

Effect of Gonadotropin Releasing Hormone Treatment on Semen Characteristics and Enzymatic Activities of Awassi Rams in Breeding and Non Breeding Seasons

Research Article

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ABSTRACT

This study was aimed to evaluate the effect of GnRH injection on semen ejaculate characteristics in Awassi rams collected in breeding and non breeding seasons. The study was conducted in late summer to early autumn when breeding activity commences, and in winter during the non breeding season. Twelve mature Awassi rams were used in this study. Animals were randomly allocated into two equal groups, the experimental group received 50 µg IM of GnRH weekly and the control group received 1 ml of saline. Libido was assessed and semen samples were collected from the Awassi rams 24 hours after IM administration. Scrotal circumference (SC) and testicular volume were measured weekly during the study period. Ejaculates were collected 24 hours after each GnRH/saline administration and assessed for volume, sperm concentration, mass and individual motility, live sperm and sperm abnormalities. Seminal plasma was assayed for the estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH). Results show that GnRH treatment improves semen volume, total ejaculated sperm and reaction time in both breeding and non-breeding seasons compared to control ($P < 0.05$). However, GnRH treatment reduces the activity of LDH in both seasons. Other seminal traits show similar values for both seasons. In conclusion, GnRH treatment of Awassi rams improved libido, semen volume, sperm numbers, but not sperm viability.

KEY WORDS Awassi rams, breeding seasons, enzymes, GnRH, semen.

INTRODUCTION

The reproductive activity of the ram appears to be influenced, in certain breeds and regions, by the season of the year (Glover *et al.* 1990; Karagiannidis *et al.* 2000), with photoperiod being the key environmental signal timing the reproductive cycle (Lincoln and Short, 1980). Sexual preparation that includes semen collection has been shown to optimize the number of spermatozoa in the ejaculate of the ram (Mekonnen *et al.* 1989). Unfortunately, the availability of teaser ewes is frequently limited out of season and AI

workers are, therefore, forced to collect semen from rams without sexual preparation. Improving semen quality and quantity in breeding and non breeding seasons of the ram ejaculate would benefit all assisted reproductive techniques used in this species.

Normal hypothalamic-pituitary-testicular axis function is critical for reproductive capacity; for this reason it has been studied and manipulated in the hope of finding a way to increase reproductive efficacy of rams.

GnRH is the key hormone of the reproductive axis because of its control of luteinizing hormone (LH) and follicle

stimulating hormone (FSH) production from the anterior pituitary gland. These gonadotropins are actively involved in male reproductive functions. GnRH has been used in rams, bulls, boars and stallions to increase sperm numbers in the ejaculate (Almquist, 1973; Zamiri and Khodaei, 2005 and Sajjad *et al.* 2007). In addition to increased sperm numbers in the ejaculate following GnRH administration, some researchers noted that treated animals had a greater libido at the time of semen collection (Gabor *et al.* 1998). Libido was assessed using quantifiable observations, such as time to initial false mount and time to ejaculation in buffalo, and time for collection in rams (Stellflug, 2002; Stellflug and Lewis, 2007). Initially, the effects on reaction time and collection time were attributed to hypothalamic-pituitary-testicular effect following GnRH administration. The aims of the current study were to evaluate the effect of GnRH on ejaculate characteristics, libido, scrotal circumference, testicular volume and some reproductive traits in rams examined in breeding and non breeding seasons.

MATERIALS AND METHODS

Animals and semen collection

The experiment was conducted in breeding season (August-October 2008) when major breeding activities commenced and in winter during the non breeding season (December-February 2009) at the Animal Research and Practice Farm of the College of The Veterinary Medicine, University of Mosul. Twelve mature Awassi rams (2-3 years of age, 45-50 kg weight) were used in this study, and were maintained on identical conditions using conventional feeding, housing and lighting conditions. All these rams were in good health. They were maintained in identical nutritional and managerial condition throughout the period of study. The animals were kept in open front barns, fed individually with concentrated mixture of 1 kg per ram per day (containing 65% barley, 33% bran, and 2% minerals and salt mixed in the farm), and were given water *ad libitum*. Rams were randomly allocated into two groups based on their ear numbers, first group were administered with 50 µg IM of GnRH (Cystorelin, Ceva Sante Animale, La Ballastiere-33501, Libourne Cedex, France) weekly for 3 months and the second group as a control received 1 ml of Normal-saline solution. Semen samples were collected from rams 24 hours after IM administration. A total number of 288 ejaculates were collected from the rams using an artificial vagina once a week 24 hours following administrations starting from August with one month rest at November ended at February 2009. The scrotal circumference (SC) was taken weekly during the study period. SC was measured with the help of a flexible tape, while the rams were restrained in a sitting position. Scrotal wool was clipped off and the testes were

