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EFFECT OF INJECTION OF HUMAN MENOPAUSAL GONADOTROPHIN AND HUMAN CHORIONIC GONADOTROPHIN HORMONE ON SPERMATOGENESIS OF ADULT MALE MICE

Shaima Ahmed Rahim

Department of Biology, College of Science for Woman, University of Babylon, Iraq
E. mail: shaima.hilla@yahoo.com

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ABSTRACT

This study was designed to investigate the effect of the widely-used gonadotrophin hormones (Human Menopausal Gonadotrophin and Human Chorionic Gonadotrophin) on spermatogenesis of adult male albino mice. Thirteen mature swiss mice of Balb/C strain were used in this study. They were divided randomly into control and treated groups. Male mice in the first and second treated groups had been injected intramuscularly with (75 IU of hMG) and (500 IU of hCG) Every 48 hr, for 35 days of this dose. While the mice of the control groups had been injected with (0.9%) normal saline in the same way. The results revealed the following significant differences , when compared with control groups : There were no significant differences in weights of the reproductive organs and there were no significant differences in average of the percentage of spermatogonia and spermatocytes, While the study reports a significant decrease ($P<0.05$) in the percentage of spermatids of animals treated with hMG hormone and a significant increase ($P<0.01$) in the percentage of spermatozoa of animals treated with hMG hormone and also A significant decrease ($P<0.01$) in the average numbers of Leydig cells for the animals treated with hMG and hCG hormones and A significant decrease ($P<0.01$) in the average diameters of epididymal tubules and the height of epithelial cells of caput epididymis tubules for two animals with hMG and hCG .

Key words: hMG, hCG, spermatogenesis, testosterone, LH, FSH, sertoli cells, leydig cells

INTRODUCTION

Gonadotropins are chemical messengers regulating physiological functions including maintenance of differentiated functions of gonads (Norman and Litwack, 1997) in all vertebrates. Gonadotrophins is one of the hormones that secreted by anterior lobe of pituitary gland include LH Luteinizing hormone (ICSH Interstitial cell stimulating hormone) and FSH (Follicle stimulating hormone) and hMG (human Menopausal Gonadotrophin) and in females secreted these hormones from second place (the placenta) for example, PMSG (pregnant Mare serum Gonadotrophin) and hCG (human Chorionic Gonadotrophin). On this basis, it can be classified gonadotrophin hormones on the two main sections:

Pituitary Gonadotrophins include FSH, LH and hMG. These hormones are manufactured in specialized cells of pituitary Adenohypophysis from Basophilic Staining Cells. Studies confirmed that FSH hormone plays a pivotal role in the initiation and maintenance of spermatogenesis, induction of proliferation of Sertoli cells and spermatogonia (Heckret et al., 1998; Walker & Cheng, 2005), LH hormone is essential for manufacturing steroid hormones through its impact on the leydig cells because there are special receptors on their membranes (Vasta et al., 2006).

As for the human menopausal Gonadotrophin (hMG) it produces naturally in the pituitary gland in the post-menopause in women because a significant decrease in the level of sex hormones because of the sharp decline in the number of Primordal Follicles and levels of estrogen and progesterone, and the lack of influence of the two inhibitors at the level of the hypothalamus and pituitary gland, which leads to a rapid rise in hormones secretion of LH, FSH from pituitary gland (Guyton & Hall, 1996). And put large amounts of hMG in women urine at menopause which contains equal proportions of LH and FSH. hMG hormone extracted, purified and injected intramuscularly because the dosage oral ineffective (Diamond et al., 1997; Smitz et al,2007; Kim & Schlegel 2008).

