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Sexual Performance and Reproductive Characteristics of Young Adult Awassi, Charollais-Awassi and Romanov-Awassi Rams

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Summary

This study was conducted to evaluate the reproductive performance of two-yr-old, sexually naïve rams of different genotypes. Eight rams of each Awassi (A), F_1 Charollais-Awassi (CA) and F_1 Romanov-Awassi (RA) genotypes were subjected to sexual performance tests by being individually exposed to two estrous Awassi ewes for five, 20-min periods. Body weight (BW), body condition score (BCS), scrotal circumference (SC) and semen characteristics were recorded every 2 wk for 2 mo prior to sexual performance testing. Awassi rams engaged in more leg-kicking bouts ($P < 0.01$) than RA rams. Mounting frequency, raising the fat tail of females, and ejaculation rate were greater ($P < 0.05$) in A than in CA and RA rams. No genotype x test day interactions were detected, however, test day influenced ($P = 0.05$) ejaculation rate. Rams of the CA genotype had greater BW ($P < 0.01$) than RA and A. The CA rams had greater SC ($P < 0.01$) than A rams and higher BCS ($P < 0.01$) than RA rams. The RA rams had greater ($P < 0.05$) semen mass motility than A and lower ($P < 0.05$) percentage of abnormal spermatozoa than A and CA rams. Additionally, semen concentration tended ($P < 0.10$) to be greater in RA than in A and CA rams. Results of the present study indicate that RA rams tend to have better semen characteristics, while Awassi rams had better sexual performance when mated with fat-tailed females than the CA and RA genotypes, which may necessitate the use of artificial insemination during crossbreeding programs.

Key words: Awassi Sheep, Sexual Performance, Semen Characteristics, Crossbreeding

Introduction

The efficiency of sheep production depends highly on reproductive performance, especially in countries where the sheep industry is important (Ibarra et al., 2000). Most reproductive-efficiency research in sheep focuses on females, due to their seasonal patterns, while giving relatively less attention to males, as they are capable of reproducing all year round. Aspects of ram sexual behavior under various conditions have been studied extensively (Price et al., 1991; Price et al., 1992; Price et al., 1994). However, to our knowledge, little information is available on sexual performance and the ability of thin-tailed rams to naturally mate with fat-tailed ewes.

Awassi, a fat-tailed breed, is the most prevalent sheep breed in the Middle East (Gootwine et al., 1992). Awassi sheep have desirable carcass traits and meat quality (Holloway et al., 1994), especially because the presence of tail allows for leaner carcasses and makes it easier to trim any undesirable fat. The presence of tail, however, causes mating difficulties by impeding copulation (Kridli and Said, 1999). This is observed mostly when using sexually naïve rams or when mating fat-tailed ewes with exotic rams.

In the present study, Awassi were crossed with mutton (Charollais) and prolific (Romanov) breeds in an attempt to combine the adaptability and hardiness of Awassi with the prolificacy and meat production abilities of Romanov and Charollais sheep, respectively. The success of such breeding programs highly depends on reproductive performance. The objective of this study was to evaluate sexual performance and reproductive characteristics of crossbred rams as compared with Awassi and to test their ability to naturally mate with fat-tailed Awassi ewes.

Materials and methods

The experiment was conducted during the autumn season at the Center of Agricultural Research and Production at Jordan University of Science and Technology located in the northern part of Jordan at 32° 34' N and an altitude of 520 m above sea level. Experimental animals were kept in open-front barns with free access to shade, water and mineral blocks. The natural breeding season occurs during the period of June through December for Awassi (Epstein, 1985), August through March for Romanov (Fahmy, 1996) and July through December for Charollais (British Charollais Sheep Society).

