Anim. Reprod., v.5, n.1/2, p.30-33, Jan./Jun. 2008

Factors affecting the reproductive performance of fat-tailed ewes inseminated with laparoscopy in the late breeding season

E. Emsen[1], C.A. Gimenez Diaz, M. Yaprak, F. Koycegiz, M. Kutluca, F.A. Aslan

Department of Animal Science, Ataturk University, 25240 Erzurum, Turkey.

Abstract

The reproductive responses to one and two inseminations of frozen-thawed semen were compared in fat-tailed ewes which had previously been used in controlled reproduction programs aimed at estrus synchronization, multiple ovulation embryo transfer, and out of breeding season. In the trials, mature dry (n = 50) ewes in progestagen-induced estrus were inseminated 54 to 56 h after sponge removal. Half of the ewes received a second insemination 6 or 8 h later. The quality of the semen was determined by evaluating sperm motility after thawing. Association between factors included in this study and sex ratio was also investigated. The previous breeding programs effected ewe's subsequent fertility and the range in lambing rates observed was from around 33% to over 75%. Higher (P < 0.05) lambing rate (56% versus 29%) and litter size (1.8 versus 1.2) indicated an advantage of two inseminations. Post-thaw evaluation of frozen semen was good indicator to achieve acceptable pregnancy rate. Sex ratio was significantly affected by the previous breeding program, one or two inseminations, and post-thaw quality of frozen semen.

Keywords: sheep, frozen semen, laparoscopic artificial insemination

Introduction

Ability to freeze ram semen has opened the door for interstate as well as international movement of semen. Laparoscopic artificial insemination (LAI) is being improved to the point where AI of sheep would be practical on a commercial basis. The success of LAI depends on events and factors that interrelate in a complex way. The main factors that influence conception rates are semen quality and concentration, type of synchronization agent, breed and physiological status of the ewes, management, time of year, and the technician's skill (Shackell *et al.*, 1990; Paulenz *et al.*, 2002).

Ewes previously included in controlled reproduction programs such as estrus synchronization, superovulation, artificial insemination and *in vivo* embryo production bring into question whether their subsequent fertility will be affected or not. Equine chorionic gonadotrophin (eCG) is one the most efficient co-treatments routinely used in controlled breeding programs, and it was reported that repeated eCG injections had negative effects on subsequent fertility of ewes (Bodin *et al.*, 1997). Similarly, the interval between recovery and subsequent pregnancy may vary in different breeds, recovery procedures, superovulation regimes, season etc. (Gimenez Diaz and Emsen, 2006). The LAI procedure is well tolerated by ewes, however conception rates are variable (Miller, 1995). In the research setting, some stresses such as surgery, handling, and anesthesia are unavoidable. When several stressful situations occur simultaneously the animal becomes compromised. This fact led us to the hypothesis that the response to the stress of LAI would be reduced later in the breeding season.

Evaluation of the post-thaw frozen semen is also necessary to determine motility, morphology, and concentration, all of which determine the concentration and volume used in the insemination dose. Numerous factors have been associated with variation in the sex ratio of mammals. Among others, these factors include nutrition, season, disease, level of gonadotropin and steroid hormones, time of insemination, social status, stress, age, and parity (Krackow, 1995; Hardy, 1997).

Consideration of these factors are important in determining the efficacy of LAI which will play a role in achieving our main interest, which is to apply a genetic improvement program to Turkish native sheep raised in eastern Anatolia. Thus, we undertook the study of factors related to the success of an LAI program carried out in the late breeding season in fat-tailed sheep. 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). In particular, we analyzed the reproductive performance of ewes previously included in different controlled breeding programs and usefulness of post-thaw evaluation and repeated insemination to achieve acceptable fertility rate. Differences in the sex ratio according to these factors were also investigated.

Materials and Methods

There were three groups of ewes that had been differently treated previously. One group of animals (n = 12) was used as donor 6-9 weeks prior to insemination

[1]Corresponding author: eemsen@atauni.edu.tr
Telephone: +90(442)231-2558
Received: November 28, 2007
Accepted: February 20, 2008

