

SEXUAL ACTIVITY, SEMEN CHARACTERISTICS AND TESTOSTERONE LEVELS IN MATURE MALE RABBITS TREATED WITH HORMONAL AND NON-HORMONAL PREPARATIONS

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SUMMARY

This study was carried out to determine the influence of human chorionic gonadotropin (hCG), L-Carnitine (LC) and Royal jelly (RJ) treatments on Sexual activity, semen characteristics and testosterone levels of mature New Zealand White (NZW) males rabbit. A total of 20 NZW bucks were randomly assigned into four groups (5 bucks/each). The 1st group bucks were left without treatment (Control group). The Rabbit bucks of the 2nd group injected IM with 50 IU of hCG / male / weekly for 6 weeks (hormonal group). While the 3rd and 4th male groups (LC and RJ) received 50 mg LC daily and 100 mg RJ /kg BW, orally, respectively for 6 weeks (non-hormonal groups). The results revealed that the bucks treated with LC and RJ had significantly decreased ($P < 0.01$) in the duration of the reaction time with prominent increase in the mating activity than hormonal treatment (hCG) and control groups. However, the semen ejaculate volume in bucks treated with LC and RJ were significantly higher ($P < 0.01$) than hCG and control groups. Also, there were no significant differences in semen pH values among groups. While, the overall mean of testosterone concentrations in rabbits treated with LC and RJ were significantly higher ($P < 0.01$) than in hCG and control groups. In conclusion, the results indicated that, both non-hormonal treatments (L-carnitine and Royal Jelly) had a positive and significant effect on reproductive activity and semen quality in mature NZW male rabbits.

Keywords: *human chorionic gonadotropin, L-Carnitine, Royal jelly, Libido, semen characteristics*

INTRODUCTION

Male rabbits with little libido and low sperm production are usually detected among rabbitries. Some sources suggested enhancement of those animals treated with GnRH or hCG (Hsu *et al.*, 1987 and Rebollar *et al.*, 1998). The application of hCG stimulates the leydig cells of testes to produce testosterone (Altoé *et al.*, 2014). Male rabbits are the most important component in reproductive success, especially when artificial insemination (AI) is used on a regular basis in rabbit farms and one male affects the fertility of several females. Human Chorionic Gonadotrophin (hCG) is a glycoprotein hormone secreted by the placenta of pregnant women. It is structurally and biologically similar to luteinizing hormone (LH), (Abdel-Raouf, 2009). However, male rabbits with little libido and low sperm production are usually detected among rabbits, and those animals treated with GnRH or hCG may be enhanced, according to Hsu *et al.* (1987) and Rebollar *et al.* (1998). Also, the leydig cells of the testes are stimulated to generate testosterone when hCG is applied (Altoé *et al.*, 2014).

L-Carnitine is a small, water-soluble particle synthesized in the liver and kidneys from the amino acids lysine and methionine with the help of vitamin C and other body components (Rebouche, 1991 and Leibetseder, 1995). However, Pirestani *et al.* (2011) found that the L-Carnitine is important not only for initiating sperm motility, boosting sperm maturation,

and improving sperm fertilization, but also for regulating Sertoli cell capacities, protecting sperm from oxidative damage, and reducing spermatogenic cell death (Abdelrazik and Agrawal, 2009). In a similar study, Al-Daraji and Tahir (2014) looked at the effects of different doses of LC on male bucks and discovered that LC improved semen quality traits such as ejaculate volume, mass, and individual motility of spermatozoa, as well as spermatozoa concentration, while decreasing the percentages of dead and abnormal spermatozoa. Furthermore, it has been demonstrated that taking LC or one of its derivatives causes an increase in testosterone levels (Abo-Ghanema *et al.*, 2012).

Royal jelly (RJ) is a milky liquid secretion produced by the pharyngeal glands of bees. Its primary function is to nourish the queen, forming an anatomically and physiologically distinct individual from the workers, with increased reproductive potential (Kohnno *et al.*, 2004). In mice and rats, RJ has a terrific effect on sperm development as well as a reduction in defective sperm (Amirshahi *et al.*, 2014 and Ghanbari *et al.*, 2015). Furthermore, El-Hanoun *et al.* (2014) discovered that RJ given in a water solution to adult male rabbits boosted libido, raised serum testosterone, and reduced reaction time in RJ groups (50, 100, and 150 mg/kg BW) compared to the control group.