pulled fully into the scrotum before measurement. Testicular volume was estimated by the amount of liquid displaced by immersing the whole scrotal sac of a standing ram into a 2-L container filled with warm water according to Archimedes law of buoyancy (Piperelis *et al.* 2008). The sexual behavior expressed as reaction time of the rams was evaluated weekly, on day of semen collection in a pen test with estrous ewes. Ewes were induced into estrus with intravaginal sponges impregnated with medroxy-progesterone acetate (Synncropart 40 mg sheep sponge, Ceva 140 Sante Animale, France) during 6 days and 300 IU of eCG (Synncropart) IM at sponge withdrawal. Rams were individually located with one estrous ewe in a 5 x 5 m pen and the time of lateral approach, mounts and ejaculation in the artificial vagina were recorded. The intervals to first mounts and ejaculation in the artificial vagina were recorded.

Semen analysis

The volume of each ejaculate was recorded and sperm concentration was determined using semen diluted with 3% NaCl, the diluted semen was placed on a hemocytometer with the sperm counted in five squares of one chamber (80 small squares with 0.2 mm volume). Methylene blue reduction test was performed immediately after semen collection as the method described by Maule (1962). Sperm motility was identified as the percentage of sperm cells that demonstrated progressive motility, from 0 to 100%, by a qualified and experienced investigator. Semen was placed on a heated (35 °C) glass slide and scoring was performed at microscopic magnification of x 200. Each sample was evaluated twice. The mean value was used for data analysis. Assessments of abnormal and normal spermatozoa (live, dead, primary and secondary defects) were performed using an eosin-nigrosin staining method. For the percent of spermatozoa with abnormal acrosomes (swollen, detached, irregular and multi-defects), fast green stain was used (Wells and Awa, 1970).

Analysis of enzymes

Samples for the estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH) were obtained by centrifugation of 5 ml of diluted (in a dilution rate 1:20) semen using N-saline solution for volume expansion at 5000 rpm for 15 min using available diagnostic kits (BIOLABO SA, 02160, Maizy, France). Determination of ALT and AST in diluted cell free semen samples were carried out by means of a spectrophotometer with photometric determination of the concentration pyruvate and oxaloacetate hydrozone formed with 2,4 dinitrophenylhydrazine as described by Reitman and Frankel (1957). ACP was determined as described by Tietz

(1999), ALP as described by King and King (1954) and LDH activity in seminal plasma free cells as described by Henry (1974).

Statistical analysis

Data were expressed as means (\pm SE) after these data were tested for normally distributed and statistical analyses were performed with the software Sigma stat (Jandel scientific software V2.0, Richmond, CA, 2004). The differences between means of the same parameter were tested by the two way (treatment \times season) analysis of variance (ANOVA) and Duncan's multiple range test.

RESULTS AND DISCUSSION

Results of the present study showed that GnRH treatment to Awassi rams improved ($P < 0.05$) some semen characteristics by increasing semen volume, sperm concentration/ejaculate (Figure 2) in both breeding and non breeding seasons (Table 1) compared with the control group (Figure 1). Other seminal parameters showed no significant differences in the breeding and non breeding seasons including pH, sperm concentration, live sperm, primary abnormalities, acrosomal defects and methylene blue reduction test in the GnRH treated group and control group. The GnRH treated group showed a significant decrease in the secondary sperm abnormalities mainly in the non breeding season compared with the control group ($P < 0.05$). Seminal parameters showed a significant ($P < 0.05$) decrease in the control group in the non breeding season including mass motility, individual motility and percent live sperm as shown in Table 1.

The effect of season on semen quality and/or day length has been studied in different breeds of rams and GnRH treatment (Glover *et al.* 1990; Ibrahim, 1997; Karagiannidis *et al.* 2000; Kafi *et al.* 2004; Deldar Tajangookeh *et al.* 2007; Makawi *et al.* 2007). The increase in semen volume, sperm concentration/ejaculate, testicular volume, and decreasing the reaction time, in the breeding and non breeding season of Awassi rams observed in this study was associated with treatment of GnRH, which agrees with the findings of Zamiri and Khodaei (2005) in Iranian fat-tailed rams.

High environmental temperatures in some countries (average of 25 °C), particularly in association with increasing day length during summer months has been demonstrated to result in a reduction in semen quality in Corriedale, and Chios rams (Perez *et al.* 1997; Karagiannidis *et al.* 2000).

