



Duration effect of fresh semen kept *in vitro* on sheep conception rate

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Abstract

Yearling Akkaraman ewes (n = 140) were apportioned into two groups and inseminated with fresh semen collected from Suffolk and Dorper rams. Bilateral intrauterine insemination (100 x 106 spermatozoa in 0.25 ml) was performed with the aid of laparoscope. Fixed time insemination was carried out 48-50 h after controlled intravaginal drug release (CIDR) removal with the aid of estrus detection using vasectomized rams. Uterine tone at the time of insemination was scored (1-3), the highest pregnancy rate achieved for ewes with score 3 (67%), followed by 59 and 47% for scores 2 and 1, respectively. General decrease in pregnancy rates were observed with increased duration of stored fresh semen over the insemination period (Fall from 73 to 57% over 120 min storage). However, a slight increase in conception rate was observed at the time of AI with the semen kept *in vitro* for a longer period (90-120 m) which might be the most appropriate time relative to ovulation.

Keywords: aged semen, Dorper, fat tailed sheep, fresh semen, Suffolk.

Introduction

Akkaraman is the most common endemic fat tailed sheep breed in Turkey accounting for 37% of the total sheep population. The breed is well known for its resistance to diseases and parasites as well as converting poor quality forage to valuable animal sourced products. The breeds' meat/carcass quality and prolificacy characteristics however are low, usually producing a single drop. Improving the carcass traits have always been one of the more important breeding goals for the Akkaraman breed in the Turkish sheep industry creating a large demand for improved terminal sire crossbreeding programs. The limiting factor for an effective mating between fat tailed Akkaraman ewes and rams from thin tailed exotic meat breeds such as Dorper and Suffolk, is the need for assistance in lifting up the fat tail of the ewe for the inexperienced exotic rams to better perform. As this process is not often practical and as unassisted copulation is mostly ineffective, artificial insemination (AI) is often essential.

AI is necessary not only for overcoming mating problems due to the fat tail issues but 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% (McKelvey, 1985; Hill *et al.*, 1998).

For artificial insemination (AI) in sheep, intrauterine semen deposition is a necessity for obtaining high lambing rates. Conception rates after intrauterine insemination are acceptable not only with freshly collected semen both also with frozen then thawed semen that has been subjected to minimal handling. Pregnancy rates of fat tailed ewes conducted from several studies ranged from 30-75% with fresh and frozen thawed semen (Emsen *et al.*, 2008, 2011; Atsan *et al.*, 2009). 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 the spermatozoa under these conditions is quickly reduced, due to the increase in the concentration of lactic acid in the ejaculate (Vivanco, 1988). It was indicated that the adverse effects of sperm aging are likely mediated by membrane changes associated with increased lipid peroxidation, with the effect of reducing numbers of viable sperm available to form a sperm reservoir in Boar semen (Kumaresan *et al.*, 2009; Am-in *et al.*, 2011). Uterine tone has also been scored during the laparoscopic artificial insemination (LAI) in this study. Gimenez-Diaz *et al.* (2012) reported that uterine tone, a measure of quality of heat, was significantly related to conception rates when it is measured at the time of intrauterine insemination. In a large scale epidemiological study, Anel *et al.* (2005) observed that the male factor significantly influenced fertility. Salamon and Maxwell (1995) proposed that ram differences in fertility could be both genetic and environmental, whereas ejaculate differences are probably due to nutrition, management and previous frequency of ejaculation (concentration of spermatozoa).

Limited information based on ram semen aging led us to conduct the present study. We hypothesized that there might be an adverse effect of increased sperm age on conception rates. Therefore, the objective of the experiment described here was to determine the effect of ram genotype, uterine tone and holding time of fresh semen on pregnancy rates in fat tailed yearling Akkaraman ewes.

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Materials and Methods

This study was approved by the animal ethics committee of the Ataturk University and was carried out on a commercial farm in Burdur, southern Turkey in April. A total of 140 Akkaraman fat tailed yearling ewes at the commercial sheep enterprise were used for the present study. The ewes had a moderate body condition score (average 2.5-3.0). Production is based on a semi-intensive system and the Akkaraman is a seasonal breed. The ewes were treated for 12 days with controlled internal drug release devices (CIDR) containing 300 mg progesterone (Eazi-Breed CIDR, Inter Ag, Hamilton, New Zealand). At pessary removal all ewes were injected intramuscularly with 500 UI of equine chorionic gonadotrophin (eCG). Estrus detection was performed with the aid of two vasectomized rams equipped with a canvas apron three times a day (6:00 a.m., 2:00 p.m. and 9:00 p.m.) for 3 days after pessary removal and insemination took place 48-50 h after CIDR removal. Time of semen collection and the subsequent insemination was recorded to ensure that the protocol for the aging process was adhered to as per the allocated groups.