The goal of this study was to see how human chorionic gonadotropin (hCG), L-Carnitine (LC), and Royal jelly (RJ) treatments affected sexual activity,

semen quality, and testosterone levels in adult NZW male rabbits.

MATERIALS AND METHODS

Study location and experimental animals:

The present study was carried out at private farm (Almarai farm) in Mallawy, El-Minia governorate (located at latitude of 28° 07' 6.00" N, longitude 30° 44' 23.99" E and about 52 meters above sea level). A total number of 20 New Zealand White (NZW) rabbit bucks aged 7-8 months with average body weight (2505±15.61 gm) were randomly assigned into four groups (5 males / group), the experiment lasted for 6 weeks. The first group bucks were left without treatment and served as control group. Rabbits bucks of the 2nd group (Hormonal group) treated with hCG (Epifasi, EIPICO, M.O.H Reg. No. 25480/2008, Egypt) 50 IU / male / IM weekly for 6 weeks, Each lyophilized ampoule contains: hCG, 5000 IU, each solvent ampoule contains: Sodium chloride 0.9% (1ml) and inactive ingredients which involve; Lactose, dipotassium hydrogen phosphate and potassium dihydrogenphosphate.

The rabbit bucks of the 3rd administered L-Carnitine 50 mg /kg BW /day / orally for 6 weeks, L-Carnitine capsules (L-Carnitine, Mepaco, M.O.H Reg. No. 20679/99, Egypt), mixed with distilled water and given orally using asyringe (5 ml). Each capsule contains 350 mg L- Carnitine (as tartrate). While in the 4th group animals administered Royal jelly 100 mg /kg BW /day / orally for 6 weeks, Egyptian fresh RJ was obtained from a local bee products company (KONOZ Company, Egypt). Frozen Royal Jelly tubes were placed at room temperature for thawing then mixed with distilled water and given orally using asyringe (5 ml).

All rabbits were housed individually in suspended galvanized wire cages provided with feeders and automatic drinking system (nipples), in semi closed farm. All animals were kept under similar managerial, hygienic and environmental conditions, with controlled light (16^{hrs} light & 8hrs dark), temperature (18 to 21°C) and relative humidity (50 to 55%). Fresh water and commercial pelleted feed were provided *ad libitum* throughout the experimental period. The pelleted commercial diet (Almarai feed) containing crude protein 18%, crude fat 3-4%, crude fiber 13-15% and metabolizable energy content 2520 K Cal/ Kg diet was used for feeding animals.

Data Collection:

Sexual behavior and Semen evaluation:

Sexual desire (libido) was estimated by recording the reaction time (seconds) which was the beginning from introducing the doe to the buck until complete ejaculation according to (Hussein *et al.*, 2012 and Seleem, 2003). Mating activity (repeat mating within

15 minutes) of each buck was determined using sexually receptive doe according to Seleem *et al.* (2006). Semen samples were collected artificially from each buck once a week for six weeks by means of an artificial vagina (handmade). The temperature of the lumen of the artificial vagina (AV) ranged from 45 to 50°C at collection (Andrade *et al.*, 2002). Bucks have been previously acclimatized to this routine. Two successive ejaculates were collected; each ejaculate was kept separately for assessment, the average values of both first and second ejaculates were determined (Khadr *et al.*, 2015). Gel ejaculates were discarded. Ejaculates containing urine, abnormal color and any deposits were discarded. Semen samples ejaculated from each rabbit buck were evaluated individually. The parameters including; semen ejaculate volume (ml), individual and mass sperm motility (%), live and dead spermatozoa (%) assessed by Eosin-Nigrosin stain technique, morphological abnormal spermatozoa (%) assessed by Alkaline Methyl violet stain, sperm-cell concentration (Nx10⁶/ml) estimated by haemocytometer were estimated according to (Salisbury *et al.*, 1978, Seleem, 2003 and Khadr *et al.*, 2015) and semen pH by using pH-paper (pH 5-9, Combi screen, Germany) according to (Hussein *et al.*, 2012). Also, live body weight at the start and the end of treatments (6 weeks) was estimated.

