Indian Journal of Animal Sciences **82** (10): 1166–1169, October 2012

Effect of laparoscopic insemination on reproductive performance of indigenous Cine Capari sheep

ORHAN KARACA[1], IBRAHIM CEMAL[2], ONUR YILMAZ[3] and MURAT YILMAZ[4]

Adnan Menderes University, Aydin, Turkey

Received: 29 November 2011; Accepted: 10 April 2012

ABSTRACT

The feasibility of use of laparoscopic artificial insemination (LAI) method was investigated in endangered indigenous Cine Capari sheep breed under conservation. Setting the mating plans and controlling the inbreeding may be possible with the use of effective artificial insemination techniques. In the present study, 23 and 46 ewes showing estrus at 2002–2003 and 2008–2009 mating seasons were laparoscopically inseminated with diluted fresh semen from 6 and 9 rams, respectively. Data were evaluated with respect to gestation length, litter size, duration between sponge removal and beginning of estrus activity (DBSR-BOA), duration between beginning of estrus activity and LAI (DBSR-LAI) and AI success rate. The least square means for mentioned traits were found as 150.3 days, 1.36, 39.98 h, 22.31 h and 69.57% for 2003–2004 lambing season and 148.70 days, 1.41, 41.18 h, 18.35 h and 63.04% for 2008–2009 lambing season, respectively. The difference between means of years were not statistically significant for all the evaluated variables except gestation length. Our results showed that LAI will be a useful tool in activities to conserve Cine Capari sheep as a genetic resource.

Key word: Laparoscopic insemination, Reproductive performance, Sheep

The use of laparoscopy for intrauterine insemination of sheep was described by Killen and Caffery (1982) firstly. Laparoscopic artificial insemination (LAI) is used throughout the world to increase the genetic merit of sheep flocks (Hill *et al.* 1998, Donovan *et al.* 2001, Killen *et al.* 1982). Currently, a pregnancy rate of 60–80% is regarded as a reasonable commercial result using LAI (Maxwell and Hewitt 1986, Gourley and Riese 1990, Marshall and Nancarrow 1993, Gil *et al.* 2003, Yilmaz *et al.* 2007, Halbert *et al.* 1990).

In the last 15 to 20 years, the pure number of Cine Capari sheep which is a regional native fat-tailed sheep breed of Aydin province has rather decreased due to backcrossing efforts of farmers. Efforts for establishing a conservation flock at Adnan Menderes University were started in 1996 for conservation of this endangered breed and definition of its some characteristics. Backcrossing of Cine Capari ewes with rams of Kivircik and prolific Sakiz (Chios) breeds has put this breed in danger of extinction (Karaca *et al.* 1999, Karaca and Cemal 2005).

This study aims to investigate the feasibility of use of LAI technique in endangered indigenous Cine Capari sheep breed under genetic conservation program.

Present address: [1-4]Department of Animal Sciences, Faculty of Agriculture (okaraca@adu.edu.tr, icemal@adu.edu.tr, oyilmaz @adu.edu.tr, myilmaz@adu.edu.tr).

MATERIALS AND METHODS

Animals and management: The experiment was carried out at the Cine Capari Conservation flock, Adnan Menderes University, Aydin, Turkey. In the present study, 23 and 46 ewes were laparoscopically inseminated with diluted fresh semen from 6 and 9 rams at 2002–2003 and 2008–2009 mating seasons, respectively. The age of rams ranged from 2 to 8 years. Animals were fed with concentrate mix and had access to a shelter, where water and salt stone complement were available.

Semen collection and preparation: Semen was collected from each ram using an artificial vagina before artificial insemination. Semen samples were evaluated for volume (ml), mass motility (on a scale of 0 (immotile) to 5 (vigorous motility)), percentage of dead sperm cells, sperm concentration and total number of spermatozoa per ejaculate. One part of evaluated semen was diluted with 3 parts of a commercial semen extender. The semen was kept in water bath at 30°C until inseminations.

Estrus synchronization: The estrus cycles of all ewes were synchronized using a 14-day treatment of progestagen vaginal sponges (40 mg FGA) and 500 IU pregnant mare serum gonadotrophin were administered (im) at the time of

sponge removal to stimulate follicular development.

