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CERVICAL INSEMINATION IN KARYA SHEEP

Orhan KARACA, Onur YILMAZ, Ibrahim CEMAL

Adnan Menderes University Faculty of Agriculture Department of Animal Science, TR-09010, Aydin, Turkey

Corresponding author email: karaca_o@yahoo.com

Abstract

The objective of this study was to investigate the feasibility of use of cervical artificial insemination (CAI) method in Karya sheep breed. Planning the mating plans and controlling the inbreeding may be possible with the use of effective artificial insemination techniques. In the present study, 89, 16 and 85 ewes showing estrus were cervically inseminated with diluted fresh semen from 13, 4 and 9 head rams at 2005, 2007 and 2009 mating seasons, respectively. Data were evaluated with respect to gestation length, litter size and AI success rate. The least square means for mentioned traits were found as 147.61 days, 1.73 and 57.30% for 2005 lambing season and 148.57 days, 1.71 and 43.80% for 2007 lambing season and 147.53 days, 1.47 and 50.59% for 2009 lambing season respectively. The difference between means of years were not statistically significant (P> 0.05) for all the evaluated variables except gestation length and litter size. These results have been close to the values indicated in the literature. Our results show that cervical insemination will be a useful tool in field conditions.

Key words: *Cervical insemination, Karya sheep, reproductive performance, sheep.*

INTRODUCTION

Artificial insemination (AI) is probably the most important technique devised to facilitate the genetic improvement of farm animals. The widespread use of AI in cattle has allowed accurate genetic evaluation and rapid dissemination of genetic merit on a national and international basis to the benefit of both breeders and consumers. For sheep producers, the technology for the control of reproduction, including control of estrus, fixed-time AI, early pregnancy diagnosis and synchronization of lambing are now available and offers possibilities in allowing lamb production to be planned in a way which is not feasible under nature (Halbert, 1990; Donavan et al., 2001; Eppleston and Maxwell, 1993). The use of AI can greatly increase the number of offspring produced per sire per year because a ram has the potential to produce enough spermatozoa to inseminate thousands of ewes. So artificial insemination is probably the most important and the first great biotechnology devised to facilitate the genetic and reproduction improvement of sheep. Insemination outcome is affected by many factors (intrinsic and extrinsic) related to female (handling, seasonality, genital morphology, etc.), male (seasonality, sperm quality, sperm conservation, etc.), farm (environmental conditions, sanitary status, handling, etc.) and the technique itself (route of application, spermatozoa/dose, technician, etc.) (Shackell et al 1990; Donovan et al 2004; Paulenz et al. 2005).

Most of insemination procedures in sheep are performed after oestrus syncronization. Penetration of the cervix is a major problem in sheep (Verberckmoes et al, 2001). Conception rates with fresh semen are good (65 to 75%). While cervical artificial insemination with fresh semen yields acceptable conception rates, the short shelf life of fresh semen coupled with a natural limitation on the number of semen doses achievable per unit time restricts the widespread use of individual sires (Gordon, 1997).

But many research reported that conception rate of artificial insemination of sheep is low in comparison with that of the cow. It is reported that the conception rates are %45 on average (Anca et al 2007).

Cervical insemination is a cheap and relatively easy method of artificial insemination. Cervical artificial insemination, using a speculum and pipette, deposits semen directly into the cervix, through the vagina. Using fresh diluted semen, it has the advantage of being a relatively simple operation and potentially large numbers of

animals can be inseminated during a short period.

In the last two decades, fat-tailed sheep breeds at western part of the Turkey were backcrossed with rams of thin-tailed breeds by farmers to form a thin tailed genotype (namely Karya) that have high reproductive and growth performance. The breeds used for crossbreeding of local genotypes are prolific Chios breed that a common breed of Greece and Turkey (known as Sakiz in Turkey) and Kivircik breed that have a good growth performance. In 1994, an open nucleus flock was established to improve performance traits in synthetic Karya sheep. Karya sheep which have high reproductive performance has been preferred by breeders in the region (Karaca et al 2009a; Karaca et al 1998, Cemal et al 2009). A breeding scheme named as Adnan Menderes University Group Sheep Breeding Program (ADÜ-GKYP) was established to improve performance traits of Karya sheep at extensive management conditions.

