Reproductive Responses of Awassi Ewes Treated with either Naturally Occurring Progesterone or Synthetic Progestagen

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ABSTRACT : The objective was to identify the appropriate form of progesterone, which exhibits compact reproductive responses in Awassi ewes during mid to late seasonal anestrous period. Forty-eight Awassi ewes were randomly allocated into four groups to be treated with 60 mg medroxyprogesterone acetate (MAP), 30 mg fluorogestone acetate (FGA), 40 mg FGA, or 600 mg progesterone sponges. After a 12 day period, sponges were removed and ewes were administered i.m. with 600 IU PMSG (d 0, 0 h). Five harnessed Awassi rams were turned-in with the ewes to detect heat. Ewes were checked for breeding marks at 6 h intervals for 5 days. Blood samples were collected from all ewes for analysis of progesterone concentrations. Pretreatment (d -13 and -12) progesterone concentrations were ≤0.2 ng/mL among all ewes and were indicative of seasonal anestrous period. On d 0, progesterone concentrations were elevated to 1.4±0.1 ng/mL in ewes received progesterone sponges only and were higher ($p<0.0001$) than those (≤0.2 ng/mL) administered MAP or FGA sponges. Progesterone concentrations returned to their basal values of <0.2 ng/mL within 24 h of sponge removal and were similar ($p>0.1$) among all ewes. Incidence of estrus was similar ($p>0.1$) among the four groups and occurred in 75% (9/12), 82% (9/11), 67% (8/12) and 58% (7/12) of the ewes receiving MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively. Estrous responses occurred 14.7, 20 and 13.6 h earlier in progesterone-sponge-treated ewes than those of MAP- ($p<0.04$), 30 mg FGA- ($p<0.01$) and 40 mg FGA-treated ($p=0.06$) ewes, respectively. Induced estrus conception rates were 50% (6/12), 55% (6/11), 50% (6/12) and 42% (5/12), out of which 4/6, 4/6, 3/6 and 3/5 lambed 151 days following d 0, and were similar ($p>0.1$) among ewes of the four treatment groups. Ewes that returned to estrus 16 to 20 days following d 0 were 5/12, 5/11, 6/12 and 4/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively, and all lambed 169 days later. Overall lambing rates were 75% (9/12), 82% (9/11), 75% (9/12) and 58% (7/12) ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively. Results demonstrate that applications of MAP, 30 mg FGA, 40 mg FGA and progesterone sponges Awassi ewes were equally effective in induction of estrus and tended to favor both types of FGA and MAP in overall lambing rates over progesterone sponges during the seasonal anestrous period. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 9 : 1257-1262)***

Key Words : Estrus Synchronization, Progesterone, MAP, FGA, Awassi Ewes

INTRODUCTION

Overall productivity of Awassi ewes in Jordan is poor. Low reproductive rates (Lubbadeh, 1986), selective breeding and environmental factors, and/or flock management practices (Goddard, 1982) may contribute to low productivity. Better nutrition can significantly improve reproductive efficiency (Cumming, 1977), but still reduced first service conception and variable fertility rates may have been the outcome of progestagen usage, seasonal and/or environmental influences in this region. Drought has been one of the major limiting factors affecting fertility of Awassi ewes for the last 3 years (Abdullah et al., 2001). According to Epstein (1982) the breeding season of Awassi ewes lasts from July to December. Due to lamb marketing constraints and to be more competitive, sheep producers recognize the need to breed at different times of the year. Under intensive management systems in Jordan, most sheep producers introduce rams in May and June. Beside nutrition, a number of strategies have been developed to stimulate and control ovarian activity in ewes aiming at increasing fertility rates. The most commonly used strategy has been proposed using intravaginal pessaries impregnated with either synthetic progestagen or naturally-occurring progesterone and PMSG (Gordon, 1971; Cunningham, 1980; Fukui et al., 1987). There is, however, little information about reproductive responses of Awassi ewes following administration of different types of progesterone/ progestagen treatments and PMSG. Administration of PMSG has long been used in conjunction with progestagen to stimulate reproductive activity of anestrous ewes (Dutt, 1953; Cunningham et al., 1980; Hamra et al., 1989). Exogenous equine gonadotropin increases incidence of estrus and ovulation and increases ovulation rate (Killeen and Moore, 1970). Despite a high degree of control of estrus and ovulation, variable reproductive performance and lower conception rates have been observed in ewes bred out-of-season (Husein and Kridli, 2001). However, it was not clear whether such variability was a function of using a synthetic progestagen rather than natural progesterone, or was a reflection of other related reproductive management parameters. The

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experimental objective was to identify the appropriate form of progesterone, which exhibit compact reproductive responses in Awassi ewes during mid to late seasonal anestrous period.

