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Short Communication

Laparoscopic artificial insemination in dairy sheep with chilled semen stored for up to 26 h

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Adult East Freisan crossbred (n = 220) and Chios ewes (n = 105) were divided into four groups and inseminated with chilled semen, which had been stored for 7, 13, 20 or 26 h at 5°C. Unilateral intrauterine insemination (50 x 10^6 spermatozoa in 0.25 ml) was performed with the aid of a laparoscope. Inseminations were carried out 48 – 52 h after pessary removal (30 mg FGA) without detecting estrus. The lambing rates after intrauterine insemination with chilled semen were found to be similar in East Fresian crossbred (40%) and Chios ewes (30%). Intrauterine insemination with chilled semen stored up to 26 h resulted in similar lambing rates; whereas, fertility of Chios ewes tended to decline with increased holding time of chilled semen. From this study, it is concluded that decreasing the storage time of chilled semen at 5°C improves pregnancy in Chios ewes and that East Fresian crossbred ewe's conception rates to intrauterine insemination with chilled semen was relatively higher than Chios ewes.

Key words: Dairy sheep, chilled semen, time of insemination, lambing rate.

INTRODUCTION

East Friesian, a German dairy sheep breed, has been used in the development of synthetic breeds in Turkey. There are four different sheep breeds obtained from crossing East Freisan with Chios and Kivircik in Turkey. The East Friesian crossbred ewes from these studies produce almost two times more milk per lactation than the pure Turkish breeds. The Chios breed is the result of crossbreeding between local sheep of the island of Chios and breeds from Anatolia, possibly the Kivircik and Daglic breeds. The Chios is non-seasonal and some ewes are reported to have two lambing in one year. Research has shown the ovulation rate to range from 2.9 to 3.3 in mature ewes. Milk production for the Chios varies from 120 - 300 kg per lactation (Hatziminaoglou et al., 1996).

Artificial insemination (AI) is limited by the short storage time (8 h) required for fresh semen. Fresh semen should be used immediately after it is collected, as the motility and viability of spermatozoa under these conditions is quickly reduced, due to the increase in the concentration of lactic acid in the ejaculate (Vivanco, 1990). Another alternative is the use of liquid semen stored between 0 and 5°C, allowing the use of semen for a longer period of time compared to fresh semen (Menchaca et al., 2005). Intrauterine insemination with chilled semen using laparoscopy makes it possible to deposit the semen directly into the uterus near the oviduct, shortly before ovulation, which produces fertility rates comparable to those obtained by natural mating (Evans and Maxwell, 1990).

The objective of the experiment described here was to determine the effect of breed and holding time of chilled semen on lambing rate and prolificacy in East Fresian crossbred and Chios ewes.

MATERIALS AND METHODS

A total of 325 East Freisan crossbred (n = 244) and Chios (n = 81) ewes of mixed ages (2 - 6 years) at a commercial sheep enterprise (Trek Farm) were used during autumn (November). East Freisan crossbred ewes have been obtained by seven years of specific selection associated with controlled breeding of the Kivircik and Chios breeds. Management is based on a semi-intensive system and the breeds are non-seasonal (estrus cycle does not depend on a season). Animals included in this study are on an accelerated lambing program of 3 lambings in 2 years. Prior to the trial animals were maintained on natural pastures. Ewes were synchronized with intravaginal pessaries containing 30 mg of fluorgestone acetate

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Table 1. Effect of breed and holding time of chilled semen on lambing rate and prolificacy.

Insemination time	n	Lambing rate	Prolificacy
A (Soon after chilling, 7 h)	89	33.8 ± 0.06	2.0 ± 0.18
B (12 h after chilling)	84	35.2 ± 0.06	1.7 ± 0.20
C (20 h after chilling)	88	40.0 ± 0.05	1.9 ± 0.16
D (26 h after chilling)	84	31.0 ± 0.06	2.1 ± 0.20
		N.S	N.S
Breed			
East Fresian Crossbred (EFC)	220	40.0 ± 0.03	1.9 ± 0.09
Chios (C)	105	30.0 ± 0.05	2.1 ± 0.16
		N.S	N.S
Insemination time *Breed			
A – EFC	64	35.8 ± 0.06	1.7 ± 0.18
A – C	25	31.8 ± 0.09	2.2 ± 0.31
B – EFC	63	42.9 ± 0.06	2.0 ± 0.17
B – C	21	27.6 ± 0.11	1.3 ± 0.37
C – EFC	57	40.6 ± 0.06	1.9 ± 0.18
C – C	31	38.6 ± 0.09	2.0 ± 0.25
D – EFC	56	39.4 ± 0.06	1.8 ± 0.19
D – C	28	22.0 ± 0.02	2.2 ± 0.36
		N.S	N.S

N.S: Not significant $p > 0.05$.

