Age at Puberty and Some Biological Parameters of Awassi and its First Crosses with Charollais and Romanov Rams

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ABSTRACT

The aim of this experiment was to determine the age at puberty of both ram and ewe lambs of three genotypes: Awassi (A), Charollais x Awassi (CA), and Romanov x Awassi (RA) F1 crosses. Twenty, six-month old lambs (10 from each sex) from each genotype were used. Ram and ewe lambs were kept in separate groups from the beginning until the end of the experiment. Birth weight and weaning weight were recorded for all animals. Body weights and body condition scores were recorded at monthly intervals between 6.5 and 12 months of age. Blood samples were collected weekly from ewe lambs and fortnightly from ram lambs to monitor progesterone and testosterone profiles, respectively. Semen characteristics and scrotal circumferences (SC) were evaluated fortnightly. Weight at puberty in ewe and ram lambs were not significantly different among the three genotypes and ranged around 35 kg and 42 kg, respectively. In ewe lambs, age at puberty was significantly higher (P< 0.01) in A (280±11.5 d) than in RA (232±11 d) and CA (255±11.5 d). Age at puberty of ram lambs was also significantly different (P < 0.01) among the three genotypes being higher in A (243±5 d) than in CA (223±5 d) and RA (226±5 d) ram lambs. Semen parameters improved with age in all genotypes. The RA ram lambs had greater semen concentration and lower abnormal spermatozoa than the other two genotypes (P< 0.05). Results of the present study indicate that crossing Awassi ewes with either Charollais or Romanov sires tends to improve reproductive characteristics of the F1 crossbreds through advancing age at puberty in both ram and ewe lambs.

Key words: Crossbreeding, Sheep, Reproduction, Puberty.

RIASSUNTO

ETÀ ALLA PUBERTÀ E CORRELAZIONE CON ALCUNI PARAMETRI BIOLOGICI DI AGNELLI DI RAZZA AWASSI E DI PRODOTTI F1 CHAROLLAIS X AWASSI E ROMANOV X AWASSI

Obiettivo di questa ricerca è stato quello di determinare l’età alla pubertà di agnelli, sia maschi che femmine, appartenenti a tre diversi genotipi: Awassi (A), Charollais x Awassi (CA) e Romanov x Awassi (RA). A tal fine sono stati utilizzati venti agnelli di sei mesi (dieci per ciascun sesso) per ogni classe genotipica. Maschi e femmine sono stati divisi in due gruppi durante l’intera durata dello studio. Si è provveduto a valutare il peso alla nascita e allo svezzamento di tutti gli animali ed inoltre sono stati registrati mensilmente, tra i 6,5 e i 12 mesi di età, il peso corporeo e il relativo Body Condition Score (BCS). Sono stati effettuati prelievi di sangue una volta alla settimana nelle femmine e con cadenza bisettimanale nei maschi tenendo così monitorato l’andamento del progesterone e del testosterone. Le caratteristiche dello sperma e la circonferenza scrotale (SC) sono state valutate ogni quindici giorni. Il peso alla pubertà non è risultato significativamente diverso tra i tre genotipi e si è aggirato sui 35 Kg nelle femmine e 42 Kg nei maschi. Nelle femmine, l’età alla pubertà è stata significativamente più alta (P<0,01) nel genotipo A (280 ± 11,5 d) rispetto a quello RA (232 ± 11 d) e CA (255 ± 11,5 d). Anche nei maschi l’età alla pubertà è risultata significativamente diversa (P < 0,01) tra i
Introduction

Reproductive efficiency is one of the main factors that determine the efficiency of production especially in countries in which the sheep industry, and particularly meat production, is important (Ibarra et al., 2000). Improvement in productive and reproductive traits of sheep can be achieved using several approaches including selection, crossbreeding or a combination of these methods. Since the efficiency of sheep production mainly depends on genetic improvements of reproduction, growth and meat production, improving such traits became a major goal for sheep breeding (Dickerson, 1970).

Awassi, the predominant sheep in the Middle East, is a dual-purpose breed (Gootwine et al., 1995) characterized by several desirable traits such as the popularity of its meat and milk, and the high adaptability to different ecosystems (ICARDA, 2003). Awassi sheep, however, have low prolificacy (Abdullah et al., 2002) and reach puberty at a later age than other breeds (Al-Molla and Kridli, 2003). Romanov sheep are characterized by early sexual maturity (3 months of age), high prolificacy and out-of-season breeding ability (Fahmy, 1996). The Charollais sheep breed is also prolific but mostly known for high lean meat production (Farid and Fahmy, 1996).

