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Endocrine Response Tests for Fertility Assessment in Awassi Rams in Jordan

AL-Omari, H.Y.[1], Ababneh, M.M.[2] and Beni-Domi, M.Q.[3]

ABSTRACT

The obective of this study was to determine testosterone profiles in fertile and subfertile Awassi rams, and to develop endocrine response tests for investigating subfertility. Nine Awassi rams were divided equally into three groups: low (LF), medium (MF) and high (HF) fertility groups. Semen quality, testicular and epididymal ultrasonic images and testosterone (T) profile were examined for all rams. Results indicated that both ultrasonographical images of testicles and semen parameters did not differ significantly among groups. Motility and concentrations of the sperms were lower in the LF (58.8±13.3 % and $0.9\pm0.5\times10^9$/ml, respectively) group. Testosterone concentrations were significantly higher in HF (2.7±0.1 ng/ml) group compared to the other groups (0.99±0.2 and 0.7±0.1 ng/ml for LF and MF, respectively). Three small doses of Gonadotrophin Releasing Hormone (GnRH) and Human Chorionic Gonadotrophin (hCG) injections resulted in significantly lower testosterone concentrations in LF (3.2±0.2 and 2.9±0.3 ng/ml, repectively) group than the other ones. In conclusion, T profile and hCG response tests can be used to determine subfertile rams that passed breeding soundness exam.

Keywords: Endocrine Response Tests, Testosterone Profile, Subfertility, Awassi Rams.

INTRODUCTION

In Jordan, Awassi sheep numbers have increased to reach about 2.25 million in 2007 (Agricultural Statistical Book, 2007). The percentage of lambs born to ewes that lambed is around 105%, while for lambs born to exposed ewes is about 87% (Thomson et al., 1989). The poor fertility or infertility of rams causes major economic losses to the sheep industry. It was estimated that 16 to 25% of breeding rams had reduced fertility in the USA (Fitzgerald and Perkins, 1993).

Fertility indicates that males are functionally capable of producing and ejaculating normal fertile sperm. In addition, the female must provide a competent reproductive system suitable for fertilization, embryo and fetal development, and finally birth of lambs (Foote, 2003). Rams infertility or subfertility may be due to poor nutrition, adverse environmental and managemental conditions, genetic factors, diseases, poor semen quality and endocrine disturbances (Bruere, 1986; Fitzgerald, 1997).

It is important to note that breeding soundness exam (BSE) of the male be determined accurately and with precision before the breeding season. The results for this exam should be made available for sheep flock owners to eliminate non satisfactory breeding rams from the flock. Potential breeding soundness of rams and bucks in

[1]Department of Animal Production, Faculty of Agriculture, Mu'tah University, Karak, 61710 Jordan, P.O. Box: 7, Email: alomari75@yahoo.com (Corresponding author).
[2]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan.
[3]Department of Animal Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid Jordan.
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Jordan begins from June to August to have lambs and kids during winter and spring seasons. However, potential breeding soundness and levels of LH, FSH and testosterone are highly augmented during short days starting from summer (Pelletier et al., 1988; Hammoudi et al., 2010). Highly fertile rams with high sex drive and good experience in copulation settle more ewes earlier and produce more twins than subfertile rams (Hulet, 1977). Unfortunately, subfertile rams are not identified until the end of breeding season when high percentage of ewes fail to conceive or become pregnant late in the breeding season leading to significant economic losses (Ott and Memon, 1980). BSE would not identify all infertile rams and the only final proof of rams' fertility is pregnancy in a significant percentage of ewes. This study was designed to determine if endocrinological testing would add more value to the current rams' BSE and identify subfertile rams. Therefore, this study has two main objectives. The first objective aims at determining testosterone (T) profiles in fertile and subfertile Awassi rams, while the second one is to develop endocrine response tests.

MATERIALS AND METHODS

Animals

Nine Awassi rams, maintained at the National Center of Agricultural Research and Extension (NCARE), Khanasry Station for Livestock Research, Northern part of Jordan at 32°30` N, 59°35` E and an altitude of 860 m above the sea level, were used in this experiment. Rams were fed concentrate diets composed of barley (68%), wheat bran (18%), soybean (12%), alfa-alfa (0.2%), table salts (1%), multiminerals (1%) and multivitamins (1%). The rams were selected and divided depending on the lambing rates from the last two breeding seasons into low (LF), medium (MF) and high (HF) fertility groups (Table 1). Allocation of treatments to the three groups of rams based on complete randomized design are presented in Figure 1.

Table 1: Means (± SEM) of age, body weight, scrotal dimensions and lambing rates in low, medium and high fertility Awassi rams groups.

Parameters	Low	Medium	High
Age (year)	4.2±0.80	2.5±0.60	3.4±0.80
Body weight (kg)	53±4.6	61.7±2.7	53.3±6.1
Scrotal length (cm)	11±0.60	10.3±0.30	10.7±0.70
Scrotal circumference (cm)	29.7±0.30	28.7±0.30	30.3±1.4
Lambing rate (%)*	$(43.9)^{a}$ (25/57)	$(66.6)^{bc}$ (36/54)	$(82.5)^{c}$ (47/57)

*Lambing rates with different superscripts are significantly different ($p<0.05$).