Laparoscopic insemination: Prior to laparoscopic insemination food and water were restricted for 24 h. Inseminations were carried out 58–63h after sponge removal. Only ewes that showing estrus were inseminated. Each ewe was restrained on an operation cradle in dorsal recumbency with the head down at an angle of 45°. After local anesthesia wide-angle endoscope was introduced into the abdominal cavity at the ventral midline about 6–10 cm cranial to the mammary gland. Each horn of the uterus were inseminated with 0.2 ml of diluted semen (1 volume of semen diluted with 3 volume of diluents) using an insemination pipette connected to a 1 ml syringe. Each ewe was inseminated totally with 0.4 ml of diluted semen that contained approximately 84.3 million spermatozoa.

Data collection and statistical analysis: Dates of synchronization, time of estrus start, insemination time, gestation length, date of lambing, and number of lambs born per ewe lambing were recorded. Pregnancy rates from laparoscopic AI were determined by using the combined information on date of lambing relative to date of AI.

Semen characteristics were analyzed using GLM (general linear model) procedure and phenotypic correlations between variables were obtained using the CORR procedure of the SAS (1999) statistical software.

RESULTS AND DISCUSSION

Semen characteristics are important variables considered in the most studies of artificial insemination (Colas 1980, Gordon 1997, Kaymakçý 2002, Ollero *et al.* 1996). Simple statistics for some semen characteristics of Cine Capari rams used for laparoscopic artificial inseminations are given in Table 1. A good mass motility with an overall mean of 4.67 was observed for semen samples.

The means of semen volume (SV), mass motility (MM), percentage of dead sperm cells (PDSC), sperm concentration (SC) and total number of spermatozoa per ejaculate (TNSPE) were 1.04, 4.83, 9.25, 1.65×10^9/ml, and 1.70×10^9 in 2003; 1.00, 4.54, 6.58, 1.63, 1.04, 4.56, 2.47, 1.71×10^9/ml, and 1.76×10^9 in 2008; 1.04, 4.67, 5.18, 1.69×10^9/ml and 1.74×10^9 in general, respectively.

Simple statistics for semen volume and sperm concentration are comparable to those reported by Saleh (1997) who established these as 0.67 ml and 4.3×10^9. However in the present study we found higher volume and lower concentration than those reported by Saleh (1997). It might be due to the breed and environmental differences.

Mass motility was found similar to that reported by Yilmaz and Karaca (2004), and was also very close to the results reported by Karagiannidis *et al.* (2000) and Saleh (1997). Mass motility score of Cine Capari rams was higher than the results reported by Fourie *et al.* (2004) and Kafi *et al.* (2004).

Sperm concentration was generaly similar to that reported by Yilmaz and Karaca (2004) and Saleh (1997). Mean percentage of dead sperm cells of Cine Capari Rams, 5.18% in general (Table 1), is lower than that reported by Saleh (1997). In this study, estrus synchronization was applied to 30 and 50 head of Cine Capari sheep in 2003 and 2008 respectively. But 23 head and 46 of Cine Capari sheep showed estrus in 2003 and 2008 respectively. For this reason, 23 and 46 ewes showing estrus were laparoscopically inseminated with diluted fresh semen at 2003 and 2008 mating seasons, respectively.

Table 1. Simple statistics for some semen characteristics of Cine Capari rams used for laparoscopic artificial inseminations

Year	Sperm characteristics	N	Means±SD (Min-Max)
2003	SV	6	1.04±0.29 (0.75–1.50)
	MM	6	4.83±0.41 (4.00–5.00)
	PDSC (%)	6	9.25±2.40 (6.00–12.00)
	SC	6	1.65±0.28 (1.38–2.16)
	TNSPE	6	1.70±0.47 (1.20–2.58)
2008	SV	9	1.04±0.41 (0.60–1.50)
	MM	9	4.56±0.53 (4.00–5.00)
	PDSC (%)	9	2.47±0.90 (1.25–4.00)
	SC	9	1.71±0.15 (1.41– 1.86)
	TNSPE	9	1.76±0.66 (1.01–2.68)
General (2003 and 2008)	SV	15	1.04±0.35 (0.60–1.50)
	MM	15	4.67±0.49 (4.00–5.00)
	PDSC (%)	15	5.18±3.79 (1.25–12.00)
	SC	15	1.69±0.20 (1.38–2.16)
	TNSPE	15	1.74±0.58 (1.01–2.68)

SV, Semen volume (cm^3); MM, mass motility; PDSC(%), percentage of dead sperm cells; SC, semen concentration ($\times10^6$/mm^3); TNSPE, total number of spermatozoa per ejaculate ($\times10^9$/ejaculate volume).

