DOI: https://doi.org/10.53350/pjmhs22164773

ORIGINAL ARTICLE

Progressive Detoriating Effect of Glucocorticoidson the Histomorphology of Seminiferous Tubules and Height of Germinal Epithelium of Testis of Albino Rats used for a Short Duration

UZMA HAMEED[1], SADIA IQBAL[2], UROOJ FATIMA[3], TANZEELA KHAN[4], HIRA AHMED[5], SHAZIA FAHMI[6]
[1]*Assistant Professor Anatomy, Dow University of Health Sciences Karachi, Pakistan.*
[2]*Assistant Professor Anatomy, Dow University of Health Sciences Karachi, Pakistan.*
[3]*Assistant Professor Anatomy, Jinnah Sind Medical University Karachi, Pakistan.*
[4]*Assistant Professor Anatomy, Dow International Medical College Karachi, Pakistan.*
[5]*Assistant Professor Anatomy, Karachi Medical & Dental College Karachi, Pakistan.*
[6]*Assistant Professor Anatomy, Karachi Medical & Dental College Karachi, Pakistan.*
Corresponding author: Uzma hameed, Email: uzma.hameed@duhs.edu.pk, Cell: +923453497208

ABSTRACT

Objective: To observe the microscopic changes in the seminiferous tubules of testis of albino rats, following the intravenous administration of corticosteroids.

Study design: Laboratory based experimental study.

Place and duration of the study: This study was conducted in the department of Anatomy, Baqai Medical University Karachi, in June 2017 for aperiod of 21 days.

Methodology:32 Adult,healthy male Albino rats equally divided into four groups were used in this study.Group A1 served as control for group B1 and received intra-peritoneal injection of 0.9 % normal saline for 7 days, Group A2 served as control for group B2 and received i.p injection 0.9 %normal saline for 14 days, whereas Group B2 and Group B3 were given i.pinjection of Dexamethsone at the dose of 7mg/kg/body weight for 7 and 14 days in a single daily doseat the lower site of the abdomen respectively.

Results: The Haematoxylin and Eosin (H&E) stained sections of testis tissue have shown marked histomorphological changes,the germinal epithelium was disorganized and there was a marked reduction in the height of epithelium Seminiferous tubules attained different shapes, congested blood vessels were also found,detachment and vacuolization of the seminiferous tubules were noted that eventually leads to atropy of the tubules.

Conclusion: Even short term used of glucocorticoids caused changes in the histomorphology of seminiferous tubules that may lead to infertility.

Keywords: Corticosteroids,Height of germinal epithelium,Seminiferous tubules.

INTRODUCTION

The increased and unnecessary use of glucocorticoids in present era due to covid pandemic has raised many questions, knowing its consequences on human body as well as reproductive health is a must to know for its consumers.

Glucocorticoids are the synthetic drugs that can be prescribe to any age group to treat many inflammatory, autoimmune conditions and an overactive immune system, such as allergies, asthma, autoimmune disorders, sepsis, inflammatory bowel disease, adrenal insufficiency, multiple sclerosis, graft-versus-host disease, rheumatoid arthritis, psoriasis, eczema, leukemias, ulcerative colitis and lymphomas,prevent acute transplant rejection and now in covid induced pneumonia and other complications[(1)].

In determining the dosage of glucocorticoids, many factors need to be consideration, including glucocorticoid versus mineralocorticoid activity, duration of action, type of preparation, and the time of day that a steroid is administered[(2)]. For example, when large doses of the hormone is required for prolonged period of time(more than a fortnight) suppression of the hypothalamic-pituitary-adrenal (HPA) axis occurs[(3)].

