Reproductive response of ewes synchronized with different lengths of MGA treatments in intrauterine insemination program

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Abstract

A total of 415 fat tailed ewes were randomly assigned to two groups to assess the effect of duration of melengestrol acetate (MGA) (9 versus 12 d) administration on reproductive parameters associated with laparoscopic artificial insemination. At the end of MGA treatment, ewes in each group were subdivided and inseminated with one of two different insemination doses (10 × 10^7 or 20 × 10^7 sperm per 0.5 ml insemination dose) of fresh diluted semen. Inseminations were carried out 11–18 h after first detected estrus. Ewes were screened for their return to oestrus from 10 to 21 days post AI and inseminated at their returned oestrus. Pregnancy diagnosis was done from approximately 55 days after insemination in both synchronized and return estrus. For short (9-day) and long (12-day) term MGA treated groups, estrus rates were 62% versus 89% (P < 0.0001), respectively. Ewes (n = 115) that returned to estrus were inseminated (7–11 h after estrus detection) with fresh diluted semen at different doses (20 × 10^7 or 40 × 10^7 or 60 × 10^7 sperm per 0.5 ml insemination dose). Pregnancy rates were 41% and 44% for short term and long term MGA treated ewes, respectively. Pregnancy rate of ewes which returned to oestrus was 53.4%. There was a significant (P < 0.05) increase in pregnancy rates (38–52% for 11–16 h; 63% for 17–18 h) when insemination was held at 17–18 h after first detected estrus following MGA treatments. Pregnancy rates were found to be similar in ewes inseminated with 10 × 10^7 (36%) or 20 × 10^7 (47%) motile spermatozoa at first AI, and 20 × 10^7 (44%) or 40 × 10^7 (59%) or 60 × 10^7 (48%) at second AI.

It was concluded that short term MGA treated ewes were recorded with lower estrus rates but was similar to pregnancy rates with long term MGA treatment. Acceptable pregnancy rates were achieved in MGA induced estrus when insemination is conducted at 17–18 h after estrus onset and with 20 × 10^7 sperm per insemination dose.

1. Introduction

The sheep industry in Turkey has been undergoing a decline due to multiple reasons. Most of the sheep breeds are of low prolificity, and profitable sheep production is difficult under the current conditions.

Fertility, litter size and lamb survival have been recognized as major factors influencing profitability of sheep production. Morkaraman is a multi purpose fat tailed breed from the eastern region of Turkey. These sheep are known for their hardiness and adaptability to the local environment, but prolificacy is low with ewes usually producing single lambs (Emsen and Yaprak, 2006). Increasing the prolificacy of the Morkaraman sheep has always been an important breeding goal. The highly prolific short-tailed breed, Romanov, has been widely used in breeding...
programs to increase prolificacy in sheep production systems worldwide. However, short tailed Romanov rams were not able to lift the fat-tail of Morkarman as is done by the Morkarman native rams.

Artificial insemination (AI) is not only a necessary technique for overcoming mating problems due to the fat tail but is also a valuable technique to improve reproductive performance and introduce new genetics. Laparoscopic AI (LAI) has evolved as one of the most viable techniques for depositing semen in the uterus of the sheep and fertility may reach up to 70% (Hill et al., 1998; McKelvey et al., 1985). However, the cost of LAI makes it impractical for the average sheep producer to use at this time.

In sheep, melengestrol acetate (MGA), a synthetic progesterone product, has been identified as a simple and cost-effective treatment for the successful induction and synchronization of estrus (Safranski et al., 1992; Jabbar et al., 1994; Daniel et al., 2001). Powell et al. (1996) compared 8-day, 11-day, and 14-day MGA supplementation for estrus response and lambing rates in out of season breeding programs and they concluded there was no difference. Based on these observations and the economics of using the product for a shorter period of time, they concluded that an 8-day course of treatment was best.

The purpose of the present study was to evaluate the oral progestagen, melengestrol acetate (MGA), for induction of estrus and subsequent fertility in Morkarman ewes in response to LAI using fresh diluted semen from 3 Romanov rams.

2. Materials and methods

2.1. Animals and management

A total of 415 multiparous Morkarman ewes were reared in eastern 39°55′N, 41°16′W Turkey and were randomly divided into two experimental groups. Animals in the groups were satisfactorily homogeneous in terms of age (2–6 year) and body condition score (BCS = 3). Scoring is done on a scale of 1–5, where 1 is emaciated (extremely thin), and 5 is very fat. All animals were dry and time from previous lambing was at least 24 weeks. Research was conducted early in the breeding season which is mid August. All ewes originated from three different flocks and were managed as a single group. Water and mineral licks were available ad libitum.