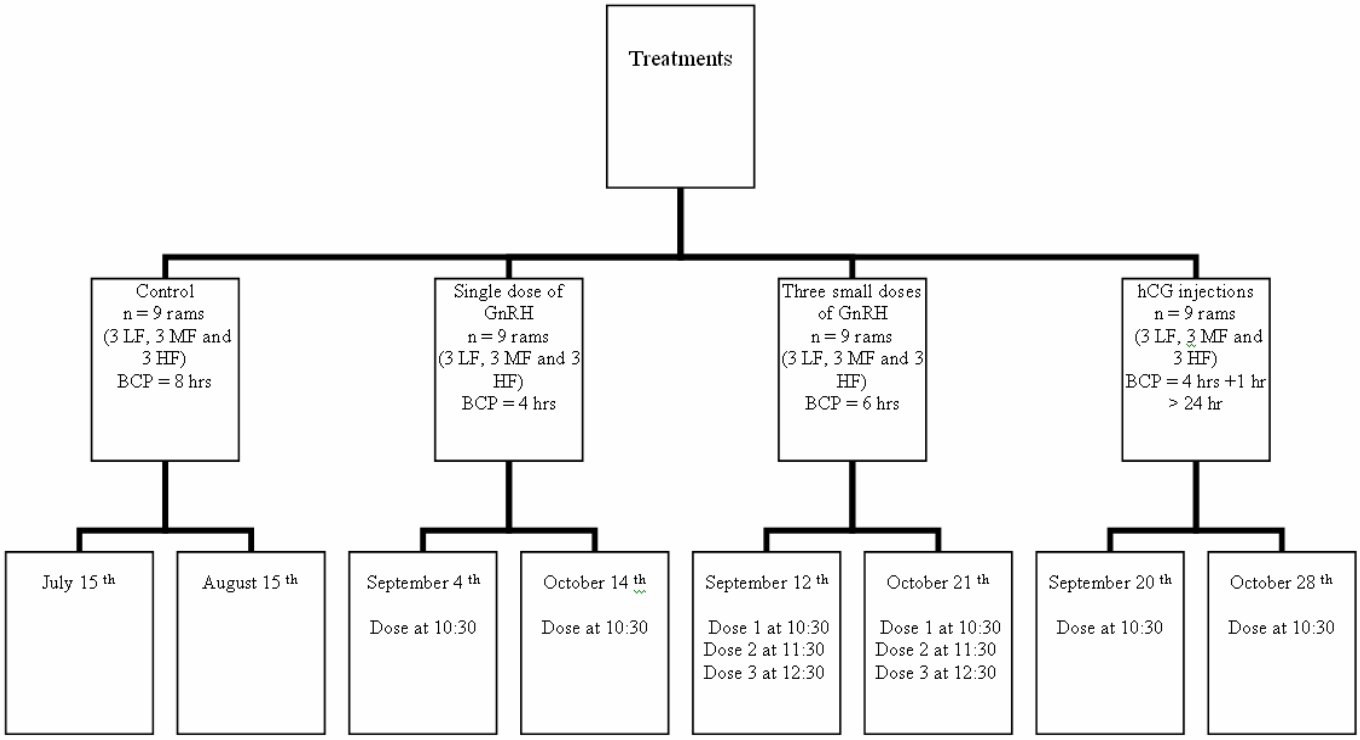

Figure 1: Experimental design showing all periods of the experiment in low (LF), medium (MF) and high (HF) fertility Awassi rams groups. BCP: blood collection period; n: number of rams.

Breeding soundness exam and ultrasonic imaging

The evaluation of the ram for potential breeding soundness consisted of 3 parts; (1) general physical examination, (2) examination of reproductive organs and (3) semen collection and evaluation.

The general physical examination includes the overall conditions of the animal, the feet, legs and eyes. Once the ram passes this initial physical exam, the reproductive organs are examined. The external genital organs were examined by palpation. The testicles were examined for size and circumference, consistency, symmetry and normal development of epididymides. The penis and urethral process were examined during semen collection.

Ultrasound imaging

A B-mode, real time portable scanner (Ultrascan 900; Ami Incorp, Canada) fitted with 7.5 MHz sector array and 5 MHz linear array transducers and connected to a printer (UPP, 110 S; Sony, Japan) was used to examine the testicles and epididymides. Each ram was placed in lateral recumbency position and the fleece of scrotum was clipped. The testis was then pulled down into scrotum. The transducer was covered with water-soluble gel and pressed against each scrotum. Both transverse and longitudinal images were made for testes and epididymides of each ram.

Semen collection and evaluation

During breeding season of Awassi sheep, rams were exposed to three consecutive stages of estrus ewes (from June 15 th to August 15 th). However, rams were banned from accessing ewes for a period of 3 to 5 days before the time of semen collection. Two ejaculates were collected

from each ram in a two-week-interval. Rams were placed on lateral recumbency and a rectal electroejaculator ram probe lubricated with Vaseline was inserted into the rectum. The semen was collected in a graduated tube and transferred immediately to the laboratory for evaluation. Semen samples were evaluated for volume, color, consistency, mass motility, individual motility, sperm concentration, percentages of live-dead spermatozoa and percentage of morphologically normal spermatozoa and specific abnormalities.

Ejaculate volume and appearance were immediately evaluated after collection through a transparent graduated test tube. Mass motility was immediately assessed after collection by adding a small drop of undiluted semen on a warmed slide and examined under low magnification (10x). The percentage of progressive forward motility of spermatozoa was estimated by diluting a drop of semen with 0.1 M sodium citrate and examined under high magnification (40x). Sperm concentration was determined by means of Neubauer haemocytometer. The sample was mixed by inverting the tube, and the red cell pipette was filled to 0.5 mark with semen. The semen was diluted with a special dilution fluid to 101 mark, and the counting chamber in the Neubauer haemocytometer was filled after adequate mixing.

