

Effect of Exogenous and Endogenous Glucocorticoids on the Spermatogenesis of Albino Rats; A Comparative Study

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Abstract

Objective: The objective of the present study was to compare the earlier possible effects of exogenous and endogenous glucocorticoids on the spermatogenesis of albino rats.

Methods: This comparative study was conducted in Baqai Medical University in the department of Anatomy from June till July 2016. Total 96 adult Sprague rats were used in this study obtained from Agha Khan University; their ages were between 10-12 weeks and their weight ranged between 180-200 gms. Exogenous glucocorticoid were given through intra peritoneal route (i.p. route) at the dose of 1.2 mg/kg/body weight and for endogenous production of cortisol, they were kept in a plastic box daily which was thermostatically maintained at 44° C for 1 hour. They were divided into three main groups and the main group was further categorized into subgroups according to their number of days. The three main groups were; A (control), B (exogenous glucocorticoid) and C (endogenous glucocorticoid). They were sacrificed at the end of experiment, testes were removed and effects of exogenous and endogenous glucocorticoids on the spermatogenesis were observed under the light microscope in H&E and PAS stained sections.

Results: Spermatogenesis was assessed by Johnsons scoring which ranges from 10 to 1. In the control group, complete spermatogenesis was observed with Johnsons score 10 while in group B and C scores were in between 10-2 and 10-3, respectively.

Conclusion: From the present study, we conclude that exogenous glucocorticoids cause earlier damage and effects all stages of spermatogenesis with decreased production of quality sperms. This animal study will help to decrease infertility rate due to excessive and unnecessary use of glucocorticoids especially in the early years of life and by taking preventive measures when exposed to high temperatures.

Keywords: Glucocorticoids, heat stress disorders, spermatogenesis, infertility.

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Introduction

Testes are paired ovoid glandular organs present in the hollow sac of the scrotum¹. Infertility could be caused by disrupted spermatogenesis that can occur due to undescended testes or

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raised temperature². Spermatogenesis is a complex series of mitotic and meiotic cell divisions in the production of the mature sperm cell. Testes function under the influence of anterior pituitary hormones³ and both testosterone and follicle stimulating hormones are required for spermatogenesis⁴. The process of spermatogenesis is also temperature dependent and occurs optimally at temperatures slightly lower than the body temperature. Increase in temperature has an effect on mammalian spermatogenesis and resultant spermatozoa⁵.

Cortisol is the principal circulating glucocorticoid in the human body and is secreted under the control of the hypothalamo-pituitary-adrenal axis⁶. It

compromise gonadal function at different stages in hypothalmo-pituitary-gonadal axis; through hypothalamus by reducing the production and release of gonadotrophic releasing hormone, the pituitary gland by inhibiting the synthesis and release of luteinizing and follicle stimulating hormones and gonads by transforming steroidogenesis and/or gametogenesis directly⁷. For proper functioning of testes, appropriate levels of glucocorticoids are necessary⁷.

Stress can be initiated by any social, traumatic, emotional, psychological, institutional or environmental stimuli, which produces a demand for adaptation⁸. Every individual is exposed to some stressor in their life the most common is the environment⁹. Every day we are exposed to unlimited environmental stressors that cause minor irritation. Extreme temperatures are also environmental stressors and can lead to discomfort¹⁰. The temperature of the testes must be maintained 4-5°C below body core temperature for effective spermatogenesis¹¹. Abnormal testicular thermoregulation is the most common reason of impaired spermatogenesis and sperm function in animals and humans with outcomes ranging from subclinical infertility to sterility¹². Failure to regulate testicular temperature can compromise sperm quality and increase the risk of infertility, as the world is going through drastic environmental changes, one is extreme temperatures and higher temperatures (>36°C) inhibit the proliferation of Sertoli cells. So, this study is specifically designed to observed the earlier changes in stages of spermatogenesis due to heat stress and to compare their effects with exogenous glucocorticoids which are used unnecessarily in our society which may also reduce male fertility by affecting the proliferation of Sertoli cells and integrity of tight junctions among Sertoli cells thereby reducing spermatogenesis¹³.