Non-pituitary Gonadotrophins include hormones that do not product from pituitary gland and usually called Anterior Pituitary like Hormones they are: Pregnant Mare Serum Gonadotrophin (PMSG) which secreted from endometrial Cup and appears in horse blood between the day (50-90) of pregnancy and his biological characteristic like FSH hormone. And human Chorionic Gonadotrophin (hCG) which secreted from Chorionic Villi of Placenta in the first week of ovulation, its effective is to LH hormone and it is working on maintain the corpus luteum, increase the secretion of sex hormones -in females and stimulate Interstitial cells in the testes to secrete androgens in males (Cole & Cuppus, 1977; Schiff et al., 2007; Zitzmann, et al., 2013; Kumari et al., 2015). These hormones belong to glycoprotein hormones and consists of α and β units, the α unit is a regular series of 89 amino acids linked to bilateral sulfur bridges, while the β unit which return to it her Specificity hormone consists of 115 amino acids for FSH hormone, 147 amino acids for LH and hCG hormone (Findlay, 1984) and carbohydrates, which are monosaccharides Mannose, Galactose, Fructose, N-acetylglucose Amine, N -acetyl Galactose Amine and sialic acid. the sialic acid is essential to total express biological activity to gonadotrophins through interact Glycoprotin hormones with receptors on the members of the target. (Cole & Cuppus, 1977) and imposes LH and FSH hormones impact on the target tissues (testis) through activation cyclic adenosine Monophosphate (cAMP), which in turn

activates certain special enzymatic systems in the appropriate target cells (Guytan & Hall, 1996; Guytan, 1987).

The use of hormonal hMG and hCG is successful treatment for the induction of spermatogenesis and sexual maturity for patients who suffer dys-function at hypo thalamaspituitary axis (Hypogonadotropic Hypo-gonadism) (Mycek et al*.,* 2000; Frashchi et al., 2009; Balen et al*.,*1997; Shoham et al*.,* 1992). Tanaka and his group (2001) found that the estimation of hCG is useful to distinguish between Primary Hypogonadism and normal gonadal function during the period of pre-puberty and estimation of GnRH is useful to distinguish between Secondary Hypogonadism and Normal Gonadal Function during the period of puberty.

Because of the prevalence of use of Gonadotrophins in the treatment of infertility nowadays by giving it to patients by injection, so this study was conducted to detect fertilization efficiency of hCG and hMG hormones as a result of being injected to adult male mice.

MATERIALS AND METHODS

Laboratory animals: This study used a white Swiss mice *Mus musculus* of Bulb/C by 30 male aged (50 -75) days, average weights of (25-35) grams obtained from the center of Baghdad embryos and infertility / Baghdad University. Put animals in a special metal cages inside the animal house of the Department of Biology / College of Science / University of Babylon in the conditioned room where the temperature ranged between 22-27° C and under constant lighting system (12hour light: 12 hours dar -kness). The animals have been given water and feed *ad libitum*.

Tested Materials: in this current study used two hormones:

1. human Menopausal Gonadotrophin (hMG) Use the product called commercially (Pergonal) and manufact -ured by Italian Serono Company, a glass ampule filled with capacity of 1 mL and a concentration of 75IU.
2. Human Choroinic Gonadotrophin (hCG) Use the product called commercially (Human Gonadotrophin) manufac-tured by the Chinese Meheco Company, a glass ampule capacity of 2 ml and a concentration of 500IU.

Experimental protocol: The laboratory animals divided into three groups of 10 animals per group, One of them was treated with hMG hormone (75 IU)of body weight and treated the second group with hCG hormone (500 IU)of body weight, either control group animals was treated with physiological salt solution a concentration of 0.9% sodium chloride, experimental animals injected intramuscularly by (18) doses for a period of (35) days every (48) hr, it has given the size of fluid injected relative to body weight.

At the end of the treatment period, male albino mice killed in with cervical dislocation. The testes and epididymis carefully collected and calculate organs weight ratio to body weight (mg \ 100 grams) of body weight and then fixed immediately in Bouins Solution for histological examination. tissues dehydrated and embedded in paraffin 5 µm thick sections and stained with hematoxylin and eosin (Bankroft & Stevens, 1982).