Glucocorticoids affects gonadal function at multiple levels in hypothalmo-pituitary-gonadal axis through hypothalamus to decrease the synthesis and release of gonadotrophic releasing hormone, the pituitary gland (to inhibit the synthesis and release of luteinizing hormone and follicle stimulating hormone) and Testis/Ovary (to transform steroidogenesis and/or gametogenesis directly [(4)]. Precise levels of glucocortcoids are required for appropriate gonadal functions [(4)].Researchers integrated that glucocorticoids act directly on the cells of testis through the classic Glucocorticoid Receptor, and in the rat testis GR is exclusively localized in Leydig cells where it is abundantly expressed and may catalyze the oxidative inactivation of glucocorticoids [(5)].

This study was design to investigate the histomorphological changes induced by the usage of glucocorticoid drug on the testis of albino rats as it is commonly prescribed drug especially in covid pneumonia n sepsis[(6)].

METHODOLOGY

This laboratory based experimental study was conducted in the Department of Anatomy Baqai Medical University (BMU), Karachi in collaboration with animal house of BMU from May to June 2017 after getting the approval from Board of Advanced Studies and Research Baqai Medical University (Ref:BMU-EC/2016-05).

32 adult Albino rats weighing 180 to 200 grams and aged 12 weeks were selected for this studyfrom the animal house of Agha Khan University (AKU), Karachi,selection criteria based on non-probability simple randam sampling.The sample size was calculated by open Epi web based calculator.All albino rats were kept under observation for one week before starting the study to assess their health in the well ventilated animal house of Baqai Medical University . They were exposed to day and night cycle at 30°C. All albino rats were housed in standard cages, fed with standard laboratory diet and tap water ad libitum**.**The rats were equally divided into 4 groups (n=8)after a week long adaption[(7)].

The grouping of animals were as follows:

Group A1=served as control for B1, fed on standard diet and were injected with 0.9% normal saline in equal volumes for 7 days.

Group A2=served as control for B2, fedon standard diet and were injected with 0.9% normal saline in equal volumes for 14 days.

Group B1=received i.p injection of Dexamethsone at the dose of 7mg/kg/body weight for 7 days in a single daily dose [(8, 9)] at the lower site of the abdomen.

Group B2= received i.p injection of Dexamethsone at the dose of 7mg/kg/body weight for 7 days in a single daily dose [(8, 9)] at the lower site of the abdomen14 days.

Dexamethasone (dexamethasone sodium phosphate) (purchased from a Agha Khan Hospital pharmacy and manufactured by OBS pharmaceuticals, Pakistan was used in the study.The route of administration was intra-peritoneal at a dose of 7mg/kg/day given daily to all animals of group B1 and B2 for 7 and 14 days respectively.

All the animals were sacrificed at the completion of respected experimental period.Before the collection of testis, neat and clean plastic jars were filled upto 3 quarter with10% buffered neutral formalin (BNF) and were labelled with the groups.Removed testis were kept in the jar for 48 hours. Each testis was cut into two equal vertical halves. The tissue was washed with the running water to remove excess fixative. Tissues were then dehydrated by passing through ascending grades of alcohol (70% - 100%) and cleared by xylene,The process of infiltration and embedding carried out in paraffin wax, 4 micron thick sections were obtained with the help of rotary microtome and allowed to float on hot water bath at 42 ^{0}C and were mounted on glass slides. Mounted tissue slides were set on hot plate at 32 ^{0}C to 40 ^{0}C for the purpose of fixing the sections on the slides and stained with Haematoxylin and Eosin (H&E) for routine histological staining.The diameter of seminiferous tubules with profiles that were round or nearly round were estimated for each animal and the mean + SD of the diameter was determined by taking the average of two diameters, D1 and D2 (perpendicular to one another). D1 and D2 were taken no more than when D1/D2 > 0.85[10]. The diameter of 3 seminiferous tubules were measured per section with the help of micrometer at 10X magnification. For each animal two sections were observed one for the right and one for the left and then mean tubular diameter was calculated for each right and left testis and then finally average mean was taken for each animal[11].