The percentage of live/dead spermatozoa and morphologically normal and abnormal spermatozoa was obtained by staining a film using eosin nigrosin stain (Dott and Foster, 1972). An equal part from eosin and nigrosin stains was placed and mixed on a slide, then one drop of diluted semen was added and mixed with stains and a thin film was made. At least 200 spermatozoa were examined under a microscope using 40x and 100x objective lenses. The number of normal sperm and the number and type of abnormal sperm were counted.

Blood collection

Using heparinized graduated tubes (10 IU heparin/tube), blood samples (5 ml) were collected at 30-min-interval for 8 h from each ram on two different occasions one month

a part (starting at 10:00 on July 15th and August 15th). Blood samples were centrifuged at 4000xg for 5 min. Plasma was separated and stored at –20^{0}C until assayed for determination of T using radioimmunoassay (Immunotech, France) (Sanford et al., 1974). Assay binding was 46%; sensitivity of assay was 0.025 ng/ml and interassay/intrassay coefficient of variations were 15% and 22 %, respectively. Mean T levels during the 8 hours collection periods in the three groups were used to establish a control profile to compare with the T levels after GnRH and hCG response tests. Therefore, T folds were measured by dividing T concentration after endocrine response tests on each group with T concentration of the control profile of the corresponding group.

Endocrine response tests

GnRH (single and multiple doses) and hCG response tests were performed in all rams at two occasions. A minnium of one week wash up period was allowed between the tests.

GnRH response tests

A: a single large dose of GnRH (15 µg) (Cystorelin®: Sanofi, Animal Health, Libourne Cedex, France) was injected intravenously, and blood samples were collected for four hours at 30-min-interval on September 4th and the same protocol was repeated on October 14th. The GnRH was injected after the second blood sample at 10:30. Blood plasma was separated and stored at -20^{0}C until assayed for T.

B: three small doses of GnRH (5 µg) were injected intravenously at one hour interval, and blood samples were collected for 6 hours at 30-min-interval. The three GnRH doses were injected after the second, fourth and sixth blood samples on September 12th, and the same protocol was repeated on October 21st at 10:30, 11:30

and 12:30, respectively. Blood plasma was separated and stored at -20^{0}C until assayed for T.

hCG response test

A single dose of hCG (1000 IU/ram) (Chorulon®: Intervet, Cambridge, United Kingdom) was intravenously injected, and blood samples were collected for four hours at 30-min-intervals on September 20th and the same protocol was repeated on October 28th, with hCG injected after the second blood sample at 10:30. Two blood samples were collected after 24 hours of hCG injection . Blood plasma was separated and kept frozen at -20^{0}C until assayed for T. Testosterone concentrations during one hour before the injection and 8 hours collection period were used as a control value to compare with the T concentrations after GnRH and hCG response tests.

Statistical analysis

All the data were analyzed using General Linear Model (GLM) procedures of the SPSS ™ program, version 11. The effect of fertility status on T profiles and endocrine response tests were analyzed using repeated measures one way analysis of variance (ANOVA). Moreover, ANOVA was also applied for semen parameters in the three fertility groups. Chi-square test was used for the lambing rates of the three groups. Post Hoc Test (Tukey HSD) was used to separate means statistically.

RESULTS

Breeding soundness exam and ultrasonic imaging

Since no definite guidelines for the scrotal circumference (SC) in Awassi rams have been established, and the scrotal circumferences found in this experiment (about 30 cm in all groups; Table 1) were not different among the three fertility groups and all are below the advocated 32 cm in other breeds, the SC criterion was excluded from the BSE of rams. All rams in the three fertility groups passed the minimum requirement of the BSE advocated by the Society of Theriogenology for rams (motility > 30% and normal morphology > 70 %, Table 2).

Table 2: Semen characteristics (mean±SEM) in low, medium and high fertility Awassi rams groups.

Parameter	Low	Medium	High
Color and consistency of ejaculate	Watery, thin creamy to creamy	Watery, cloudy, thin creamy to creamy	Cloudy, thin creamy, creamy to thick creamy
Ejaculate volume (ml)	0.5±0.2	0.8±0.1	0.8±0.1
Mass motility (0-5)	2.0±0.7	2.3±0.2	4.0±0.3
Individual motility %	58.3±13.3	69.2±6.7	76.7±2.5
Sperm concentration (X10^9/ml)	0.9±0.5	1.24±0.4	2.0±0.7
Total sperm output/ejaculate (X10^9)	0.8±0.5	1.0±0.4	1.7±0.8
Live spermatozoa %	49.7±11.5	75.6±3.3	71.8±5.9
Normal spermatozoa %	93±1.5	94.3±0.7	87.7±4.1

Ultrasonographical appearance of the testes and related structures of all rams were normal (Figure 2). The testicular parenchyma appeared as a homogenous and moderately echogenic structure. The mediastinum testis appeared as a centrally located hyperechoic line in the longitudinal section. The tail of the epididymides was more heterogeneous structure and less echogenic than the testis in all groups.