program (Donor, DON); second group of animals (n = 15) had undergone an insemination program in the anestrus season (June-July) of the same year but did not conceive (Open Ewes, OE); third group of animals (n = 13) had been treated with sponge (30 mg) and eCG (400 I.U.) three weeks before the trial (Exogenous Hormone Treated, EHT); and the fourth group of animals (n = 10) had never been treated with any exogenous hormones and never undergone an LAI program (CONTROL, CON). For 30 d prior to the insemination and for 6 weeks prior to the expected lambing date, ewes were offered concentrate (500 g/ewe/day) and dried grass hay (1.5 kg/ewe/day). Water and mineral licks were available ad libitum. Four groups of animals were treated with vaginal sponge containing 30 mg fluorgestone acetate (FGA; Chrono-gest, Intervet, Boxmeer, The Netherlands) for 12 d. Immediately following sponge removal, ewes received an injection of 600 IU, i.m. eCG. Vasectomized rams were introduced to better synchronize ewes and to mark ewes as they came into heat at the rate of 5 rams per 100 ewes. The animals were screened for estrus beginning at 24 h after sponge removal and continuing up to 60 h. Screening was performed every day at 9:00 am and 9:00 pm. Animals that did not show any mating marks by 60 h were not inseminated. Marked ewes received i.v. injection of an anesthetic cocktail containing 2 cc Ketasol (Indus Pharma, Karachi, Pakistan) + 0.04 cc Romphun (Bayer) and were inseminated 54 to 56 h after sponge removal with frozen thawed semen containing 120×10^6 motile spermatozoa. Half of the ewes received a second insemination 6 or 8 h after the first insemination. The quality of the semen was determined by evaluating sperm motility after thawing. Frozen semen loaded into 0.25-mL French straws (IMV International, Minneapolis, MN), was thawed in a water bath at 37°C for 30 s to evaluate post-thaw motility. Post-thaw sperm motility was assessed subjectively using a phase contrast microscope. Sperm motility > 40%, > 20% or < 20% were classified as satisfactory, acceptable and poor, respectively.

One experienced laparoscopic AI operator performed the inseminations. The following procedure was used: the ewes were placed in laparoscopic cradles and kept in dorsal recumbent position while 2 trocars (10 and 5 mm) were inserted at 5 cm on either side of the midline to allow for the introduction of a laparoscope (Richard Wolf GmbH, Knittlingen, Germany) and an insemination pipette (Aspic UA 091, IMV, L'Aigle, France), respectively. Compressed air was insufflated into the abdominal cavity immediately before insemination. Semen was then deposited into the lumen of the mid portion of each uterine horn.

Data were analyzed by analysis of variance (MINITAB). Statistical differences between means of estrus, lambing rate, and litter size were tested using Duncan's multiple range tests. Differences in occurrence of sex ratios among groups were analyzed by the Chi-square test. For all statistical analyses, the minimum significance level was $P < 0.05$. Results are presented as untransformed arithmetic means ± SEM.

Results

Table 1 shows the distribution of the occurrence of estrus in ewes after sponge removal. It can be seen that in each category, onset of estrus occurred mostly between 30 and 48 h. Neither the difference in the percentage of occurrence of estrus nor onset of estrus after sponge removal among ewes was significant.

Table 1. Distribution of the occurrence of estrus in ewes.

	n	% Estrous response (Mean ± SEM)	Onset of estrus (h), after sponge removal (Mean ± SEM)
Physiologic status			
DON	12	91.6 ± 8.7	34.7 ± 3.0
CON	10	90.0 ± 9.5	44.0 ± 4.5
OE	15	80.0 ± 7.8	36.6 ± 2.9
EHT	13	100.0 ± 8.3	42.2 ± 2.9
		NS	NS

NS: not significant ($P > 0.05$). DON, donor; CON, control; OE, open ewes; EHT, exogenous hormone treated.

Our current study showed that animals previously inseminated (OE) during the anestrus season of the same year had significantly ($P < 0.05$) higher lambing rate (Table 2). Additionally, ewes receiving second sponge inserts after three weeks (EHT) had lower lambing rate than that observed in the ewes (OE) treated with sponge six months ago.

Two inseminations were superior ($P < 0.05$) to one for lambing rate and prolificacy. Significant differences ($P < 0.01$) were found for the post-thawing evaluations in terms of the lambing rate (7.6, 56.2 and 63.9%, respectively) and there was a tendency ($P < 0.1$) for an increase in the litter size when post-thaw motility was improved from poor to satisfactory. Variation in the sex ratio in response to physiological conditions of ewes, repeated insemination, and post-thaw semen quality were significant ($P < 0.05$). There was an increase in the sex ratio (proportion of males) when the factors included in this study are more favorable (ewes for first LAI and satisfactory result of post-thaw semen evaluation) and practical (one versus two inseminations).