Sexually naïve, two-yr-old rams of three different genotypes were used in this study. Eight rams of each Awassi (A) and its first crosses with either Charollais (CA) or Romanov (RA) sheep were subjected to sexual performance tests by being individually exposed to two estrous Awassi ewes (fat-tailed) for five, 20-min periods. Thus, each ram was tested five times, each on a different test day. Thirty, mature Awassi ewes were synchronized to exhibit estrus on five test days, each two days apart (six ewes/occasion). Vaginal progestogen sponges (40 mg flourogestone acetate sponges, Ceva, France) were inserted for 12 d. Six hundred IU of PMSG were injected intramuscularly at the time of sponge removal. Only four of the six ewes were used on each test day. The reason for synchronizing six ewes to exhibit estrus on each test day was to ensure having the four required estrual females. The four ewes to be used in the testing procedures were detected to be in estrus by mature, experienced Awassi rams.

Sexual performance testing started one week after the end of blood sampling and semen evaluation. Sexual performance tests were conducted during the morning hours between 0700 and 1200. Four estrual ewes were placed in two 6 x 6 m pens (two ewes/pen). A third similar pen, where the observers stood, separated the two test pens. Ewes were kept unrestrained in the pens. Each ram was individually evaluated by exposing it to the two estrual ewes for 20 min on each test day. Two rams were tested simultaneously while the remaining rams were kept about 20 m away with visual barriers between them and the test pens. Rams were randomly selected for testing, whereby each pen was used to test rams of all genotypes on each test day.

Observations were recorded for each ram throughout the 20-min period. Collected observations were similar to those reported by Kridli and Said (1999) and Kridli and Al-Yacoub (2006) and included bouts of leg kicking, anogenital sniffing, mount attempts (both front feet off the ground without complete positioning of the ram on the ewe's rump), mounts without ejaculation, frequency of raising the fat tail of ewes, and ejaculation frequency.

Testosterone concentration, semen characteristics, body weight (BW), body condition score (BCS) and scrotal circumference (SC) were recorded for each ram every 2 wk for 2 mo (September and October) before sexual performance was evaluated. Blood samples were collected via jugular venipuncture into heparinized tubes, centrifuged and stored at –20° C until analyzed for testosterone concentration by RIA (Immunotech, France). Blood samples were collected at 0700 followed by semen collection and evaluation at around 0800.

Scrotal circumference was measured using a flexible tape at the widest scrotal diameter. Semen samples were collected using a battery-operated electro-ejaculator [as it does not require previous training of ram lambs (Belibasaki and Kouimtzis, 2000)] and a series of short electrical stimuli (approximately 5 sec) were administered at 20 sec intervals (Buckrell et al., 1994). Two ejaculates were collected and discarded on the first day of semen collection to remove any poor quality sperms present, due to rams being sexually inactive prior to the start of the experiment. Ejaculate volume was determined immediately after collection using a transparent graduated vial. Mass motility was assessed as a percentage by viewing one drop of semen at low magnification (40x) as described by Al-Ghalban et al. (2004). An aliquot of semen was diluted in a physiological saline solution containing 0.01 percent mercury chloride at 1:400 (semen:diluent) for hemocytometric determination of concentration and percentage of abnormalities (Chemineau et al., 1991). The same person evaluated BCS, measured SC, operated the electro-ejaculator and evaluated the semen samples throughout the study.

Data were analyzed by analyses of variance for completely randomized design using the "general linear model" procedure of SAS. Semen characteristics, BW, BCS, SC and sexual performance data were submitted to a repeated measures multivariate model by the "repeated" statement, to evaluate the effect of the within-subject "sampling day" factor, and the between-subject "genotype" factor. Differences were tested by a pairwise *t* test using the "least square means" statement. Because the study included count data, log (x + 1) transformation was conducted on all sex-

ual performance parameters. However, the actual sexual performance data are presented with means being separated based on transformed data. Simple correlations were conducted among the various variables for all rams within all classes. All analyses were conducted using the General Linear Model procedure for SAS (SAS, 1997).