Histological study

Histological slides examined using a Compound Microscope and recorded different measurements using Ocular Micrometer calibrated with Stage Micrometer. The percentage of (Spermatogenia, Spermatocytes, Spermatids and Spermatozoa) is calculated for 12 seminiferous tubules for each animal (Patra & wadsworth, 1991) and 20 reading for leydig cells as well as 20 reading for the diameters of seminiferous tubules, diameters of epididymal tubules(caput) and height of epithelial cells for caput epididymal tubules for each animal also. photographing was achieved by using Olympus model DB2 – N180 microscope which provided by computer type L

Statistical analysis: The data were expressed as Mean ± SEM, Statistical analysis was performed with ANOVA followed by Post-Hoc Tukey multiple range tests using the Statistical Package for the Social Sciences (SPSS/version 17.0/) for Windows. ($P < 0.01$), ($P < 0.05$) considered statistically significance

RESULTS AND DISSCUTION

In this study, the weight of testis of experimental animals was not significantly affected compared with the control group, but the treatment caused a decline not significant for average weights of testes for the two treatment groups and likely that this decline is due to a decline in LH, FSH content responsible for stimulate manufacturing testosterone hormone from leydig cells by feedback mechanism which leads to low level and reduction in the size of the testes and atrophy , this study was In agreement with(Thomas et al., 1994). It is known that the testosterone plays a key role in the descent of the testicles into the scrotum, development of puberty and sexual maturity and Setting of threshold for feedback reactions between the pituitary and the testis (Lunn et al., 1994; Saito et al., 2000; Raheem, 2014). While the results showed not significant changes in the rate of weights for each head and tail of the epididymis for both treatments, because the possible change in testosterone hormone level during the treatment period was not enough to change the weight of the epididymis significantly, it is known as that the epididymis growth and activity is regulated mainly by testosterone hormone (Amman, 1989; Robaire et al., 2007).

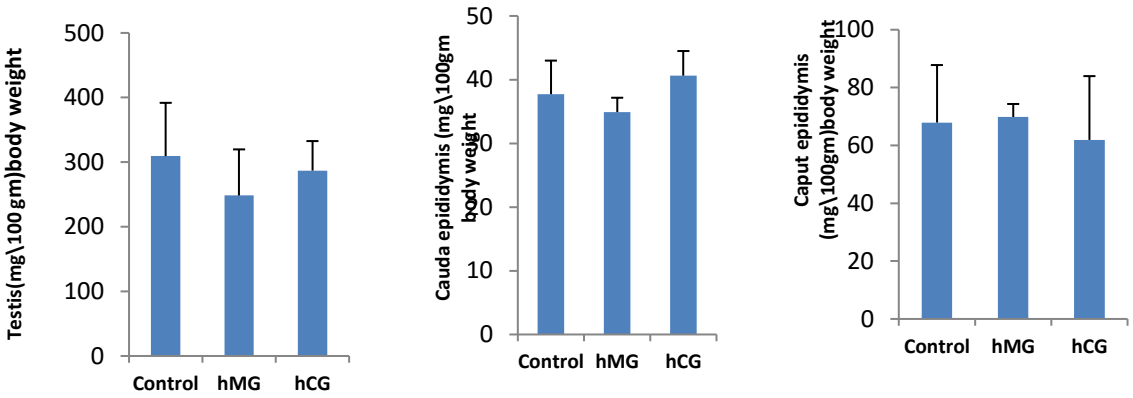


Figure 1: Effect of Injection (hMG and hCG) hormone in sex organs weight mg / 100 g body weight. Results are presented asMean ± SEM

The results of current study showed that there were no significant differences in the percentage rates of spermatogonia and spermatocytes, may be due to the reason that the mitosis of spermatogonia and formation of spermatocytes does not need to hormone stimulation as for meiosis of primary spermatocytes to secondary spermatocytes and then sperm formation requires presence of testosterone hormone (Hussain et al., 2012; Ganong, 1989).

