*Journal of Chemical Health Risks*

Journal of Chemical Health Risks (2015) 5(4), 295–300

**ORIGINAL ARTICLE**

# Long-term Effects of Betamethasone on Epididymal Tissue, Epididymal Sperm Counts and Fertility in Male Mice

**Jalaleddin Gouyandeh[1*], Mehrdad Modaresi [2], Sara Mansouri [2], Fahimeh Yaghoubieh najafabadi [3]**

[1] Young Researchers and Elite Club, Najafabad Branch, Islamic Azad University, Najafabad, Iran.

[2] Department of Animal Science, Faculty of Agriculture, Islamic Azad University, Khorasgan Branch, Isfahan, Iran.

[3] Department of Medicine, Najafabad Branch, Islamic Azad University, Najafabad, Iran.

*(Received: 6 June 2015 Accepted: 8 August 2015)*

**KEYWORDS**

Betamethasone;

Epididymal sperm;

Fertility;

Mice

**ABSTRACT:** Recent studies show that synthetic glucocorticoids alter testicular homeostasis, In this regard, the influence of Betamethasone as a glucocorticoid widely used on histological changes, epididymal sperm counts and fertility was explored in male mice. The study sample (50 mice) was allocated to 3 treatment groups, placebo and a control group. Control group was no injected. Only normal saline was given to the placebo group and Betamethasone (0.1, 0.5 and 1 mg/kg) was injected to the treatment group in peritoneum for 20 days (every other day). After treatment periods, two mice from each group were selected to measure fertility and each male with two females mice were kept for 15 days. After two weeks, the female mice were sedated and the number of embryos in the uterine horn was counted. However, epididymal sperm was counted in others mice by preparation epididymal suspension. The data analysis was done in SPSS through the Duncan's multiple ranges test. Evaluation of epididymal sections under the microscope showed difference between the group treated in epididymal tissue sections and the control group. This means that the amount of spermatozoa in treated groups with Betamethasone was lower than in the control group. Epididymal sperm and the fertility rate in all doses of Betamethasone significantly decreased compared with the control group. However, increasing the dose of Betamethasone fertility rate also non-significantly decreased. It seems that glucocorticoids like the Betamethasone affect testicular function and spermatogenesis Causes reduced fertility and have adverse effects on male reproduction.

* Corresponding author: jalal.gouyandeh@off.iaun.ac.ir (J. Gouyandeh).

## INTRODUCTION

Glucocorticoids are strongly immune suppressive and anti-inflammatory; thus, these are among the most commonly drugs prescribed worldwide [1]. Betamethasone is a synthetic glucocorticoid that readily crosses the placenta and suppresses the fetal pituitary adrenocortical axis [2]. Betamethasone reduces of binucleate cell number and placental lactogen and fetal weights in sheep [3]. The preceding research showed that in the male, the increased glucocorticoids concentration occurs before a decreas in testosterone concentration [4]. Evident chronic placental deficiency is not the cause of growth constraint resulting from the frequent maternal treatments with Betamethasone. On the contrary, it can be triggered by placental transport impairment in certain nutrients or changes in hormonal mediators of fetal development [5]. Some researchers stated that in preterm neonates, Dexamethasone and Betamethasone can similarly decrease the incidence of main neonatal mortality and morbidities. Nevertheless, it seems that Dexamethasone is more effective in decreasing the interventricular hemorrhage rate than Betamethasone [6]. In 2012, researchers examined effect of steroidal anti-inflammatory medications on fertility and viability of equine semen. They concluded that while Dexamethasone led to a premature reduction of total sperm, in sperm velocities and progressive motility, the fertility and sperm membrane integrities did not show any difference [7]. Even though a single sequence of prenatal corticosteroid management apparently reduces preterm infants' morbidity [8], effects of frequent use of corticosteroids is not understood [9]. And the other hand no study on the effect of Betamethasone on male reproduction is done. According to the evidence, the current study on male mice aimed to explore long-term outcomes of treatment by Betamethasone on fertility.

