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REPRODUCTIVE PERFORMANCE OF MALE BOUSCAT RABBITS AS AFFECTED BY LONG-TERM TESTOSTERONE PROPIONATE OR HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION *

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ABSTRACT

Thirty, nine month old, male Bouscat rabbits were used in this research to study the long-term ,15 wks, effects of testosterone propionate (TP) or human chorionic gonadotropin (hCG) administration on reproductive performance. Rabbits were randomly divided into five equal groups (6 males each) and housed individually in standard batteries. The first two groups were injected with TP (5 and 10 mg/kg/day) while the other three groups were injected with hCG (200, 400 and 800 IU/animal/day). Semen was collected weekly using the artificial vagina and evaluated during the pre (3wks) and post (15 wks) treatment. The criteria under study were: reaction time, ejaculate volume, motility, sperm concentration/mm^3 and seminal fructose level.

The results showed no significant differences between all treatments in the reaction time and also pre and post treatments. TP administration resulted in impaired motility in both doses, while there were no obvious trends in all hCG doses. There were a highly significant differences ($P<0.01$) in ejaculate volume in both treatments due to the increase in gel part. Sperm concentration showed significant decrease in number at the end of the experiment to reach 10% of the pre-treatment values. Both doses of TP administration didn't result in any significant increase in fructose level in the liquid part of semen. Similarly, medium and high doses of hCG had no effect on fructose level. In contrast, the low dose of hCG resulted in significant increase in fructose secretion after the fifth week of injection. In conclusion, hCG can be more safe for treatment or use than TP. Therefore, additional precautions should be considered with TP usage. .

Keywords: Rabbits; Testosterone propionate; hCG; Reaction time; Semen Quality.

INTRODUCTION

Gonadal steroids as well as gonadotropin hormones are used for treatment of testes dysfunctions (Kawakami *et al.*, 1997 and 2000 in dogs), improve fertility (Tawfeek *et al.*, 1994 in rabbits) and as contraceptive. In 1970, the use of estrogen/progestins combination was introduced as contraceptive pills for females. Thereafter, several hormonal treatments were applied on males in a similar manner for contraception. Exogenous testosterone injection alone or with progestins suppresses gonadotropin secretion; consequently reduce sperm concentration in men (Zhengwei *et al.*, 1998 and McLachlan *et al.*, 2002).

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In man as well as in laboratory animals, the injection of testosterone (T) and its esters increases serum androgen level and might affect intratesticular androgen level (Abdel-Raouf and Paulsen 1987). This kind of treatments causes impairment in spermatogenesis with subsequent drop in sperm concentration (oligospermia or azoospermia) in the ejaculate.

On the other hand, chorionic gonadotropin stimulates Leydig cells to secrete androgen. Accordingly, it was found that human chorionic gonadotropin (hCG) increases androgen level in the testicular veins at certain doses, consequently, it affects fertility (Abdel-Raouf, and Paulsen 1987; El-Gaafary *et al.*, 1994 and Yamani *et al.*, 1994) and spermatogenesis. Therefore, the aim of the present study is to compare the effect of long-term administration (15 wks) of testosterone propionate TP or hCG in different doses on seminal characteristics in normal adult male Bouscat rabbits.

MATERIALS AND METHODS

1-Experimental animals:

Thirty, nine month old, pure male Bouscat rabbits, purchased from the research farm, college of Agriculture, Assiut University, were used in this study. Animals were tagged carefully by tattooing and then housed individually in standard batteries. Feed (rabbit's standard diets) and water were available ad lib., throughout the experimental period (3wks pre treatment and 15 wks post treatment).

2-Experimental design and treatments:

Rabbits were divided into five groups, 6 males each. Two doses of TP (low and high) and three doses of hCG (low, medium and high) were used. The first group (TPL) was injected with 5 mg /kg/day, while the second group (TPH) was injected with 10 mg/kg/day. The other three groups were given hCG; 200 (CGL), 400 (CGM) and 800 (CGH) IU/animal/day. Testosterone propionate was obtained from Eli Lilly Co., Indianapolis, Ind., USA, while hCG (Coriantin) was purchased from the Nile Drugs Co., (Cairo production) under the license from Ormonotera, Milan, Italy. Drugs were injected intramuscular in the front muscle of the thigh. Injection time was carried out at 9:00 AM and following semen collection on the proposed day. Treatments were continued for 8 successive weeks, but since slight changes were obtained, treatment was continued for 7 more weeks.