One of the most important problem in Aydin finding stud ram for breeding season. If this technique become available in a widespread manner can be overcome a shortage of stud rams. Hereby farms will be able to record keeping more accurate pedigree records. This study aims to investigate the feasibility of use of cervical insemination technique in Karya Sheep under open nucleus breeding program.

MATERIALS AND METHODS

The research was made 2005, 2007 and 2009 mating season at Karya sheep elite flock, Adnan Menderes University Group Sheep Breeding Program (ADU-GKYP), Aydin, Turkey. Its objective was the sheep artificial insemination with fresh diluted semen, by the cervical method. In the present study, 89, 16 and 85 ewes showing estrus were cervically inseminated with diluted fresh semen from 13, 4 and 9 head rams at 2005, 2007 and 2009 mating seasons, respectively. The age of rams are ranged from 3 to 5 years. Animals were fed with concentrate mix and had access to a shelter, where water and salt stone complement were available.

Oestrus cycles of all ewes was synchronised with progestagen sponges for 14-day treatment of progestagen vaginal sponges (40 mg FGA, Chronogest CR, Intervet) with an 500 IU pregnant mare serum gonadotrophin (Chronogest CR) given (i.m.) at the time of sponge removal to stimulate follicular development.

Semen was collected from each ram using an artificial vagina before artificial insemination. Immediate after semen collection, the semen was examined. Semen samples were evaluated for volume (SV, ml), mass motility (MM), percentage of dead sperm cells (PDSC,%), sperm concentration (SC) and total number of spermatozoa per ejaculate (TNSPE). Simple statistics for some semen characteristics of Karya rams used for cervical inseminations were given in Table 1. One part of evaluated semen were diluted with three part of a commercial semen extender (Laiciphos, IMV, Sark Kemikal, Istanbul, Turkey). The semen was kept in water bath at 30°C until inseminations.

Inseminations were carried out 50-51 h after sponge removal. Only ewes that showing estrus was inseminated. After cervix making evident with a speculum which was equipped with a light source, the fresh diluted semen was inoculated inside of cervix, in 0.5 ml. doses.

Data 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 DISCUSSIONS

Semen characteristics are important variables considered in the most studies of artificial insemination (Colas, 1980; Gordon, 1997; Kaymakçi, 2002; Ollero et al, 1996). Simple statistics for some semen characteristics of Karya rams was given in Table 1. In the present study all ejaculate characteristics had similar to previous studies (Yilmaz and Karaca, 2004, Yilmaz et al., 2009).

Table 1. Simple statistics for some semen characteristics of Karya rams used for cervical inseminations

Years	Variable	N	Mean±SD	Min.	Max.	CV (%)
2005	SV	13	0.95±0.360	0.50	1.50	38.01
	MM	13	4.62±0.650	3.00	5.00	14.09
	PDSC (%)	13	12.6±3.032	9.75	20.50	24.07
	SC	13	1.42±0.206	1.04	1.77	14.54
	TNSPE	13	1.38±0.625	0.52	2.65	45.44
2007	SV	4	1.13±0.250	1.00	1.50	22.22
	MM	4	5.00±0.000	5.00	5.00	0.00
	PDSC (%)	4	2.81±0.774	1.75	3.50	27.52
	SC	4	1.82±0.093	1.72	1.94	5.12
	TNSPE	4	2.06±0.571	1.72	2.91	27.75
2009	SV	9	0.96±0.288	0.50	1.30	30.11
	MM	9	4.67±0.500	4.00	5.00	10.71
	PDSC (%)	9	3.31±0.527	2.75	4.25	15.94
	SC	9	1.60±0.153	1.37	1.81	9.55
	TNSPE	9	1.51±0.442	0.82	2.26	29.23