## MATERIALS AND METHODS

In this study 50 male mice from Balb/c breed and in weight range of 30±5 gr were prepared and kept for two weeks in similar condition with free access to food, water, normal light and appropriate temperature (20-25 ċ) and moisture. These favorite conditions were continued for whole period of study. Mices randomly were divided in five groups with ten replication including placebo, 3 treatment groups and a control group**.** The group of control in order to achieve normal levels of epididymal spermatozoa and ultimately fertility in a similar situation with treatment groups, but were held without injection in the duration trial. Placebo group in order to ensure that non-effect of injection of the test results and the comparison with the control group, these daily rates 0/3 mL of normal saline was injected. Every other day, Betamethasone (1, 0.5, 0.1 mg/kg) was injected to the treatment groups in peritoneum for 20 days. After this period, in order to assess the fertility (Based on the number of embryos) two male mice from each group were selected and each male mice with two females mice to mate for 15 days in individual cages kept. After 15 days, the female mice were anesthetized and the number of embryos in the uterine horn was countedand were compared with control group. However, the rest of the male mice were anesthetized and testes were dissected. Then the epididym of the testes was separated and epididymal sperm counts by preparation suspension epididymal with optical microscop and lens 40 by hemocaytometer slides were performed [10]. Other testicular'sepididymal was separated for preparation Cross sections histological by microtome device and stained with hematoxylin-eosin [11]. At the end the data analysis was done in SPSS program through the Duncan's multiple ranges test.

## RESULTS

### *Evaluation of epididymal tissue sections*

Evaluation of epididymal sections under the microscope showed dissimilarity between the control (Figure 1) and the group treated with Bethamethasone (Figure 2) in epididymal tissue sections, this means that the amount of spermatozoa in this section is less than the control group. While Betamethasone were not causes tissue destruction epididym.

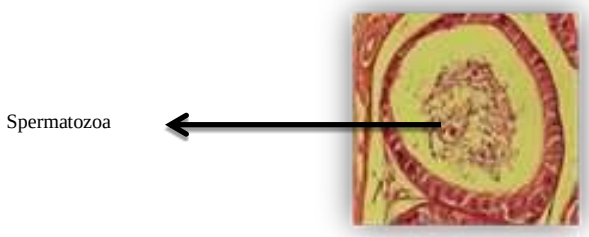


**Figure 1.** The epididymis cross section in the control group with a magnification of 400

**Figure 2.** The epididymis cross section in the group treated with Bethamethasone with a magnification of 400

### *Rate of epididymal sperm*

Epididymal sperm counts showed that significant differences were between the mean levels of epididymal sperm in the treated groups and the control group, As the amount of epididymal sperm significantly reduced when Betamethasone (doses of 0.1, 0.5 and 1 mg/kg) was injected than the control group ($P < 0.05$) (Figure 3).

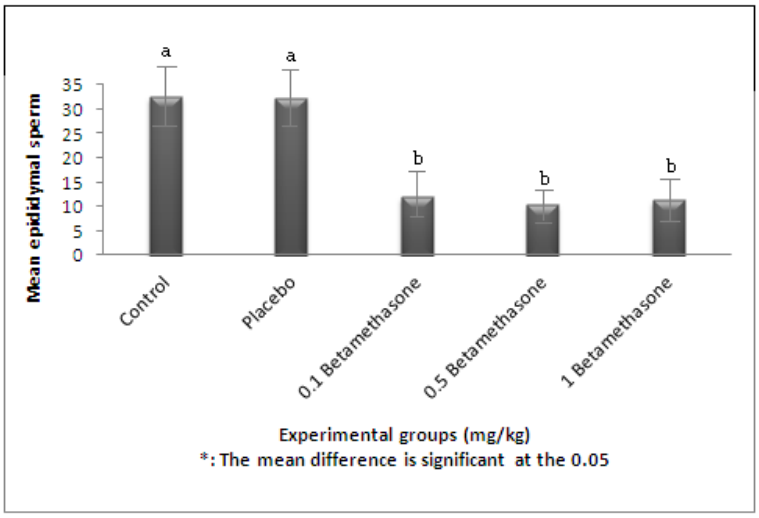


**Figure 3.** Change in epididymal sperm levels in experimental groups.
In each column means no similar letters is significant