3-Studied parameters:

3.1 Reaction time:

It was measured by determining the time (seconds) required for the treated male to show any sign of sexual behavior (mating attempts, mounting and / or ejaculation) after introducing a receptive female.

3.2 Semen collection:

Semen samples were collected at weekly intervals during the experimental periods. Samples were collected from each animal for 3 successive weeks before applying any treatments. These samples were evaluated and used as self control for each treatment. Males were trained to serve on artificial vagina with suitable temperature (45-50C^{o}), pressure and lubrication. Thereafter, semen samples were evaluated:-

3.3 Ejaculate volume:

Measured directly from the calibrated collection tube (gel was separated from the fluid portion and measured separately).

3.4 Motility:

Motility was examined by placing a small drop of fresh semen on a clean warm slide, then diluted with two drops of phosphate buffer (pH 7.4) at the same temperature and covered with cover slide. Examination was done under the microscope to the nearest 10%.

3.5 Sperm concentration:

Sperm number was counted by using the heamocytometer.

3.6 Fructose Concentration (initial):

In less than a minute after semen collection, 0.1-0.2 ml. from the fluid part of semen sample was deproteinized by sodium hydroxide and zinc sulphate. The deproteinized filtrate was then stored at -20 C^{o} until assay. Fructose concentration (mg/100ml semen) was determined according to Mann (1946).

4-Statistical Analysis:

The Statistical analysis was conducted using SAS; General Linear Model (GLM; SAS, 1996). Means were separated by LSD when treatments showed significant differences.

RESULTS

1-Sexual behavior (reaction time):

The effect of long-term TP or hCG administration on sexual behavior as determined by the reaction time show insignificant differences during the whole experimental period. The range of the reaction time pre and post treatment was 3.6 ± 0.36 to 6.0 ± 0.17 seconds.

2-Ejaculate volume (gel part):

The present results show that TP treatment in both doses resulted in highly significant increase ($P<0.001$) in the gel volume just one week after treatment. The average of gel volume pre-treatment was 0.3 ml then increased to 0.7 ml just one week post treatment, Fig.(1A). Starting from the second week post injection, the volume greatly increased significantly compared to the pre-treatment volume throughout the entire experimental period. It is interesting to mention that some animals ejaculated up to 13 ml of the gel.

Human chorionic gonadotropin in low doses resulted in significant increase ($P<0.05$) in the gel volume which sustained significant up to the 7^{th} week, then sporadically increased (with low significance) in the 11^{th}, 13^{th}, and 15^{th} weeks. In between the previous weeks, gel was still higher than the pre-treatment level but it was not significant. In the other two treatments, data show that hCG in the medium dose resulted in a significant increase ($P<0.01$) in gel volume during the first and second post treatment weeks, Fig. (1B). Thereafter, the averages were almost near to the pre-treatment averages.

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Also, the high dose of hCG resulted in significant increase (P<0.05) in the gel volume during the whole treatment period with the exception of a few and sporadically weeks that showed non-significant differences. It is clear that TP resulted in great increase in the gel volume due to the effect of exogenous androgen on the accessory reproductive gland. Meantime, hCG resulted in an increase of the gel component in the ejaculate due to the increase of endogenous androgen secreted by the testis in response to the injected gonadotropin. In the high dose, the testis almost continuously respond to the effect of hCG .The response in the low dose continued for the first 7 weeks of treatment and was sporadically thereafter, while in the medium doses the response took place during the first two weeks and was abolished thereafter.

3-Ejaculate volume (fluid part):

No significant differences were found among treatments in the fluid part volume, Fig. (1C and D).