SV: Semen volume (cm^3). **MM:** Mass motility**. PDSC (%) :** Percentage of dead sperm cells. **SC:** Semen concentration ($x10^6/mm^3$).
TNSPE: Total number of spermatozoa per ejaculate ($x10^9$/ejaculate volume)

The general mean values for SV, MM, PDSC and TNSPE were found as 0.98, 4.69, 7.88, 1.54 and 1.53, respectively.

SV, PDSC and TNSPE had lower value and MM and SC had higher value than Yilmaz and Karaca 2004, who working in the same genotype, in this study. Although SV, MM, PDSC and TNSPE were greater than Yilmaz et al, 2009, SC value was less than the same literature. Semen volume and sperm concentration are comparable to those reported by Saleh (1997) who established 0.67 ml and 4.3 × 109. However in the present study had higher volume and lower concentration to those reported by Saleh (1997). It might be due to the breed and environmental differences.

Phenotypic correlation coefficients for some semen characteristics were given Table 2. The correlation between SV with TNSPE, and SC with MM and SC with TNSPE were found to be positive and significant. But the correlation between SC with PDSC and TNSPE with PDSC were found to be negative and significant. The correlation between semen volume with total number of sperm per ejaculate and sperm concentration with total number of sperm per ejaculate were found to be positive and significant, in agreement with previous reports (Yilmaz and Karaca, 2004, Karagiannidis, 2000).

Table 2. Phenotypic Correlations among semen characteristics (N=26)

	SV	MM	PDSC (%)	SC
MM	-0.04^{NS}			
PDSC (%)	-0.27^{NS}	-0.34^{NS}		
SC	0.29^{NS}	0.45^{*}	-0.72^{**}	
TNSPE	0.93^{**}	0.11^{NS}	-0.45*	0.59^{**}

NS=Non-significant, **=**P< 0.05, ****=**P< 0.001

The correlation coefficient between semen volumes with sperm concentration did not differ significantly in this work in agreement with Yilmaz et al. 2009, but this result are not consistent with previous studies (Karagiannidis, 2000, Yilmaz and Karaca, 2004).

Least square means and standard errors for some semen characteristics of Karya rams were given in Table 3. The mean values for PDSC and SC except the other semen characteristics were significantly differed between years (P< 0.01). All semen characteristics were not affected ram age.

Although there are statistically significant changes in SC, TNSPE is an interesting result that there is appears to be a statistically insignificant. These results are not consistent with previous studies (Yilmaz et al, 2009, Yilmaz and Karaca 2004, Saleh, 1997)

Table 3. Least square means of semen characteristics

Variable	N	SV	MM	PDSC (%)	SC	TNSPE
Years		**P=0.614**	**P=0.344**	**P=0.000**	**P=0.002**	**P=0.126**
2005	13	0.95±0.090	4.54±0.15	12.6±0.618	1.42±0.049	1.37±0.155
2007	4	1.13±0.162	5.00±0.27	2.81±1.115	1.81±0.089	2.06±0.280
2009	9	0.96±0.108	4.67±0.18	3.31±0.743	1.60±0.059	1.51±0.187
General	**26**	**1.01±0.071**	**4.74±0.119**	**6.24±0.492**	**1.61±0.039**	**1.65±0.124**

SV: Semen volume (cm^3). **MM:** Mass motility**. PDSC (%) :** Percentage of dead sperm cells. **SC:** Semen concentration ($x10^6/mm^3$).
TNSPE: Total number of spermatozoa per ejaculate ($x10^9$/ejaculate volume).

The success of cervical insemination according to years was summarized in Figure 1. Success rates were found 57.30%, 43.80% and 50.59% in 2005, 2007 and 2009 respectively.