Blood sampling:

Blood samples were collected by veni puncture from the jugular vein into collection non-heparinized tubes and centrifuged at 4000 r.p.m for 15 minutes, and then serum was obtained and stored at -20°C till assay. Blood samples collected weekly (Before the morning feeding about 8-10 A.M) to measure testosterone hormone. The testosterone level was assayed by ELISA kit (Enzyme immunoassay for the quantitative determination of testosterone concentration (TESTO-RIA- CT, Louvain-La-Neuve, Belgium).

Statistical analysis:

Statistical analysis of the data obtained in the study was performed by using the Statistical Package for the Social Sciences (SPSS) computer programs (2006), method of analysis (Snedecor and Cochran, 1982). Significant differences among sub-class means were analyzed by Duncan's multiple range tests (Duncan 1955). Differences between the groups were calculated with the ANOVA test.

RESULTS

Libido and mating activity:

Effect of hormonal and non-hormonal treatments on sexual desire (libido) and mating activity of rabbit males are presented in Table 1. The results revealed that the non- hormonal treatments in LC and RJ groups significantly (P<0.01) decreased the duration of the reaction time with prominently increase in the

mating activity than *hCG* and control groups. Also, the mating activity of groups LC and RJ were significantly ($p < 0.01$) higher than *hCG* and control groups.

Table 1. Effect of hormonal (*hCG*) and non-hormonal (LC, RJ) treatments on the libido (Reaction time/ Sec) and mating activity of mature NZW male rabbit during 6 weeks of treatment (Mean \pm SEM; n=5)

Groups	Control (G1)	<i>hCG</i> (G2)	LC (G3)	RJ (G4)	Sig
Libido (Reaction time/ Sec)	18.83 ^a \pm 0.48	17.84 ^a \pm 0.42	8.38 ^b \pm 0.13	8.68 ^b \pm 0.42	**
Mating activity (during 15 minutes)	2.00 ^b \pm 0.11	2.26 ^b \pm 0.13	2.70 ^a \pm 0.12	2.83 ^a \pm 0.09	**

Different superscript letters indicate significance within the same row, NS = No Significant. **= probability ($P < 0.01$).

Semen evaluation:

Data presented in Table 2, shows the ejaculate volume (ml), mass motility (%) and sperm viability (Live sperm %) of rabbit bucks treated with LC and RJ which were significantly ($P < 0.01$) higher than those treated with *hCG* and control groups. However, the sperm individual motility (%) of bucks treated with LC and RJ were significantly ($P < 0.05$) higher than those of treated group with *hCG*, without significant difference among the other treatments. While, there were no significant differences in semen pH values between groups.

The data of the sperm cell concentration ($N \times 10^6/ml$) in G3 and G4 presented in Table 2, were

significantly ($P < 0.05$) higher than G2. Additionally, the registered sperm abnormalities (%) in LC and RJ groups were (14.20 and 13.95) significantly ($P < 0.01$) lower than that in the control (17.75) and *hCG* (21.66) groups, respectively. The sperm cell concentration ($N \times 10^6/ml$) of rabbit bucks treated with LC and RJ were significantly ($P < 0.05$) higher than G2. The present results showed that the treatment with LC and RJ induces a significant ($P < 0.05$) increase in the ejaculate volume (ml), mass and individual motility (%), sperm live (%), sperm cell concentration and abnormality (%). However, no significant differences among treatments in the pH of semen were found.