This trial is part of a crossbreeding experiment aiming at improving reproductive efficiency and meat production of Awassi through crossing with Romanov and Charollais sheep breeds, respectively. The long-term objective of this experiment is to produce synthetic breeds that combine the adaptability of Awassi and the prolificacy and meat productivity of Romanov and Charollais sheep. Along the course of producing such breeds, evaluating offspring reproductive performance, particularly age at puberty, is essential in order to reduce generation intervals. For this reason, this study was conducted to evaluate age at puberty, biological parameters and semen characteristics of Awassi and its crosses with Charollais and Romanov sheep breeds.

Material and methods

Animals and treatments

The study was conducted at the Agricultural Center for Research and Production at Jordan University of Science and Technology located in the northern part of Jordan (semi-arid) at 32°N and an altitude of 510 m. Awassi ewes were mated with either Charollais or Romanov sires. Twenty lambs (10 males and 10 females) of similar ages from each genotype [Awassi (A), F1 Charollais x Awassi (CA) and F1 Romanov x Awassi (RA)] were identified at weaning (60 days of age) to be used in this study. The experimental lambs were chosen based on age with as little variation in weights as possible. Birth and weaning weights were recorded for all animals. Ram and ewe lambs were kept in separate groups from the beginning until the end of the experiment. Each group was maintained in an outside pen with an access to an open-front barn and fed according to NRC (1985) recommendations depending on their stage of growth. The offered rations consisted of wheat straw, barley, soybean meal, wheat bran, corn and mineral and vitamin premix.

Body weight and body condition score

Body weight and body condition score (BCS) were recorded at the beginning of the experiment (6.5 months of age) and at monthly intervals until 12 months of age. Body condition scores were manually evaluated by palpating the fullness of muscling and fat cover over and around the vertebrae in the loin area using a score system of 1 to 5 (1 being emaciated and 5 being obese).

Blood collection, testosterone and progesterone assays

Blood samples were collected weekly from ewe...
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lambs and fortnightly from ram lambs. Samples were collected via jugular venipuncture and loaded into heparinized tubes (5 I.U heparin/tube). Plasma was harvested by centrifugation at 3000 rpm for 15 min within an hour of collection. Plasma was stored at -20 °C until analyzed. All plasma testosterone samples were run in a single assay having a CV of 5.6%. Similarly, all progesterone samples were run in a single assay having a CV of 7.3%. Both hormones were analyzed using radioimmunoassay kits (Immunotech, France).

Puberty

Ewe lambs were considered to be pubertal upon sustained elevation in progesterone concentration above 1 ng/ml over two consecutive blood collections. A 1 ng/ml progesterone concentration is indicative of luteal function (Chagas e Silva et al., 2003). Ram lambs were considered to be pubertal based on elevated testosterone concentrations (2 ng/ml) and the presence of normal spermatozoa in the ejaculate with at least 30% mass motility over two consecutive semen collections. This testosterone level was adopted as Dufour et al. (1984) reported a 2.5 ng/ml average testosterone concentration in Suffolk rams. For animals to reach puberty, their circulating hormones have to reach the adult levels.

Semen collection and evaluation

Semen samples were collected fortnightly using an electroejaculator as it does not require previous training of ram lambs (Belibasaki and Kouimtzis, 2000). Samples were evaluated for volume (using graduated vials) and mass motility (ranging from 0% = total immobility to 100% = rapid wave motion). Evaluation of semen concentration and abnormal sperm percentage were performed later in the lab using a haemocytometer with the aid of a microscope. semen evaluation was conducted using similar procedure to that described by FAO (1991). Scrotal circumference (SC) was evaluated at the time of semen collection using a flexible tape around the widest point of the testes (maximum circumference of the paired testes).

Statistical analysis

Male and female data were independently analyzed as a completely randomized design using the “general linear model” procedure of SAS statistical package (SAS, 1997). The differences between genotype levels of birth weight, weaning weight, age at puberty, weight at puberty and BCS at puberty were tested by a pairwise t test using the “least square means” statement. Scrotal circumference and semen characteristics 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 (SAS, 1997). Simple correlations between age, weight, SC and BCS at puberty were calculated.