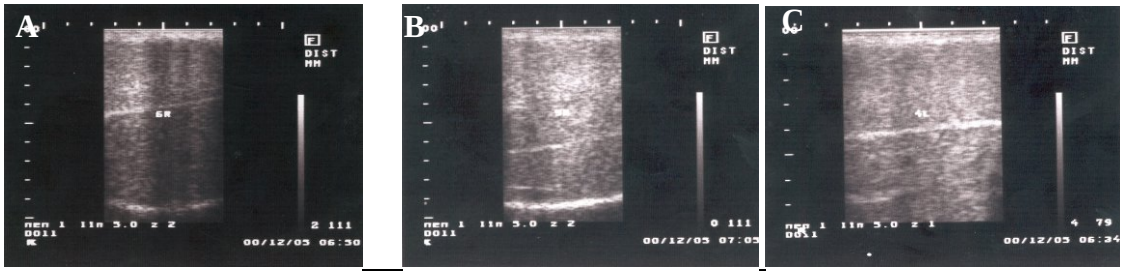

Figure 2: Ultrasonic images in the longitudinal section of the testes of rams in low (A), medium (B) and high (C) fertile groups showing the testicular parenchyma and mediastinum (using 5 MHz linear array transducer).

Semen evaluation

Semen characteristics in the LF, MF and HF groups are presented in Table 2. Few WBC's were present (< 10 per 20x field) in all semen samples collected from the three fertility groups. Although there were no significant differences in semen parameters among the three fertility groups, sperm concentration and motility in the HF group (2.0±0.7 x 10^9/ml and 76.7±2.5, respectively) were numerically higher than the LF and MF groups (0.9±0.5 x 10^9 / ml, 58.3±13.3 and 1.24±0.4 x 10^9/ml, 69.2±6.7, respectively).

Testosterone profiles

Testosterone concentrations during the 8 h collection period in the LF, MF and HF groups are presented as mean±SEM in Table 3. Mean plasma baseline T concentrations among groups differed ($P < 0.05$) significantly and were 0.30±0.04, 0.20±0.05 and 1.4±0.2 ng/ml for LF, MF and HF groups, respectively. The statistical analysis indicated that mean T concentrations in the HF group were significantly ($P < 0.05$) higher than the other two groups with no difference between the LF and MF groups. Testosterone concentrations were not different between the first and second collection periods. Moreover, mean peak concentrations were 2.4±0.2, 1.3±0.1 and 3.6±0.1 ng/ml for the LF, MFand HF groups, respectively (Figure 3). Two peaks were found at 10:00 and 17:00 for the LF group, and at 13:30 and 17:30 for the MF group. However, three peaks were found at 10:30, 14:30 and 16:30 for HF group (Figure 3). There were no significant differences among the three groups in the number and duration of peaks.

Table 3: Mean (±SEM) testosterone concentrations (ng/ml) in the profile, in response to a single dose of GnRH, three small doses of GnRH and hCG injection in low, medium and high fertility Awassi rams groups.

Group	GnRH response tests		hCGinjection	Profiles		
	One dose	Three doses		1st Collection period	2nd Collection period	Control
Low	2.7±0.4	3.2±0.2[a]	2.9±0.3[a]	0.97±0.2[a]	1.0±0.2[a]	0.99±0.2[a]
Medium	3.2±0.4	5.0±0.2[b]	4.8±0.3[b]	0.6±0.2[a]	0.7±0.2[a]	0.7±0.1[a]
High	3.0±0.5	5.7±0.4[b]	5.4±0.3[b]	3.1±0.2[b]	2.4±0.2[b]	2.7±0.1[b]

[a,b] Means with the same column with different superscripts differ ($p<0.05$).