2.2. Estrus synchronization treatments

Ewes in group I (n = 286) were supplied with 150 g of ration containing 0.125 mg of melengestrol acetate (MGA\textsuperscript{B}) 200 premix, Pharmacia & Upjohn Company, Kalamazoo, MI 49001, USA) twice (8 h intervals) daily for 12 days. In group II (n = 130) ewes were offered the same amount of ration containing MGA premix for twice (8 h intervals) daily for 9 days. The experiment was conducted at the same time as the 12 day protocol starting 3 days earlier than the beginning of the 9 day protocol.

Treatment diets were mixed mechanically to provide one homogenous mixture of 0.125 mg per 150 g of ration and packed in 50 kg concentrate bags. Vasectomized rams fitted with a crayon-marking harness were introduced to increase synchrony of the ewes and to mark ewes as they came into heat, at the rate of 5 rams per 100 ewes. The animals were observed for estrus beginning at 24 h after cessation of MGA feeding and continuing up to 144 h in both short and long term MGA treated groups. Observation from onset of estrus was performed every day at 9:00 am and 9:00 pm. Animals that did not show any mating marks at 144 h were not inseminated. Ewes marked by the vasectomized rams received an i.v. injection of anesthetic cocktail containing 2cc Ketaset (Indus Pharma, Karachi, Pakistan) + 0.04cc Rompun (Bayer) and were inseminated 11–18 h after first estrus with freshly diluted semen with 10 (n = 152) or 20 × 10^7 (n = 182) motile spermatozoa/0.5 ml. Return rates were screened from 10 to 21 days from first estrus and insemination was repeated for return animals (n = 115) with a volume of 0.5 ml and 20 (n = 30), 40 (n = 40), or 60 × 10^7 (n = 45) motile spermatozoa. Pooled semen from three Romanov rams, collected with an artificial vagina, was used. Semen was extended at 35 °C in an Andromed-based extender (MINITUBE, Germany) and maintained at 30 °C in a water bath for a maximum of 2 h until intrauterine inseminations. One experienced laparoscopic AI operator performed the inseminations. The duration of restraint of the ewe and time to completion of insemination were recorded and categorized as optimum (A = 1–4 min) or far from optimal (B = 4–8 min). All ewes were scanned transabdominally by using a real-time ultrasound scanner equipped with a 5 MHz linear-array transducer (Pie Medical, 100 Falco. Vet) at day 55 post insemination of synchronized and return estrus.

2.3. Statistical analysis

The chi square analysis of MINITAB was used to analyze the effects of three different farms, duration of MGA treatments, sperm concentrations, rams, time of insemination and time in cradle on estrus and pregnancy rates. A model that included all main effects was tested. As factors were found to be non-significant, they were excluded from the

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**Table 1**

Percentage and distribution of the occurrence of estrus in ewes treated with short and long term MGA.

<table>
<thead>
<tr>
<th></th>
<th>Estrus rate (%)</th>
<th>Number of animals exhibiting estrus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;24 h</td>
<td>24–36 h</td>
</tr>
<tr>
<td>Long term MGA</td>
<td>286</td>
<td>254/286 (89\textsuperscript{b})</td>
</tr>
<tr>
<td>Short term MGA</td>
<td>130</td>
<td>80/130 (62\textsuperscript{b})</td>
</tr>
</tbody>
</table>

Columns in each category with different superscripts differ P<0.0001.
model. Farm effects were found not significant therefore it was omitted from the statistical analysis.

3. Results

The traditional melengestrol acetate (MGA) synchronization protocol (0.125 mg/head/twice daily for 12 days) used to induce estrus in fat tailed ewes resulted in significantly higher ($P < 0.0001$) estrus response of 89% (254/286) compared to ewes treated with MGA for only 9 days (62%; 80/130). Estrus of ewes treated with long and short term MGA protocols were observed in the first 6 days of MGA cessation. The distribution of estrus following short and long MGA treatments were similar and not well synchronized (Table 1). Similar conception rates were obtained with traditional (44%; 112/254) and short term (41%; 33/80) MGA protocols.

There was no significant effect of sire or farm on pregnancy rates for MGA treated ewes as well as ewes inseminated in their natural cycles. The time of insemination affected the pregnancy rates which were higher when the insemination was carried out 17–18 h after first detected estrus ($P < 0.05$) for first AI. With the ability to precisely determine the onset of estrus, the optimal time for artificial insemination should be determined. In the current study the appropriate time of intrauterine insemination following administration of MGA was 17–18 h after first detected estrus. There was significantly higher ($P < 0.05$) pregnancy rates for ewes inseminated with $20 \times 10^7$ motile spermatozoa/0.5 ml than those inseminated with $10 \times 10^7$ motile spermatozoa/0.5 ml but no significant difference between 20, 40 or $60 \times 10^7$ motile spermatozoa/0.5 ml in second AI (Table 2).