Results

Awassi rams had greater bouts of leg kicking ($P < 0.01$) while CA had greater mount attempts ($P < 0.05$) than RA rams (Table 1). Mounting frequency, raising the fat tail of females, tail raising per mount and ejaculation rate were greater ($P < 0.01$) in A than in CA and RA rams (Table 1). Correlations were detected ($P < 0.01$) between mounting and ejaculation rate (r = 0.4) and tail raising and ejaculation rate (r = 0.8). Pre-copulatory behavior (kicking and sniffing) was moderately correlated ($P < 0.05$) with ejaculation rate (r = 0.41).

There was no ($P > 0.10$) genotype x test day interaction for any of the sexual performance parameters. Test day, however, influenced mounting frequency ($P < 0.05$) and tended to influence ($P = 0.10$) ejaculation rate (Table 2). Mounting frequency declined from 21.8 ± 2.9 mounts per 20 min on day 1 to 7.5 ± 2.9 mounts per 20 min on day 5 (Table 2). Ejaculation rate increased from 0.1 ± 0.1 on day 1 to 0.4 ± 0.1 on day 5. Even though it was not significant, kicking frequency and mount attempts declined while sniffing frequency, tail raising and tail raising per mount increased as test day advanced.

Rams of the CA genotype were heavier ($P < 0.01$) than RA and A (Table 3). Additionally, CA rams had greater SC ($P < 0.01$) than A rams and higher BCS ($P < 0.01$) than RA rams (Table 3). Correlations existed ($P < 0.001$) between BW and BCS (r = 0.67), BW and SC (r = 0.79) and between BCS and SC (r = 0.59).

There was no ($P > 0.10$) test day effect on testosterone concentrations and any semen characteristic of rams. Testosterone concentrations were greater ($P < 0.05$) in RA than A rams while CA were intermediate (Table 4). Testosterone concentrations were correlated ($P < 0.01$) with BW (r = 0.51) and SC (r = 0.58). Ejaculate volumes did not differ ($P > 0.05$) among genotypes (Table 4). The RA rams had greater ($P < 0.05$) mass motility than A and lower ($P < 0.05$) percentage of abnormal spermatozoa than A and CA rams. Additionally, semen concentration tended ($P < 0.10$) to be greater in RA than in A and CA rams.

Table 1. Sexual performance (mean ± SE) of Awassi (A), Charollais-Awassi (CA) and Romanov-Awassi (RA) rams averaged over five test days[1].

	Genotype		
Variable[2]	A (n=8)	CA (n=8)	RA (n=8)
Leg kicking	9.8^{a} ± 1.4	6.6^{ab} ± 1.4	3.3^{b} ± 1.4
Anogenital sniffing	9.1 ± 1.6	6.1 ± 1.6	7.0 ± 1.6
Mount attempts	2.6^{de} ± 0.7	2.8^{d} ± 0.7	0.7^{e} ± 0.7
Mounts	17.6^{a} ± 1.9	9.1^{b} ± 1.9	6.9^{b} ± 1.9
Tail raising	3.9^{a} ± 0.5	0.3^{b} ± 0.5	0.1^{c} ± 0.5
Ejaculation	0.4^{a} ± 0.1	0.0^{b} ± 0.1	0.0^{b} ± 0.1
Tail raising per mount	0.23^{a} ± 0.02	0.03^{b} ± 0.03	0.01^{b} ± 0.03

[1] Sexual performance testing was performed on 5 test days (20 min. ram^{-1} day^{-1})
[2] There were no test day x genotype interactions.
[abc] Means within the same row with different superscripts differ ($P < 0.01$)
[de] Means within the same row with different superscripts differ ($P < 0.05$)

Discussion

This study aimed at evaluating sexual performance characteristics of Charollais-Awassi and Romanov-Awassi as compared with Awassi rams in order to determine the capabilities of crossbred males to naturally mate with the fat-tailed Awassi ewes.