Northern Iraq is regarded as a dry region with a hot summer (40-50 °C). Results of the present study show that semen volume, sperm concentration, and mass motility of Awassi rams were superior in late summer and autumn

compared to that obtained in winter in both groups including GnRH treatment and control groups. Similarly, Ibrahim (1997) in a study with Chios crossbred rams reared in the United Arab Emirates found that semen quality was not reduced during hot months of summer. These findings suggest that the semen quality of Awassi rams is not affected by summer high temperature in northern Iraq. This study is the first to report the seasonal changes with GnRH treatment of Awassi rams reared in northern Iraq. The results of the present study show that Awassi rams have continuous and acceptable spermatogenic activity during breeding and non breeding seasons.

However, seasonal variations in semen characteristics are observed. Semen of superior quality was produced in late summer and throughout autumn in the GnRH treated and control groups.

Scrotal circumference of Awassi rams treated with GnRH and the control groups showed no significant difference in both breeding and non breeding seasons as shown in Table 2. While testicular volume showed a significant increase in GnRH treated group compared to control group in the non breeding season.

The Testicular volume showed a significant increase ($P < 0.05$) in Awassi rams treated with GnRH in the non breeding season compared to the control group and GnRH treated group in the breeding season. This increase in testicular volume and not in scrotal circumference could be attributed to the increase in the volume of tail of epididymis as GnRH increases sperm concentration/ejaculate in both breeding and non breeding seasons (Adams *et al.* 1996).

Reaction time (libido) of Awassi rams treated with GnRH (10.67 \pm 1.47 seconds) showed a significant ($P < 0.05$) improvement in the breeding and non breeding seasons by expressing shortened reaction time per seconds in collecting semen samples with artificial vagina. A marked elevation in the semen volume, sperm concentration/ejaculate, testicular volume and sexual activity (expressed by reaction time) were observed in GnRH treated group coincides with the commencement of the breeding season (from late August towards November) and in non breeding season (from late November to early March) in Awassi rams used in the current study which is similar to the findings of Gabor *et al.* (1998) and Sajjad *et al.* (2007).

A significant effect of GnRH treatment by decreasing the secondary sperm abnormality of Awassi rams was observed in the current study ($P < 0.05$).

The use of GnRH treatment in conjunction with semen collection has been shown to optimize the number of spermatozoa/ejaculate in the ejaculate of the Awassi ram semen. This result is in agreement with the findings of Almqvist (1973) who found shown to optimize the number of spermatozoa in the ejaculate of the bull.

Table 1 Mean (\pm SE) values of semen parameters in breeding and non breeding seasons in GnRH treated and control groups of Awassi rams

Groups (6 rams in each group)	No. of ejacu- lates	Vol- ume (mL)	Sperm con- centration X10 ⁹ sperm/m L	Sperm con- centration X10 ⁹ sperm/ej aculate	Ph	Mass motility (%)	Indi- vidual motil- ity (%)	Live sperm (%)	Abnormal sperm (%)		Abnor- mal acro- somes (%)	Methylene blue reduc- tion test (min)
									primary	secondary		
GnRH in breeding season	72	1.52 ^a \pm 0.07	1.72 \pm 0.06	2.01 ^a \pm 0.61	6.68 \pm 0.06	95.1 ^a \pm 2.13	94.2 ^a \pm 3.65	89.5 \pm 0.76	2.3 \pm 0.02	9.3 \pm 1.82	0.98 \pm 0.02	2.3 \pm 0.44
Control in breeding season	72	1.1 ^b \pm 0.07	1.56 \pm 0.11	1.2 ^b \pm 0.24	6.52 \pm 0.51	92.8 \pm 0.83	92.0 \pm 1.78	85.83 \pm 2.01	2.83 \pm 0.39	15.5 \pm 0.65	1.3 \pm 0.10	2.61 \pm 0.44
GnRH in non breeding season	72	1.2 ^a \pm 0.26	1.52 \pm 0.02	2.42 ^a \pm 0.69	6.67 \pm 0.11	91.6 \pm 2.47	90.6 \pm 1.66	87.18 \pm 1.35	2.4 \pm 0.01	10.6 ^a \pm 1.32	1.01 \pm 0.03	2.35 \pm 0.15
Control in non breed- ing season	72	0.96 ^b \pm 0.09	1.43 \pm 0.05	1.18 ^b \pm 0.15	6.45 \pm 0.177	84.16 ^b \pm 1.53	82.3 ^b \pm 1.05	81.66 \pm 1.05	2.79 \pm 0.37	15.15 ^b \pm 1.94	1.0 \pm 0.01	2.72 \pm 0.31

Means for the same parameter with different superscripts (a, b) within each column are significantly different ($P < 0.05$).