Results and discussion

Puberty in ewe lambs

Higher birth weights (P < 0.01) were recorded for CA than for A and RA ewe lambs. The A ewe lambs, however, had higher weaning weights (Table 1). Similar results for birth weight were reported by Momani Shaker et al. (2002). Age at puberty in ewe lambs was significantly affected (P < 0.01) by genotypes (Table 1). Puberty occurred earlier in RA (232 d) and CA (255 d) compared with A (280 d) ewe lambs. Age at puberty in sheep ranges from 4 to 15 months with an average of 7.5 months (225 d) (Hunter, 1982). Awassi ewe lambs reach puberty at around nine months of age (Al-Molla and Kridli, 2003). Crossbreeding accelerated the onset of puberty in the present study. Variation in age at puberty may be attributed to genetic differences such as breed and crossbreeding, whereas inbreeding tends to delay and crossbreeding tends to accelerate the onset of puberty (Hunter, 1982). Romanov ewe lambs reach puberty earlier than lambs of most other European breeds (Cornu and Cognie, 1985). Our results show that puberty occurred around three weeks earlier in RA than in CA ewe lambs.

Puberty in crossbreds in the present study was delayed compared to what was reported in the literature (Belibasaki and Kouimtzis, 2000; Al-Molla and Kridli, 2003). This may have been caused by several reasons including breeds, birth date and temperature. The experimental animals were born between November and December. By the time these ewe lambs were old enough to reach puberty...
Table 1. Weights, age at puberty and body condition scores (BCS) in Awassi (A), Romanov x Awassi (RA) and Charollais x Awassi (CA) ewe lambs (Mean±SE).

| Genotype | Item (n.=10) | RA (n.=10) | CA (n.=10) | A (n.=10) |
|----------|-------------|------------|------------|-----------|
|          | Birth weight kg | 4.5 ±0.24  | 5.4 ±0.25  | 4.3 ±0.25 |
|          | Weaning weight " | 18.5 ±1.0  | 20.6 ±1.1  | 23.3 ±1.1 |
|          | Age at the beginning of sampling d | 188±6 | 191±6 | 184±6 |
|          | Weight at puberty kg | 34.9±1.7 | 35.3±1.8 | 36.5±1.9 |
|          | Age at puberty d | 232±11 | 255±11.5 | 280±11.5 |
|          | BCS at puberty | 3.2±0.2 | 3.4±0.2 | 3.3±0.2 |

*B* Means within the same row with different superscripts differ significantly (P<0.01).

Table 2. Correlation coefficients between variables tested at puberty for ewe and ram lambs.

| Ewe lambs | Age at puberty | BCS at puberty¹ |
|-----------|----------------|-----------------|
| Weight at puberty | 0.53a | 0.86a |
| Age at puberty | 0.39 | |

| Ram lambs | Age at puberty | BCS at puberty¹ | SC² |
|-----------|----------------|-----------------|-----|
| Weight at puberty | 0.35 | 0.75b | 0.69b |
| Age at puberty | 0.1 | 0.56a |
| BCS at puberty | | 0.63a |

¹ BCS = Body condition score.
² SC = Scrotal circumference at puberty.
* Superscript above correlation coefficients indicates significance level (P<0.05).

As reported in the literature, day length was increasing. In addition to that, summer temperature in Jordan is higher than where Charollais and Romanov breeds originated, which may have delayed the onset of puberty due to heat stress. Weight and BCS at puberty in ewe lambs were similar among the three genotypes (Table 1) ranging around 35 kg. The lack of significance in body weight in our study may be due to the fact that heterosis resulted in faster growth rate in the crossbreds. These crossbred ewe lambs reached the designated weight required to initiate puberty at a younger age. Age at puberty was moderately correlated (P<0.05) with body weight at puberty (Table 2).

**Puberty in ram lambs**

Similar birth weights were recorded for the three genotypes while weaning weights were higher (P<0.05) for A than for CA and RA ram lambs (Table 3). Age at puberty in ram lambs significantly differed (P<0.05) among genotypes (Table 3). The RA and CA ram lambs reached puberty earlier than A (226, 223 and 243 days of age, respectively).
PUBERTY IN AWASSI AND CROSSBRED LAMBS

These results are in agreement with previous research in which crossbreds reached puberty earlier than purebreds (Hunter, 1982). Our data indicate that crossbreeding accelerated the onset of puberty. Romanov is a breed noted for high reproductive performance. Fahmy (1990) reported that some Romanov ram and lambs may reach puberty at the extremely young age of 2.5 to 3 months.