In each tubular cross-section, the height of the epithelium was measured four times at right angles in each tubule. In this way, mean epithelial values were obtained. The height of the seminiferous epithelium was estimated in three tubules per section and mean was calculated and measured from the basal membrane towards the lumen of the tubule[12]. For each animal two sections (one from Rt + one from Lt) were analysed by this method.A comparison was done between the control and treated group.

The statistical significance of differences in the categorical variables between the experimental and control groups were analyzed using SPSS 21.Arithmetic means were calculated and compared using student 't' test and one way ANOVA (analysis of variance) with post hoc tukey tests. The p-value of <0.05 was considered significantand <0.001 was considered highly significant.

RESULTS

The testicular tissues were carefully observed under the microscope.the diameter of seminiferous tubules and height of germinal epithelium were subjected to through histological examination.

The sections of both testisrevealed parenchymal structure of the testis were composed of round or oval seminiferous tubules. The seminiferous tubules were bounded by basal lamina, lined by stratified germinal epithelium, consisting of two types of spermatogenic cells (germ cells and sertoli cells). No vacuolization or detached basement membrane was observed.

Seminiferous tubules were separated from each other by interstitial spaces. These spaces were mostly triangular in shape consisting of leydig cells and very small blood vessels

MORPHOMETRIC RESULTS

Mean thickness of germinal epithelium (µm) in group A1 at 10X was 175.50+5.22. (Table 1)

Mean thickness of germinal epithelium (µm) in group A2 at 10X was 176.77+5.39. (Table 1)

Mean diameter (µm) of seminiferous tubule in group A1 at 10X was 444.44+9.35.

(Table 1)

Mean diameter (µm) of seminiferous tubule in group A2 at 10X was 444.44+9.35.

(Table 1)

The animals in group B1 were active for the first three days of experiment then they gradually lost their appetite. They developed alopecia of the whole body, became lethargic with weak response to external stimuli.

Histological examination of testes of group B1, stained with haematoxylin and eosin showed the seminiferous tubules, that attained different shapes (fig 2A). The germinal epithelium was disorganized, there was thining of germinal epithelium and the tubules showed debris within the lumen because of sloughing of germ cells as well as pyknotic nuclei (fig 2B), varying degree of germ cells degenerative changes occurred, ranging from loss of elongated spermatids, disorganization of germ cell layers, detachment and sloughing to vacuolization of the seminiferous tubules, contributing to eventually atrophy (Fig 2C). Characteristic multinucleate giant cells were also detected. Percentages of eccentric tubules were significantly increased (fig 2D). The interstitial tissue exhibited groups of leydig cells and congested blood capillaries (fig 2D).

Mean thickness of germinal epithelium (µm) at 10X was 114.89+5.22. (Table 1)

Mean diameter (µm) of seminiferous tubule at 10X was 392.67+3.57. (Table 1)

The animals in group B2 were active first three days of experiment then they gradually lost their appetite and thirst. They also lost interest in surroundings during last week of experiment. They developed alopecia of the whole body, became lethargic with weak response to external stimuli. They had difficulty in initiating movement. On gross examination the size of testis were appeared shrunken in comparison with control and group.2 animals out of 8 in this group died on day 12 and day 14.

Histological examination of testis of group B2, stained with haematoxylin showed the seminiferous tubules were surrounded by thickened tunica albuginea, exhibiting deformed sertoli cells, being detached from abnormal basal lamina, marked reduction in the germ cells causing dilatation intercellular spaces (fig 3A). spermatogonia manifest vacuolated cytoplasm and pyknotic nuclei (fig 3B). The effects are much severe in spermatid differenciation , whereas there is a complete loss of elongated spermatids and accordingly of spermatozoa, which denotes that the spermatogenesis was held at the stage of rounded spermatids formation under the effect of dexamethasone treatment (Fig 3C). The lumen of the affected tubule contained sloughed germ cells (fig 3D). The interstitial tissue exhibited groups of leydig cells and congested blood capillaries (Fig 3D).Interstitial spaces were wide in almost all area and leydig cells were scanty with marked vacuolization (fig 3D).