(Chrono-gest, Intervet, Boxmeer, The Netherlands). In the fourteenth day, at the same time of the removal of the pessaries, 400 I.U. of Equine Chorionic Gonadotrophin (eCG), were injected intramuscularly.

Pooled semen from three East Fresian cross rams and two Chios rams, collected with artificial vagina, was used. Semen was extended at 35°C in an Andromed-based extender (MINITUBE, Germany) to a concentration of 50 X 10^6 spermatozoa/0.25ml. Andromed is a yolk-free extender consisting of aqua bidest, fructose, glycerol, citric acid, phospholipid buffers and additional antibiotics.

Extended semen was chilled to 5°C in 2 – 3 h within a water jacket. Chilled semen, rewarmed at the time of insemination to 35°C, was used for laparoscopic artificial insemination and a single uterine horn was inseminated without examining ovaries. Animals in each breed were randomly allocated to four groups according to time of storage of chilled semen: (A; East Fresian cross: 65; Chios: 27) 7 h, (B; East Fresian cross: 64; Chios: 21) 13 h, (C; East Fresian cross: 56; Chios: 31) 20 h or (D; East Fresian cross: 56; Chios: 28) 26 h. Estrus of treated animals was not detected and insemination took place 48 – 52 h after pessary removal in each group. Animals in the groups were satisfactorily homogeneous in terms of genotype, age and body condition score. Data collected included lambing rate and prolificacy. The data were analysed using the least square methodology of the GLM procedure, fitting a two-way model with a fixed effect of breed and holding time of chilled semen (7, 13, 20 or 26 h) plus the interaction effect.

RESULTS AND DISCUSSION

The lambing rate obtained in this study using chilled semen did not differ for the ewes inseminated with semen stored for up to 26 h. No statistical significance ($p > 0.05$) has been found between lambing rates in Chios and East Fresian crossbred ewes, despite the fact that the latter was recorded with higher pregnancy rates on each occasion. Overall fertility dropped 5.3% as the chilled semen was stored up to 26 h. Fertility of Chios ewes decreased more dramatically than East Friesian crossbred ewes when the storing-insemination interval was 26 h. Prolificacy was not affected by breed, or holding time of semen (Table 1).

Our current study agrees with Fair et al. (2005) who reported ewe breed has not been shown to have a major effect on pregnancy rates following laparoscopic artificial insemination. However, fertility results for the East Fresian crossbred ewes (40%) and Chios ewes (30%) inseminated with chilled semen are different to those from other breeds such as Churra breed (milk breed of the North-West of Spain), Manchega, Latxa, Merino, Lacaune, Sarde, etc. (Anel et al., 2005) which is due to multiple factors. In contrast, Fernandez-Abella et al. (2003) observed similar lambing rates (45.3% and 20.0% at 46 h and 50 h after pessary removal, respectively) with our current study.

The motility of spermatozoa is known to be influenced by the diluent used. Milczewiski (2000) investigated the effect of different types of extenders and reported that Citrate-yolk extended semen resulted with higher pregnancy rate (85.7%) in intrauterine insemination with chilled semen for 8 h at 5°C. In our current study, we used yolk-free extender with Glycerol which was found significantly inferior by Milczewiski (2000). Furthermore, it has been shown that glycerol decreases fertility in sheep when the semen is stored at 5°C (Abdelhakeam et al.,

1991) and accelerates the acrosome reaction in ram spermatozoa (Slavik, 1987).

Fiser and Batra (1984) reported that storage of ram sperm for longer times (18 h) at 5°C has resulted in a decline in progressive motility (5% decrease) and kinetic ratings (0.5 point decrease). It was hypothesized that storage of ram sperm in the manner presented here at 5°C for times greater than 20 h, may have a detrimental effect and result in a decline (9%) in the overall lambing rate.