Material and Methods

This study was conducted in the animal house of Baqai Medical University in the department of Anatomy after the research approval from Board of Advanced Studies and Research from June 2016 till July 2016, the duration of study being 21 days. In this study about ninety-six (96) adult albino rats weighing 180 to 200 grams and aged 10 to 12 weeks were purchased from the animal house Agha

Khan University. All healthy albino rats of same age and weight were selected and any animal found unhealthy or dead during study was excluded. All albino rats were kept in a well-ventilated room for one week prior to studying in order to acclimatize them and to assess their health. They had access to 8 hours daylight and 16 hours darkness at a controlled temperature of 30°C. All albino rats were housed in standard cages, fed with standard laboratory diet and tap water ad libitum. Dexamethasone (decadron®) (dexamethasone sodium phosphate) purchased from Agha Khan pharmacy and manufactured by OBS pharmaceuticals Pakistan, was used as exogenous glucocorticoid in the study. This was a comparative study and was designed to compare the earlier outcomes of the exogenous and endogenous glucocorticoid exposed groups. Animals were randomly divided into three main groups (A, B and C) and each group had 24 rats. Each group was divided into subgroups according to the number of days which were 1, 7 and 14 days, 8 animals were kept in each subgroup. For identification purpose their tails were marked by different colours. Group A was divided into A1 and A2. A1 was control for Group B and their subgroup and A2 was control for Group C and their subgroups. A1 group was given 0.9% saline in equal amount as for group B and its sub groups. Sub-group A2 were kept in the plastic box for 1 hour maintained at room temperature (30°C) supplied with same diet and water as given to other groups and were sacrificed next day of exposure¹⁴.

GROUP B was the exogenous glucocorticoid exposed group. Rats were fed on basal diet and divided into three sub groups B1, B2 and B3 according to the number of days they received glucocorticoid exogenously. They received intra peritoneal injection of Dexamethasone at the dose of 7 mg/kg/body weight for 1, 7 and 14 days respectively. Single daily doses were given at the lower site of the abdomen. The dosing was done in the morning and the rats were sacrificed next day of their exposure.

GROUP C was the heat exposed group which released cortisol in response to heat stress. Rats were fed on basal diet and divided into three sub groups C1, C2 and C3 according to the numbers of days they were exposed to heat stress. They were

kept in plastic box (which was 2 feet in height, length and width) with thermostatically controlled heater at the top and were exposed to heat for 1, 7 and 14 days respectively, for 1 hour at the temperature of 44°C. They were provided with water and food during the heat procedure and were sacrificed next day of their exposure¹⁵.

Their health status and activities were recorded on daily basis. Rats were anaesthetized by ether in a glass container and then sacrificed after exposure to exogenous or endogenous glucocorticoids on the next day.

Spermatogenesis were assessed by Johnsons scoring method 16. For each animal, two slides (one from left and one from right testes) were taken and observed at 40X magnification under a light microscope. Ten tubules (rounded) were observed and their mean was calculated. Each tubule was given a score ranging from 1 to 10. The tubules having no activity were scored as 1 and those with complete activity (at least five or more spermatozoa in the lumen) scored 10. This method includes from 1 - 10 scores as follows:

10. Complete spermatogenesis with many spermatozoa; the thickness of the germinal epithelium is normal with smooth surface.
9. Distortion seen in epithelium but still many spermatozoa present.
8. Only a few spermatozoa seen.
7. Many spermatids but no spermatozoa.
6. Less than 5 spermatids but no spermatozoa
5. Many spermatocytes but no spermatozoa or spermatids.
4. Less than 5 spermatocytes but no spermatozoa or spermatids.
3. Only spermatogonia cells seen.
2. Only Sertoli cells present.
1. No cells in the seminiferous tubule.

In this study the data was stored and analysed using SPSS (Statistical Package for Social Sciences) version 21.0. For comparison student 't'

test and one way ANOVA (analysis of variance) with post hoc tukey tests were applied.

Results

As explained earlier, Johnsons scoring was used to observe spermatogenesis, which includes scores from 10-1 and results are given in percentages. In the control group A1 and A2, all stages of spermatogenesis were observed with score of 10. But in the groups B1, B2, B3, scores were in between 10 to 5, 10 to 3 and 8 to 2, respectively. These scores are significant in comparison with control group A1 (Table 1).