Mean thickness of germinal epithelium (µm) at 10X was 95.96+5.59. (Table 1)

Mean diameter (µm) of seminiferous tubule at 10X was 330.80+4.67. (Table 1)

Mean thickness of germinal epithelium (µm) of seminiferous tubules in group B1 and B2 was significantly decreased (p 0.001 and p 0.001) when compared with the group A1 and A2. (Table 2)

Mean diameter (µm) of seminiferous tubules in group B1 and B2 was significantly decreased (p 0.001 p 0.001) when compared with group A1 and A2. (Table 2)

Table 1: Mean Thickness Of Germinal Epithelium (µM) And Mean Diameter Of Seminiferous Tubules (µM) In Different Groups

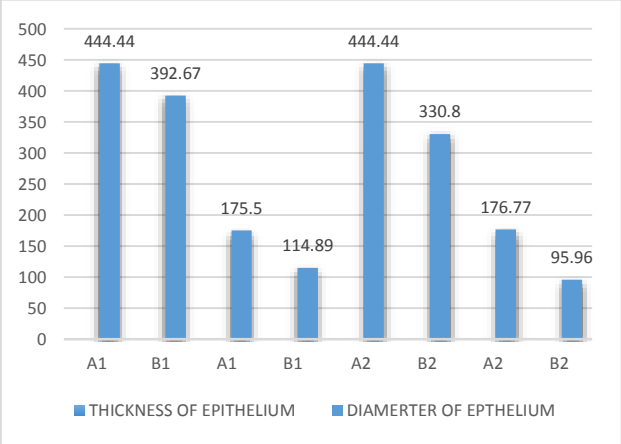
Groups	Experiment done	Thickness Of Germinal Epithelium 10x µM	Diameter Of Seminiferous TubulesµM
A1(N=8)	I.p normal saline given for 7	175.50+5.22	444.44+9.35
A2(N=8)	I.p normal saline given for 14 days	176.77+5.39	444.44+9.35
B1(N=8)	I.p glucocorticoid for 7 days	114.89+5.22	392.67+3.57
B2(N=8)	I.p glucocorticoid for 14 days	95.96+5.59	330.80+4.67

Mean ± SEM (Standard Error of Mean)

Table 2: Analysis Of Differences In Germinal Epithelium (µm) Andanalysis Of Differences Of Seminiferous Tubules (µm) Between Different Groups

Comparison of groups	Statistical analysis	Difference between groups	P-value
A1 and b1	Difference in germinal epithelium	60.61	0.001*
A2 and b2	Difference in germinal epithelium	79.54	0.001*
A1 and b1	Difference in seminiferous tubules	51.76	0.001*
A2 and b2	Difference in seminiferous tubules	113.63	0.001*

* highly Significant



Graph 1: Bar Chart Showing Comparison Of Mean Values Of The Thickness Of Epithelium Andamong Diameter Of Seminiferous Tubules The Control Group A1,A2 And Experimental Groups B2 And B3

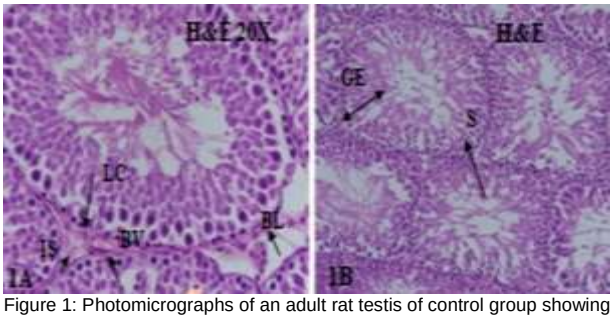


Figure 1: Photomicrographs of an adult rat testis of control group showing

1A Leydig cells (LC) in interstitial space(IS),basal lamina (BL)and blood vessel(BV) (20X objective and 10X eye piece)
1B Seminiferous tubules(S) lined by stratified cuboidal epithelium(GE) (10X objective and 10X eye piec

