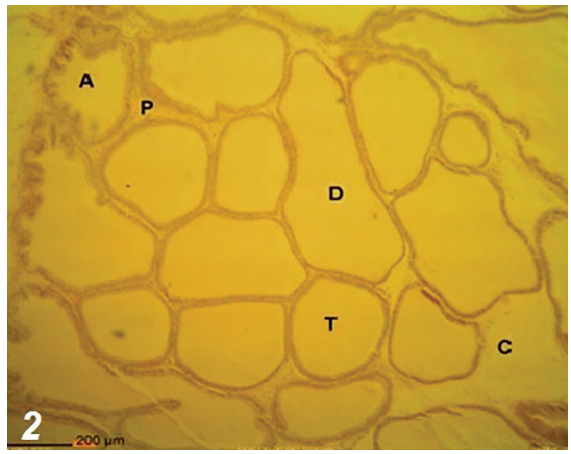


Figure 2. The ventral lobe of rat prostate gland, the control group (X4, H&E). The folded secretory units in peripheral area (P), alveolar (A), tubular units (T), ducts (D), and the central area of lobe (C).

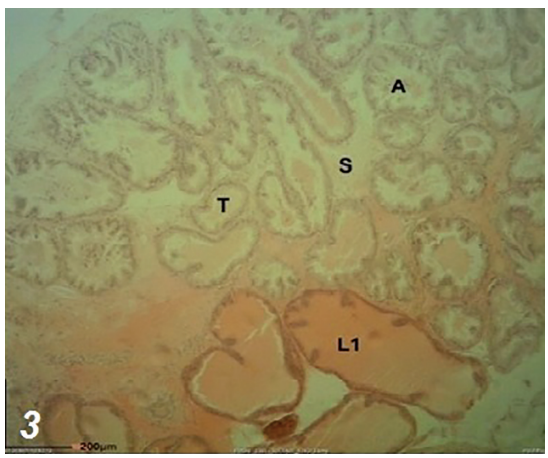


Figure 3. The dorsal lobe of rat prostate gland in control group (X4, H&E). The folded secretory units (A) dispersed in the entire lobe.

Figure 4. The ventral lobe of rat prostate gland in photoperiod group (X4, H&E). The increasing of peripheral folded secretory units in long-photoperiod group (arrows) is considerable.

metrical and histochemical reactions of different lobs of normal rat prostate are different. The ventral and dorsal have more parenchyma than other lobes, and the folded secretory units were concentrated peripherally in ventral lobe (Figure 1 & 2) while they were diffuse in dorsal lobe (Figure 3). Folded secretory units in long photoperiod group increased but decreased in heat stress group. These changes were more visible in the ventral lobe (Figure 4). Histological results showed that the proportion of parenchyma to stroma has changed in different groups. This proportion decreased by heat stress (Figure 5); however, it increased by long-photoperiod regime (Figure 6). These changes were more visible in the lateral lobes.

PAS Reaction: PAS staining showed that all secretory cells in different prostate lobes have positive PAS reaction in normal rat prostate; however, the staining intensity were maximum and minimum in ventral and anterior lobes, respectively. The tubular secretory units have more reaction to PAS staining than alveolar units. The results also indicated that PAS reaction increased by long-photoperiod regime; however, it decreased by heat stress.

Micrometrical Results: The micrometrical results showed that the epithelial thickness of secretory units were changed in different groups. It increased in long-photoperiod group (G2), while it decreased in heat stress group (G3). The most significant changes were seen in lateral lobe type 2 (Table 1).

The lumen diameter of secretory units increased

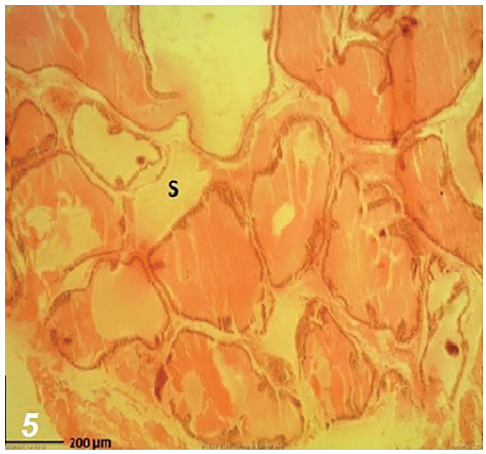


Figure 5. The lateral lobe 1 of rat prostate gland in heat stress group (X4, H&E). The decreasing of parenchyma (secretory units) and epithelium thickness and increasing of stroma (S) are considerable.