In addition to evaluating sexual performance, we also examined other biological variables and seminal characteristics of rams from the three genotypes.

Most sexual performance parameters were better in Awassi compared with the crossbred males. Awassi rams engaged in more leg-kicking behavior than RA, while anogenital sniffing was similar among genotypes. Pre-copulatory behavior (leg-kicking and anogenital sniffing) was correlated with ejaculation rate, which supports the hypothesis by

Table 2. Effect of test day on sexual performance (Mean ± SE) of Awassi, Charollais-Awassi and Romanov-Awassi rams exposed to estrous Awassi ewes for 20 minutes on five occasions[1].

	Test day					
Variable	1	2	3	4	5	P
Leg kicking	8.4 ± 2.3	7.8 ± 2.3	6.6 ± 2.3	7.2 ± 2.3	4.1 ± 2.3	> 0.05
Anogenital sniffing	6.7 ± 2.1	6.8 ± 2.1	8.3 ± 2.1	8.8 ± 2.1	9.6 ± 2.1	> 0.05
Mount attempts	3.3 ± 1.0	2.3 ± 1.0	2.7 ± 1.0	1.3 ± 1.0	1.1 ± 1.0	> 0.05
Mounts	21.8 ± 2.9	9.1 ± 2.9	11.3 ± 2.9	8.3 ± 2.9	7.5 ± 2.9	< 0.005
Tail raising	1.2 ± 0.9	1.1 ± 0.9	1.5 ± 0.9	2.0 ± 0.9	1.9 ± 0.9	> 0.05
Ejaculation rate	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	= 0.10
Tail raising per mount	0.04 ± 0.05	0.12 ± 0.05	0.09 ± 0.05	0.15 ± 0.05	0.17 ± 0.05	> 0.05

[1] Sexual performance was conducted on five test days each two days apart (20 min. ram^{-1} day^{-1}).

Table 3. Body weights, body condition scores and scrotal circumferences (mean ± SE) of Awassi (A), Charollais-Awassi (CA) and Romanov-Awassi (RA) rams averaged over the experimental period[1].

	Genotype		
Variable[2]	A (n=8)	CA (n=8)	RA (n=8)
Body weight (kg)	70.3[a] ± 0.3	78.7[b] ± 0.3	70.5[a] ± 0.3
Body condition score	3.9[cd] ± 0.1	4.1[c] ± 0.1	3.7[d] ± 0.1
Scrotal circumference	30.9[d] ± 0.3	33.0[c] ± 0.3	32.5[cd] ± 0.3

[1] Data were collected every 2 wk for 2 mo.
[2] There were no test day x genotype interactions.
[ab] Means within the same row with different superscripts differ ($P < 0.01$)
[cd] Means within the same row with different superscripts differ ($P < 0.05$)

Price et al. (1992) that the frequency of pre-copulatory behavior in rams reflects their underlying sexual motivation.

There are many factors that affect sex drive and sexual performance. These factors include: season of year, genetics, breed differences, hormonal influence, post-weaning management, temperature and nutrition (Mickelsen et al., 1982). The fact that Awassi rams outperformed CA and RA rams could be attributed to genetic factors, particularly those related to the presence of fat-tailed females during sexual performance testing. This is supported by the higher frequency of mounting, tail-raising, ejaculation and tail-raising per mount observed in the Awassi rams. When mounting, Awassi rams have a side approach to females (stand behind the ewe and slightly to the side) while CA and RA rams position themselves directly behind females before attempting to mount. This approach is essential for successful mating. As Awassi males "kick up" to mount females, they raise the fat tail with their front legs thus increasing the probability of penile intromission. This difference in approach led to differences in tail-raising and ejaculation rate among genotypes.