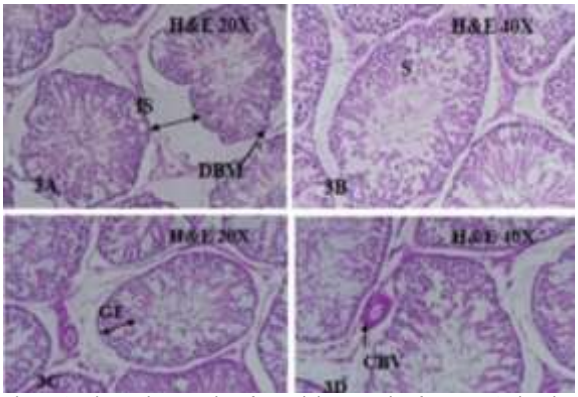


Figure 2: Photomicrographs of an adult rat testis of group B2 showing

2A distorted basal membrane and wider interstitial spaces (IS) (20X objective and 10X eye piece)
2B different shapes of seminiferous tubules (ST) bounded by (DBM) (40X objective and 10X eye piece)
2C reduced height of germinal epithelium (GE) and marked vacuolation (V) (20X objective and 10X eye piece)
2D congested and dilated blood vessel(CBV) with scanty leydig cells (LC). (40X objective and 10X eye piece)

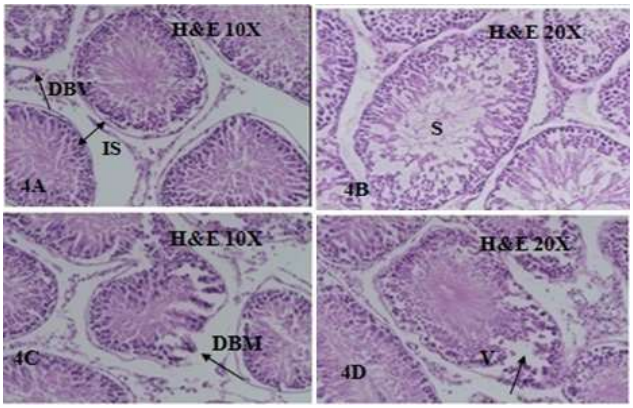


Figure 3: Photomicrographs of an adult rat testis of group B3 showing

3A Dilated and congested boodvessel(DBV) with increased interstitial spaces(IS)(10X objective and 10X eye piece)
3B Wavy appearance of basal lamina(BL) with distorted shapes of seminiferous tubules(ST) (20X objective and 10X eye piece)
3C shrunken and distorted seminiferous tubules (ST), bounded by distorted basal membrane (DBM) and lined by accentuated germinal epithelium(GE) (10X objective and 10X eye piece)
3D more marked vacuolation (V) within the epithelium scanty leydig cells (LC)(20X objective and 10X eye piece)

DISCUSSION

The present study was conducted to observed the effects of glucocorticoids on the testis of albino rats.Dexamethasone is a synthetic glucocorticoid whose potent to suppress the immune system is 20 to 30 times more greater than other steroids reported by Scharkman etl.,2013[14]. People used this medicine without knowing the fact that it also affects the fertility of the animals as well as the human being by affecting the histomorphological changes damage the testicular tissue.

In this study, seminiferous tubular diameter of the experimental groups B1 and B2 was reduced as compare to control group A1 and A2 respectively,and this reduction was statistically significant with the (P value<0.05). However silva et al.,2014[15] found that diameters of seminiferous tubules were markedly increased with atropy of testis and absence of elongated spermatozoa in rats after a single intra-peritoneal injection which is not accordance with our study.

In the present study, examination of the section of the testis of the dexamethasone exposed rats showed the seminiferous tubules attained different shapes and had lost the common distribution of their epithelial lining with appearance of several layers of dark type spermatogonia. The lumina of the affected tubules contained exfoliated germ cells, these results were in agreement with the findings of Turecki et al.,2016[16] who attributed the exfoliation of these cells to retraction of the cytoplasmic processes of Sertoli cells so the cells became loosely arranged and were easily sloughed off. (Halberg F et al.,2010)[17] proposed that decreased seminiferous tubular diameter is probably due to inhibition of spermatogenesis which results in decreased sperm count.

