Comparative study of Equine Chorionic Gonadotropine and Licorice Extract (Glycyrrhiza Glabra) on the some Reproductive Traits in Awassi Ram Lambs

Salah M. Al-Shammary1, Ihsan H. Al-Timimi1 and Ahmad A. Al-An1

1 Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad
2 Chief Researcher, Ministry of Agriculture Baghdad, Iraq
E-mail: therio-ial@yahoo.com
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Summary
This study was conducted to find out the effect of licorice extract and eCG on some reproductive traits in Awassi ram lambs. This study was conducted on State Board for Agriculture Researches, Animal Researches station. Twenty four Awassi ram lambs at the age of 67-77 days were randomly divided into four groups according to the lambing date as follow: Group (A) was injected with eCG 300 I.U/lamb/week. Group (B) was treated with licorice 300 mg/kg B.W/week orally. Group(C) also was treated licorice 400 mg/kg B.W/week orally. Group (D) were kept as a control group. Testes measurements were taken at the 12th week of the study period. The level of testosterone hormone was measured at monthly intervals. Age of puberty, number of mounts/successful ejaculation were recorded. Testes measurements length and depth of right and left of animal in groups B and C showed significantly (P< 0.05) higher values than the control and group A. Width of the right and left testes of lambs in all groups did not record significant differences. Circumferences of the testes showed higher (P< 0.05) values in group B compared to other groups. Serum testosterone concentration increased in all animals during the experimental periods but group B and C showed significantly (P< 0.05) higher values than other groups. Age of puberty and sperm production of the lambs in the control group showed significantly (P < 0.05) higher values compared with all groups, but group A recorded significantly (P< 0.05) higher values compared with groups B and C, while groups B and C recorded significantly (P< 0.05) lower age of puberty compared with groups A and control. The number of Mounts/successful ejaculate decreased with progress of age in all groups, while the control group recorded significantly (P< 0.05) higher number than other treated groups. Group B recorded significantly (P< 0.05) lower values than A and C groups.

Keywords: eCG, Licorice, Awassi ram, Awassi Lambs.

Introduction
Nutritional management is one of the aspects that has been used to improve animals fertility (1). Previous studies suggested that nutritional supplements such as vitamins, cotton seeds and lupine improved reproductive performance of sheep (2). Recent studies have been directed for food organization by using herbs and medical plants as a therapeutic for many diseases and a base to avoid the side effects of drugs and hormones and to provide an alternative sources or support the chemical medicine (3 and 4).

This first sources of these medical plants are to get benefit from them, which are considered as a natural and economic source in order to rise the nutritive value of the diet, improve the economic input for production and consumer safety (5).

Licorice (Glycyrrhza glabra Linn) is one of the plants that have many important nutritional components (6). It has been used since the 2100 before Christ for disease treatment (7). Licorice extract has many phytochemical compounds (8). Treatment with licorice extract improved semen quality and sexual activity in Awassi rams due to the role of licorice as anti-oxidant agent (9), increase testis volume and sperm production (10) improvement of mass activity and individual motility and decrease of dead and abnormal sperms (11 and 12). This study was to evaluate the effect of licorice on the age of puberty in Awassi lamb rams.
Materials and Methods

Twenty four healthy Awassi male lambs at age of 67-77 days and body weight (20.10±1.20 kg) were used. These lambs were divided equally (according body weight) into four groups. They were also supplied freely with tap water and mineral blocks.

Radioimmunoassay of testosterone is a competition assay. Samples or calibrators are incubated with $^{125}$I-labeled testosterone in antibody coated tubes. After weaning measurements (length, depth, width and circumferences) of testes for all groups at the end of the period of the experiment by using a vernia and a regle.

The data were analyzed by using Complete Randomized Design (C.R.D) in one way a nova (13).

Results and Discussion

The length of the R and L testes showed no differences between group B and C, however, and showed significantly (P < 0.05) higher values than those of group A and the control group. The results of group A and the control group represented no significant differences between them (Table, 1).

The result of the depth of the right testes of the animals in the group B recorded significantly (P<0.05) higher values compared with groups A and control, while group C showed significantly (P<0.05) higher value in the depth of the right testes compared with control group. While the depth of the left testes of animals in the group B and group C significantly (P<0.05) higher values compared with group A, while no significant differences were obtained between groups A and the control.

The circumferences of the testes of the animals in the group B showed significantly (P<0.05) higher values compared with groups A and control (table, 1), while group C showed numerically higher values than group A and control , but no significant differences between groups A and control was existed.

Testes size (length, width, depth and circumference) increased with body weight and age progress (14). Animals in groups B and C showed significant (P<0.05) higher values than the groups A and control in length, depth of right and left testes and circumference in group B only, the increase in the most testes measurements in the treated groups B and C could be due to licorice extract contents such as vitamins, minerals and volatile fatty acids specially vitamins E and selenium (15). Natural health (16) showed that vitamin E and volatile fatty acids have vital role in biological process occurs in the testes, because testes are organs of male reproductive system and contain a large numbers of tissues of high efficiency to produce steroids and semen, thus the testes grow better as a result of such process and they correlated with the growth in these tissues.