Table 2. Effect of hormonal (*hCG*) and non-hormonal (LC, RJ) treatments on semen parameters of mature NZW male rabbit for 6 weeks of treatment (Mean \pm SEM; n=5)

Semen Evaluation	Control (G1)	<i>hCG</i> (G2)	LC (G3)	RJ (G4)	Sig
Ejaculate Volume (ml)	0.38 ^b \pm 0.0	0.43 ^b \pm 0.01	0.61 ^a \pm 0.01	0.62 ^a \pm 0.01	**
pH	7.30 \pm 0.04	7.22 \pm 0.03	7.24 \pm 0.02	7.24 \pm 0.05	NS
Mass Motility %	55.02 ^b \pm 3.53	56.00 ^b \pm 3.35	68.66 ^a \pm 3.08	73.66 ^a \pm 1.71	**
Individual Motility %	52.78 ^{ab} \pm 4.54	46.39 ^b \pm 2.65	62.77 ^a \pm 4.05	58.33 ^a \pm 1.27	*
Live Sperms %	73.59 ^b \pm 2.37	72.80 ^b \pm 0.47	83.03 \pm 1.02a	81.99 ^a \pm 1.82	**
Sperm-cell concentration ($N \times 10^6/ml$)	325.72 ^{bc} \pm 14.27	312.00 ^c \pm 16.66	395.05 ^a \pm 24.02	373.33 ^{ab} \pm 10.61	*
Sperm Abnormalities %	17.75 ^b \pm 0.54	21.66 ^a \pm 0.20	14.20 ^c \pm 0.19	13.95 ^c \pm 0.55	**

Different superscript letters indicate significance within the same row, NS = Not- Significant. *= probability ($P < 0.05$) **= probability ($P < 0.01$)

Testosterone concentrations (ng/ml) in serum of NZW male rabbits during hormonal and non-hormonal treatments:

The recorded levels of testosterone hormone (ng/ml) during the experimental period at 2, 4, 6 weeks and overall mean, were presented in Table 3. After two weeks (W2) of treatments, the results

showed that the *hCG* group had significantly ($p < 0.05$) higher level of testosterone than control and LC groups, but no significant difference compared to RJ group. While, LC and RJ had no effect on testosterone levels throughout the first two weeks of treatment.

Table 3. Effect of hormonal (*hCG*) and non-hormonal (LC, RJ) treatments on testosterone levels (ng/ml) of mature NZW male rabbits during 6 weeks of treatment (Mean \pm SEM; n=5)

Groups	Control (G1)	<i>hCG</i> (G2)	LC (G3)	RJ (G4)	Sig
2 Weeks	1.59 ^b \pm 0.19	2.72 ^a \pm 0.09	1.86 \pm 0.27	2.21 ^{ab} \pm 0.23	*
4 Weeks	1.74 ^b \pm 0.28	1.52 ^b \pm 0.27	3.07 ^a \pm 0.16	3.22 ^a \pm 0.22	**
6 Weeks	1.94 ^b \pm 0.06	1.54 ^c \pm 0.13	3.12 ^a \pm 0.08	2.96 ^a \pm 0.08	**
Overall mean	1.76 ^b \pm 0.10	1.93 ^b \pm 0.06	2.68 ^a \pm 0.15	2.80 ^a \pm 0.15	**

Different superscript letters indicate significance within the same row, NS = Not- Significant. *= probability ($P < 0.05$) **= probability ($P < 0.01$)

At the fourth week (4W) of treatment, testosterone level was significantly ($p < 0.01$) higher in LC and RJ groups than in control and *hCG* groups. Also, the testosterone level after six weeks (6 W) of the treatment had significantly ($p < 0.01$) higher in LC and RJ groups than in control and *hCG* groups.

DISCUSSION

The decrease in reaction time (libido) and increase mating activity in males treated with LC and RJ (non-hormonal treatments) may be due to the increasing of testosterone level (Table 3) compared with *hCG* (hormonal treatments) and control groups. Where, there is a clear relationship between high testosterone level and improvement of libido and sexual activities of rabbit males (Hafez and Hafez, 2000 and Kamel *et al.* 2009). Also, the delayed reaction time and low mating activity in *hCG* and control groups seems to be due to low in testosterone levels during experimental period. The present results are in agreement with those reported by Seleem *et al.* (2006) who found that, supplementation of LC to the rabbit bucks significantly improved libido (reaction time) and mating activity (number of mating during 15 minutes). However, El-Hanoun *et al.* (2014) found that the RJ administered to adult male rabbits has a positive effect on libido while the reaction time was reduced in RJ groups (50,100 and 150 mg/kg BW) in comparison with control group, furthermore, the authors stated that reducing in reaction time might be due to the increase of testosterone level in the rabbits treated with RJ, which had higher levels of testosterone hormone than the control animals.