As for the significant decrease ($p<0.05$) in the percentage rate of spermatids in experimental mice (which injected with the hMG hormone) in comparing with control and hCG hormone groups, the reason it has come back that the treatment with the FSH hormone increases androgen levels within the testis (Matikainen et al., 1994) by the possibility of stimulating the number of testicular LH receptors (Weinbauer et al., 1994; Krishnamurthy et al., 2001) , and the metabolic state of the Sertoli cell change as a result of exposure to high concentrations of FSH, as it metabolite testosterone to estradiol E2 by Aromatase enzyme (Qin & Lung, 2000). Both testosterone and estradiol have a direct effect in the negative feedback regulation to GnRH in hypo-thalamus and to FSH in the area of anterior pituitary which leads to decline in concentration of FSH (Amann, 1983; Chimento et al., 2014). Since the FSH hormone is the cardinal hormone responsible for the production and maturation of sperm, spermeiogenesis to spermatids and it is also participate in the final stages of Spermeiogensis and maturity of the epididymis (Ben - Rafael et al., 2000; Shaughnessy et al., 2010), basing on this, the low-lying levels from it cause regressive for the Spermeiogensis process and Meiosis and disruption for the Sertoli cells for her work in the production of cAMP and ABP.

In the present study, there are significant increase ($p<0.01$) in the percentage rate of sperm in treated mice with hMG hormone in comparing with control and hCG hormone groups, this is due to the low level of FSH in the anterior pituitary which leads to the reduction of numbers Sertoli cells which causes a decrease secretion inhibin (Pinilla et al., 1994; Dungen et al., 1989). Studies indicated that the inhibin possesses a major role in the negative control to FSH at pituitary level so that it works as embolic regulator to FSH hormone, and as an indicator for the maturity of the seminiferous tubule (Kolb et al., 2000; Siegel et al., 2013). On this basis, a low-lying level of the vital effective Inhibin sufficient to cause increased in secretion of FSH from the pituitary (Klaij et al., 1994) which leads to increased production of sperm process, which is made by hormonal interaction between the hypo-thalamus and Adenohypophysis and gonadotrophin cells (Sertoli cells, spermatogenic cells and leydig cells) (Ross et al., 1995, Ramaswomy & Weinbauer, 2014).