While in the group C1, C2, and C3, scores ranged in between 10 to 3, 10 to 3 and 10 to 3 respectively. However, differences in percentages in each group were noted (Table 1).

When the scores were compared between control and the experimental groups and between Group B and their subgroups (Fig 1) and Group C and their subgroups (Fig B), there was significant difference in their percentages and scoring.

Discussion

The present study was conducted on albino male rats to compare the earlier effects of exogenous and endogenous glucocorticoids on the spermatogenesis of albino rats.

Spermatogenesis was assessed by Johnson's scoring which has score from 10-1. In present study, in the experimental group B1 (exogenous treated group), 37.5% of cases had Johnson Score 10, 37.5% of cases had score 9, 12.5% of cases had score 7 and 12.5% of cases had score of 5. In B2, 12.5% had score 10, 12.5% of cases had score 9, 25% cases had score 7, 25% of cases had score 5, 12.5% had score 6 and 12.5% had score 3. In B3, 12.5% of cases had Johnson's Score 8, 37.5% of cases had score 6, 12.5% of cases had score 5, 25% of cases had score 3 and 12.5% of cases had score 2. Group B score was in between 10 and 2. These results were in agreement with the

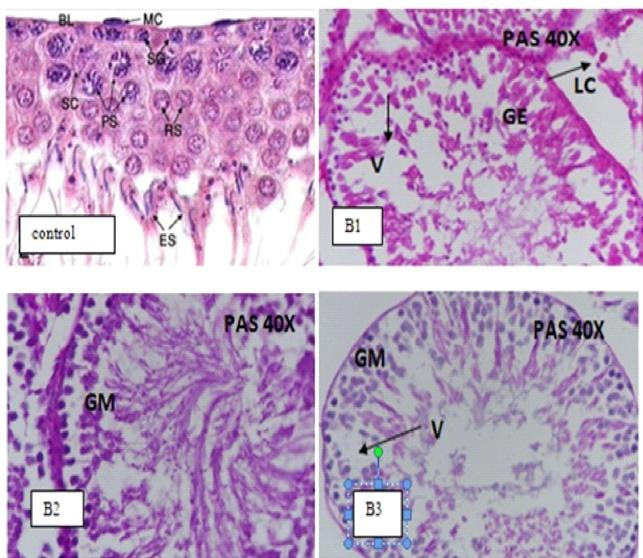


Fig 1. Germinal epithelium (GE) of control group consist of different stages of germ cells spermatogonia (SG), primary spermatocytes (PS), round spermatids (RS) and elongated spermatids (ES). Germ cells are in layer with sertoli cells (SC) interposed between them. photograph of an adult rat testis of groups B1, B2 and B3 showing shrunken and distorted seminiferous tubules (S), bounded by distorted basal membrane (DBM) and lined by accentuated germinal epithelium (GE) depletion and marked vacuolation (V) and wider interstitial spaces (IS) with scanty leydig cells (LC). GM shows decreased spermatogenesis.

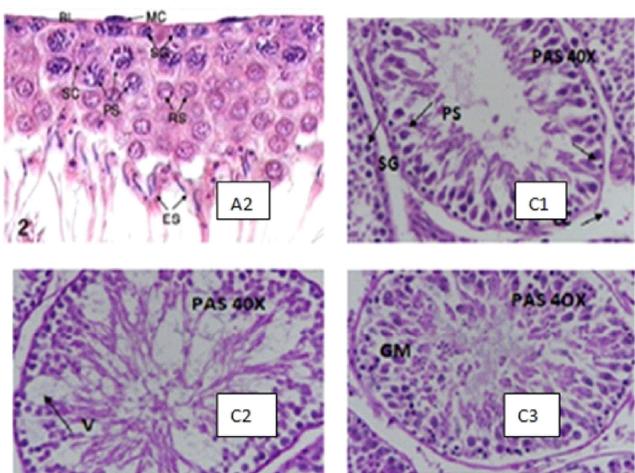


Fig B

Fig B Germinal epithelium (GE) of control group consist of spermatogonia (SG), primary spermatocytes (PS), round spermatids (RS) and elongated spermatids (ES). Germ cells are in layer with sertoli cells (SC) interposed between them. control, C1, C2 and C3 showing seminiferous tubules (S), C1 bounded by distorted basal membrane (DBM) and lined by accentuated germinal epithelium (GE) depletion and vacuolation (V), wide interstitial spaces with scanty leydig cells (LC). Germinal epithelium showing decreased spermatogenesis.