The improvement in semen characteristics of bucks treated with LC may be due to vital role of LC not only in acquiring sperm motility, supporting sperm maturation and improving sperm fertilizing ability, but also in controlling Sertoli cell functions and protective spermatozoa against oxidative harmful, decreasing apoptosis of spermatogenic cells and reducing sperms accumulation (Abdelrazik and Agrawal, 2009). However, increased ejaculate volume and higher sperm numbers of rabbits receiving LC were reported by Jacyno *et al.*, (2007). Also, LC can advance fatty acid and energy production by transporting fatty acids into mitochondria for β -oxidation, consequently enhance ATP production (Vanella *et al.*, 2000), this produce energy is utilized for sperm respiration and motility (Aliabadi *et al.*, 2012). LC is required for energy metabolism which supports sperm motility, maturation and the spermatogenic process by providing easily available energy to sperms (Cheah and yang, 2011).

Regarding to the positive effect of RJ on semen parameters, would be due to the presence of complex composition of a lot of nutrient factors (as antioxidants, water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes and hormones, etc.) which make RJ have powerful

characteristics as a natural product and this is reflected on fertility and increased testosterone of males. El-Hanoun *et al.* (2014) indicated that increased ejaculate volume of adult males treated with RJ may be due to increased secretion of seminal fluids from the sex accessory glands due to increased testosterone level. Abdel Hafiz and Muhamad (2008) concluded that RJ contains fructose, and RJ acids which increase sperm motility.

RJ contains vitamin C, vitamin E (Bayer, 1990) and arginine (Boselli *et al.*, 2003), vitamins E and C are well- certified antioxidants and have been shown to reduce free-radicals that induce damage to sensitive cell membranes of the spermatogenic epithelium (Ebisch *et al.*, 2006). Also, vitamin E improved semen quality parameters (sperm count, motility and normal morphology) and testosterone in male rats (Oyeyemi *et al.*, 2015). Arginine plays an important role as essential amino acid in spermatogenesis; it is a biochemical precursor in the synthesis of putrescine, spermidine and spermine, which are necessary for sperm motility (Cheah and yang, 2011).

The higher level of testosterone at the second week (W2) of treatments in *hCG* group may be due to the stimulating effect of *hCG* which have LH-like activity on the interstitial cells. The subunits of *hCG* and LH are structurally similar and they act on the same receptor on Leydig cells (Madhukar and Rajender, 2009), LH induce Leydig cells to synthesize and produce testosterone (Yang *et al.*, 2012). On the other hand, in an experiment on rats, which received *hCG* a significant increase in testosterone concentration had been taken place (Damber *et al.*, 1981). While, LC and RJ did not significantly improve testosterone level during the first two weeks of treatment, this may be due to the short limited period (at 2W).

The high levels of testosterone during 4W, 6W and overall mean of the period of treatment in males treated with LC may be attributed to the role of LC in transports of fatty acids to the mitochondria to generate energy in the form of ATP needed by the cells to perform their functions (Bhat *et al.*, 2010). On the other hand, the positive effect of RJ on testosterone concentrations during 4W, 6W and overall mean of the period of treatment may be due to the RJ have hormonal activities which improve reproductive performance and fertility of males, in addition to the RJ contain mineral salts. Some studies reported that RJ is containing testosterone and have steroid hormone-type activities (Hidaka *et al.*, 2006 and Bogdanov, 2017). When RJ was administered to male rats, it increased both of LH and testosterone production (El-Banby, 1987).