Table, 2 shows that the level of testosterone was increased in the animals of all groups. Testosterone level in animals of group B showed significantly difference (p<0.05) compared with the control group at the 8th week of the studied period, while at the 12th week this level showed significantly difference (p<0.05) compared with all groups of the studied period.

The testosterone level increased progressively with age, testosterone levels in all animals increased because the animals were under growth ,but the level of testosterone in treated groups (B and C) significantly (P<0.05) increased in comparison with the groups A and control this could be due to effect of nutrient contents in licorice extract to be as an antioxidant defensive system and during its direct effect on interstitial cells in the testes (2).The increase in the body weight has an effect on hypothalamus pituitary plexus and lead to increase secretion of testosterone hormone (17). Production and secretion of testosterone accompanied with an increase in testes weight, the testosterone level increases with age until it reached puberty and sexual maturity as anabolic hormone (18).

Age of puberty in the control group was significantly (P<0.05) longer as compared with the other groups. Group A recorded significantly (P<0.05) higher values compared with group B and group C (Table, 3).

The significant differences in the age of puberty of the studied animals could be due to
treatment with Licorice which cause an increase in appetite and enhance digestion, which leads to increase the metabolism for nutrients like amino acid, glucose and volatile fatty acids. Nutrition is the main limiting factor for reproduction in animal production system (19), nutrition appears to be the major modulators of sexual activity in small ruminants. However feed, additives can enhance puberty (20). The treatment with licorice extract improved semen quality (9). This could be due to the role of licorice as anti-oxidant agent, which might improve the stages of spermatogenesis, maintained LH receptors and increase FSH and testosterone concentrations (21).

Licorice improving the physical properties of semen ; individual and mass motility for the animals treated with licorice root extract (17). There is link between better nutrition and higher sperm producing in the testes of the animals(20). The significant differences in the age of puberty of the animal in group A compared with control group could be due to eCG lead to improve semen traits of the ram semen ejaculate volume, mass activity and individual motility, sperms concentration, sperm livability rates and decrease the rate of sperms abnormality (22), also lead to increase testosterone level in blood plasma and increased rams semen ejaculation volume (23). The age of puberty of Awassi ram lamb was about 8.5 months (about 255 days) (24) while Ghannam et al., (25) recorded (232-235) days as the age of puberty in Awassi lambs in Iraq.

The results of (Table 4) indicated that the number of mounts/successful ejaculation of the treated groups were significantly (P<0.05) lower than the control group, the lower number of mounts may be due to the treatment with eCG or licorice which results in an increase in testosterone hormone and increased in their sexual behavior which improves libido rams (1). However, the treatment with eCG has less effect on the sexual behaviors than that of licorice, this difference was significant (P< 0.05) between group B and A. (4) showed that treatment with licorice extract improved sexual activity in adult rams, this may be due to the role of licorice as anti-oxidant agent, which might improve the stages of spermatogenesis, maintained LH receptors and increase FSH and testosterone concentrations (22). It is well known that both sexual behavior and pheromone production are androgens dependent (26). The high level of their hormones would increase testis volume, sperm production and the high level of testosterone also improves libido of treated rams with licorice.

### Table 1: Effect of Licorice extract or equine Chorionic Gonadotropin eCG on Testes measurement (cm) of Awassi male lambs (means ± SE).

| Parameter                  | Treatment       | Group A         | Group B         | Group C         | Control         | LSD Value |
|----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|
| Length R.T                 |                 | 11.37±0.55      | 14.07±0.54      | 13.3±0.89       | 11.80±0.58      | 1.596     |
| Width R.T                  |                 | 19.37±0.94      | 20.6±0.5        | 19.90±1.10      | 18.60±0.83      | 2.07      |
| Depth R.T                  |                 | 52±2.64         | 58.60±2.01      | 56±4.00         | 49±2.49         | 6.43      |
| Circumferences of the testes|                 | 24.62±2.62      | 29±4.36         | 26.60±1.61      | 23.9±1.05       | 3.67      |
| Length L.T                 |                 | 11.75±0.83      | 13.40±0.60      | 13.3±0.46       | 11.70±0.59      | 1.45      |
| Width L.T                  |                 | 18.37±0.94      | 20.10±0.53      | 19.60±0.97      | 18.10±0.78      | 1.931     |
| Depth L.T                  |                 | 47.50±3.97      | 55.80±2.41      | 56.40±2.72      | 49.60±2.73      | 6.91      |

Different letters mean significant differences among treatments at one period at 5% level. RT=right testis, LT=left testis.
Table 2: Effect of Licorice extract or eCG on Testosterone Hormone (ng.mol/L) of Awassi male lambs (means ± SE).

| Period Week | Treatment | Group A | Group B | Group C | Control | LSD Value |
|-------------|-----------|---------|---------|---------|---------|-----------|
| 0           |           | 0.0998±0.04 a | 0.2772±0.06 A | 0.2848±0.20 A | 0.0148±0.03 A | 0.29 N.S   |
| 4           |           | 0.253±0.16 a  | 0.845±0.30 A  | 0.6648±0.55 A  | 0.1446±0.06 A  | 0.77 N.S   |
| 8           |           | 1.455±0.80 ab | 2.706±0.67 A  | 1.208±0.48 A  | 0.655±0.46 B  | 1.53       |
| 12          |           | 2.182±0.23 c  | 10.458±1.89 A | 4.628±1.33 B  | 0.75±0.71 C  | 1.48       |

Different letters mean significant differences among treatments at one period at 5% level.