study of Elshennawy WW et al., 2011¹⁷, he explained that after administration of dexamethasone spermatogonia was severely compromised, they lost their normal shapes and showed features of necrotic cells. Spermatogonia are particularly affected by physical and chemical toxicants because of their mitotic activity, and they are easily damaged as compared to sertoli, leydig cells and spermatids. Oei et al., 2014¹⁸ explained that dexamethasone treatment causes more destruction to spermatocytes and spermatids, that reflect the disturbances in the microenvironment of the sertoli cells and also affect the protein synthesis mechanism necessary for the germ cells differentiations. The glucocorticoids inhibit the testicular steroidogenesis by an indirect action on the hypothalamus and pituitary gland and by a direct action on leydig cells. As suggested by Nelson et al., 2003¹⁹, the dexamethasone acts on the gonadotropic cells of pituitary gland former and had influence on FSH regulation.

Dexamethasone treated groups revealed poor spermatogenesis according to results obtained by Johnson's scoring. Differences in the Johnson's scoring in dexamethasone treated rats may be associated with the induction of apoptosis in testicular germ cells, additionally, dose dependent structural alterations may have been a result of the apoptotic effects of dexamethasone on germ cells, thus spermatogenic defects might result not only from a direct effect of dexamethasone on germ cells apoptosis, but also from changes of the Sertoli cells microenvironment. Wahbah et al., 2010⁶ found that glucocorticoids affect both steroidogenesis, spermatogenesis and cause spermatogenic arrest at a certain level of germ cells. He also reported that glucocorticoid receptor has been found on leydig cells, early pachytene and zygotene, primary spermatocytes, peritubular myoid cells, and fibroblast. FSH & testosterone receptors are located on sertoli cells. These hormones influence the germ cells by regulating Sertoli cell function. Glucocorticoids act directly on sertoli cells and/or on germ cells and induce retardation of testicular development. In confirmation with the above results sertoli

Table 1. Results of Johnsons score.

JOHNSONS SCORING FOR SPERMATOGENESIS	A1	A2	B1	B2	B3	C1	C2	C3
Complete spermatogenesis with many spermatozoa; the thickness of the germinal epithelium is normal with smooth surface (score=10)	24 100%	24 100%	3 37.5%	1 12.5%	0 0 %	2 25%	1 12.5%	2 25%
Many spermatozoa present but germinal epithelium disrupted (score=9)	0 0 %	0 0 %	3 37.5%	1 12.5%	0 0 %	3 37.5%	1 12.5%	2 25%
Only a few spermatozoa(score=8)	0 0 %	0 0 %	0 0 %	0 0	1 12.5%	1 12.5%	0 0 %	1 12.5%
No spermatozoa but many spermatids (score=7)	0 0 %	0 0 %	1 12.5%	2 25%	0 0 %	0 0 %	3 37.5%	1 12.5%
No spermatozoa but few (< 5 spermatids) (score=6)	0 0 %	0 0 %	0 0 %	2 25%	3 37.5%	0 0 %	0 0 %	0 0 %
No spermatozoa or spermatids but many spermatocytes(score=5)	0 0 %	0 0 %	1 12.5%	1 12.5%	1 12.5%	1 12.5%	1 12.5%	1 12.5%
No spermatozoa or spermatids but few (<5)spermayocytes(score=4)	0 0 %							
Spermatogonia are the only germ cell present(score=3)	0 0 %	0 0 %	0 0 %	1 12.5%	2 25%	1 12.5%	2 25%	1 12.5%
No germ cells but Sertoli cells are present(score=2)	0 0 %	0 0 %	0 0 %	0 0 %	1 12.5%	0 0 %	0 0 %	0 0 %
No cells in tubules section(score=1)	0 0 %							

cells provide structural and nutritional support to all the different stages of spermatogenic cells that lie in close contact with them..

