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

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Submitted: 5/4/2022; Accepted: 19/6/2022; Published: 6/7/2022

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 Gonadotropin (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 (Kohn et al., 2004). In mice and rats, RJ has a horrific 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 80° 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. Rabbit 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 (16hrs 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± SEM; n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (G1)</th>
<th>hCG (G2)</th>
<th>LC (G3)</th>
<th>RJ (G4)</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Libido</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Reaction time/Sec)</td>
<td>18.83±0.48</td>
<td>17.84±0.42</td>
<td>8.38±0.13</td>
<td>8.68±0.42</td>
<td>**</td>
</tr>
<tr>
<td><strong>Mating activity</strong></td>
<td>2.00±0.11</td>
<td>2.26±0.13</td>
<td>2.70±0.12</td>
<td>2.83±0.09</td>
<td>**</td>
</tr>
</tbody>
</table>

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 x 10⁶/ml) in G3 and G4 presented in Table 2, were significantly (P<0.05) higher than G2. Additionally, the registered sperm abnormalities (%) in hCG 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 x 10⁶/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 (Means± SEM; n=5)

<table>
<thead>
<tr>
<th>Semen Evaluation</th>
<th>Control (G1)</th>
<th>hCG (G2)</th>
<th>LC (G3)</th>
<th>RJ (G4)</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate Volume (ml)</td>
<td>0.38±0.0</td>
<td>0.43±0.01</td>
<td>0.61±0.01</td>
<td>0.62±0.01</td>
<td>**</td>
</tr>
<tr>
<td>pH</td>
<td>7.3±0.04</td>
<td>7.22±0.03</td>
<td>7.24±0.02</td>
<td>7.24±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Mass Motility %</td>
<td>55.02±3.53</td>
<td>56.00±3.35</td>
<td>68.66±3.08</td>
<td>73.66±1.71</td>
<td>**</td>
</tr>
<tr>
<td>Individual Motility %</td>
<td>52.78±4.54</td>
<td>46.29±2.65</td>
<td>62.77±4.05</td>
<td>58.33±1.27</td>
<td>*</td>
</tr>
<tr>
<td>Live Sperms %</td>
<td>73.59±2.37</td>
<td>72.80±0.47</td>
<td>83.03±1.02a</td>
<td>81.99±1.82</td>
<td>**</td>
</tr>
<tr>
<td>Sperm-cell concentration (N x 10⁶/ml)</td>
<td>325.72±14.27</td>
<td>312.00±16.66</td>
<td>395.05±24.02</td>
<td>373.33±10.61</td>
<td>*</td>
</tr>
<tr>
<td>Sperm Abnormalities %</td>
<td>17.75±0.54</td>
<td>21.66±0.20</td>
<td>14.20±0.19</td>
<td>13.95±0.55</td>
<td>**</td>
</tr>
</tbody>
</table>

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 at2, 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±SEM; n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (G1)</th>
<th>hCG (G2)</th>
<th>LC (G3)</th>
<th>RJ (G4)</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Weeks</td>
<td>1.59±0.19</td>
<td>2.72±0.09</td>
<td>1.86±0.27</td>
<td>2.21±0.23</td>
<td>*</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>1.74±0.28</td>
<td>1.52±0.27</td>
<td>3.07±0.16</td>
<td>3.22±0.22</td>
<td>**</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>1.94±0.06</td>
<td>1.54±0.13</td>
<td>3.12±0.08</td>
<td>2.96±0.08</td>
<td>**</td>
</tr>
<tr>
<td>Overall mean</td>
<td>1.76±0.10</td>
<td>1.93±0.06</td>
<td>2.68±0.15</td>
<td>2.80±0.15</td>
<td>**</td>
</tr>
</tbody>
</table>

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-Hanounet 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 spermatooza 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.

ACKNOWLEDGEMENTS

The authors express their gratitude to the academic staff of the Department of Animal Production at Al-Azhar University (Assiut branch), as well as engineer/Yousef Aziz Beshay, the owner of Almarai farm, for his assistance in providing the rabbits needed to conduct the experiment and all other available facilities within his farm.

REFERENCES


النشاط الجنسي وخصائص السائل المنوي ومستويات هرمون التستوستيرون في ذكور الأرانب الناضجة المعالجة

⇒ الكيميائية ومستويات هرمون التستوستيرون في ذكور الأرانب الناضجة المعالجة

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هـدفت هذه الدراسة إلى تقييم مقارنة تأثيرات الهормون المشيمى البشري (hCG) والمعاملات الغير هرمونية (Royal Jelly و L-Carnitine) على النشاط الجنسي وخصائص السائل المنوي ومستويات هرمون التستوستيرون في ذكور الأرانب الناضجة وتشمل هذه التجربة

إلى عدد 20 ذكر ناضج جنسيًا من سلالات الأرانب البرازيلية الأبيض. تم تقسيمهم عشوائيًا إلى أربعة مجموعات (5 ذكور لكل مجموعة) كالتالي: مجموعة (1) وهي المجموعة الضعيفة (كانت بدون أي معاملات). و مجموعة (2) مجموعة من (hCG) لمدة 15 دقيقة عشية لمن الذكور لمدة 3 أسابيع. مجموعة (3) تم تجريبيها باستخدام الـ hCG (100 ملغ/كم من وزن الجسم يوميا لمدة 3 أسابيع). مجموعة (4) تم تجريبيها باستخدام الـ RJ (RJ) وLC (LC) بجرعة 100 ملغ/كم من وزن الجسم يوميا لمدة 3 أسابيع. أظهرت النتائج أن المعاملات الغير هرمونية (hCG JS) وLC (LC) قادرة على تحسين النشاط الجنسي. و من جهة أخرى، لم تؤثر معالمة hCG (hCG JS) والتستوستيرون في الورم مع المعالمة مع LC (LC) على بشكل معوي في قيم درجة الجموحة للسائل المنوي بين جميع المجموعات. بالنسبة لتركيز هرمون التستوستيرون، كانت المتوسط العام لكثير من المعالمة مع LC (LC) أعلى بشكل معوي مقارنة مع مجموعة الـ RJ (RJ) وLC (LC) إلى أن المعاملات الغير هرمونية (إل. كارتين، أو الروايل جيلي) كان لها تأثيرات إيجابية على الآداء الجنسي وجودة السائل المنوي في ذكور الأرانب الناضجة.

الكلمات الدالة: الهورمون المشيمى البشري، الكرتاني، غذاء مكتات النحل، الرغبة الجنسية، تقييم السائل المنوي