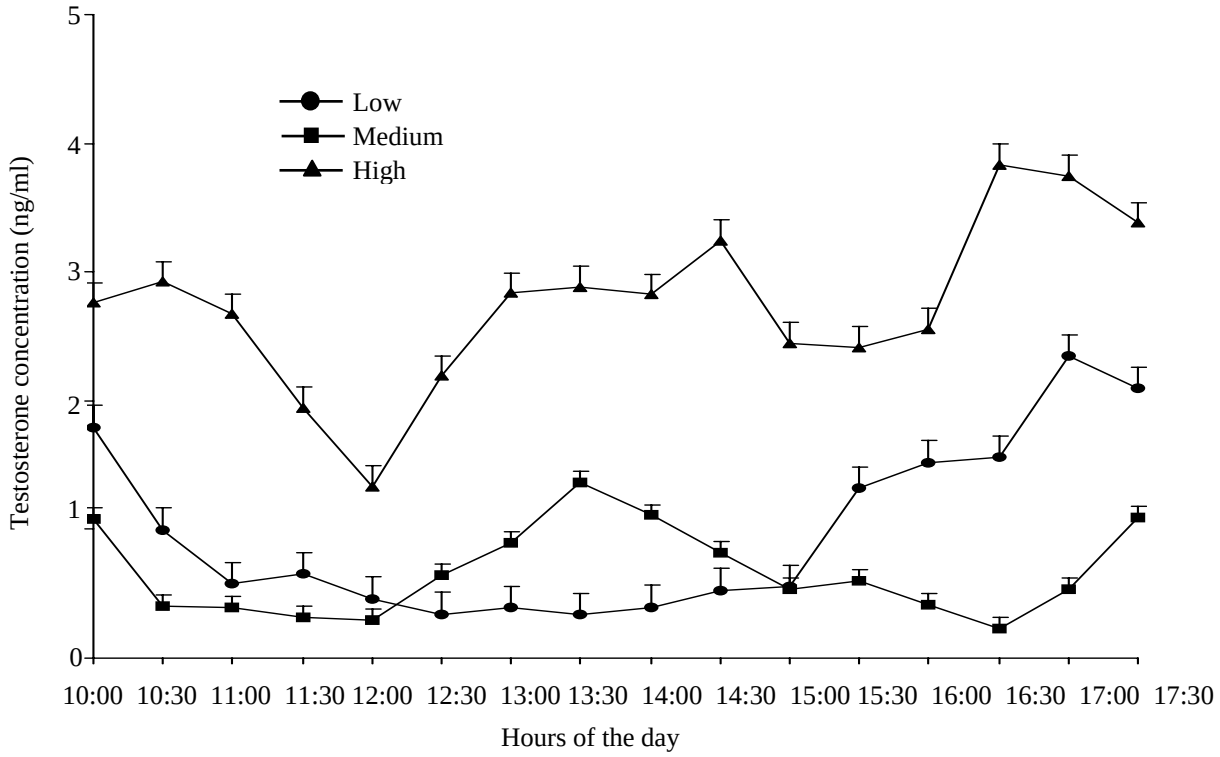

Figure 3: Mean (±SEM) testosterone concentrations in low, medium and high fertility Awassi rams groups during the 8-hour collection period.

Testosterone responses to a single GnRH injection

Plasma T concentrations in all groups increased in response to a single dose of GnRH within 30 to 60 min after injection and high concentrations were maintained throughout the collection period (Figure 4). Mean T levels in reponse to a single dose of GnRH were 2.7±0.4, 3.2±0.4 and 3.0±0.5 ng/ml for the LF, MF and HF groups, respectively (Table 3). There were no significant differences among mean T concentrations after GnRH injection and the mean T concentrations in the control profile. However, mean T concentrations in the LF, MF and HF groups 60-min after GnRH injection until the end of the collection period (3.0±0.4, 3.5±0.3 and 3.4±0.2 ng/ml, respectively) were three, five, and one folds higher than mean T concentrations in the control profiles (0.99±0.2, 0.7±0.1 and 2.7±0.1 ng/ml, respectively) (Figure 4).

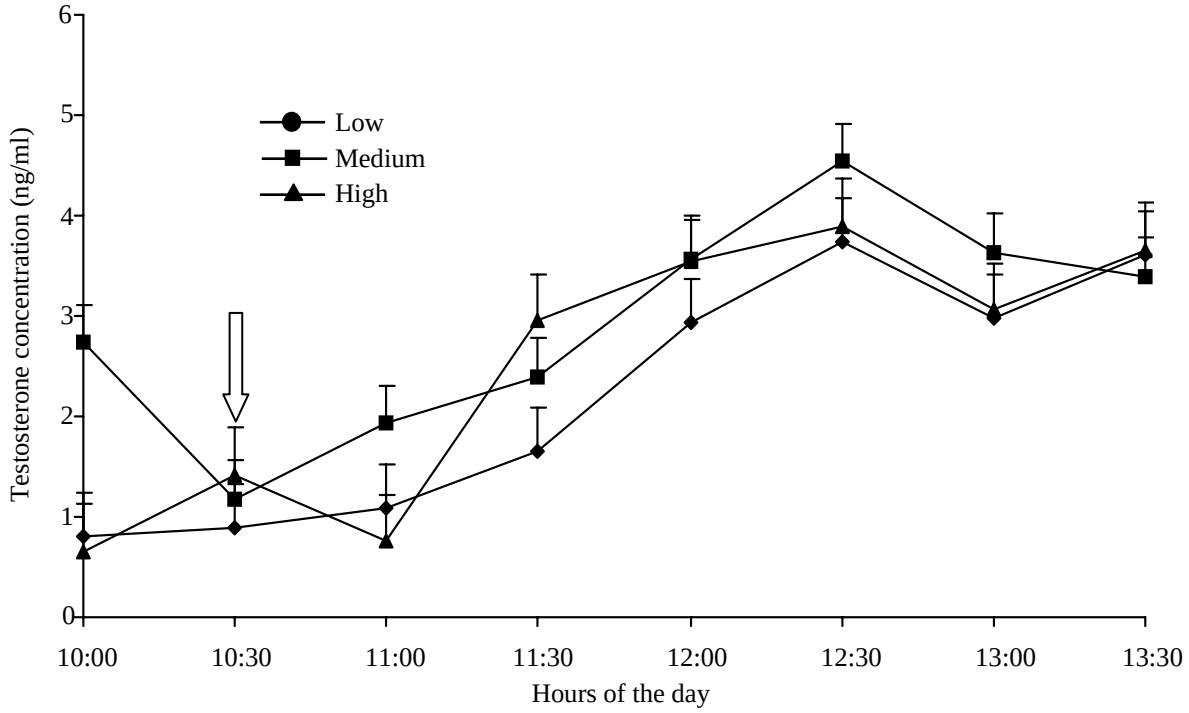

Figure 4: Mean (±SEM) testosterone concentrations in response to a single dose of GnRH in low, medium and high fertility Awassi rams groups. Arrow indicates the time of GnRH injection.