Weight at puberty was similar among the three genotypes ranging around 40 kg (Table 3). Previous studies reported puberty in Libyan fat-tailed rams to occur at 40 kg of body weight (Madani et al., 1989). Other breeds (such as Debouillet) reach puberty at heavier body weights (Shirley et al., 2001). Pure Romanov rams have much lower mature body weights than pure Charollais while pure Awassi are intermediate. Puberty is reached when sheep reach 40 to 50% of their mature body weight (Bearden and Fuquay, 1997). Crossbred goats have higher growth rates (Zaman et al., 2002) which can be attributed to heterosis. The three genotypes reached puberty at similar weights but their ages were different. This indicates that body weight is more important than age in determining the onset of puberty.

Weight at puberty was significantly correlated (P<0.05) with SC and body condition score (Table 2). The correlation between weight at puberty, SC and BCS could be explained by growth of the ram lambs. This is in agreement with previous research (Duguma et al., 2002; Fourie et al., 2002).

**Scrotal circumference and testosterone concentration**

Scrotal circumferences were significantly lower (P<0.05) in A than in RA and CA lambs throughout the sampling period (Figure 1). Scrotal circumference differs among breeds of sheep (Belibasaki and Kouimtizis, 2000; Kridli et al., 2002) being higher in crossbred than purebred lambs. There was a significant (P<0.05) linear sampling day effect with respect to scrotal circumference (Figure 1).

Testosterone concentration increased as the experiment advanced (Figure 2). The overall testosterone concentration differed significantly (P<0.05) among genotypes with RA and CA having higher concentrations than A lambs. Testosterone concentrations did not differ among genotypes during the first few weeks of the experiment. The difference in SC noted above was reflected in testosterone concentration in favor of crossbred ram lambs. Testosterone concentrations increase linearly with age and may differ between pure- and crossbred rams (Fahmy, 1997) and among breeds of sheep (Dickson and Sanford, 2005).

**Semen characteristics**

Semen characteristics are reported in Figures 3 to 6. Sampling day significantly affected (P<
No genotype by sampling day interactions were detected regarding semen characteristics. Ejaculate volume was similar among genotypes (Figure 3). The lack of significance among breeds with respect to ejaculate volume, despite the difference in scrotal circumference, 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).

Figure 4 shows mass motility of spermatozoa.
In general, RA lambs had numerically higher values than the other two genotypes. Mass motility was influenced by sampling day (P< 0.05). All semen and spermatozoa traits improve with age (Rege et al., 2000). Abnormal spermatozoa were influenced by genotype and sampling day (Figure 5). The RA lambs had significantly lower (P< 0.05) abnormal sperm percentage than CA and A lambs. Abnormality percentage decreased as the experiment advanced.

Semen concentrations were also influenced by genotype and sampling day (Figure 6). During most of the experiment, semen concentration was significantly higher (P< 0.05) in RA than the other two genotypes. This difference in semen concentration resembled the difference in testos-
Testosterone greatly influences spermatogenesis (sperm output) as it is involved in mitotic division of spermatogonia (Lincoln, 1989). Similar relationship between testosterone concentration and sperm output was reported by Dickson and Sanford (2005). Spermatozoa output also has medium to high relationship with testicular measurements (Rege et al., 2000). 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).

Semen quality at puberty is poor with high proportion of abnormalities and low motility, but it improves significantly within few months following puberty with the rate of transition from poor to good semen being dependent on breed (Court, 1976). Differences in semen quality among genotypes in the present study are probably related to the more fertile and prolific nature of the Romanov breed (Dufour et al., 1984).

Figure 5. Sperm abnormality percentages in Awassi (A), Charollais x Awassi (CA) and Romanov x Awassi (RA) ram lambs (error bars indicate ±SE).

Figure 6. Sperm concentration (x 10^7 sperm/ml) in Awassi (A), Charollais x Awassi (CA) and Romanov x Awassi (RA) ram lambs (error bars indicate ±SE).
Conclusions

Crossing Awassi with Romanov improved reproductive performance in the crosses through advancing age at puberty in males and females and improving semen quality in males. Crossing Awassi with Charollais advanced age at puberty in males and females but had no influence on semen quality in males compared with Awassi.

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