

Reproductive performance of hormonally-treated anestrus Awassi ewes

R.T. Kridli¹, M.Q. Husein, H.A. Muhdi and J.M. Al-Khazaleh

Dept. of Animal Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid 22110, Jordan.

Abstract

An experiment was conducted to evaluate the effect of administering melatonin alone or in combination with a progestagen and equine chorionic gonadotropin (eCG) on reproductive performance and inducing estrus in Awassi ewes. Fifty-nine, multiparous, winter-lambing Awassi ewes, ranging from 3 to 6 years of age, were randomly assigned during the late anestrus period (Spring) to one of six treatment groups as follows: no treatment (CON; n = 9), melatonin implant only (M; n = 10), flourogestone acetate (FGA) sponge only (P; n = 10), melatonin implant plus FGA sponge (MP; n = 10), FGA sponge plus eCG (PE; n = 10), or melatonin implant plus FGA sponge and eCG (MPE; n = 10). Ewes in P, MP, PE, and MPE groups were fitted with intravaginal progestagen sponges for 14 days. On the day of sponge removal, each ewe in the PE and MPE groups received a 600-IU dose of eCG. Ewes in the M, MP, and MPE groups received subcutaneous melatonin implants (18 mg melatonin) 36 days before sponge insertion. Estrus expression was lower ($P < 0.001$) in the CON and M groups than in the remaining groups. The interval to onset of estrus was shorter ($P < 0.001$) in the PE and MPE ewes compared to ewes in the remaining treatments. Ewes in the P, PE, and MPE groups lambed earlier ($P < 0.01$) than those in the remaining groups. Fecundity during the induced estrus was greater ($P < 0.05$) in the PE and MPE groups than the CON, M, and MP groups. The overall lambing rate during the two estrous cycles following sponge removal was similar among all groups. The overall prolificacy and litter birth weight were greater ($P < 0.05$) in the groups that received eCG. Results of the present study indicate that melatonin alone failed to induce estrus in a manner similar to FGA-primed ewes. Melatonin administration in combination with FGA and eCG can be successfully applied to improve induced estrus reproductive parameters in sheep.

Keywords: Awassi sheep, eCG, estrus, FGA sponges, melatonin.

Introduction

Reproductive seasonality in ewes is characterized by changes in behavioral, endocrine, and

ovulatory patterns (Epstein, 1985; Rosa and Bryant, 2003). Seasonal reproduction in sheep is mainly regulated by photoperiod through melatonin secretion along with other environmental factors such as temperature, nutrition, and social relationships (Arendt, 1998).

Prolonged administration of exogenous melatonin during the anestrus season leads to an early onset of the breeding season (Misztal *et al.*, 2002). Melatonin implants have the ability to advance and condense the breeding season in ewes from a wide range of breeds with different patterns of seasonality (Laloties *et al.*, 1997; Abecia *et al.* 2005). The treatment must begin within the effective period, which depends on the normal date of seasonal onset of estrus for the breed (Wheaton *et al.*, 1990; Haresign, 1992b). Haresign *et al.* (1990) suggested that the optimum implantation of melatonin is around 60 days before the normal onset of estrus for a given breed. The response of ewes to melatonin treatment depends on breed (Abecia *et al.*, 2006), season, and location (Abecia *et al.*, 2005).

Fat-tailed Awassi is the local breed of sheep in Jordan and is the most important breed in the semi-arid regions of the near east countries (Epstein, 1985). It has several desirable traits such as the popularity of its meat and milk and the high adaptability to different ecosystems. Resistance to diseases, tolerance to extreme temperatures, ability to walk long distances for grazing, strong flock instinct, and endurance of adverse management and feeding conditions have encouraged sheep producers to raise this breed (Thomson *et al.*, 2003). However, a negative characteristic of the Awassi breed is low fertility (Hamadeh *et al.*, 2001). Under natural conditions, the Awassi sheep breeding season occurs during the summer and fall (Epstein, 1985). Mating mostly occurs between late June through early September, allowing ewes to lamb between late November and early February (Thomson *et al.*, 2003). Breeding ewes before this period results in lambing before the cold winter months.