Testosterone responses to three GnRH injections

In all groups, plasma T concentrations increased within 60-min of the first GnRH injection. In the LF and MF groups, T concetrations continued to increase after the second GnRH dose but decreased one hour after the second injection to rebound after the third GnRH dose. High T concetrations were maintained thereafter until the end of the collection peroid (Figure 5). In contrast, in the HF group, plasma T concentrations peaked within 60-min of first GnRH injection, and remained at this level after second GnRH dose, then started to decline after the third GnRH dose until the end of the collection peroid. Plasma T concentrations in the HF group were higher than those of the other two groups (Figure 5). In the LF group, mean T concentration was significantly ($p < 0.05$) lower (3.2±0.2 ng/ml) than those of the other

two groups with no differences between the MF (5.0±0.2 ng/ml) and HF (5.7±0.4 ng/ml) groups. Mean T concentrations 60- min post-GnRH injection were significantly higher than pre-GnRH or mean T concentrations in the control profile ($p < 0.05$). Moreover, mean T concentrations in the LF, MF and HF groups 60-min from the first GnRH injection until the end of collection period (3.4±0.1, 5.0±0.2 and 5.9±0.4 ng/ml) were four, seven and two folds higher than mean T concentrations in the control profile (0.99±0.2, 0.7±0.1 and 2.7±0.1 ng/ml), respectively (Figure 5).

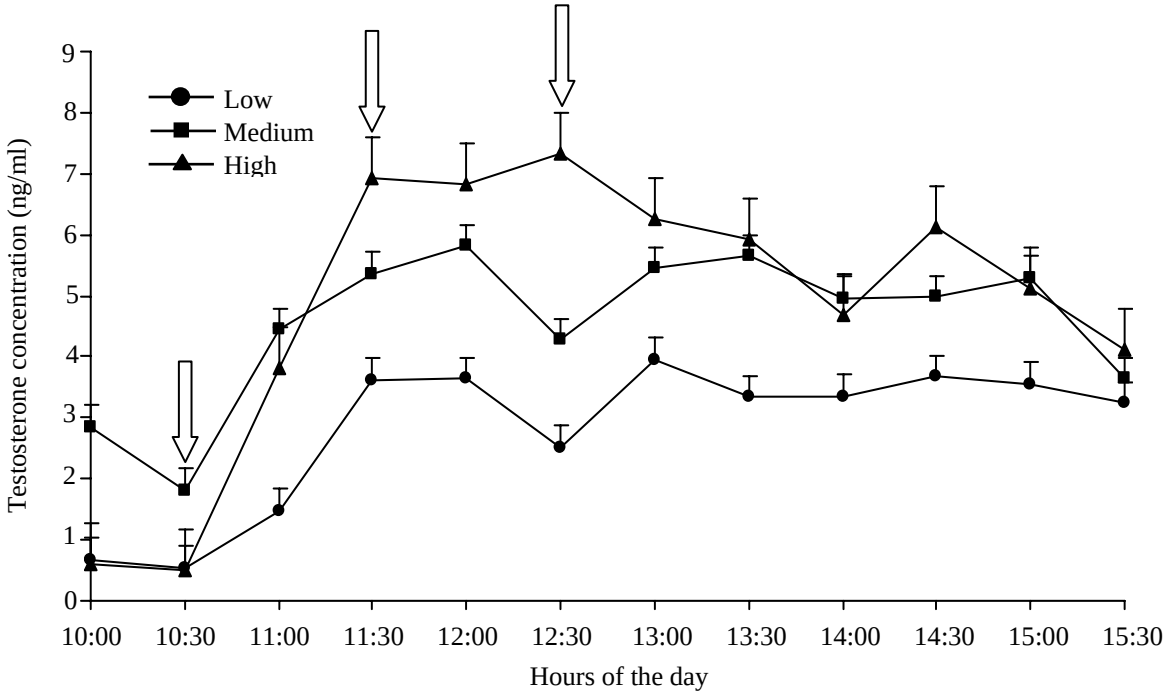

Figure 5: Mean (±SEM) testosterone concentrations in response to three doses of GnRH in the low, medium and high fertility Awassi rams groups. Arrows indicate the time of GnRH injections.

hCG response test

Testosterone concentrations increased significantly ($p < 0.05$) after hCG injection (2.9±0.3, 4.8±0.3 and 5.4±0.3 ng/ml) compared with those of the control profile (0.99±0.2, 0.7±0.1 and 2.7±0.1 ng/ml) for LF, MF and HF groups, respectively. Mean T concentrations 30-min after hCG injection in the LF, MF and HF groups were three, seven and two folds higher than the mean T concentrations in the control profile, respectively. However, plasma T concentrations in LF group showed delayed and lower response to hCG injection for one hour, then T concentration increased hereafter until the end of the collection period. Peak T concentrations were recorded 60-min post-injection in the MF and HF groups and high T concentrations were maintained until the end of collection period in all groups (Figure 6). Mean T concentrations in response to hCG injection were significantly lower in the LF group than other two groups ($p < 0.05$; Figure 6). However, after 24 h T concentration was comparable to the other two groups.