Table 2. Reproductive performance and sex ratio in ewes.

	n	% Lambing Rate (Mean ± SEM)	Prolificacy (Mean ± SEM)	Sex ratio (% of males)
Physiologic status				
DON	11	36.4 ± 14.8[a]	1.3 ± 0.3	56[ab]
CON	9	40.0 ± 21.9[a]	1.3 ± 0.3	89[c]
OE	12	75.0 ± 14.2[b]	1.3 ± 0.2	44[a]
EHT	13	33.3 ± 14.2[a]	1.8 ± 0.3	67[b]
Insemination				
Single	23	28.9 ± 9.4[a]	1.2 ± 0.1[a]	72[a]
Double	22	56.2 ± 10.6[b]	1.8 ± 0.2[b]	57[b]
Post-Thaw Test				
Poor	11	7.6 ± 14.7[a]	1.0 ± 0.6	0[a]
Acceptable	18	56.3 ± 10.6[b]	1.3 ± 0.2	57[b]
Satisfactory	16	63.9 ± 11.1[b]	1.6 ± 0.2	87[c]
		**	NS	*

[a,b,c]Means within columns, by category, not followed by the same letter are significantly different.
NS: Not significant ($P > 0.05$);*$P < 0.05$; **$P < 0.01$.

Discussion

The FGA impregnated sponge inserts in late breeding season produced estrus rates that were similar to those observed in middle of the breeding season (96-100%; Emsen and Yaprak, 2006). Furthermore, the timing of estrus after sponge removal in our study was consistent with observations after using the same hormonal treatment in the middle of the breeding season (31-41 h; Emsen and Yaprak, 2006). Thus, delaying estrus synchronization to the late breeding season (January) did not result in any significant decrease in the percentage of animals in estrus and was not associated with any differences in time to estrus after sponge removal compared to those obtained in the middle of the breeding season.

Even though it was stated by Jerram (1987) that LAI is about as stressful as shearing, when performed correctly by a veterinarian, there is no guarantee that animals do not experience stress while they are handled before and after insemination. Higher lambing rate obtained in previously inseminated animals was assumed that stress responses of this group of ewes experienced of human handling earlier might be more tolerable than those have not experienced at all. Besides, there could be indirect effect of high-responder ewes from previous LAI conducted in out of season. These groups of animals had expressed estrus but did not conceive when they were on pasture. The results from previously treated ewes confirm previous reports of good reproductive performance of mature ewes housed indoors in controlled environments and inseminated with fresh extended semen (Langford, 1982; Langford *et al*., 1982).

In practice, repeated use of eCG treatment for induction of ovulation is generally followed by decreasing fertility in goats (Roy *et al*., 1999b). It was emphasized that such negative effects were even more dramatic after a second eCG treatment in the same breeding season (Baril *et al*., 1992). In the current study, two groups of animals received eCG injections in three (EHT) or six-eight week (DON) intervals in the same breeding season. Lower lambing rate in the resynchronized group (EHT) of animals co-treated with eCG in 21 day intervals are in agreement with Roy *et al.* (1999a) who reported that anti-eCG antibody concentration in plasma of eCG treated ewes is one risk factor for infertility after AI. This negative effect was not observed in ewes used as donors (DON) 6-9 weeks before the insemination (Table 2). Our results are supported by the report that ^{125}I-eCG binding measured 25 days after eCG injection was associated with the decreased lambing rates after repeated eCG treatments (Baril *et al*., 1992; 1996).

Physiologically, an altered sex ratio might be achieved by facilitating or inhibiting the transport of either X- or Y-bearing sperm through the reproductive tract, preferential selection of sperm at fertilization, or sex-specific death of embryos after fertilization (Hardy, 1997). William (1987) emphasized that in cases where AI is preceded by hormone treatment, the sex ratio (proportion of males) is lower and gonadotropin has a pre-conceptional sex-selective effect in human.

Acute psychological stress in relation to repeated insemination 6-8 h after first insemination resulted in a lower proportion of male compared to single insemination. This is similar to cases in humans reported by Hansen *et al.* (1999). The results also showed a statistically significant dependence of the offspring sex ratio on the semen concentration (increased with two inseminations) and semen motility. Jacobsen *et al.* (2000) indicated that no important association exists between particular semen characteristics and a female-biased offspring sex ratio, while Zorn *et al*. (2002) noted that negative changes in sperm motility may be involved in the sex ratio modifications. Our current study showed that sperm motility was the main factor determining sex ratio by increasing proportion of males from poor to satisfactory. Similarly, many researchers indicated that a decrease in semen quality led to a decrease in the sex ratio (proportion of males) among newborn infants in many populations (Carlsen *et al*., 1992; Møller, 1996; James, 1997).

In conclusion, estrus of fat-tailed ewes can be successfully induced in late breeding season (January). Significant variation with respect to lambing rate found among ewes with different physiologic conditions is an important result of this study to emphasize on consideration of ewe physiologic history before they are included in the LAI program. Higher reproductive performance in ewes inseminated two times led to the question of whether higher dose of spermatozoa or repeated insemination caused an increase in both lambing rate and litter size. Thus, further research is required. Evaluation of motility of frozen-thawed semen provided a useful indication of lambing rate and litter size. Overall result of this study has shown that sex ratio is highly dependent on the factors included in this study.