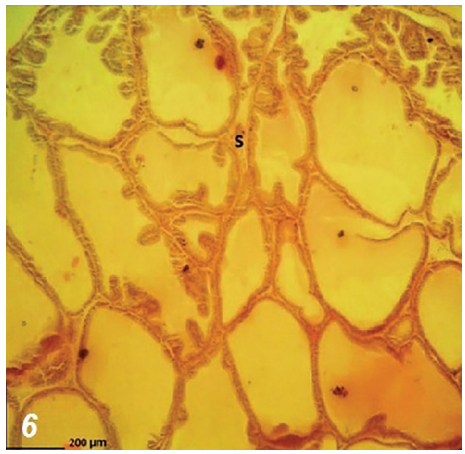
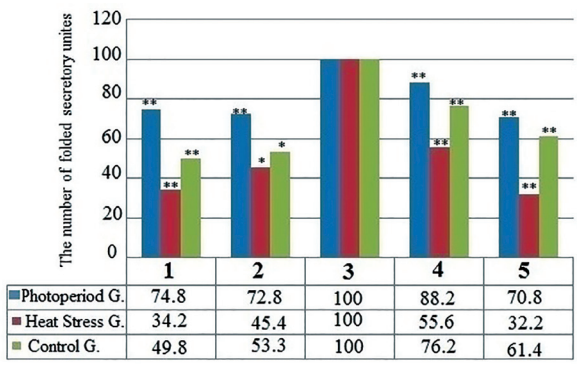


Figure 6. The lateral lobe 1 of rat prostate gland in photoperiod group (X4, H&E). The increasing of parenchyma (folded secretory units) and epithelium thickness and decreasing of stroma (S) are considerable.



	1	2	3	4	5
Photoperiod G.	74.8	72.8	100	88.2	70.8
Heat Stress G.	34.2	45.4	100	55.6	32.2
Control G.	49.8	53.3	100	76.2	61.4

Figure 7. The number of folded secretory units in different lobes and groups (mean+SD). Ventral lobe (1), dorsal lobe (2), cranial lobe (3), lateral lobe (type1) (4), lateral lobe (type2) (5). (*) Indicate a significant difference ($p<0.01$). (**) Indicate a significant difference ($p<0.001$).

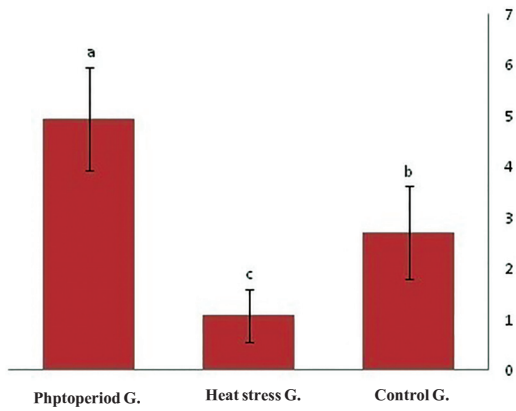


Figure 8. The serum testosterone (n/mg) in different groups. Different letters (a-c) indicate a significant difference ($p<0.01$).

in long-photoperiod group (G2) compared to heat stress group (G3) and control group (G1). The most significant changes were observed in lateral lobes (Table 2).

The number of secretory cells increased in heat stress group (G3), while they decreased in long-photoperiod group (G2), because cell size was increased. The most significant changes were observed in dorsal and lateral lobe type 1 (Table 3).

The proportion of parenchyma to stroma changed in different groups. It increased in long-photoperiod group (G2), while it decreased in heat stress group (G3) (Table 4).

The number of folded secretory units increased by long-photoperiod regime, whereas heat stress had an adverse effect (Figure 7).

Testosterone: The serum testosterone level increased by long-photoperiod regime and it decreased by heat stress (Figure 8).

Discussion

Androgen plays an essential role in embryonic development and in adult prostate (Bartsch et al., 2002; Schroder, 1994; Thomson, 2001). Androgens promote the growth and differentiation of prostate cells through ligand activation of the androgen receptor (AR) (Zhu and Kyprianou, 2008). Prostate secretory cells have androgen receptor and they are continuously stimulated by androgen in order to

survive and function (Chatterjee, 2003), so that after castration which androgen cease, the apoptosis will occur in rat prostate epithelial cells (Kyprianou and Isaacs, 1988; Schroder, 1994). The photoperiod and temperature are important factors which affect the androgen levels (Carballada, 2006). Photoperiod is also an important factor for regulating the reproductive activity (Anne Grocock, 1981; Shimizu, 2003). Reproductive activity in long-day animals can be stimulated by a long light period (e.g. 16h) and followed by a shorter dark period (e.g. 8h). Carballada et al. (2006) reported that the percentage of apoptotic cells increased in animals which were maintained for 6, 8, or 12 weeks in a short photoperiod. This study shows that photoperiod has an important effect on increasing rat prostate parenchyma and its secretory activity. The results of the present study showed that prostate parenchyma was affected by a long-photoperiod (16h light and 8h dark) which has conformity with increasing of serum testosterone. The number of secretory cells in 50m length of secretory units wall decreased in the photoperiod group because the secretory cells size increased. The dorsal lobe of the prostate Golden hamster has more response to long-photoperiod regime than ventral lobe (8h dark, 16h light) (Carballada, 2006). It has also been shown that the ventral lobe of castrated rat prostate undergoes more changes than the dorsal lobe (Banerjee et al., 1995). The results of the present study showed that ventral lobe of rat prostate has more changes to long-photoperiod regime than dorsal lobe, which is consistent with Carballada (2006) in Golden hamsters and Banerjee et al. (1995) in rats. The finding of present study showed that the cranial lobes of rat prostate is an active lobe, while most researchers suggested that cranial lobe is an inactive lobe and it is not considered as a part of rat prostate (Jesik et al., 1982; Wylot et al., 2004 Hernandes, et al., 2006).