4-Motility:

The effect of TP and hCG treatment on sperm motility is presented in Fig. (2 A and B). In general, TP administration resulted in impaired motility in both doses. Nevertheless, this significant (P<0.05) impairment started earlier in the TPH group (7th week) than the TPL group (10th week). For the TPL group, the impairment continued and the significance increased during the following weeks till the end of the experiment. Although the motility in the TPH group was lower than the pre-treatment period and observed until the end of the 15th week, however, this was not regularly significant due to the high individual variations. On the other hand, the low dose of hCG resulted in significant (P<0.05) improvement of motility in the 4th and 5th weeks, but this was followed by reduction in the percent of motile spermatozoa which became significant during the 8th, 9th, and 10th week. The impairment was very sharp between the 14th and 15th week. The medium dose of hCG resulted in a significant increase (P<0.05) in the percentage of motile sperm during the 4th week and the significant decrease (P<0.05) on the 11th and 13th weeks. The other semen samples show slightly lower or equal percent of motile sperms compared to the pre-treatment. The high dose of hCG resulted in slight insignificant variations in the percent of motile spermatozoa. The TP administration resulted in a clear cut and apparent impairment of sperm motility than the low and medium doses of hCG, while the high dose was not effective.

5-Sperm concentration:

The concentration of spermatozoa per unit volume shows almost the same trend as the total sperm concentration per ejaculate, Fig., (2C and D). This mainly because the fluid part didn't show great variation. Both TP and hCG treatments resulted in significant decrease (P<0.05) in sperm count which was late, less steep and less significant in hCG group. The averages of pre-treatment sperm number were 339.2±44.8, 372.9±54.5, 380.0±39.9, 401.0±29.0 and 308±56.3 x 10^3 sperm/mm^3 in the TPL, TPH, CGL, CGM and CGH respectively. There were no significant differences in pre-treatment values among the different groups. Neither TP nor hCG administration resulted in azoospermia in any of the treated animals.

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TP in both doses resulted in significant decrease in sperm count already by the second post-injection week, (176.7±37.3 and 178.0±27.5 x 10^3 sperm/mm^3 in TPL and TPH, respectively). Starting from the 6th post-treatment collection, sperm count was significantly ($P<0.05$) lower than the pre-treatment average and continued to decrease to reach about 10% of the formal level by the 15th week,(43.0±16.7 and 40.4±38.0 x 10^3 sperm/mm^3in TPL and TPH, respectively). The low and medium doses of hCG resulted in variations in sperm count similar to that obtained by TP. However significant decrease ($P<0.01$) in sperm number was observed one week earlier in CGM and one week later in CGL. Another difference is that the significance was greater in the CGM than the TP. By the end of the 15th week, sperm concentrations reached about 10 % of the pre-treatment level.

6-Fructose level:

The results in Fig. (3A and B) show that both doses of TP administration didn't result in any significant increase in fructose level in the liquid part of semen, although, there were slight variations under and above the central line. Similarly, medium and high doses of hCG had no effect on fructose level. On contrast, the low dose of hCG resulted in significant increase ($P<0.05$) in fructose secretion after the fifth week of injection, and continued for 7 successive weeks. Also, another rise was re-observed in the 14th week.

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DISCUSSION

The previous studies showed that the administration of testosterone alone or in a combination with other hormones, decrease sperm production in many animals in addition to men (Zhengwei *et al.*, 1998 ; McLachlan *et al.*, 2002 and Meriggiola *et al.*, 2002). The present results show reduction in

sperm count/mm^3 in TP as well as hCG administrations. The reduction in sperm concentrations almost reached the maximum in both treatments and in all doses around week 10 after injection. The percent changes compared to the pre-treatment period were 15.9 and 15.8% in TPL and TPH groups and 15.7, 22.4, 17.2% in CGL, CGM and CGH groups, respectively. Although, a significant decrease in sperm count was observed in all groups, however, azoospermia was not attained in this study. The drop in sperm production is actually due to the direct suppression of gonadotropin from the pituitary gland by the exogenous testosterone through the negative feed back mechanism or indirectly through the testosterone produced by the testes in response to hCG. The percent changes in sperm count at the end of the experiment (15 weeks) were almost 10 % compared to the pre-treatment period (100%) in all groups. This reduction in sperm concentrations indicated that most of the studied males showed an oligospermia as a result of the applied treatments. Sperm number reflected by the percent change showed a steady decrease until week 10 and 11 in the hCG and TP group , respectively. Sperm number rebounded early in both treatments for one week then returned to decline again till week 15. A similar result was found in monkeys treated with testosterone, where the sperm number dropped at week 14, thereafter, sperm count transiently rebounded until week 21 of the study, (Weinbauer, *et al.*, 2001). The unfavorable rebound effects in both studies might reflect the neuroendocrine regulation of spermatogenesis in rabbits and non-human primate model. Even though oligospermia was achieved in this study, however, males from all treatments showed a strong sexual behavior throughout the experimental period. This was obvious from the reaction time .and proves that the presence of testosterone is crucial in such these studies to prevent the loss of sexual desire and maintain the activity of the male accessory glands.