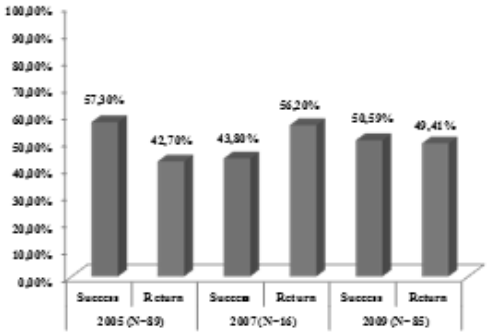

Figure 1. Success of cervical insemination according to years

Cervical insemination using fresh semen gives a higher pregnancy rate than cervical insemination using frozen-thawed semen (Donovan et al., 2004); 76% compared with 46% (Irish breed) and 36% (Norwegian breed). The pregnancy rate obtained following cervical insemination with fresh diluted semen is less than Evans and Maxwell, 1987 (65-75%). However in the present study had higher insemination success than reported previous studies (Rojero et al. 2009; Anca et al., 2007). These differences might be due to the breed and environmental differences.

Simple statistics for gestation length and litter size were given in Table 4.

Table 4. Simple statistics for gestation length and litter size from successful insemination

Year	Variable	N	Mean±SD	CV (%)	Min	Max
2005	GL	51	147.61±3.75	2.54	133.00	153.00
	LS	51	1.73±0.666	38.58	1.00	3.00
2007	GL	7	148.57±1.27	0.86	147.00	150.00
	LS	7	1.71±0.756	44.10	1.00	3.00
2009	GL	43	147.53±3.91	2.65	131.00	152.00
	LS	43	1.47±0.592	40.38	1.00	3.00

GL: Gestation length, **LS**:Litter size

General mean of litter size was 1.64 for all the years. The highest litter size was 1.73 in 2005. Least square means and standard errors for gestation length and litter size for Karya ewes which were cervically inseminated in 2005, 2007 and 2009 years were given Table 5.

Table 5. Least square means of gestation lenght and litter size from successful insemination

	N	GL	LS
Year		**P=0.457**	**P=0.357**
2005	51	147.52±0.564	1.76±0.095
2007	7	149.17±1.419	1.60±0.238
2009	43	147.23±0.626	1.55±0.105
Dam Age		**P=0.116**	**P=0.013**
2	34	148.68±0.829	1.40±0.139
3	24	149.17±0.885	1.45±0.149
4	17	146.87±1.006	1.95±0.169
5>	26	147.18±0.784	1.72±0.132
General		**147.97±0.536**	**1.63±0.090**

GL: Gestation length, **LS**:Litter size

The difference between means of years for gestation length were not statistically significant (P>0.05). The results show that years have a significant effect on litter size (P< 0.05). The gestation length obtained following cervical insemination with diluted fresh semen is similar to that previously reported under same conditions in Çine Çapari Sheep in Turkey (Karaca et al., 2009b). Litter size obtained from this study had higher value than Karaca et al., 2009a. The results show that litter size is higher than the other Turkish sheep breeds.

CONCLUSIONS

Artificial insemination (AI) of sheep is an advantageous management practice aimed at the genetic improvement at farm level and a programme of genetic selection. Furthermore AI has the potential for a significant impact on the sheep breeding industry. The main role of AI in sheep production is to increase the rate of genetic improvement and AI also contributes to achieving other goals, e.g. allowing extensive use of the best available rams, therefore increasing selection pressure and the rate of response to selection.

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. It is an effective, easy and cheap method of cervical insemination with diluted fresh semen.

It is important to determine the feasibility of use of cervical insemination technique in field conditions. The use of reproductive technologies such as artificial insemination in

animal breeding programs is very important tools. Our results show that cervical insemination can easily use in field conditions, while not a very high success planning the mating plans and controlling the inbreeding may be possible with the effective use of this technique.

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