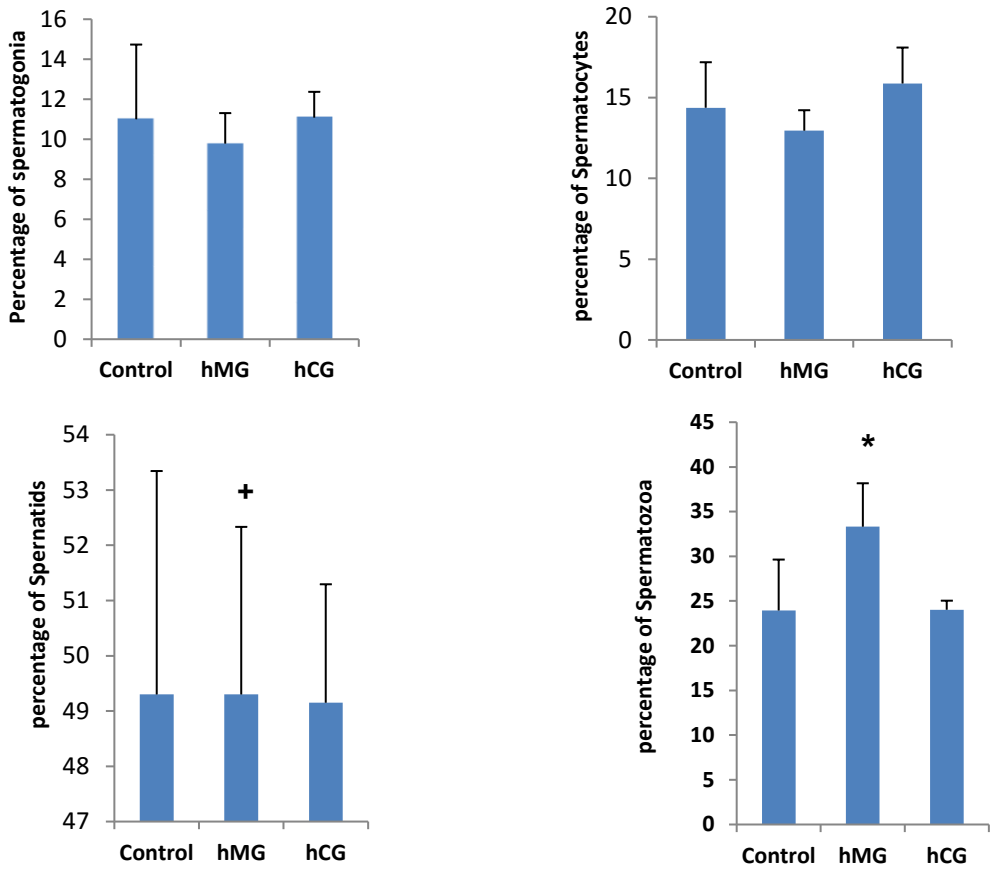


Figure 2: Effect of Injection (hMG and hCG) hormone in percentage of spermatogenic cells, Results are presented as Mean ± SEM, *P<0.01, + p<0.05 compared to the control

The current study revealed that the numbers of leydig cells in experimental mice (which injected with hMG)were significantly (P<0.01) less than the control group, The reason may be that the treatment of trophic hormones (hCG, LH) causing increased production of testosterone from the testis of various species through their effect on cAMP, which is an intermediary for the transfer of the trophic hormone on steroids manufacturing in the testis (Viho & Ruokonen, 1974; Gaytan et al., 1994).on the other hand, Matikainen and his groups 1994, noted that the FSH hormone possesses indirect catalytic effect on function of leydig cells which cause increased LH receptors and mRNA levels and steroidal response. The injection of exogenous hormones (LH) lead to increased production of testosterone from leydig cells which inhibits the secretion of LH from hypothalamus and anterior pituitary (Amann, 1983), leading it to atrophy leydig cells to produces the little from testosterone. The low-lying levels of the testosterone hormone reduce the secretion of seminal fluid and slows movement of sperm which leads to accumulating inside the testis and thus produces undesirable microenvironment for spermatogenes is (Qin & Lung, 2000).

As for the significant decline (p<0.01) in the numbers of leydig cells to hCG hormone group as compared to the control, in vivo studies have indicated that the treatment with the hCG hormone cause an increase secretion of testosterone, estrogen and androstedione (Campbell et al., 1974). Schulz and his groups1994 explained that the treatment with the hCG hormone and 17β estradiol increases GnRH content in some areas of the brain and content of Gn in the anterior pituitary thus the sensitivity of the steroidal system to gonadotrophin sufficient to raises androgen levels in plasma. High concentrations of the testosterone inhibit secretion of the GnRH on the level of hypothalamus and this in turn causes a decrease in the secretion of LH from the anterior pituitary gland, and leads to degeneration of leydig cells (which negatively affects in testosterone production).

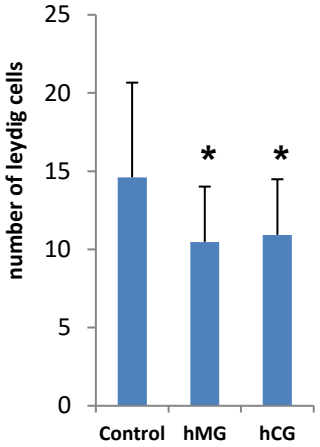


Figure 3: Effect of Injection (hMG and hCG) hormone in number of leydig cells, Results are presented as Mean ± SEM, *P<0.01 compared to the control.