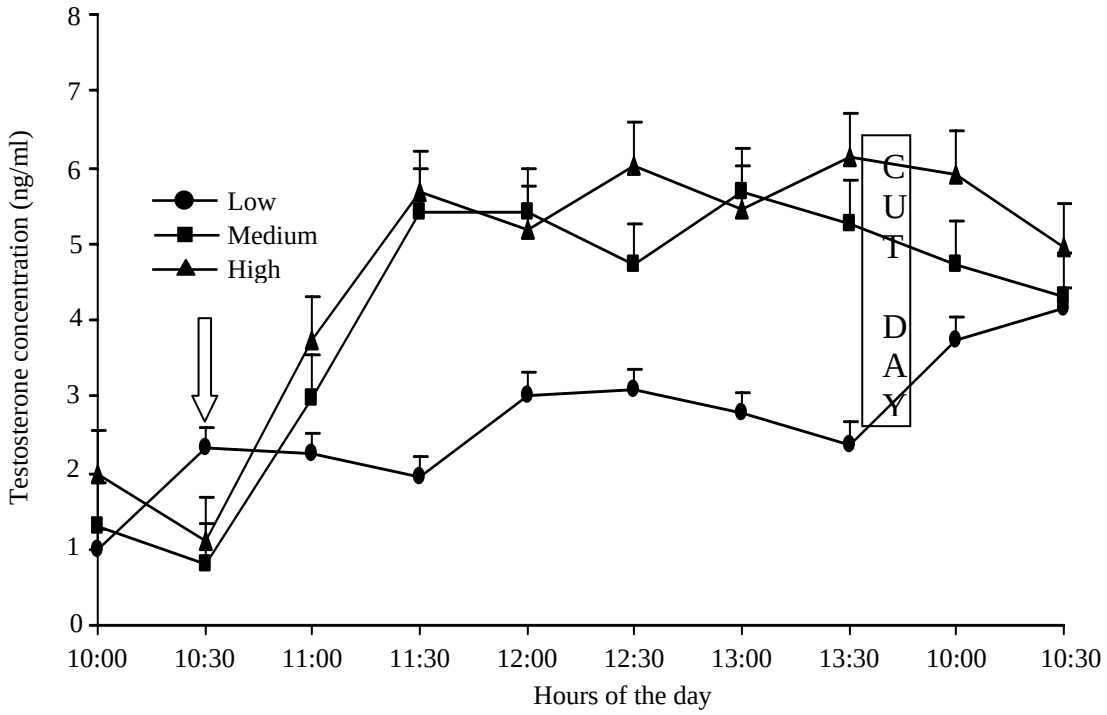

Figure 6: Mean (±SEM) testosterone concentrations in response to hCG injection in the low, medium and high fertility Awassi rams groups. Arrow indicates the time of injection of hCG. The cut text box for the two samples collected after 24 hour from injection of hCG.

DISCUSSION

To classify a male as potentially satisfactory breeder, it must meet minimum standards of reproductive soundness, including physical examination and semen quality. However, a classification of potentially satisfactory breeders does not necessarily assure good fertility and only breeding trials can prove adequacy. No definite guidelines have been established for minimum scrotal circumference in Awassi rams. Recommendations from the current study have been made for a minimum of 30 cm in mature rams, immediately before the breeding season. However, further studies are necessary to establish the minimum SC during the non- breeding and breeding seasons. In the present study, semen quality among the three groups was similar and met the minimum standards to consider the rams as potentially satisfactory breeders. Semen characteristics of Awassi rams in Northen Jordan were similar to those reported for Awassi rams in Egypt and lower than those reported in other breeds (Ott and Memon, 1980; Mann and Lutwac-Mann, 1981; Taha et al., 2000).

Mean T concentrations in the present study were significantly higher in the HF group than the other two groups. This is contradictory to Moore et al. (1978), who demonstrated that mean T concentrations in high and low fertile Romney rams during the spring were not different after a single sexual stimulation. However, mean T concentrations were different after multiple sexual stimulation. In our study, the rams were exposed to estrous ewes earlier during the current breeding season, which might have caused the difference in T concentrations. Furthermore, including the MF group in our study might have caused better grouping of animals.

In addition, our criteria in grouping rams depended on lambing rates and not on twining or triplets rates.

Testosterone concentrations in Awassi rams of our study were slightly lower than those previously reported for other breeds, but were similar to those reported for imported Awassi rams in Egypt (Taha et al., 2000). In our study, T levels ranged around 2.7±0.1 ng/ml which is quiet close to the imported Awassi rams in Egypt that have been found to be within the range 3.5±0.38 ng/ml. Our present finding, therefore, suggests that a level of 2.7 ng/ml is satisfactory for breeding rams. In the present study, T release was multiphasic with multiple peaks occurring between 5 to 12 hours after dawn (light on) (10:00 to 17:00). Most workers who had studied the release of T and LH showed a circadian rhythm with highest activity after dawn and during the afternoons (Lincoln and Peet, 1977; Ortavant et al., 1982). This is in accordance with our study, in which T peaks coincided with morning at 10:00 and afternoon at 16:00 to 16:30 samples. In the present study, T peaks did not show a rhythm but demonstrated that there is a higher probability of finding peaks of T at one part of the day rather than at another. Variation encountered among the peaks of the three groups could be due to different fertility classification or body activity (Ortavant et al., 1982).

Testosterone concentrations increased within 30 to 60 min in response to a single GnRH injection in the three fertility groups. Testosterone concentrations rose 3 to 5 folds above the control values of profile in LF and MF fertile groups. This is in accordance with the observations that LH and T concentrations rose above the control values within 30 to 40 min after a single dose of GnRH in rams (Wu et al., 1987). A single GnRH injection response test was unable to differentiate means T concentrations among the three groups of fertility ($p>0.05$). This is in accordance with the observation that a single GnRH injection response test was unable to differentiate between fertile and subfertile stallions (Roser and Hughes, 1992).