MATERIALS AND METHODS

Animals

Forty-eight 3- to 6-yr-old Awassi ewes, weighing 54±2 kg with a condition score of 2.5 to 3.5 (scale=0 lowest to 5 highest), were used in a study conducted at the Agricultural Center for Research and Production at Jordan University of Science and Technology (32°33'N, 35°51'E). Ewes last lambing dates ranged from Dec. 15 to Feb. 15 and had their lambs been weaned on May 17. Ewes were fed 1.2 kg alfalfa hay and 0.5 kg concentrate mixture per ewe per day. Trace mineral salt and water were available on an *ad libitum* basis.

Experimental design

Ewes were randomly allocated into four groups of 12 ewes each to be treated with intravaginal pessaries of 60 mg medroxyprogesterone acetate (MAP, Synchron sponge, Farvet, Bladel Holand), 30 mg fluorogestone acetate (FGA, Sanofi Animal Health, Libourne Cedex, France), 40 mg FGA or 600 mg progesterone sponges prepared as described by Husein et al. (1996). Sponges were inserted on May 24 and were removed 12 days later on June 5 at 08:00 (d 0 and 0 h). At the time of sponge removal ewes received an i.m. injection of 600 IU PMSG (Sanofi Animal Health, Libourne Cedex, France). At 0 h five harnessed Awassi rams were turned-in with ewes, which were checked for breeding marks at 6 h intervals for 5 days. Rams remained with ewes for two consecutive cycles. The second estrus was determined by changing the color of crayons on the harnesses and inspecting the flock twice daily for fresh marks.

Blood sampling and hormone assay

Blood samples were collected via jugular veinpuncture from all ewes one day prior (d -13) to sponge insertion, at the time of sponges insertion (d -12) and at the time of sponge removal (d 0). Blood samples were also collected once daily from d 0 until d 4 and then on alternate days thereafter until d 18 to verify progesterone concentrations and for pregnancy diagnosis. All blood samples (5 mL each) were drawn form the jugular vein into heparinized tubes (5 IU/mL) and centrifuged soon thereafter. Plasma was pipetted and stored at -20°C until assayed (Coat-A-Count, Diagnostic Products Corporation, DPC, Los Angeles, CA) for progesterone concentrations. Sensitivity was 0.1 ng/mL and intraassay coefficient of variation was 3.3%.

Statistical analysis

Data were analyzed using SAS/STAT ANOVA procedures (1990). Means±SE are presented in text and tables. Onset of estrus was considered to have occurred 3 h before observation of the breeding mark. Effects of PMSG treatment on incidence of estrus, induced estrus conception and pregnancy rates were analyzed using Chi-square test. Effects of treatment on various intervals were tested using the least square means of the GLM procedures. Progesterone concentrations were analyzed for the effect of treatment and time using the repeated-measures procedure of GLM. Induced estrus conception rate was defined as the number ewes bred by rams within 5 days following d 0 and became pregnant based upon sustained progesterone levels of >4 ng/mL on d 18. Overall pregnancy rate/lambing rate was defined as the proportion of ewes that became pregnant and lambed in two consecutive estrous cycles. Ewes lambing up to 155 days following sponge removal were considered to have conceived from mating at induced estrus. Ewes lambing following d 155 until d 170 were considered to have conceived form mating at a spontaneous estrus (second cycle).

RESULTS AND DISCUSSION

Progesterone concentrations prior to and following sponge removal and induced estrus conception

All but one ewe had plasma progesterone concentrations of <0.2 ng/mL prior to pessary insertion (d -13 and -12). Plasma progesterone concentrations from this ewe (from 30 mg FGA group), which had a mean concentration of 6.1 ng/mL throughout the blood sampling period, were excluded from statistical analysis. Differences in progesterone concentrations before pessary insertion were not significant ($p>0.1$) among ewes of the four groups. None of the ewes among the four treatment groups were cycling at the start of the experiment. Additionally, the initial lower levels of progesterone among all ewes may reveal absence of their luteal function. Ewes of the present study received progestagen/progesterone sponges on May 24, which were removed on June 5. Absence of cyclicity at this time of the year indicates that ewes may have been in mid-seasonal anestrous period. Epstein (1982) reported that cyclic activities of Awassi ewes cease from May to July. Similarly, seasonal absence of estrual activities have been reported previously (Husein and Kridli, 2001) in Awassi ewes in June and July.