Least square means and standard errors for some reproductive parameters and simple statistics of pooled data for reproductive parameters for Cine Capari ewes which were laparoscopically inseminated in 2003–2004 and 2008–2009 years are given in Table 2. The means for gestation length, litter size, duration between sponge removal and beginning of estrus activity (DBSR-BOA), duration between beginning of estrus activity and laparoscopic AI (DBSR-LAI) and AI success rate were 149.23 days, 1.39, 40.49 h, 19.59 h and 65.21%, respectively. The duration between sponge removal and beginning of estrus varied between 16.16 and 58.55 h and have a coefficient of variation as 23.4%.

The difference between means of years was not statistically significant (P>0.05) for all the evaluated variables except gestation length. The insemination success obtained as 69.57% and 63.04% in 2003–2004 and 2008–2009 lambing period, respectively, are closer to results reported by Yamaki *et al.* (2003). Our results were also similar to LAI success reported by Yamaki *et al.* (2003). But success rate in this study was lower than the results reported by Evans

Table 2. Least square means and standard errors and simple statistics for reproductive parameters of laparoscopically inseminated Cine Capari ewes

Variable	Basic statistics		Year 2003–2004		Year 2008–2009		Sign
	N	Mean±SD (Min-Max)	N	Mean±SE	N	Mean±SE	(Pr>F)
Gestation length (day)	43	149,23±2,200 (143–153)	16	150.13±0.53	27	148.70±0.41	0.039*
Litter size	51	1,39±0,600 (1–3)	22	1.36±0.13	29	1.41±0.11	0.772^{NS}
DBSR-BOA(h)	67	40,49±9,470 (16,16–58,55)	21	38.98±2.07	46	41.18±1.40	0.380^{NS}
DBBOA-LAI(h)	67	19,59±8,220 (3,59–40,44)	21	22.31±1.76	46	18.35±1.19	0.067^{NS}
AI success rate (%)	69	65,21±47,980 (0–100)	23	69.57±10.06	46	63.04±7.11	0.598^{NS}
Synchronization success (%)	80	0,86±0,346 (0–100)	30	0,77±0,06	50	92.0±0,05	0.550^{NS}

*: P<0.05; NS, nonsignificant (P>0.05); LAI, laparoscopic artificial insemination; DBSR-BOA(h), duration between sponge removal and beginning of oestrus activity (h); DBBOA-LAI(h), duration between beginning of oestrus activity and laparoscopic AI (h).

(1988) and Kühholzer *et al.* (1997). Bonev *et al.* (2005) reported that the AI success was 76% with frozen ram semen. It might be due to the breed, semen characteristics, semen evaluation and environmental differences.

The mean values of gestation length were significantly different (P<0.05); however, litter size, DBSR-BOA, DBSR-LAI and AI success rate were not significant in this research, in disagreement with previous report (Kühholzer *et al.* 1997). Duration between sponge removal and beginning of estrus activity and duration between beginning of estrus activity and laparoscopic AI did not have significant effect on the AI success. Donovan *et al.* (2001) reported that the timing of AI did not have any effect on pregnancy rates. The mean values of duration between sponge removal and beginning of estrus activity and duration between beginnings of estrus activity were 42.79 h and 39.37 h, and 17.52 h and 20.60 h for successful and unsuccessful LAI respectively. These values were not significant in the present study, and were in agreement with previous report by Donovan *et al.* (2001).

In conclusion, the very low level of fertility obtained when frozen-thawed semen is used for cervical insemination in sheep has stemmed widespread interest/uptake of AI by the sheep sector. The alternative, LAI technique, is an effective method of insemination with fresh or frozen-thawed semen, but cost of application limits its use. It is important to determine the feasibility of use of LAI technique in genetic conservation programs. In combination with modern reproductive technologies there is potential to use AI, embryo transfer and stored germplasm (genetic resource banks) to support conservation measures for the maintenance of genetic diversity in threatened species. Our results showed that laparoscopic insemination will contribute to the conservation activities in Cine Capari sheep in future. Planning the mating plans and controlling the inbreeding may be possible with the effective use of this technique.

REFERENCES

Bonev G B, Vassilev N Y, Dimitrov M A, Yotov S A and Penche P. 2006. Adjustment of echography and laparoscopic insemination to the reproductive particularities of Pleven Blackhead sheep. *Bulgarian Journal of Veterinary Medicine* **9(1)**: 61–65.

Colas G. 1980. Seasonal variation of semen quality in adult Ile-de France rams 2. Fertilizing ability and its relation to qualitative criteria in vitro. *Journal of Reproduction and Fertility Supplement* **2**: 89–101.

Donovan A, Hanrahan J P, Lally T, Boland M P, Byrne G P, Duffy P, Lonergan P and Neill D J. 2001. AI for sheep using frozen-thawed semen. *End of project report* (Project number: ARMIS–4047).