Heat Stress: It has been shown that cancer cells are relatively sensitive to heat stress. It has been reported that heat treatment (43oC) increased the expression of heat shock protein 70 (hsp70), and it increased apoptosis. Hsp70 is a protein that protects cells against heat damage (Nakanoma et al., 2001). It has been shown that increasing the temperature caused apoptosis in rat epididymis (Jara et al., 2002). The apoptosis in rat epididymis and ventral prostate lobes increased with age (Jara et al., 2004). The results of the present study showed that serum testosterone levels reduced with heat stress and subsequent prostate tissue also underwent changes, so that, the thickness of the epithelium and ratio of parenchyma to the stroma decreased. The number of secretory cells in 50m length of secretory units wall increased because the cell size decreased. Heat stress has often caused apoptosis which was inconsistent with the present results. It should be noted that in most studies cell culture is used for showing heat stress effects (Nakanoma et al., 1998). The maximum lumen diameter of tubular secretory units were seen in long-photoperiod regime group that it is consistent with the statements of Fink et al. (2005); they reported that the lumen diameter of secretory units increased with increasing of prostate activity.

The results of the present study showed that long-photoperiod increased prostate parenchyma and activity, while heat stress has inverse effects.

Acknowledgments

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اثرات فتوپریود و استرس گرمایی بر ساختار هیستومتریک غده پروستات موش صحرایی

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چکیده

زمینه مطالعه: اطلاعات کافی در مورد ساختار هیستومتریکی لوب‌های مختلف غده پروستات رت و همچنین اثر استرس دمایی و فتوپریود برساختار لوب های مختلف در دسترس نمی باشد. **هدف:** تعیین تغییرات بافتی هریک از لوب های پروستات رت تحت تاثیر تغییرات فتوپریود و استرس گرمایی. **روش کار:** بدین منظور ۱۵ سر رت نر نژاد ویستار در سه گروه تقسیم شدند: گروه کنترل، رت ها در شرایط ۱۲ ساعت روشنایی و ۱۲ ساعت تاریکی و دمای $25^{o}C$، گروه استرس دمایی، رت ها در شرایط ۱۲ ساعت روشنایی و ۱۲ ساعت تاریکی و در دمای $42\pm1^{o}C$ به مدت ۴ الی ۵ ساعت در هر روز و گروه فتوپریود، رت ها در شرایط ۱۶ ساعت روشنایی و ۸ ساعت تاریکی در دمای $25^{o}C$ نگه داری شدند. پس از ۳۰ روز، از لوب های مختلف نمونه گیری و برش هایی با ضخامت ۵µ الی ۶ تهیه و با رنگ های PAS و هماتوکسیلین- ائوزین رنگ آمیزی شدند. **نتایج:** نتایج میکروسکوپی نشان داد که ساختار میکرومتری و واکنش هیستوشیمایی لوب های مختلف پروستات رت در حالت طبیعی متفاوت است. نسبت پارانشیم به داربست توسط استرس گرمایی کاهش ولی با افزایش دوره روشنایی افزایش می یابد. بیشترین میزان تغییرات در لوب شکمی مشاهده شد. ضخامت اپیتلیوم، قطر حفره و تعداد واحدهای ترشحی با افزایش دوره روشنایی (۱۶ ساعت روشنایی و ۸ ساعت تاریکی) افزایش اما در گروه استرس گرمایی کاهش می یابد. تعداد سلول های ترشحی به دلیل کاهش در اندازه سلول توسط استرس گرمایی افزایش می یابد. این تغییرات در گروه دوره روشنایی طولانی به دلیل افزایش اندازه سلول بر عکس بود. تعداد واحدهای ترشحی چین خورده توسط فتوپریود طولانی افزایش داشت اما استرس گرمایی اثر متضاد داشت ($p\leq0/001$). تستوسترون سرم در گروه فتوپریود طولانی افزایش اما در گروه استرس گرمایی کاهش یافت ($p<0/01$). **نتیجه‌گیری نهایی:** این مطالعه نشان داد که فتوپریود طولانی و استرس گرمایی تأثیر معنی داری به ترتیب در افزایش و کاهش پارانشیم پروستات رت دارد.

واژه های کلیدی: استرس گرمایی، هیستومتریک، فتوپریود، پروستات رت

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