Table 2 Mean (\pm SE) values of reproductive traits in breeding and non breeding seasons in GnRH treated and control groups of Awassi rams

Groups	Reaction time (seconds)	Scrotal circumference (cm)	Testicular volume (cm ³)
GnRH in breeding season	10.67 ^a \pm 1.47	33.66 \pm 0.62	670.8 ^a \pm 33.79
GnRH in non breeding season	13.5 \pm 3.73	31.25 \pm 0.45	766.67 ^a \pm 18.08
Control in breeding season	15.1 ^b \pm 2.35	32.1 \pm 0.61	475.11 ^b \pm 38.98
Control in non breeding season	17.5 ^b \pm 2.81	30.1 \pm 0.31	462.33 ^b \pm 17.28

Means for the same parameter with different superscripts (a, b) within each column are significantly different ($P < 0.05$).

The beneficial effects of GnRH noted in the present study can be attributed to the GnRH injection by causing a surge in luteinizing hormone (LH) and testosterone, 30 and 60 min, respectively, after administration in the dog (Purswell and Wilcke, 1993). The testosterone surge following GnRH administration is the proposed cause for the improvement in libido (Foote, 1978). GnRH is produced by the hypothalamus and signals the release of LH and FSH from the anterior pituitary. Luteinizing hormone acts as a trigger for testosterone release by the leydig cells in the testicle. Administration of exogenous anterior pituitary. Luteinizing hormone acts as a trigger for testosterone release by the leydig cells in the testicle. Administration of exogenous GnRH to

male rams induces a dose dependent release of LH immediately following treatment (Sieme *et al.* 2004). Although GnRH has not been shown to improve semen quality in the immediate post-administration period, it has been shown to improve semen quality over longer periods as no significant differences between semen parameters in the GnRH treatment group in breeding and non breeding seasons.

The mean activity of LDH enzyme estimated in the GnRH treated and control groups showed a significant difference ($P < 0.05$) between the two groups in the breeding season and non breeding season (45.9 \pm 28.8 and 77.9 \pm 30.8 vs. 117.0 \pm 5.2 and 131.8 \pm 5.0, respectively). Other enzymatic activities showed no significant differences between

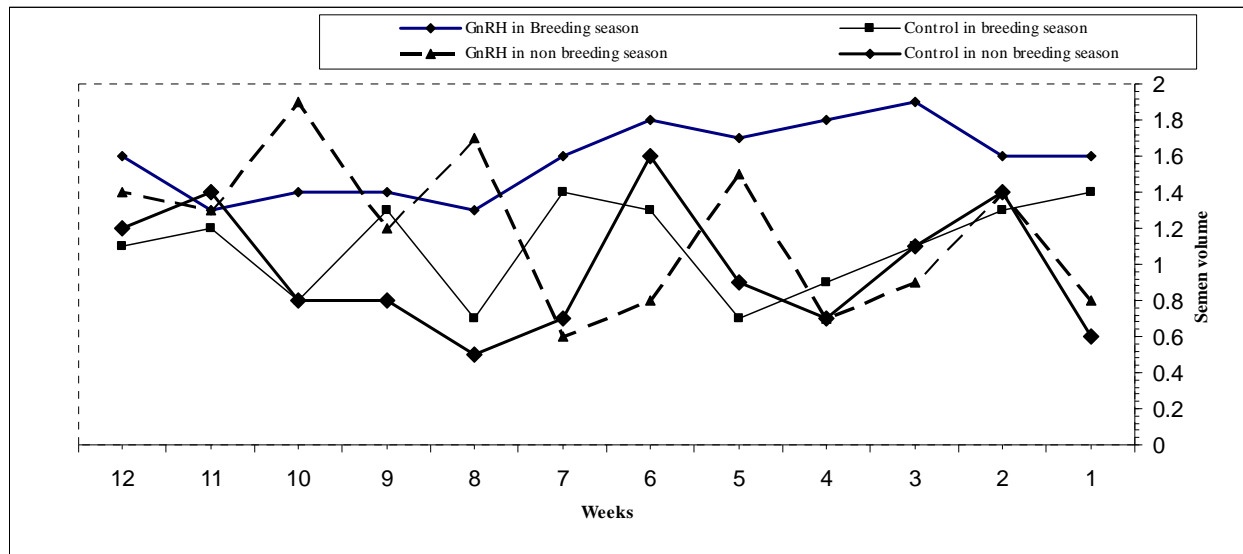


Figure 1 Semen volume of Awassi sheep in GnRH treated and control groups in breeding and non breeding seasons