To our knowledge, no experiments have been conducted regarding the use of melatonin implants to improve reproductive efficiency of Awassi sheep. The current study was designed to investigate the effects of administering melatonin alone or in combination with a progestagen and equine chorionic gonadotropin (eCG) on reproductive performance and inducing estrus in Awassi ewes.

¹Corresponding author: rkridli@just.edu.jo
Phone: +962-2-7201000 ext. 22213; Fax: +962-2-7095069
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Materials and Methods

The study was conducted during the months of March (spring) through October of 2004 at the Center of Agricultural Research and Production at the Jordan University of Science and Technology located in the northern part of Jordan at 32° 30' N and an altitude of 520 m above sea level.

Fifty-nine, multiparous Awassi ewes, ranging from 3 to 6 years of age and weighing 43.2 ± 5.7 kg (mean \pm SEM), were used. Animals were offered a balanced ration composed of 0.5 kg of concentrate mixture and 1 kg wheat straw per head per day. In addition, animals were allowed range grazing during the day. All experimental animals had free access to water, shade, and trace mineral blocks.

The experimental ewes were placed in one group and randomly allocated to one of six treatments in a completely randomized design. The six treatment groups were: no treatment (CON; $n = 9$), melatonin implant only (M; $n = 10$), flourogestone acetate (FGA) sponge only (P; $n = 10$), melatonin implant plus a FGA sponge (MP; $n = 10$), FGA sponge plus eCG (PE; $n = 10$), or melatonin implant plus a FGA sponge and eCG (MPE; $n = 10$).

Ewes in the M, MP, and MPE group received melatonin implants (Regulin®, CEVA Sante Animale, France) on March 8th, 50 days before the anticipated breeding season (Day-50) as recommended by CEVA Sante Animale. Melatonin implants were administered subcutaneously near the base of the ear using an applicator gun. Each Regulin® implant contained 18 mg of melatonin. Ewes in the P, PE, MP, and MPE groups were fitted with intravaginal FGA sponges (40 mg, CEVA) on April 14th (Day-14). Sponges were treated with a broad spectrum antibiotic (0.5 ml) in order to prevent microbial growth. Sponges were withdrawn on Day 0 at which time a 600-IU dose of eCG was administered to each ewe in the PE and MPE groups. Ewes in the remaining groups received normal saline solution (no eCG).

Blood collection started on the day of melatonin implant insertion (Day-50). Weekly blood collection was conducted from Day-50 until the day of intravaginal FGA sponge insertion (Day-14) and once more on Day 0. Blood samples were collected via jugular venipuncture into heparinized tubes. One hundred microliters containing 5 IU of heparin were pipetted into each blood tube just before each blood collection. Blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma was harvested by using sterilized, plastic, disposable Pasteur pipettes into plastic vials and stored at -20 °C until assayed for progesterone. Progesterone was quantified by radioimmunoassay (RIA; Diagnostic Products Corporation, LA, CA, USA). All samples were run in a single assay. Assay sensitivity was 0.1 ng/ml and the

intra-assay CV was 3%.

Eight fertile, experienced, mature Awassi rams were introduced to all groups at the time of FGA sponge removal (Day 0) and remained with the groups for 51 days (3 cycles). Rams were fitted with marking harnesses and allowed to interact with the group of ewes. Detection of estrus (1 hour observation time) was performed every 6 hours for 6 days starting on Day 1 following sponge withdrawal. Estrus was detected by ram seeking behavior and breeding marks. Estrus in "marked" ewes was estimated to have started 3 hours before the actual observation. Crayons installed on the ram harnesses were scrubbed off on daily basis to remove any attached manure.

Ewes lambing up to 155 days following Day 0 were considered to have conceived at induced estrus. On the other hand, ewes that lambed more than 155 days following Day 0 were considered to have conceived from mating during the following estrous cycles (the cycles following induced estrus). Lambing rate was calculated as the number of lambed ewes as a percentage of ewes exposed to rams. Fecundity was calculated based on the number of lambs born per exposed ewe while prolificacy was calculated as the number of lambs born per lambed ewe.

Data were analyzed as a completely randomized design using a General Linear Model procedure of SAS (SAS, 1997). The effect of treatment on the interval to onset of estrus, the number of lambs born, prolificacy, fecundity, and litter birth weight was tested by using the pairwise t-test with a "least square means" statement. Data regarding estrus expression, lambing rate, and the number of multiple births were analyzed by Chi-square (SAS, 1997).