In the present study, the basal lamina surrounding the seminiferous tubules showed irregularties, disruption and wavy appearance especially in B1 and B2 groups. This finding is

reported by (Witorscl et al.,2016)[18], he explained the basal lamina plays an important role in maintaining substance transportation between interistial tissue and spermatogenic epithelium and in maintaining the structural and functional integrity of tissues, similar explaination had been given by (Zhang TY et al.,2013)[19] he explained that detached basement membrane structure has been associated with severe functional impairment of the testis, this destruction might subsequently affect transportation of oxygen , hormone, nutrition and metabolites.

In groups B1 and B2 there was distorted shape of seminiferous tubules, along with marked vacuolation and sloughing of germ cells, pyknotic nuclei were also visible. The diameter of seminiferous tubules was also reduced with decreased thickness of germinal epithelium. These results agreed with the findings of (Pramanik P et al.,2012)[20] who explained glucocorticoids receptors (GRs) of sertoli cells are necessary to support normal testicular functions and dexamethasone decreases the concentrations of the receptors . Animals lacking of (GRs) showed morphological changes, including low number of sertoli cells, low number of spermtocytes and spermatids and decreased seminiferous tubule formation.

In this study the height of germinal epithelium was significantly decreased in the experimental groups B1 and B2 as compare to control. These finding was coincides with the result of (Wahbah 2010)[8]who also reported the decreased thickness of germinal epithelium.(Nelson et al.,2003)[21] found detachment (appearance of breakingoff of cohorts of spermatocytes from the seminifeousepithelim), sloughing (release of clusters of germ cells into the lumen of the seminiferous tubules which decreased the height of the germinal epithelium and these results agreed with the present study.

In the present work, damage of sertoli cells was evident following dexamethasone administration, dilated intercellular spaces and loss of contact between germ cells is apparently due to sertoli cells disturbance which also lead to loss of these germ cells and conclusively to the destruction and reduction in germinal epithelium, same results were found in the study of (Revollo JR et al.,2009)[22].

In the present study the surrounding basal lamina of the tubules was detached with irregular wavy appearance,The basal lamina plays an important role in maintaining substance transportation between interstitial tissue and spermatogenic epithelium and in maintaining the structural and functional integrity of tissue, same findings also described by (Hu G-X et al.,2008)[5]. Altered basement membrane structure has been associated with severe functional impairment of the testis, this destruction might subsequently affect of oxygen, hormone, nutrition and metabolies explained by (Dhar S et al.,2014)[23].

CONCLUSION

It can be concluded from the above discussion and results that glucocorticoids caused destruction and distortion of the architecture of testis. The damage observed wasincreased with duration of the drug

This animal study will help to decrease infertility rate due to excessive and unnecessary use of glucocorticoids especially in the early years of life.

Author's contribution: Dr Uzma Hameed:focal person to conceive the idea to conduct the researched data collection,Dr Sadia Iqbal: literature search, Dr Urooj Fatima: data feeding and analysis, Dr Tanzeela Khan: drafting of the article, Dr Hira Ahmed and Dr Shazia Fahmi: statistical expertise.

Conflict of interest: This study has no conflict of interest to be declared by any author.