***Rate of fertility (Based on the number of embryos)***

The results of the study of fertility based on the number of embryos in the uterine horns, showed that significant difference were between the mean fertility rate in the treated group and the control group, as fertility was compared at Betamethasone doses of 0.1, 0.5 and 1 mg/kg with the control group significantly reduced (P <0.05) and With increasing doses of Betamethasone, fertility rates non-significantly reduced (P >0.05) (Figure 4).

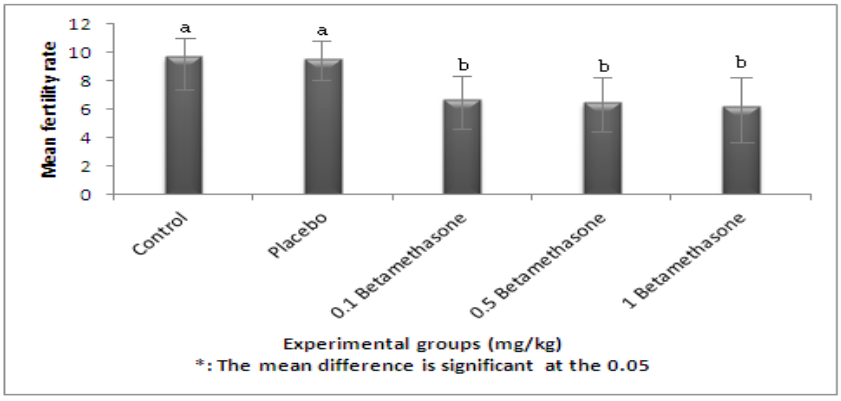


**Figure 4.** Change in fertility rate in experimental groups. In each column means no similar letters is significant

## DISCUSSION

The testis's functional unit is seminiferous tubule in mammals. Proliferation and differentiation of germ cell through an elaborate process is called spermatogenesis, which results in production and spermatozoa release from the testis. Spermatogenesis relies on dynamic interactions between the germ cells and the sertoli cells of the seminiferous epithelium as well as hormonal stimulation [12]. Decrease in spermatogenesis in the testis and ultimately reduce in the amount of epididymal sperm can be affected by many factors including reduce the amount of testosterone, FSH and increase apoptosis in testicular germ cell [13]. Also khlkute in 1977 reported that it is likely a combination affect directly on the testes or the other parts of genitals and prevented of sperm production [14]. Glucocorticoids are strongly immunosuppressive and anti-inflammatory [1]. And Since glucocorticoids can suppress the immune system are also reduced cellular immune, As a result cells against environmental factors, stress and etc are more sensitive and thus of the apoptosis probability was increased. The other hand research has shown that addition of physiological apoptosis, other factors, such as toxins and some of the chemical drugs is that cause apoptosis in testicular germ cells [15]. Increased serum concentrations of glucocorticoids induced by stress leads to inhibition of enzyme activity testosterone builder and decreased leydig cell activity and thus reduced testosterone production, testicular germ cells and primary spermatocytes in androgen-deprivation show the highest of apoptosis [16]. So according to the documents and results of this study can be said that Betamethasone (Synthetic glucocorticoid) probably is able to be either directly, to affect the activity of testis Spermatogenesis. via effects on protein expression of apoptotic germ cells, causing their apoptosis and impairs cell division, impact on Spermatogenesis and reducing sperm, Therefore as mentioned increase apoptosis testicular germ cell impairs in Spermatogenesis and reduces the testicular function and based on the results of this study, Betamethasone with a direct impact on testicular cells and increase apoptosis, reduced of testicular Spermatogenesis function, as a result, the fertility rate decreases. On the other hand, one of the results of this study seen oligonucleotides sperm (low sperm count) in the treatment groups. The oligonucleotides sperm that is the state, sperm concentration (sperm count) in semen is low and this phenomenon in treatment groups led to significantly reduced rates of fertilization and fertility, which can be sign long-term effects of Betamethasone on spermatogenesis.