Progesterone concentrations at the time of pessary removal were 1.4±0.1 ng/mL in progesterone-sponge-treated ewes, reflecting exogenous treatment levels, which differed ($p<0.0001$) significantly from those (<0.2±0.02 ng/mL) administered MAP or FGA sponges. There were no differences in progesterone concentrations among ewes

treated with either MAP or FGA sponges. Lower progesterone concentrations at the time of sponge removal in MAP- or FGA-treated ewes do not reflect exogenous progesterone levels. In fact, MAP or FGA represent synthetic progestagens, which do not cross react with progesterone assays, allowing only endogenous progesterone.

Within 24 h of sponge removal, progesterone concentrations fell to 0.2±0.02 ng/mL or were basal in all ewes ($p>0.1$) and remained low until d 4 (Figure 1). On d 4 progesterone levels increased in 11/12, 10/11, 12/12 and 11/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively. Apparently, ovulation occurred in these ewes based upon a subsequent rise in progesterone levels. In these ewes, progesterone reached highest levels of 7.6±0.3 ng/mL between d 10 and 14. Type of progesterone pessary did not influence progesterone concentrations between d 10 and 14 ($p>0.1$). Progesterone concentrations remained elevated through d 18 in 6/12, 6/11, 6/12 and 5/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively, out of which 4/6, 4/6, 3/6 and 3/5 lambed 151 days following d 0 (Table 1). The remaining 2/6, 2/6, 3/6 and 2/5 ewes from those, which conceived at first induced estrus, did not lamb by 151 days. The most likely reason may be a reduction in survivability of embryos. Reduced fertility following the use of synthetic progestagen may have been attributed to many factors including dosage (Haresign, 1978), type (Crosby et al., 1991), method of preparation/impregnation (Gordon, 1971) and season of the year (Faure et al., 1983). Treatment with PMSG increased the total number of lambs born per ewe lambing ($p<0.05$) from mating at induced estrus compared to that of ewes lambing from a spontaneous estrus (Tables 1 and 2). Sixty-four (9/14) percent of ewes that lambed had twins and was greater ($p<0.001$) than the 36% (5/14) of ewes which had births of singles. Boland and Gordon (1973) reported increased number of multiple ovulation and higher lambing rate following progestagen-PMSG treatment in anestrous ewes.

Ewes that did not conceive from mating at induced estrus were 5/12, 5/11, 6/12 and 7/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively. Of these ewes, two (one from MAP and one from progesterone sponge groups) did not have full incidence of ovulation and had a short-lived increase in progesterone level from d 4 until d 8. The short-lived increase in progesterone may be secreted by luteal tissues (Leagan et al., 1985), resulting from ovulation of immature follicles. Another possibility may be insufficient gonadotropic support for the CL in terms of pulse frequency during seasonal anestrus. This phenomenon occurs more often in seasonal anestrous ewes (Oldham and Martin, 1978).