REFERENCES

1. Arora S, Singh P, Basu P, Haldar C. Effect of exogenous gonadal steroids on reproductive functions of the Indian pygmy field mouse Mus terricolor. Journal of Endocrinology and Reproduction. 2014;18(1):29-36.
2. Witorsch RJ. Effects of elevated glucocorticoids on reproduction and development: relevance to endocrine disruptor screening. Crit Rev Toxicol. 2016;46(5):420-36.
3. Berglund C. Cortisol: Why â€œThe Stress Hormoneâ€• Is Public Enemy No. 1: 5 simple ways to lower your cortisol levels without drugs. See: https://www psychologytoday com/blog/the-athletes-way/201301/cortisol-why-the-stress-hormone-is-public-enemy-no-1. 2013.
4. Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. Minerva endocrinologica. 2010;35(2):109.
5. Hu G-X, Lian Q-Q, Lin H, Latif SA, Morris DJ, Hardy MP, et al. Rapid mechanisms of glucocorticoid signaling in the Leydig cell. Steroids. 2008;73(9):1018-24.
6. Cohen S, Janicki-Deverts D, Doyle WJ, Miller GE, Frank E, Rabin BS, et al. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proceedings of the National Academy of Sciences. 2012;109(16):5995-9.
7. Dolatabadi AA, Zarchii SR. The effect of prescription of different Dexamethasone doses on reproductive system. Biomedical Research. 2015;26(4):656-60.
8. Wahbah NS, Ahmed FE, Hassan EZ. Histological study of the effect of exogenous glucocorticoids on the testis of prepubertal albino rat. Egypt J Histol. 2010;33:353-64.
9. Orazizadeh M, Khorsandi LS, Hashemitabar M. Toxic effects of dexamethasone on mouse testicular germ cells. Andrologia. 2010;42(4):247-53.
10. Saalu LC, Akunna GG, Oyewopo AO. The Histo-morphometric Evidences of Vernonia amygdalina Leaf Extract-induced Testicular Toxicity. Int j morphol. 2013;31(2):662-7.
11. Pelt AC. Glucocorticoids: effects, action mechanisms, and therapeutic uses. Nova Science; 2011.
12. Sadi-Guettaf H, Bekkouche FH. Dexamethasone: Impact on Testicular Activity. compare. 2014;220:3.
13. Nirupama M, Yajurvedi HN. Durational effects of chronic stress on the testicular damage and its reversibility in albino rat. Eur J Exp Biol. 2013;3(5):229-39.
14. Schakman O, Kalista S, Barbe C, Loumaye A, Thissen JP. Glucocorticoid-induced skeletal muscle atrophy. The international journal of biochemistry & cell biology. 2013;45(10):2163-72.
15. Silva EJR, Vendramini V, Restelli A, Bertolla RP, Kempinas WG, Avellar MCW. Impact of adrenalectomy and dexamethasone treatment on testicular morphology and sperm parameters in rats: insights into the adrenal control of male reproduction. Andrology. 2014;2(6):835-46.
16. Turecki G, Meaney MJ. Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review. Biological psychiatry. 2016;79(2):87-96.
17. Halberg F, CornÃ©lissen G, Cegielski N, Hillman D, Halberg Francine SO, McCraty R, et al. Circadian dysfrequentia of cortisol, melatonin, DHEA, testosterone and estradiol. Noninvasive Methods in Cardiology, Brno, Czech Republic Brno: Faculty of Medicine, Masaryk University. 2010:9-22.
18. Witorsch RJ. Effects of elevated glucocorticoids on reproduction and development: relevance to endocrine disruptor screening. Critical reviews in toxicology. 2016;46(5):420-36.
19. Zhang TY, LabontÃ© B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. Neuropsychopharmacology. 2013;38(1):111-23.
20. Pramanik P, Biswas S. Traffic noise: a silent killer of male gamete of albino rats. Al Ameen J Med Sci. 2012;5(1):82-9.
21. Nelson HS, Leung DYM, Bloom JW. Update on glucocorticoid action and resistance. Journal of allergy and Clinical Immunology. 2003;111(1):3-22.
22. Revollo JR, Cidlowski JA. Mechanisms generating diversity in glucocorticoid receptor signaling. Annals of the New York Academy of Sciences. 2009;1179(1):167-78.
23. Dhar S, Seth J, Parikh D. Systemic side-effects of topical corticosteroids. Indian journal of dermatology. 2014;59(5):460.