Semen consists of two parts; sperm cells from the testis and seminal plasma from the accessory glands. In the present study, TP treatment predominantly increased gel volume in both doses, while hCG sporadically increased the volume of the gel part in the low and high doses and only in the first 2 post treatment weeks in the medium dose. This may be due to the high dose of exogenous testosterone which resulted in significant ponderal increase in the weight of all the accessory genital glands. Meantime, hCG didn't result in any or slight insignificant ponderal changes in these animals .This increase is directly due to the effect of exogenous testosterone on the accessory gland and indirectly through the increase of endogenous androgen secreted from the testis in response to the injected gonadotropin (hCG). On the other hand, the liquid part didn't show any significant variations due to either TP or hCG administration

It is well known that fructose secretion from the accessory glands is under the effect of testosterone hormone. Mann (1964) reported that castration of rabbits resulted in disappearance of fructose from the ejaculate, but the post castration fall can be prevented or restored by testosterone implantation. On the other hand, Kosiniak and Lesiniak, (1976) failed to find an increase in the fructose level after injecting 50 mg TP intramuscularly at two days intervals. Therefore, it seems that there is a fructose threshold

since the exogenous testosterone (TP) or the endogenous hormone that resulted from hCG injection didn't produce any significant rise of seminal fructose in this study. In conclusion, both TP and hCG resulted in severe oligospermia in adult rabbits.

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الاداء التناسلى فى ذكور ارانب البوسكات و تأثير حقن بروبيونات التيستيستيرون والحاثة المشيمية البشرية

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أجريت الدراسة على ثلاثين أرنبا ذكرا بالغا من سلالة البوسكات (٩ شهور) والتى قسمت عشوائيا الى خمس مجموعات متساوية بكل منها ستة ذكور. تم حقن المجموعتين الأولى والثانية ببروبيونات التستستيرون بجرعة قدرها ٥ ثم ١٠ ملجم/يوم على التوالى بينما حقنت المجموعات الثالثة والرابعة والخامسة بجرعات قدرها ٢٠٠، ٤٠٠، ٨٠٠ وحدة دولية/حيوان/يوم على التوالى. علما بأن الحقن العميق كان يتم فى عضلات الفخذ الأمامية. ولقد تم جمع السائل المنوى باستخدام المهبل الإصطناعى بمعدل قذفه واحدة أسبوعيا ، ولمدة ثلاث أسابيع متتالية قبل الحقن والتى اعتبرت مرجعا لمقارنة تأثير العقاقير المستخدمة على خصائص القذفة المنوية ثم استمر الجمع بعد الحقن لمدة ١٥ أسبوعا. وكانت الصفات تحت الدراسة: وقت التفاعل ، حجم القذفة ، حركة وتركيز الحيوانات المنوية وتركيز سكر الفركتوز فى بلازما السائل المنوى.

ولقد أظهرت النتائج وجود اختلافات غير معنوية بالنسبة لوقت التفاعل. وقد أدى الحقن بالهرمون الخصوى بجرعتيه الى خفض حركة الحيوانات المنوية ، ولم يلاحظ اى تغيير معنوى فى حجم الجزء السائل من القذفة المنوية عند استخدام اى من العقارين المستخدمين فى حين لوحظ وجود زيادة معنوية فى حجم الجزء الجيلاتينى عند استخدام الهرمون الخصوى بجرعتيه. ولقد انخفض تركيز الحيوانات المنوية انخفاضا معنويا باضطراد مع تركيز العقاقير المستخدمة ووصل التركيز فى نهاية التجربة الى قرابة ١٠% من التركيز قبل الحقن. هذا ولم يلاحظ اى زيادة معنوية فى مستوى الفركتوز باستثناء الجرعة المنخفضة من الحاثة المشيمية البشرية (hCG). وخلص الباحث الى ان استخدام (hCG) أكثر أمانا من بروبيونات التستستيرون والتى تتطلب استخدامها مزيدا من الاحتياطات.