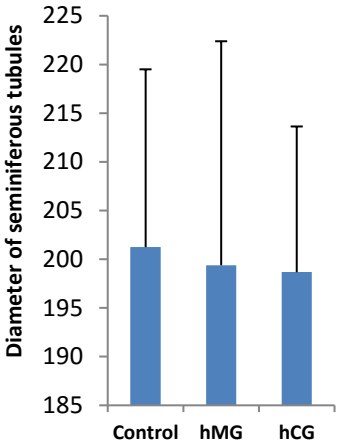


Figure -4: Effect of Injection (hMG and hCG) hormone diameter of seminiferous tubules, Results are presented as Mean ± SEM

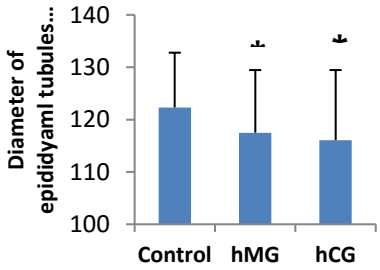


Figure 5: Effect of Injection (hMG and hCG) hormone in diameter of epididymal tubules (caput), Results are presented as Mean ± SEM. *P<0.01 compared to the control.

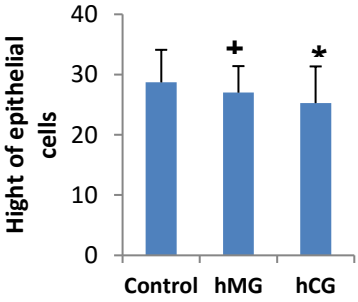
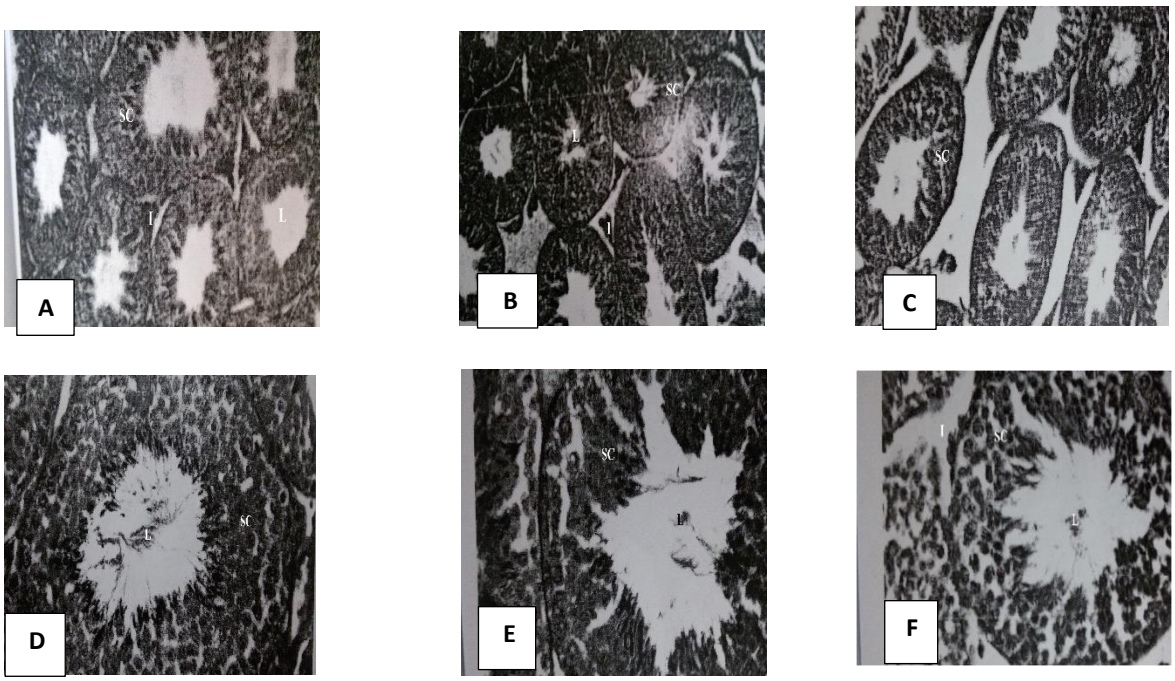
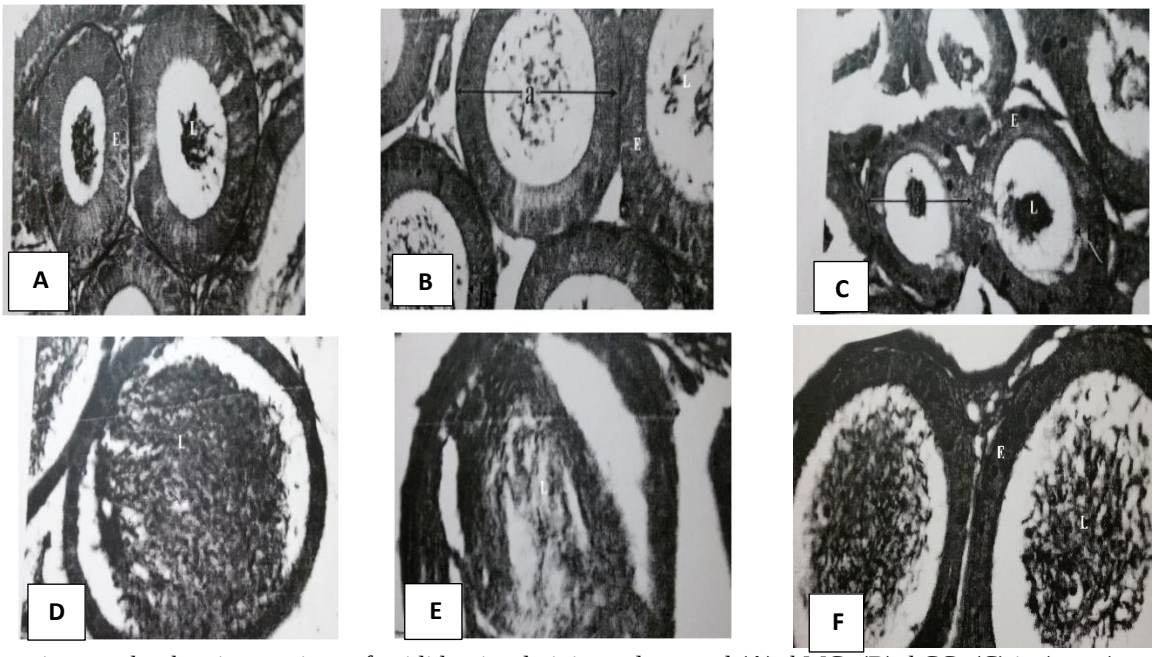