Results

Basal progesterone concentrations (< 0.40 ng/ml) were detected throughout the sampling period until the time of ram introduction. Estrus expression following ram introduction differed ($P < 0.001$) among the treatment groups (Table 1). The groups that were not treated with FGA sponges (CON and M groups) had a lower number of ewes expressing estrus than the remaining treatments. The percentage of ewes expressing estrus in this experiment was 73%. The interval from sponge withdrawal to the onset of estrus differed ($P < 0.001$) among treatments (Table 1). Ewes in the PE and MPE groups expressed estrus earlier than those in the CON, M, and P groups while ewes in the MP group were intermediate.

Lambing rates from mating at induced estrus are presented in Table 1. Lambing rate from conception during the first cycle following ram introduction significantly differed ($P < 0.05$) among treatments and was greater in the MPE than the CON, M, and MP groups while the P and PE groups were intermediate.



Table 1. Reproductive performance of Awassi ewes treated with various hormonal combinations during the spring.

Variable	Treatment ¹					
	CON (n = 9)	M (n = 10)	P (n = 10)	MP (n = 10)	PE (n = 10)	MPE (n = 10)
Expression of estrus	2(22%) ^b	4(40%) ^b	9(90%) ^a	9(90%) ^a	9(90%) ^a	10 (100%) ^a
Interval to onset of estrus (hours)	54.0 ± 6.5 ^{bc}	55.5 ± 4.6 ^b	49.3 ± 3.1 ^{bc}	43.3 ± 3.1 ^c	33.3 ± 3.1 ^a	34.3 ± 2.9 ^a
Lambing rate	1 (11%) ^d	1 (10%) ^d	5 (50%) ^{de}	2 (20%) ^d	5 (50%) ^{de}	8 (80%) ^e
Fecundity (lambs born per mated ewe)	0.11 ± 0.2 ^{de}	0.1 ± 0.2 ^{de}	0.5 ± 0.2 ^{ef}	0.2 ± 0.2 ^{de}	0.7 ± 0.2 ^{fg}	1.2 ± 0.2 ^g
Prolificacy (lambs born per lambing ewe)	1.0 ± 0.6	1.0 ± 0.6	1.0 ± 0.3	1.0 ± 0.4	1.4 ± 0.3	1.5 ± 0.2
Multiple births (No.)	0/1 ^d	0/1 ^d	0/5 ^d	0/2 ^d	2/5 ^e	3/8 ^e

¹Treatments:

CON: no treatment.

M: melatonin implants.

P: flourogestone acetate (FGA) sponges.

MP: melatonin implants plus FGA sponges.

PE: FGA sponges plus equine chorionic gonadotropin (eCG).

MPE: melatonin implants plus FGA sponges and eCG.

^{a,b,c} Means within the same row with different superscripts differ (P < 0.001).^{d,e,f,g} Means within the same row with different superscripts differ (P < 0.05).

Table 2. Reproductive performance from mating during 3 consecutive cycles in Awassi ewes treated with various combinations of hormonal treatments.

Variable	Treatment ¹					
	CON (n = 9)	M (n = 10)	P (n = 10)	MP (n = 10)	PE (n = 10)	MPE (n = 10)
Lambing ewes (No.)	7	6	7	8	6	8
Barren ewes (No.)	2	4	3	2	4	2
Lambs (No.)	7	6	7	8	8	12
Lambing rate (%)	78%	60%	70%	80%	60%	80%
Lambing date (days following ram introduction)	178 ± 3 ^b	172 ± 3 ^b	159 ± 3 ^a	169 ± 3 ^b	152 ± 3 ^a	154 ± 3 ^a
Fecundity (lambs born per mated ewe)	0.78 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	1.2 ± 0.2
Prolificacy (lambs born per lambing ewe)	1.0 ± 0.1 ^c	1.0 ± 0.2 ^c	1.0 ± 0.1 ^c	1.0 ± 0.1 ^c	1.3 ± 0.2 ^{cd}	1.5 ± 0.1 ^d
Total litter weight (kg)	4.4 ± 0.4 ^c	4.2 ± 0.4 ^c	4.4 ± 0.4 ^c	4.2 ± 0.3 ^c	5.0 ± 0.4 ^{cd}	5.7 ± 0.3 ^d

¹Treatments

CON: no treatment.