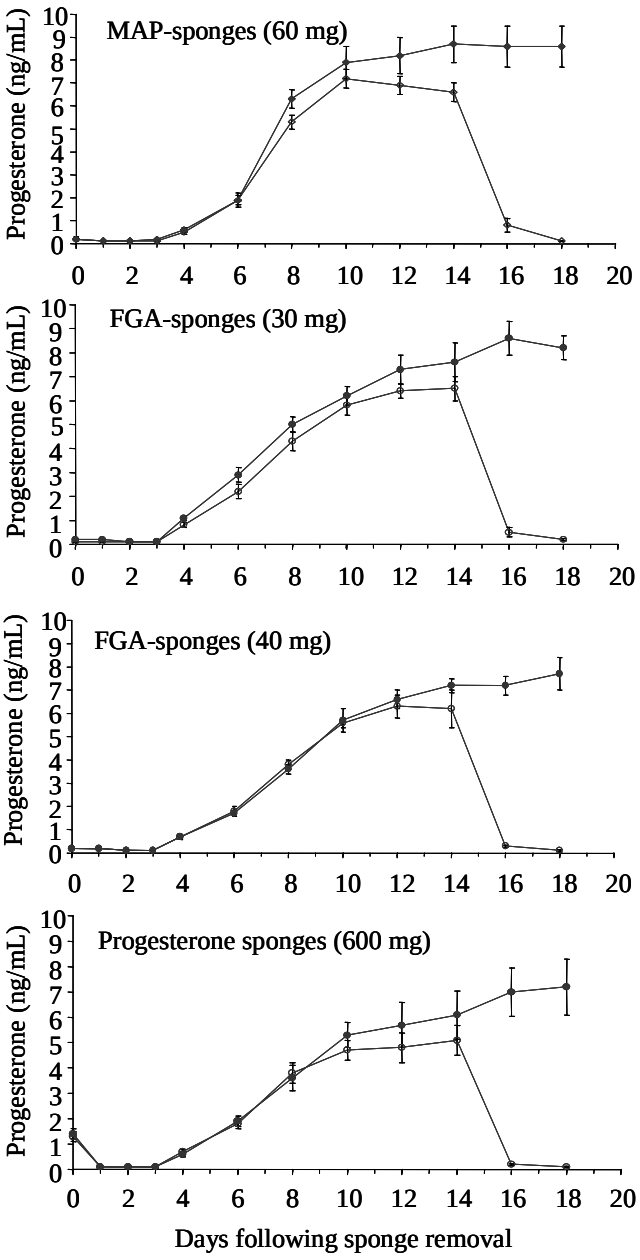

Figure 1. Plasma progesterone profiles following sponge removal in MAP-sponge-treated, 30 mg FGA-sponge-treated, 40 mg FGA-sponge-treated and 600 mg progesterone-sponge-treated Awassi ewes. In pregnant (●) ewes progesterone concentrations increased and remained elevated through day 18; in non-pregnant (○) ewes progesterone concentrations increased and then dropped after day 14.

Estrus response

Following d 0, induced estrus was detected in 9/12, 9/11, 8/12 and 7/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively. Occurrence of estrus in the present study averaged 70% (range=58 to 82%) and was similar ($p>0.1$) among ewes of the four treatment groups, which is reasonable during the seasonal anestrous

Table 1. Reproductive responses of Awassi ewes treated with 60 mg MAP, 30 mg FGA, 40 mg FGA or 600 mg progesterone sponges and PMSG

Item	MAP (60 mg)	FGA (30 mg)	FGA (40 mg)	Progesterone (600 mg)
Ewes bred at induced estrus[1]	9/12[a]	9/11[a]	8/12[a]	7/12[a]
Interval to onset of estrus[2]	42.9±4.4[ac]	48.2±4.4[ad]	41.8±4.7[ae]	28.2±5.0[b]
Induced estrus conception rate[3]	(50%) 6/12[a]	(55%) 6/11[a]	(50%) 6/12[a]	(42%) 5/12[a]
Lambing rate[4]	(33%) 4/12[a]	(36%) 4/11[a]	(25%) 3/12[a]	(25%) 3/12[a]
Twining rate[5]	2/4[a]	3/4[a]	2/3[a]	2/3[a]
Total lambs born[6]	1.5±0.3[a]	1.8±0.3[a]	1.7±0.3[a]	1.7±0.3[a]

[a] Numbers or values within row with similar superscript are similar (p>0.05).
[b,c] Numbers or values within row with different superscript are different (p<0.05).
[b,d] Numbers or values within row with different superscript are different (p<0.01).
[b,e] Numbers or values within row with different superscript are different (p=0.06).
[1] Ewes marked within 5 days following sponge removal.
[2] Hours from pessary removal to first observation of the breeding mark.
[3] Occurring based upon progesterone concentrations on d 18.
[4] Ewes lambing from breeding at first induced estrus.
[5] Ewes lambing from breeding at first induced estrus with twins.
[6] Per ewe lambing from breeding at first induced estrus.