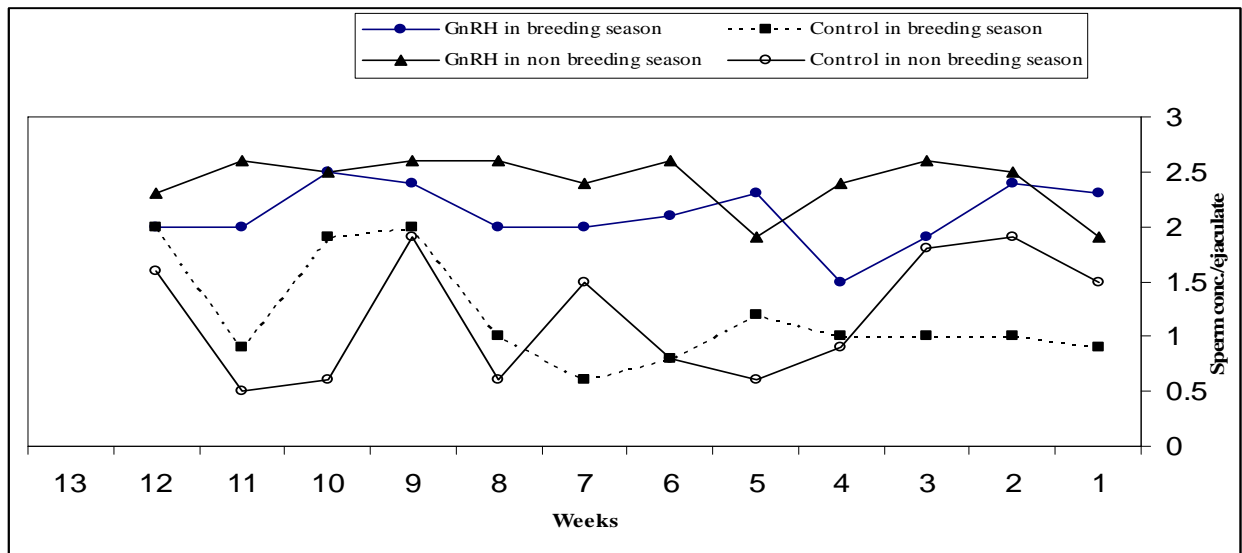


Figure 2 Sperm concentration/ejaculate of Awassi sheep in GnRH treated and control group in breeding and non breeding seasons

Awassi rams treated with GnRH and control groups in both breeding and non breeding seasons including ALT (85.4 ± 15.2 and 90.8 ± 8.8 vs. 93.2 ± 17.4 and 114.1 ± 9.4), AST (135.9 ± 5.3 and 167.9 ± 29.7 vs. 175.2 ± 27.9 and 280.3 ± 49.9), ACP (278.3 ± 9.4 and 351.6 ± 42.4 vs. 236.3 ± 41.6 and 319.1 ± 63.8) and ALP (1281.5 ± 131.6 and 1831.9 ± 138.2 vs. 3063.3 ± 96.4 and 2012.2 ± 130.8). Pursel *et al.* (1968) reported that one of the consequences of acrosomal damage is the leakage of enzymes from the sperm. The leakage of four enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP); revealed a positive correlation between enzyme release and sperm cell integrity and acrosomal damage (Azawi *et al.* 1990a; Chauhan *et al.*

1994). Enzyme release has generally been recognized as an indicator of cellular injury whereby membranes become inactivated or destroyed resulting in the loss of cellular material (Yousef *et al.* 1998). The present results revealed no significant differences in the activities of AST, ALT, ACP, and ALP in the seminal plasma of GnRH treated and control groups. Therefore, the stability in the activities of these enzymes coincided with the unaffected semen quality and viability of treated rams with GnRH and control groups in the breeding and non breeding seasons found in the present study. The significant decrease in the LDH activity in Awassi rams treated with GnRH could be due to the significant increase in semen volume and sperm concentration/ejaculate observed in this study. Roussal and Stallcup

(1965) and Azawi *et al.* (1990b) reported that the activity of seminal plasma LDH was negatively correlated with each of ejaculate volume and sperm concentration. In conclusion, GnRH treatment of Awassi rams improved semen volume, sperm concentration/ejaculate, testicular volume and libido.

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