The duration of restraint of the ewe affected pregnancy rates in the groups where animals were inseminated in the second estrus post treatment while restraint time did not result in any decline of pregnancy rates of ewes inseminated during the MGA induced estrus (Table 3).

4. Discussion

Our current findings disagree with Powell et al. (1996) who concluded that an 8-day course of treatment was best compared to 11-day and 14-day. However, the level and duration of treatment with MGA may not have provided adequate progesterone priming for Morkaraman sheep as it was previously used for predominantly Rideau Arcott crossbred ewes (Keefe and Wichtel, 2000). Estrus rates obtained in the short term (62%) MGA protocol were found lower than the estrus response reported for Morkaraman ewes (100%) which received 0.25 mg MGA/head/daily and 500 I.U. PMSG 5 h after MGA supplementation cessation (Emsen et al., 2005). Thus it can be suggested that combining MGA treatment with gonadotropins could cause a better estrus response in the Morkaraman ewes in the early breeding season. Table 1 shows the distribution of estrus following short and long MGA treatments. These results were similar to that of Quispe et al. (1994) who reported that 90% of MGA treated ewes were in estrus within 5 days after MGA treatment.
Findings of pregnancy rates were in agreement with results reported by Castonguay et al. (2002) who reported that pregnancy rates of 45% were achieved for Dorset ewes inseminated (intrauterine) with fresh semen 76 h after last MGA feeding in an out of season program. Atsan et al. (2007) highlighted the advantage of using MGA in sheep breeding programs due to its cost effectiveness compared to other synchronization agents. Keefe and Wichtel (2000) treated ewes with MGA, with and without PMSG and mated naturally. Pregnancy rates were 43.3% and 31%, respectively. In our current study, MGA was evaluated in a LAI program and pregnancy rates were similar to the finding which was obtained with MGA plus PMSG protocol in a natural mating breeding program (Keefe and Wichtel, 2000). However, we speculate that MGA treatment is not advisable for fixed time insemination due to its low synchrony of estrus. To achieve acceptable fertility in the flock overall; ewes that reretained to estrus were reinseminated and pregnancy rates were found to be 53.4%.

Pregnancy rates decreased with a low insemination dose \(10^5\) motile spermatozoa, whereas a higher insemination dose \(20 \times 10^5\) increased pregnancy rates and this result is in agreement with other researches (Maxwell and Salamon, 1993; Martin and Watson, 1976). Higher doses of spermatozoa \(40 \times 10^7\) or \(60 \times 10^7\) were not recorded with significant increase in pregnancy rates. Thus, it can be recommended that the minimum necessary for laparoscopic AI in fat tailed breeds is \(20 \times 10^7\) motile spermatozoa.

Murray and Ward (1993) emphasized the importance of minimizing stress by the LAI operator who should attempt to minimize the time each ewe spends in a cradle, and the optimum time for restraint is suggested as 2–3 min (Hill et al., 1998). In the current study duration of restraint in a cradle was categorized as optimum (1–4 min) or longer than optimum (4–8 min) and there were no differences in pregnancy rates of ewes restrained longer than optimal time in MGA treated groups. Moreover, MGA treatment has been associated with lowered cortisol (Purchas et al., 1971) and hence may have a positive effect on fertility in stressful situations.

5. Conclusion

This work showed that the short term MGA treatment was effective for obtaining similar pregnancy rates compared with long term protocol. However, higher estrus response with long term treatment indicated the advantage of higher pregnancy outcomes with higher number of ewes included in LAI program. In addition, these data indicated that pregnancy rates were improved when ewes were inseminated at 17–18 h after the first observed estrus. Laparoscopic artificial insemination based on estrus detection in MGA treated ewes enable producers to achieve acceptable pregnancy rates.

References


### Table 3

Effect of insemination dose, sire and restraint time in cradle on pregnancy percentage of ewes artificially inseminated in second estrus post MGA treatment.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Second estrus post treatment</th>
<th>Insemination dose (\times 10^7)</th>
<th>Ram</th>
<th>Time in cradle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>ET 1</td>
</tr>
<tr>
<td>Pregnancy rates (%)</td>
<td>61/115 (53.4)</td>
<td>13/30 (44)</td>
<td>24/40 (59)</td>
<td>22/45 (48)</td>
</tr>
</tbody>
</table>

Rows with different superscripts in each category differ \(P<0.05\).