The present results showed that the males treated with *hCG* exhibited decrease in testosterone concentration after 4 and 6 weeks of treatment (Table 3) compared to the other groups, it seem that *hCG* did not improve and may reduce testosterone level as

general due to ant hormone produced by the body as an immune reaction, this negative effect is reflected on sexual activity (libido and mating activity) additionally did not improve or gradually decrease weekly semen parameters which include ejaculate volume, mass motility, individual motility, live sperm and sperm concentration and increased abnormal spermatozoa during 3, 4,5 and 6 weeks of the treatment .

The negative effect of *hCG* on reproductive activity and semen parameters may be due to the repeated injection induce the body to produce anti-*hCG* as antibodies. Some studies indicated that repeated administration of *hCG* to mares has been shown to result in antibody production and decreased clinical responsiveness to the hormone (Roser *et al.*, 1979).

CONCLUSION

The obtained results indicated that, both non-hormonal treatments (L-carnitine and Royal Jelly) had a positive and significant effect on reproductive performance in male NZW rabbits since semen characteristics were improved. On the other side, the hormonal treatment (*hCG*) had a negative effect on sexual desire and semen characteristics of mature males.

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النشاط الجنسي وخصائص السائل المنوي ومستويات هرمون التسترون في ذكور الأرناب الناضجة المعالجة بمستحضرات هرمونية وغير هرمونية

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هدفت هذه الدراسة إلى تقييم ومقارنة تأثير الهرمون المشيمي البشري (hCG) والمعاملات الغير هرمونية (Royal و L-Carnitine) على النشاط الجنسي وخصائص السائل المنوي ومستويات هرمون التسترون في ذكور الأرناب الناضجة وإشتملت هذه التجربة على عدد ٢٠ ذكر ناضج جنسيا من سلالة الأرناب النيوزيلندي الأبيض، تم تقسيمها عشوائيا الى أربعة مجموعات (٥ ذكور لكل مجموعة) كالتالي: مجموعة (١) وهي المجموعة الضابطة (كنترول) وكانت بدون أية معاملات، مجموعة (٢) عوملت بالحقن باستخدام الهرمون المشيمي البشري (hCG) بمعدل ٥٠ وحدة دولية/حيوان حقن عضلي/أسبوعيا لمدة ٦ أسابيع، مجموعة (٣) تم تجريعها باستخدام إل-كارنيتين (LC) بجرعة ٥٠ ملجم/كجم من وزن الجسم يوميا لمدة ٦ أسابيع، مجموعة (٤) تم تجريعها باستخدام الرويال جلي (غذاء ملكات النحل) (RJ) بجرعة ١٠٠ ملجم/كجم من وزن الجسم يوميا لمدة ٦ أسابيع. أظهرت النتائج أن المعاملات الغير الهرمونية (LC و RJ) خفضت بشكل معنوي من مدة وقت رد الفعل أو الرغبة الجنسية (وهو الوقت اللازم من تقديم الأنثى للذكر وحتى إتمام عملية القذف) مع زيادة معنوية في نشاط التزاوج (وهي عدد مرات التزاوج خلال مدة ١٥ دقيقة) مقارنة مع المعاملة الهرمونية (hCG) و المجموعة الضابطة. وكان حجم القذف في الذكور المعاملة مع LC و RJ أكبر بشكل معنوي من تلك الموجودة في مجموعة hCG والمجموعة الضابطة. بينما لم تكن هناك فروق معنوية في قيم درجة الحموضة للسائل المنوي بين جميع المجموعات. وبالنسبة لتركيز هرمون التسترون كان المتوسط العام لتركيز هرمون التسترون في الذكور المعاملة مع LC و RJ أعلى بشكل معنوي مقارنة بمجموعة hCG والمجموعة الضابطة. تشير النتائج الحالية إلى أن المعاملات الغير هرمونية (إل-كارنيتين أو الرويال جلي) كان لها تأثيرات إيجابية على الأداء الجنسي وجودة السائل المنوي في ذكور الأرناب الناضجة.

الكلمات الدالة: الهرمون المشيمي البشري، الكارنيتين، غذاء ملكات النحل، الرغبة الجنسية، تقييم السائل المنوي