## CONCLUSIONS

According to the results of this study seem to Betamethasone reduces testicular function through different mechanisms and have a negative effect on male reproduction. So, should be considered of possible side effects of using different doses of this drug.

## ACKNOWLEDGMENTS

This experiment was performed in khorasgan branch of Islamic Azad University. We would also like to sincerely thank Dr. Mehrdad Modaresi, Ms. Sara Mansouri, Dr. Akbar Pirestani and Mr. Mohammad Bagher Amini for their contributions to this study.

## REFERENCES

1. Julia C.B., 2006. Glucocorticoids: exemplars of multi-tasking. Br J Pharmacol. 147(1), 258-268.

2. Challis J.R.G., Davies I.J., Benirschke K., Hendrickx A.G., Ryan K.J., 1974. The effects of dexamethasone on plasma steroid levels and fetal adrenal histology in the pregnant rhesus monkey. Endocrinology. 95(5), 1300-1305.

3. Challis J.R.G., Braun T., Li S., Moss T.J.M., Newnham J.P., Gluckman P.D., Sloboda D.M., 2007.

Maternal betamethasone administration reduces binucleate cell number and placental lactogen in sheep. J Endocrinol. 194(1), 337-347.

4. Hardy M.P., Gao H.B., Dong Q., Chai W.R., 2005. Stress hormone and male reproductive function. Cell Tissue Res. 322(1), 147-153.

5. Moss T.J., Nitsos I., Harding R., Newnham J.P., 2003. Differential effects of maternal and fetal betamethasone injections in late-gestation fetal sheep. Journal of the Society for Gynecologic Investigation. 10(8), 474-479.

6. Elimian A., Garry D., Figueroa R., Spitzer A., Wiencek V., Quirk J.G., 2007. Antenatal betamethasone compared with dexamethasone (Betacode Trial). Obstet Gynecol. 110(1), 26-30.

7. Fioratti E.G., Villaverde A.I.S.B., Melo C.M., Tsunemi M., Papa F.O., Alvarenga M.A., 2012. Influence of steroidal anti-inflammatory drugs on viability and fertility of equine semen. Journal of Equine Veterinary Science. 32(12), 771-775.

8. Roberts D., Dalziel S., 2007. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Obstet Gynecol. 109(1), 189-190.

9. Peltoniemi O.M., Kari M.A., Tammela O., 2007. Randomized trial of a single repeat dose of prenatal betamethasone treatment in imminent preterm birth. Pediatrics. 119(2), 290-298.

10. Hashemi, M., Hosseini, S. Reproductive Physiology: Reproduction of Applications in Domestic Animal, 3rd ed, Publications of Farhang Jaame: Tehran, 2001.

11. Maleki E., 2006. Technical methods and devices used in pathology. Med & Lab Engineering Magazine. 69, 5-8.

12. Boekelheide K., Fleming S.L., Johnson K.J., Patel S.R., Schoenfeld H.A., 2000. Role of Sertoli cells in injury-associated testicular germ cell apoptosis. Experimental Biology and Medicine. 225(2), 105-115.

13. Zamiri M.J., Physiology of Reproduction, 2rd ed, Haghshenass Publications, 2009.

14. Khlkute S.D., 1977. Effects of hibiscus rosa sinesis on spermatogenesis and accessory reproductive organ in rat. Planta Medica. 31(2), 127-135.

15. Allard E., Boekelheide K., 1996. Fate of germ cells in 2, 5-hexandione-induced testicular injury. II. Atrophy persists due to a reduced stem cell mass and ongoing apoptosis. Toxicol Appl Pharmacol. 137(2), 149-156.

16. Khorsandi L., Hashemitabar M., Orazizadeh M., 2008. The study of Bax protein expression in Dexamethasone induced apoptosis in spermatogenic cells in mice. Medical Science Journal of Islamic Azad University Tehran Medical Branch. 18(3), 141-148.