Acknowledgments

The authors thank Ileana Wenger (DVM) for her help in the linguistic revision of this manuscript.

References

Baril G, Remy B, Vallet JC, Beckers JF. 1992. Effect of repeated use of progestagen-PMSG treatment for estrus control in dairy goats out of breeding season. *Reprod Domest Anim*, 27:161-168.

Baril G, Remy B, Leboeuf B, Beckers JF, Saumande J. 1996. Synchronization of estrus in goats: the relationship between eCG binding in plasma, time of occurrence of estrus and fertility following artificial insemination. *Theriogenology*, 45:1553-1559.

Bodin L, Drion PV, Remy B, Brice G, Cognie Y, Beckers JF. 1997. Anti-PMSG antibody levels in sheep subjected annually to estrus synchronization. *Reprod Nutr Dev*, 37:651-660.

Carlsen E, Giwercman A, Keiding N, Skakkebæk NE. 1992. Evidence for decreasing quality of semen during past 50 years. *Br Med J*, 305:609-613.

Emsen E, Yaprak M. 2006. Effect of controlled breeding on the fertility of Awassi and Red Karaman ewes and the performance of the offspring. *Small Rum Res*, 66:230-235.

Gimenez Diaz CA, Emsen E. 2006. Fertility of donor ewes following surgical collection of embryos. *In*: Proceedings of the 7th International Ruminant Reproduction Symposium, 2006, Wellington, New Zealand. Wellington: The Symposium. pp.4-5.

Hansen D, Møller H, Olsen J. 1999. Severe periconceptional life events and the sex ratio in offspring: follow up study based on five national registers. *Br Med J*, 319:548-549.

Hardy ICW. 1997. Possible factors influencing vertebrate sex ratios: an introductory overview. *Appl Anim Behav Sci*, 5:217-241.

Jacobsen R, Bostofte E, Skakkebæk NE, Hansen J, Møller H. 2000. Offspring sex ratio of subfertile men and men with abnormal sperm characteristics. *Human Reprod*, 15:2369-2370.

James WH. 1997. Secular trends in monitors of reproductive hazard. *Hum Reprod*, 12:417-421.

Jerram PJS. 1987. Animal welfare considerations of laparoscopic A.I. *N Z Vet J*, 35:157-157.

Krackow S. 1995. Potential mechanisms for sex ratio adjustment in mammals and birds. *Biol Rev*, 70:225-241.

Langford GA. 1982. Influence of PMSG and time of artificial insemination on fertility of progestogen-treated sheep in confinement. *J Anim Sci*, 54:1205-1211.

Langford GA, Ainsworth L, Wolynetz MS. 1982. Reproductive response of progestogen-treated sheep in confinement to a single and double insemination. *J Anim Sci*, 54:12-17.

Miller SJ. 1995. Artificial breeding techniques - sheep. *In*: Compendium of approved procedures. Armidale: CSIRO Division of Animal Health. pp.58:71-58:83.

Møller H. 1996. Change in male: female ratio among newborn infants in Denmark. *Lancet*, 348:828-829.

Paulenz H, Adnoy T, Fossen OH, Soderquist L, Berg KA. 2002. Effect of deposition site and sperm number on the fertility of sheep inseminated with liquid semen. *Vet Rec*, 150:299-302.

Roy F, Combes B, Vaiman D, Cribiu EP, Pobelt T, Deletang F, Combarnous Y, Guillou F, Maure lMC. 1999a. Humoral immune response to equine chorionic gonadotropin in ewes: association with major histocompatibility complex and interference with subsequent fertility. *Biol Reprod*, 61:209-218.

Roy F, Maurel MC, Combes B, Vaiman D, Cribiu E P, Lantier I, Pobel T, Deletang F, Combarnous Y, Guillou F. 1999b. The negative effect of repeated equine chorionic gonadotropin treatment on subsequent fertility in Alpine goats is due to a humoral immune response involving the major histocompatibility complex. *Biol Reprod*, 60:805-813.

Shackell GH, Kyle B, Littlejohn RP. 1990. Factors influencing the success of a large scale artificial insemination programme in sheep. *Proc N Z Soc Anim Prod*, 50:427-430.

William J. 1987. The effect of hormones on the sex ratio of infants following artificial insemination *Ann Human Biol*, 14:39-47.

Zorn B, Sucur V, Stare J, Meden-Vrtovec H. 2002. Decline in sex ratio at birth after 10-day war in Slovenia: brief communication. *Hum Reprod*, 17:3173-3177.