Table 3: Effect of Licorice extract or eCG on Age of Puberty (days) of Awassi male lambs (Means ± SE).

| Treatment | Total average |
|-----------|---------------|
| Group A   | 171.83±3.91 b | 149.33±1.5 c | 153.50±3.08 C | 207.00±3.97 a | 170.417±4.98 |

Different letters mean significant differences among treatments at one period at 5% level.

Table 4: Effect of Licorice extract or eCG on number of Mounts/successful ejaculation of Awassi male lambs (Means ± SE).

| Period week | Treatment | Group A | Group B | Group C | Control | LSD Value |
|-------------|-----------|---------|---------|---------|---------|-----------|
| 21          |           | 2.20±0.12 b | 1.80±0.10 C | 2.00±0.08 Bc | 2.70±0.14 A | 0.20      |
| No. of lambs| 3         | 6       | 6       | 1       |         | --        |
| 22          |           | 2.00±0.13 b | 1.70±0.12 C | 1.90±0.12 bc | 2.56±0.16 A | 0.21      |
| No. of lambs| 5         | 6       | 6       | 1       |         | --        |
| 23          |           | 1.90±0.08 b | 1.70±0.10 C | 1.80±0.08 bc | 2.41±0.14 A | 0.24      |
| No. of lambs| 5         | 6       | 6       | 3       |         | --        |
| 24          |           | 1.80±0.11 b | 1.60±0.06 C | 1.70±0.12 B  | 2.34±0.12 A  | 0.22      |
| No. of lambs| 6         | 6       | 6       | 4       |         | --        |

Different letters mean significant differences among treatments at one period at 5% level.

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دراسة مقارنة لتأثير محولات القند المشيمية للفرس (eCG) و مستخلص عرق السوس على بعض الصفات التكاثرية لذكور الحملان العواسية

صلاح مهدي الشمري 1
احسان حمودي التميمي 1
أحمد علاء الدين العاني 4

1. فرع الجراحة والتوليد - كلية الطب البيطري - جامعة بغداد
2. محطة البحوث الحقلية - وزارة الزراعة - العراق

الخلاصة

اُجريت هذه الدراسة لمعرفة تأثير مستخلص عرق السوس والـ eCG على بعض الصفات التكاثرية في الحملان الذكري، أربع وعشرون من ذكور الحملان العواسية بـ 74 - 77 يوم قُسّمتْ بشكل عشوائي إلى أربع مجموعات طبقاً لوزن الجسم كالآتي: مجموعة (A) خيمتْ بـ 300 وحدة دولية eCG حامل إسبوعي. مجموعة (B) عولجتْ بعرق السوس 300 ملغ/كم² وزن الجسم إسبوع عن طريق الفم. Also أُعطيت كثرة الحيوانات (C) مجموعه 400 ملغ/كم² وزن الجسم إسبوع عن طريق الفم. مجموعة (D) كمجموعة سيطرة. أُخذت قياسات الخصية في الأسبوع الثاني عشر من التجربة، أضاً قُقياس مستوى هورمون التستستيرون فقلم تم قياسه كل شهر. عمر البلوغ الجنسي وانتاج السائل المنوي وعدد مرات الصعود الناجح تم قياسها. تأثير الدراسة بينت أن: قياسات الخصى (طول وعمق) في المجموعتين C و B أظهرت فروقات إحصائية (P<0.05) أعلى من مجموعة A و السيطرة بينما لم تسجل باقي المجموعات فروقاً هامة. أظهرت المجموعة B قيم أعلى بشكل إحصائي. محيط الخصية أظهر فروقات هامة. أظهرت جميع المجموعات الأخرى (P<0.05) في المجموعة B مقارنة بـ كل المجموعات الأخرى. محيط الخصية أظهر فروقات (P<0.05) بين المجموعة (B) بـ جميع المجموعات الأخرى. المحيط الجنسي وانتاج السائل المنوي في المجموعة السيطرة أظهرت فروقات معنوية (P<0.05) بين المجموعات A و C. فجوات معتدلة (P<0.05) بين المجموعات A و B. والسيطرة. عدد مرات الصعود الناجح ارتفع بشكلية مع تقدم العمر في كافة المجموعات، أظهرت مجموعة السيطرة فجوات قليلاً (P<0.05) في العدد مقارنة بـ كافة المجموعات، بينما سجلت مجموعة فجوات قليلاً (P<0.05) في ثلاثة مجموعات A و B و C.

الكلمات المفتاحية: هورمون مصل الفرس الحامل، عرق السوس، الذكور العواسية.