Although its effect was not evaluated in this study, previous studies (Fernandez-Abella et al., 2003), have shown that the time of insemination after pessary removal is another important factor affecting fertility, especially when ewes are inseminated with chilled semen at 5°C. Robinson et al. (1989) indicated that unilateral intrauterine insemination is as effective as bilateral in ensuring high fertilization rates; however, inseminating only a single uterine horn instead of both uterine horns in this study could be another factor in the decreased fertility. Utilizing low insemination dose (50 X 10^6) might have decreased the lambing rate, as the cooling process seems to reduce the transport capacity of the spermatozoa at a rate of 10 to 35% per day of storage (Evans and Maxwell, 1990). Milczewski et al. (2000) recommended that higher pregnancy rates (69.56%) can be obtained with at least 250 million spermatozoa per dose of 0.4 ml suspension in intrauterine inseminations of ewes.

Conclusion

It is possible to chill the ram semen for a period as long as 26 h at 5°C, when using intrauterine insemination. Success in chilled/stored semen use depends on a lot of confounding factors such as temperature of storage, composition of the extender, number of sperm inseminated, time of insemination after pessary removal, number of inseminations, etc. In the current study, we have chosen to focus on duration of storage and breed of sheep. To obtain and increase in overall fertility, unilateral intrauterine insemination, dose of insemination and insemination time after pessary removal should be investigated in further studies.

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REFERENCES

Abdelhakeam, AA Graham EF, Vazquez IA (1991). Studies on the presence and absence of glycerol in unfrozen and frozen ram semen: fertility trials and the effects of dilution methods on freezing ram semen in the absence of glycerol. Cryobiology, 28: 36-42.

Anel L, Kaabi M, Abroug B, Alvarez M, Anel E, Boixo JC, De La Fuente LF, De Paz P (2005). Factors influencing the success of vaginal and laparoscopic artificial insemination in churra ewes: a field assay. Theriogenology, 63: 1235–1247.

Evans G, Maxwell WMC (1990). Conservación de semen durante corto tiempo. *In*: Inseminación Artificial en Ovejas y Cabras. Evans, G., and M. C. Maxwell W. (eds). Editorial Acribia, Zaragoza, España, pp. 119-122.

Fair S, Hanrahan J, O'Meara C, Duffy P, Rizos D, Wade M, Donovan A, Boland M, Lonergan P, Evans A (2005). Differences between Belclare and Suffolk ewes in fertilization rate, embryo quality and accessory sperm number after cervical or laparoscopic artificial insemination. Theriogenology, 63: 1995-2005.

Fernandez-Abella D, Preve MO, Villegas N (2003). Insemination time and dilution rate of cooled and chilled ram semen affects fertility. Theriogenology, 60: 21-26.

Fiser PS, Batra TR (1984). Effect of equilibration time at 5 ◦C and photoperiod on survival of ram spermatozoa frozen in straws. Can. J. Anim. Sci. 64: 777-780.

Hatziminaoglou I, Georgoudis A, Zervas N, Boyazoglu J (1996). Prolific Breeds of Greece. Chap 3.3, Prolific Sheep. (M.H. Fahmy, ed.), CAB International, University Press, Cambridge, p. 542.

Menchaca A, Pinczak A Queirolo D (2005). Storage of ram semen at 5 °C: effects of preservation period and timed artificial insemination on pregnancy rate in ewes. Anim. Reprod. 2: 195-198.

Milczewski V (2000). Different ways of sheep artificial insemination using cooled semen. Sci. Agraria, 1: 83-95.

Milczewski V, Kozicki LE, Luz SLN, Neves JP (2000). Intrauterine and cervical artificial insemination in sheep using cooled semen. Arch. Vet. Sci. 5: 35-39.

Robinson JJ, Wallace JM, Aitken RP (1989). Fertilization and ovum recovery rates in superovulated ewes following cervical insemination or laparoscopic intrauterine insemination at different times after progestagen withdrawal and in one or both uterine horns. J. Reprod. Fertil. 87: 771-782.

Slavik T (1987). Effect of glycerol on the penetrating ability of fresh ram spermatozoa with zona-free hamster eggs. J. Reprod. Fertil. 79: 99-103.

Vivanco MHW (1998). Inseminación artificial en ovinos. *In*: Memorias del Seminario Internacional: Aplicación de Técnicas Biotecnológicas en la Reproducción de Ovinos y Caprinos. Chapingo, Edo. de Méx., México. pp. 135-194.