Mounting frequency declined with the advancement of test day. This is attributed to the improvement in ejaculation rate as ejaculations are normally followed by periods of sexual inactivity (Price et al., 1992) resulting in lower mounting frequency. The improvement in ejaculation rate over the successive test days is caused by the experience gained by each ram during the testing procedure. According to Price et al. (1991), libido in virgin rams is lower when first exposed to females than during subsequent exposures. Additionally, virgin rams exhibit an improvement in sexual performance after the first and the second exposures to estrous females to a level comparable to that of experienced males (Price et al., 1991).

Body weights and BCS vary among breeds of sheep (Dawson, 2002), as influenced by body conformation. In this study, CA were heavier than A and RA and had greater BCS than RA rams. This is due to the fact that Charollais is a meat breed (Farid and Fahmy, 1996) compared with the prolific Romanov (Fahmy, 1996) and the dual-purpose Awassi (Gootwine et al., 1992). Scrotal circumferences were lower in A than in CA lambs. Scrotal circumference differs among breeds of sheep (Belibasaki & Kouimtzis, 2000) being higher in crossbred than purebred lambs. The significant correlation between BW, SC and BCS is in agreement with previous research (Duguma et al., 2002; Fourie et al., 2002).

Testosterone concentrations may differ between pure and crossbred rams (Fahmy, 1997) and among breeds of sheep (Dickson and Sanford, 2005). In the present study, RA crossbred rams had higher testosterone concentrations than Awassi rams. Despite differences in SC, ejaculate volume was similar among genotypes. This may be due to the use of electrical stimulation for semen collection. The use of electroejaculation increases ejaculate volume due to stimulating the accessory sex glands (Bearden and Fuquay, 1997). Even though larger ejaculate volumes are obtained when using electrical impulses as compared with using artificial vaginas, the total number of sperms produced and the fertilizing capacity of sperms are about the same using both methods (Bearden and Fuquay, 1997).

Semen characteristics followed the same trend as did testosterone concentrations being in favor of the RA genotype. Semen mass motility was higher in RA than A while the percentage of abnormal spermatozoa was lower in RA than the other genotypes. The RA rams tended to have greater semen concentration than the other genotypes. These differences in semen characteristics may be related to differences in testosterone concentrations among genotypes. As scrotal circumference increases, sperm output increases (Langford et al., 1987). Therefore, scrotal circumference can be used as an index for sperm production in sheep (Toe et al., 2000). Differences in

Table 4. Testosterone concentration and semen characteristics (mean ± SE) of Awassi (A), Charollais-Awassi (CA) and Romanov-Awassi (RA) rams averaged over the experimental period[1].

	Genotype		
Variable[2]	A (n=8)	CA (n=8)	RA (n=8)
Testosterone (ng/ml)	4.7[b] ± 0.5	5.7[ab] ± 0.5	6.3[a] ± 0.5
Semen volume (ml)	0.9 ± 0.2	1.1 ± 0.2	1.3 ± 0.2
Mass motility (%)	59[b] ± 6.5	76[ab] ± 6.5	90[a] ± 6.5
Semen concentration (109)	1.40[d] ± 0.5	1.8[d] ± 0.4	2.9[c] ± 0.4
Abnormality (%)	21[b] ± 2.8	18[b] ± 2.8	7[a] ± 2.8

[1] Data were collected every 2 wk for 2 mo.
[2] There were no test day x genotype interactions.
[ab] Means within the same row with different superscripts differ ($P < 0.05$).
[cd] Means within the same row with different superscripts tend to differ ($P < 0.1$).

semen quality among genotypes in the present study may be related to the more fertile and prolific nature of the Romanov breed (Dufour et al., 1984).

Conclusion

In conclusion, results of the present study indicate that even though CA and RA crossbred rams have good body conformation and semen characteristics, they have difficulties when naturally mating with fat-tailed Awassi females, which may necessitate the use of artificial insemination during crossbreeding programs. The sexual performance of CA and RA crossbred males may have been improved had they been exposed to docked or thin-tailed females.

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