Evans G. 1988. Current topics in artificial insemination of sheep. *Australian Journal of Biological Sciences* **41**: 103–16.

Fourie P J, Schwalbach L M, Neser F W C and Van der Westhuizen C. 2004. Scrotal, testicular and semen characteristics of young Dorper rams managed under intensive and extensive conditions. *Small Ruminant Research* **54**: 53–59.

Gil J, Lundeheim N, Söderquist L and Rodriguez-Martinez H. 2003. Influence of extender, temperature and addition of glycerol on post-thaw sperm parameters in ram semen. *Theriogenology* **59**: 1241–55.

Gordon I. 1997. *Controlled Reproduction in Sheep and Goats*. (Ed.) Gordon Ian. 450. CAB International, Wallingford, UK.

Gourley D D and Riese R L. 1990. Laparoscopic artificial insemination in sheep. *Veterinary Clinics of North America*: *Food Animal Practic*e **6**: 615–33.

Halbert G W, Dobson H, Walton J S, Sharpe P and Buckrell B C. 1990. Field evaluation of a technique for transcervical intra-uterine insemination of ewes. *Theriogenology* **33**: 1231–34.

Hill J R, Thompson J A and Perkins N R. 1998. Factors affecting pregnancy rates following laparoscopic insemination of 28,447 Merino ewes under commercial conditions: A survey. *Theriogenology* **49(4)**: 697–709

Kafi M, Safdarian M and Hashemi M. 2004. Seasonal variation in semen characteristics scrotal circumference and libido of Persian Karakul rams. *Small Ruminant Reserach* **53(1–2)**: 133–39.

Karaca O and Cemal I. 2005. One of the our indigenous sheep breeds under risk of extinction as genetic resource: Cine Capari. *Hasad Hayvancilik Dergisi* **20**: 14–20.

Karaca O, Cetiner S and Cemal I. 1999. Some characteristics and conservation possibilities of Cine Capari sheep breed as genetic resource. *Proceedings of International Animal Husbandry'99 Congress*. pp. 558–63. 21–24 September 1999. Izmir.

Karagiannidis A, Varsakeli S, Alexopoulos C and Amarantidis I. 2000. Seasonal variation in semen characteristics of Chios and Friesian rams in Greece. *Small Ruminant Research* **37**: 125–30.

Kaymakçý M. 2002. *Üreme Biyolojisi*.M. Kaymakçý (Ed). 305. Ege Üniv. Ziraat Fak. Yayýnlarý, Ýzmir.

Killen I D and Caffery G J. 1982. Uterine insemination of ewea with the aid of laparoscope. *Australian Veterinary Journal* **59**: 95

Kühholzer B, Besenfelder U, Müller S, Reichenbach H D and Brem G. 1997. Laparoscopic insemination of seasonally anoestrous ewes by a simplified method under field conditions. *Reproduction Domestic Animals* **32**: 309–12.

Marshall J T A and Nancarrow CD. 1993. Changes in saccharide binding sites on the surface of ram spermatozoa following freeze thawing. *Proceedings of Australian Society for Reproductive Biology*. pp. 65 (Abstract). Australia.

Maxwell W M C and Hewitt L J, 1986. A Comparison of vaginal, cervical and intra-uterine insemination of sheep. *Journal of Agricultural Science* **106**: 191–93.

Ollero M, Muino-Blanco T, Lopez-Perez M J and Cebrian-Perez J A. 1996. Viability of ram spermatozoa in relation to the abstinence period and succesive ejaculations. *International Journal of Andrology* **19**: 287–92.

Saleh A I. 1997. Seasonal variation in semen quality of local and crossbred rams raised in the United Arab Emirates. *Animal Reproduction Science* **49**: 161–67.

SAS, 1999. PC SAS user's guide: statistics. SAS Inst. Inc., Cary N.C.

Yamaki K, Morisawa M, Ribadulla A and Kojima J. 2003. Sheep semen characteristics and artificial insemination by laparoscopy. *Tohoku Journal of Agricultural Research* **54**: 17–26.

Yýlmaz O and Karaca O. 2004. Seasonal changes in sperm characteristics of Chios and Karya type rams. *Proceedings of 4th National Animal Science Congress.* pp.130–35. Isparta.

Yilmaz O, Karaca O and Yarali E. 2007. Changes in semen characteristics during the Storage of ram semen. *Proceedings of 5th National Animal Science Congress.* pp.109 (abstract). Van.