Dolatabadi et al., 2015²⁰ studied the effect of dexamethasone on proteins responsible for apoptosis in mice germ cells and their results showed that glucocorticoid compounds such as dexamethasone results in apoptosis & disruption of spermatogenesis by affecting pro-apoptotic proteins such as Fas L and Bax protein. Kothari S et al., 2015²¹ explained that dexamethasone induced apoptosis, destruction in spermatogenesis and significant decreased in number of spermatocytes in laboratory rats is because of pro-apoptotic proteins such as Fas L.

In group C1 (heat treated group) 25% of cases had Johnson's score of 10, 37.5% of cases had score 9, 12.5% of cases had score 8, 12.5% of cases had score 5 and 12.5% of cases had

Johnson's score 3. In C2 12.5% of cases had Johnson's score 10, 12.5% of cases had score 9, 37.5% of cases had score 7, 12.5% of cases had score 5 and 25% of cases had score 3. In C3 25% of cases had score 10, 25% of cases had score 9, 12.5% of cases had score 8, 12.5% of cases had score 7, 12.5% of cases had score 5 and 12.5% of cases had Johnson's score 3 so group C score ranged in between 10 and 3. These results are consistent with the study results of Brito LFC et al., 2004²². A gradually increasing quantity of abnormal spermatids was observed in his study, in relation with an intensification of the structural transmutations identified by typical ring formation in the nucleus. The degenerative changes in germ cells varied considerably.

Alves MBR et al., 2016²³ explained that we can predict the pattern of degeneration in different stages of spermatogenesis and in the susceptible

cells if we know the time interval between exposure and autopsy through the rats. The current study shows that in addition to cells in the last step of differentiation, the primary spermatocytes at different stages of meiotic process are also vulnerable to heat, and even if they do not show immediate destructive changes after the exposure, they do degenerate after they have attained a certain step in their development. The absence of structural abnormality shortly or immediately after exposure revealed that heat may target some metabolic activity that cause apparent damage until the cell achieves a particular stages in its development. Degenerating primary spermatocytes contain PAS positive material in their cytoplasm may be an expression of metabolic damage. Hence, it is proved that the meiotic process is highly affected by heat.

Kumar H et al. 2016²⁴ proposed that raised testicular temperature causes inhibition of spermatogenesis in stress exposed groups, which is consistent with results of the present study.

Arun S et al., 2016¹³ explained that heat stress compromise functional capacity of the spermatocytes and if scrotal temperature raised above the normal value, more damage to DNA strands occur which leads to disruption of spermatogenesis. He also showed that there may be a temperature threshold, influenced by time exposure, above which germ cells degeneration is initiated. This too coincides with the present study results. It concluded that primary spermatocyte stage was the most sensitive and vulnerable to the temperature range of 41 to 43°C, and that the early stages of spermatid development are also heat sensitive, as agreed by the present study. The most resistant population of cells was spermatogonia, suggesting that spermatogonia became heat sensitive during mitosis. Long duration of heat exposure, resulted in morphological changes in spermatogenic and steroidogenic cells of the testes indicating permanent damage. Chronic stress induces apoptosis in sperms and Leydig cells and glucocorticoids, which was secreted at higher concentrations under stress, cause apoptosis of spermatogonia. As explained by Alves MBR et al, 2016²³, middle stages of spermatogenesis showed limitations to the adverse effect of heat, which is consistent with the present study.

Connection of Sertoli cells with their basal lamina is required for its barrier function and is therefore important for maintaining spermatogenesis. Exposure to 42°C appears to have affected cells at all stages of spermatogenesis. The effects of 40°C were more selective and 38°C showed no measurable alterations of testicular cell populations. Al-Damegh et al., 2014²⁵ explained that the antioxidant system of testes showed under functioning due to raised temperature and also caused increased lipid peroxidation within the germ cells. The major factor that impairs spermatogenesis in testes is scrotal hyperthermia and it is also associated with oxidative stress, followed by apoptosis of germ cells.

Conclusion

It has been concluded from the present study that exogenous glucocorticoid causes more distortion and destruction at different stages of spermatogenesis at the same day of exposure and becomes more aggressive if it continues for longer duration. It causes irreversible changes as compared to endogenous glucocorticoid, which is released in response to heat stress and also affects spermatogenesis at the same day of exposure but changes are reversible as adaptations appear within the cell. This study will help as glucocorticoid exposure either used exogenously or endogenously.

Conflict of Interests

Authors have no conflict of interests and received no grant/funding from any organization.

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