M: melatonin implants.

P: flourogestone acetate (FGA) sponges.

MP: melatonin implants plus FGA sponges.

PE: FGA sponges plus equine chorionic gonadotropin (eCG).

MPE: melatonin implants plus FGA sponges and eCG.

^{a,b} Means within the same row with different superscripts differ (P < 0.01).^{c,d} Means within the same row with different superscripts differ (P < 0.05).

The total number of lambs born to ewes conceiving at induced estrus was 28. Fecundity was greater (P < 0.05) in the MPE and PE groups than the CON, M, and MP groups (Table 1). Prolificacy was similar among treatments (Table 1). The number of

multiple births was greater (P < 0.05) in the PE and MPE groups. No multiple births were observed in the CON, M, P, and MP groups.

The overall lambing rate (from mating at induced estrus and the following cycles) was 42/59 (71%) and was



similar among all treatments [7/9 (78%), 6/10 (60%), 7/10 (70%), 8/10 (80%), 6/10 (60%), and 8/10 (80%) in the CON, M, P, MP, PE, and MPE groups, respectively; Table 2]. The number of lambs born per serviced ewe was similar among treatments and was numerically greater in MPE group compared to the other treatments. The number of lambs born per lambing ewe was greater ($P < 0.05$) in the MPE than the P and MP groups. No treatment effects were observed for the number of ewes that were rebred. No multiple births were obtained from mating during the 2nd and 3rd cycles. Ewes in the P, PE, and MPE groups lambing earlier ($P < 0.01$) than those in the remaining groups (Table 2). The majority of CON, M, and MP ewes that were mated during the last 2 cycles had delayed lambing compared to the remaining treatments. The overall litter birth weight was greater ($P < 0.05$) in the groups that received eCG (PE and MPE).

Discussion

Sheep are seasonal breeders although the degree of seasonality varies among breeds (Rosa and Bryant, 2003). The breeding season in Awassi ewes lasts from June to December (Epstein, 1985) with the majority of ewes bred between late June and early September (Zarkawi, 1997; Thomson *et al.*, 2003). The present study was conducted in March and April, which represent the anestrus period in Awassi ewes.

Progesterone concentrations on Day -50 indicate that none of the animals were cycling at the time of melatonin implantation. Behavioral estrus was detected in 73% of the ewes in response to treatment. Of those, 92.5% of the ewes treated with FGA sponges expressed estrus while only 31.5% of the non-FGA treated ewes expressed estrus within 6 days of ram introduction. Regardless of treatment, estrus expression was similar among FGA-treated ewes, indicating that progesterone priming was effective at inducing follicular development during late anestrus and resulted in elevated estrogen production. Similar estrus expression (97%) was reported by Kridli and Al-Khetib (2006) in ewes primed with progestagen.

Abecia *et al.* (2005) reported that melatonin administration results in a higher percentage of cyclic ewes at ram introduction while Wheaton *et al.* (1990) reported that estrus was detected at similar time in ewes that had received melatonin implants and those had not. The CON and M groups had similar responses to the ram introduction despite the fact that they failed to respond in a manner similar to the FGA-primed ewes. Treating ewes with FGA sponges accelerated the mechanism of follicular growth and development.

In the present study, ewes exhibited estrus from 24 to 84 hours following sponge removal. These results are in agreement with other research (Lunstra and Christenson, 1981). The interval to detected estrus was shorter in ewes treated with eCG. The intervals to

detected estrus in the present study are in agreement with those reported by Vinales *et al.* (2001) in which the interval to estrus occurrence in ewes treated with medroxy progesterone acetate (MAP) sponges alone was 49 hours. Ewes treated with progestagen and eCG have shorter intervals to the onset of estrus compared with ewes treated with a progestagen alone (Kridli and Al-Khetib, 2006). Administration of eCG reduces the variation in the intervals from implant removal to estrus and ovulation (Cline *et al.*, 2001).