Figure6: Effect of Injection (hMG and hCG) hormone in height of epithelial cells of epididymal tubules, Results are presented asMean ± SEM. *P<0.01, + p<0.05, compared to the control.



Photomicrographs showing sections of testes administered: control 100X(A), hMG 100X (B), hCG 100X (C), control 400X(D), hMG 400X (E), hCG 400X(F), stained with H&E. I = Interstitium; L = Lumen; SC = Spermatogonic cell



Photomicrographs showing sections of epididymis administered: control (A), hMG (B), hCG (C) in (caput),control (D), hMG (E), hCG (F) in(cauda), stained with H&E at mannification 400X. L= Lumen; E=Epithelial tissue

While diameters of epididymal tubules have shown significant decreased ($p<0.01$) in treated animal with hCG, hMG compared with the control group, The reason for this decline is attributed to low level of testosterone as result of negative feedback to gonadotrophin , Some studies have shown that disorder of testosterone production and his decline leads to reduction of diameters of the epididymal tubules in rats (Nair et al., 2002), it is known that testosterone controls on development of the epididymis and stimulates manufacture specialized proteins necessary for differentiation and Capacitation testicular sperm (Jasta et al., 2002). On the other hand, the current study revealed that treatment with hMG and hCG hormone resulted in a significant decrease in the height of epithelial cells of caput epididymis it might be due to the low level of the testosterone due to declining numbers of leydig cells in these animal tubules. In conclusion, the current results indicated that hCG hormone enjoy few side effects associated with it use, especially in histological structure of the testis.

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