In the present study, T concentrations increased 30 to 60 min after the first dose of GnRH and it rose 2 to 7 folds above the control values of the profile in the three fertility groups. This increase in plasma T concentration in response to GnRH injection is in agreement with that found previously in rats and rams (Clayton et al., 1980; Sharpe, 1982, 1984). In the present study, three GnRH injections (5 µg/injection) showed significant differences in mean T concentrations between the low fertile group and the other two groups ($p<0.05$). The increase in T concentration in response to three GnRH injections might have been due to upregulation of GnRH receptors at pituitary gland where LH stimulates Leydig cell function to secrete more T (Clayton, 1982). In stallions, Roser and Hughes (1992) demonstrated that three GnRH injections showed differences between fertile and subfertile stallions. They found that subfertile stallions had 2 to 4 folds higher plasma concentrations of gonadotropins, but similar T concentrations compared to fertile stallions suggesting a problem on the pituitary concentration, but not the testicular concentration. The disparity between our study and the study of Roser and Hughes (1992) might be caused by the site of endocrinological dysfunction.

Our results indicated that hCG response test showed significant differences in mean T concentrations between the LF group and the other two groups ($p<0.05$). The responses of plasma T to injection of hCG were similar to those found in human males, bulls, rabbits and rats (Saginor and Hortin, 1969; Katongole et al., 1971). In our study, T concentration rose 2 to 7 folds above the control values of profile in all groups. This is in agreement with the observation that T concentration rose 3 to 5 folds above the control values 30 min after hCG injection in rams (Falvo et al., 1975). The prolonged increase in T concentrations seen in the present study

may be due to a fact that hCG with its LH like activity has a direct effect on Leydig cell function and the long half life of hCG and continual activation of LH receptors on the Leydig cells (Ismail, 1976). Moreover, the delayed response to hCG injection in LF group could be due to the presence of impaired testicular function. This is in agreement with Roser (1995), who found poor T response to hCG in subfertile stallions when compared to fertile stallions. Thus, Leydig cell function is partially impaired so that T concentrations increased slowly.

Both ultrasonographical images of the testicles and semen parameters did not show differences among the three fertility groups of rams. However, sperm concentration and sperm motility showed insignificant decline in the LF group. This is in accordance with the data obtained in hCG response test suggesting abnormalities at the testicular origin. The ultrasonographical images of the testicles were in agreement with that reported by Ahmad et al. (1991) of normal ultrasonographic appearance of the testicles and epididymides. It might be that advanced degree of testicular pathology is needed before ultrasonography can be used in diagnosis of such cases. Histopathological examination of the testicles or testicular biopsies might be of vital importance to find the precise nature of the testicular abnormalities, and to classify male animals as subfertile (Costa et al., 2007).

In conclusion, both T profile and hCG response test can be used to determine subfertile rams that passed the BSE. Further studies measuring LH, repeating conditions of the experiment during the transitional period or just before breeding season of Awassi rams are needed.

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إختبارات الإستجابة الهرمونية لتقييم الخصوبة عند كباش العواسي في الأردن

حسن يوسف العمري[1] ومحمد محمود عبابنه[2] ومصطفى قاسم بني دومي[3]

ملخـص

تهدف هذه الدراسة إلى تحديد مستويات هرمون التستوستيرون عند كباش العواسي الخصبة وضعيفة الخصوبة والى تطوير اختبارات الاستجابة الهرمونية من اجل تشخيص ضعف الخصوبة عند الكباش. تم استخدام 9 من كباش العواسي وتم تقسيم هذه الكباش إلى ثلاث مجموعات متساوية هي: منخفضة، متوسطة، عالية الخصوبة. في هذه المجموعات تم فحص السائل المنوي، صور الخصيتين والوعاء الناقل باستخدام السونار، ومستوى هرمون التستوستيرون. بالرغم من عدم وجود اختلافات دلالية بين هذه المجموعات في صور السونار ومقاييس تحليل السائل المنوي إلا أن تركيز ($0.9\pm0.5\times10^9$/ml) وحركة الحيوانات المنوية (% 58.8 ± 13.3) كانت اقل في المجموعة المنخفضة الخصوبة عن المجموعتين المتوسطة والعالية الخصوبة. ارتفعت مستويات هرمون التستوستيرون معنويا في المجموعة عالية الخصوبة (2.7 ± 0.1 ng/ml) بالمقارنة مع المجموعتين منخفضة الخصوبة (0.99 ± 0.2 ng/ml) ومتوسطة الخصوبة (0.7 ± 0.1 ng/ml). لقد كان هناك دلالة اختلافية على انخفاض مستوى هرمون التستوستيرون في المجموعة المنخفضة الخصوبة بالاستجابة للجرعات الثلاثية لهرمون GnRH (3.2 ± 0.2ng/ml) وهرمون (hCG) (2.9 ± 0.3 ng/ml). بالاعتماد على ما سبق، نستنتج انه يمكن اعتماد مستوى هرمون التستوستيرون خلال تذبذبه الزمني واختبار الاستجابة لهرمون (hCG) لتحديد الكباش ضعيفة الخصوبة، التي تجاوزت اختبارات الكفاءة التناسلية.

الكلمات الدالة: اختبارات الاستجابة الهرمونية، تذبذب تستوستيرون، ضعف الخصوبة، كباش العواسي.

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1) قسم الإنتاج الحيواني، كلية الزراعة، جامعة مؤتة، ص.ب 7، الكرك 61710، الأردن.

2) قسم العلوم الطبية البيطرية الإكلينيكية، كلية الطب البيطري، جامعة العلوم والتكنولوجيا الأردنية، اربد، الأردن.

3) قسم الإنتاج الحيواني، كلية الزراعة، جامعة العلوم والتكنولوجيا الأردنية،إربد، الأردن.

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