Ewes were randomly allocated to two groups according to the breed of rams; 93 ewes were inseminated with semen from a Suffolk ram and 47 ewes with semen from a Dorper ram. After collection of semen from only one ram per breed, each ejaculate was evaluated for volume and motility. Ejaculates of more than 0.5 ml and good motility (>70%) were used for pooling procedure. Semen was extended at 35°C in OviPro® (Minitüb, Tiefenbach, Germany) and maintained at 30°C in a water bath. Ewes inseminated with freshly diluted semen containing 100 x 10⁶ motile spermatozoa per 0.25 ml. Laparoscopic artificial insemination (LAI) was performed by a single experienced LAI operator 48-50 h. after CIDR withdraw. All ewes were fasted and had no access to water for 18 h before laparoscopy. Ewes received i.v. injection of an anesthetic cocktail containing 2 cc

Ketasol (Indus Pharma, Karachi, Pakistan) + 0.04 cc Romphun (Bayer) before insemination and 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 both the uterine horns. At the time of laparoscopic AI, uterine tone was scored as no tone (1), moderate tone (2) or intense tone (3). Uterine tone is determined as a measure of quality of heat conducive to the implantation of the zygote. All ewes were scanned trans abdominally 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.

To determine the effect of ram breed, semen aging and uterine tone on conception rate, Kruskal-Wallis and Mann-Whitney U tests were applied. SPSS v.20 software was used under license to Ondokuz Mayıs University. Binary logistic regression was used to determine the effect of semen aging on conception rate. Chi-Square dependency test was used to determine the dependency of breed, semen aging and uterine tone on conception rate.

Results

The conception rate obtained in this study using fresh diluted semen did not differ significantly for the ewes inseminated with semen from different breeds of ram. The conception rates related to the uterine tone score at the time of intrauterine insemination were not statistically different. Ewes with a uterine tone score of 3 had an average 67% conception rate while scores 1 and 2 resulted with 47 and 59%, respectively. Overall conception rate dropped by 16% over a 90 min fresh semen ageing period (Table 1).

Table 1. Effect of ram breed, uterine tone and semen aging on conception rate of fat tailed ewes.

Breed	n	Conception rate (%) $\bar{X} \pm S\bar{X}$	X ²
Suffolk	93	0.59 ± 0.05	3.108
Dorper	47	0.57 ± 0.07	1.043
Sig.		0.848	
Dependency (%)		1.8	
Semen aging (min)		$\bar{X} \pm S\bar{X}$	
0-29	33	0.73 ± 0.08 ^a	6.818
30-59	41	0.66 ± 0.07 ^a	4.122
60-89	35	0.39 ± 0.08 ^b	1.400
90-119	24	0.57 ± 0.09 ^b	0.167
Sig.		0.024	
Dependency (%)		25	
Uterus Tone		$\bar{X} \pm S\bar{X}$	
1	43	0.47 ± 0.07	5.233
2	37	0.59 ± 0.08	1.324
3	15	0.67 ± 0.13	1.667
Sig.		0.744	
Dependency (%)		6.2	

^{a,b}different letters in the same column indicate significant difference (P < 0.05). Sig: significant.



Binary logistic regression was used to determine the effect of semen aging on conception rate, where correct classification rate was assessed at 83%. The odds ratio determined that with every 30 min intervals of semen ageing protocols used in the present study in insemination, the likelihood of reduced conception increased by 47, 105, 302 and 806% respectively.

The same regression analysis method was also used to determine the effect of uterine tone on conception rates and the model showed no significance between the two factors. Correct classification rate was assessed at 64% for this analysis. The dependency of semen ageing to successful conception rate were found to be significant (25%).

Discussion

Results of the present study revealed that decrease in sperm function was associated with an increase in holding time which was in agreement with previous studies reported in cattle (O'Flaherty *et al.*, 1999) and buffalo (Kumaresan *et al.*, 2006). Variations in the fertility of rams have been reported after cervical inseminations with fresh semen (Paulenz *et al.*, 2002; Anel *et al.*, 2005) and after laparoscopic inseminations with frozen semen (Eppleston *et al.*, 1986, 1991; Maxwell, 1986; Eppleston and Maxwell, 1995). Our current study indicates that fresh diluted semen from Suffolk and Doper rams resulted with 59 and 57% conception rates, respectively (Table 1). Although seasonality is less marked in males than in females, changes in testicular volume, hormonal profiles, sexual behaviour and semen quality that affect the reproductive performance of rams have been reported (Casao *et al.*, 2010). Olah (2010) reported that the largest scrotal circumference (SC) was measured in spring for the Suffolk (35.8 cm) while for the Dorper breed, the largest values measured in the summer. Olah (2010) also indicated that in all seasons, a medium positive significant correlation was found between body weight and scrotal circumference. Suffolk ram (98 kg) used in this study was heavier than Dorper ram (92 kg), this may have been the reason for the slightly better performance of the Suffolk semen.

In our study, the uterine tone at the time of laparoscopic insemination was found not to have a linear (positive) association with conception rates, which was contrary to the findings of Gimenez-Diaz *et al.* (2012). Our results are in agreement with Pervage *et al.* (2009) who reported that the number of live spermatozoa and the sperm motility decreased with increasing age of semen. It was hypothesized that incubating ram sperm in the manner presented here at 30°C for times greater than 60 min, may have a detrimental effect and result in a decline (46%) in the overall conception rate.

In conclusion, using fresh diluted ram semen stored at 30°C within 60 min of collection gave acceptable results when used for intrauterine insemination. In the current study, we focused on duration of semen storage and the uterine tone of the

ewe at the time of insemination. Pregnancy rate decreased when ewes were inseminated with aged semen kept *in vitro* for more than 60 min. Further studies related to semen ageing and uterus tone are needed.

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