Lambing rate following mating at induced estrus can be used as an effective indicator for the profitability of estrus synchronization in ewes. Induced-estrus lambing rate for all treatments combined in the present study was very low (22/59; 37%). This is lower than previously reported in other studies (Lalotiotis *et al.*, 1997; 1998) due to the lower lambing rates of the CON, M, and MP groups. Lambing rate was greater in the MPE than the CON, M, and MP groups. The low lambing rates in the CON and M groups were expected due to the low expression of estrus in these groups. However, no explanation is available at present for the lower lambing rate in MP ewes, especially considering that ewes treated with a progestagen alone had similar lambing rates to ewes that received eCG. The lower lambing rate in the MP group resulted in more ewes bred during the following cycles and led to the delayed lambing of this group.

The total number of lambs born to ewes that conceived at induced estrus was 28. All multiple births occurred within the PE and MPE groups. Rajkumar *et al.* (1989) and Haresign (1992a), however, reported a significant increase in ovulation rate and litter size in seasonally anestrus ewes treated with melatonin. The number of lambs born to ewes bred at induced estrus in the present study was greater in MPE group compared with the remaining groups. Noel *et al.* (1999) reported that melatonin pretreatment prior to the FGA-eCG synchronization treatment at the end of anestrus significantly enhanced ovulation rate of Suffolk ewes, resulting from improved follicular development. The use of melatonin and progestagen treatment promotes ovarian activity by increasing the number of follicles and rate of ovulation during seasonal anestrus in the ewe (Wheaton *et al.*, 1990). The use of melatonin associated with FGA and eCG has been shown to improve fecundity due to ovulation of more follicles (Noel *et al.*, 1999).

A major function of melatonin is to coordinate seasonal changes in reproductive activity (Hazlerigg, 2001). The mean litter size per lambing ewe (prolificacy) from mating at induced estrus was similar among treatments. In contrast, Lalotiotis *et al.* (1998) reported that Chios ewes treated with a progestagen and eCG and ewes treated with melatonin plus a progestagen and eCG differed significantly in mean litter size per lambing ewe that conceived during the first estrus after sponge removal (1.3 vs. 1.5). This



contradiction may have occurred because Awassi sheep generally have lower fertility (Hamadeh *et al.*, 1996) than Chios crossbred ewes. Haresign *et al.* (1990) reported an increase in litter size following melatonin implantation. This probably resulted from an increased ovulation rate. It is unclear whether the increase in mean litter size is due to an increase in the ovulation rate or a reduction in embryo mortality (Haresign *et al.*, 1990). The higher overall prolificacy observed in the MPE ewes in the present study (1.5 ± 0.1) could be the result of one or more of the following factors: an increase in ovulation rate, improved embryonic survival, or an improvement in luteal function (Abecia *et al.*, 2001). Melatonin administration reduces estradiol negative feedback and allows continued LH pulsatility and promotion of follicular growth and estradiol secretion (Wheaton *et al.*, 1990). This higher prolificacy led to the greater litter birth weight observed in this group.

The overall lambing rate (over 3 cycles) did not differ among groups (71%) and was similar to that (76.8%) reported by Laliotis *et al.* (1998). Lambing occurred earlier in the P, PE, and MPE groups. It appeared that ewes in the remaining groups responded to the ram effect and were bred during the subsequent cycles. The response of ewes to melatonin treatment may vary according to nutrition, breed, season, and the latitude where the study was conducted. In Mediterranean sheep breeds, there may be an interaction between nutrition and melatonin administration. Forcada *et al.* (2002) reported an increase in ovulation rate in melatonin-treated ewes and especially in those at a low rather than high plane of nutrition. Exogenous melatonin treatment could improve ovulation rate in ewes with a moderately low body condition score at the beginning of the breeding season.

Implications

Results of the present study indicate that melatonin alone failed to induce estrus in a manner similar to FGA-primed ewes upon ram introduction. Melatonin administration in combination with FGA and eCG can be successfully applied to improve induced-estrus reproductive parameters in sheep. The fact that Awassi ewes undergo a short period of seasonal anestrus may have reduced the effects of administering melatonin especially when FGA sponges were administered. However, further studies with greater animal numbers are needed to investigate such effects before any definite conclusions can be drawn.

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