Table 2. Responses of Awassi ewes from rebreeding (second estrus) 16 to 20 days following 60 mg MAP, 30 mg FGA, 40 mg FGA or 600 mg progesterone sponges and PMSG treatment and overall lambing rate

Item	MAP (60 mg)	FGA (30 mg)	FGA (40 mg)	Progesterone (600 mg)
Ewes returning to estrus[1]	5/12[a]	5/11[a]	6/12[a]	4/12[a]
Ewes lambed from rebreeding[2]	5/12[a]	5/11[a]	6/12[a]	4/12[a]
Overall lambing rate[3]	(75%) 9/12[a]	(82%) 9/11[a]	(75%) 9/12[a]	(58%) 7/12[a]

[a] Numbers or values within row with similar superscript are similar (p>0.05).
[1] Occurring 16 to 20 days following sponge removal.
[2] Occurring 169 days following sponge removal.
[3] Occurring from breeding at first and second estrus following sponge removal.

period (Table 1). Occurrence of estrus in ewes treated with either synthetic progestagen or natural progesterone in conjunction with PMSG varied from 47% (Robison and Moore, 1967) to 80% or greater (MacDonnel and Crowley, 1978; Cunningham et al., 1980; Husein et al., 1998). Although, all ewes were primed with progestagen/progesterone in conjunction with gonadotropin, 30% of the ewes did not exhibit estrus. The absence of estrus may be due to inadequate estradiol secretion by the ovarian follicles, indicating incomplete follicular growth and development (Baird and McNeilly, 1981; Quirke et al., 1981). Another possibility may be a diminished ram effect because rams were maintained in closer proximity to ewes at the start of the experiment. Other contributing factors may have been the milking status of some ewes and negative lactational/metabolic effects since one-week interval between weaning and sponge insertion was shorter than past trials. Approximately 25% of the ewes were still lactating during the period in which sponges were in place. Moreover, heat stress may have been another contributing factor since the prevalent environmental temperature during the entire trial ranged from 35 to 41°C.

Estrous responses occurred in progesterone-sponge-treated ewes 14.7, 20 and 13.6 h earlier than those occurring in MAP- (p<0.04), 30 mg FGA- (p<0.01) and 40 mg FGA-treated (p=0.06) ewes, respectively (Table 1). Intervals to onset of estrus in the present study resembled those reported recently for progestagen-PMSG treated Awassi ewes during the non-breeding season (Abdullah et al., 2001). Intervals also were close to those previously reported for Coopworth ewes by Shackell (1991) who found shorter intervals in ewes treated with progesterone (29.6 h) than MAP (42.4 h) and FGA (40.4 h) during the breeding season. Shorter intervals in progesterone-sponge-treated ewes may be due to faster pituitary endocrine response to natural progesterone rather than synthetic progestagen. The delay in occurrence of estrus in ewes treated with synthetic progestagen (MAP or FGA) sponges may be attributed to the existence of more progestagen residuals following sponge removal (Simonetti et al., 2000). The clearance rate of progestagen residuals may have been slow following sponge removal, and the pituitary would, therefore, have been under the influence of elevated progesterone levels for a longer period.

Rebreeding, non-return and overall lambing rates

Ewes that returned to estrus and were rebred at a spontaneous estrus 16 to 20 days following d 0 were 5/12, 5/11, 6/12 and 4/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively, all of

which lambed 169 days later. There were no differences ($p>0.1$) in number of ewes returning to estrus among the four treatment groups. All ewes rebred at a spontaneous estrus produced a single lamb each. Overall lambing rate of 72.3% was obtained from mating at both first and second estruses and was similar ($p>0.1$) among the four treatment groups (Table 2). The remaining 2/12, 2/11, 3/12 and 4/12 ewes treated with MAP, 30 mg FGA, 40 mg FGA and progesterone sponges, respectively, neither bred at a spontaneous estrus nor lambed. Percentages of ewes that lambed tended ($p=0.07$) to favor FGA and MAP sponges over progesterone sponges. Synthetic progestagen is more potent than natural progesterone (Shelton et al., 1967), implying that perhaps sponges of more than 600 mg progesterone may be needed to match those of FGA or MAP sponges (Hamra et al., 1989). A pregnancy rate of 70% was obtained following a 12 day 750 mg progesterone and PMSG treatment at sponge removal in ewes inseminated using frozen-thawed semen (Husein et al., 1998).

In conclusion, results from the present study demonstrate that MAP, 30 mg FGA, 40 mg FGA and progesterone sponges in conjunction with PMSG and the ram effect were equally effective in inducing estrus in Awassi ewes during the seasonal anestrous period. Application of such estrus synchronization protocols tended to favor both types of FGA and MAP in overall lambing rates over progesterone sponges.

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