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Effect of extender type and cold storage of fresh semen on reproductive indices of Karakul ewe following fixed time artificial insemination

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ABSTRACT

Semen quantity and quality are affected by transportation and frequent semen collection in ram when they are transported from nucleus flock to target farms for *in situ* artificial insemination (AI) of ewes with fresh semen. The current study was designed to introduce the best method of fresh semen preservation for fixed time AI in Karakul sheep. The adult Karakul ewes were allocated to 6 groups (30 ewes in each group) during the breeding season and each group was inseminated with one of the following treatments, viz. fresh semen without extender, fresh semen diluted with Tris-egg yolk or milk, cooled semen (stored at 10°C for 8 h) without extender, and cooled semen diluted with Tris-egg yolk or milk. Lambing and fecundity rates were the highest when ewe was inseminated with undiluted fresh semen (71.42 and 85.71%, respectively). These reproductive indices in ewes, which were inseminated with diluted fresh semen or undiluted cooled semen were significantly lower than those recorded in inseminated ewes with undiluted fresh semen. The type of extender and storage of diluted semen at 10°C for 8 h did not significantly affect lambing rate of Karakul ewe. Results suggested that use of undiluted fresh semen and diluted fresh semen in milk or Tris-egg yolk extenders are preferable for fixed time AI in ewes of genetic improvement center and Karakul sheep flocks located near to this center, respectively.

Key words: Ewe, Extender, Fertility, Karakul, Semen, Timed AI

Artificial insemination (AI) has been considered as a rapid and appropriate reproductive technique to genetic merit dissemination and genetic improvement facilitation (Gibbons and Cueto 2011, Valergakis *et al.* 2010). AI with frozen ram semen has resulted in relatively poor fertility with intra-cervical insemination method and demanded high costs and well trained technicians with intrauterine deposition method. The use of non-frozen semen in sheep sounds a promising alternative to frozen semen that causes serious damage to ram spermatozoa and impair fertility. The fresh semen can be used immediately after collection or preserved for short periods at low temperatures (cooled semen at 15°C for 8 h and chilled semen at 5°C for 12 h) and in diluents including compounds that help to delay the production of reactive oxygen and prolong fertile life of spermatozoa (Gibbons and Cueto 2011). Skim milk based extenders has given satisfactory results in preservation of ram semen, as compared to Tris based extenders (Ari *et al.* 2011). In Tris-egg yolk extender, low density lipoprotein and phospholipids such as phosphatidycholine preserve the sperm membrane against cold shock (Bergeron *et al.* 2004, Bergeron and Manjunath 2006, Kulaksiz *et al.* 2012). In skim milk extender, the casein micelles protect ram spermatozoa from cold by reducing sperm lipid loss and maintaining sperm motility and viability (Bergeron *et al.* 2007).

The Persian Karakul, one of the most economically important skin and meat-breed of sheep reared in Iran (Kafi *et al.* 2004), is a fat-tailed sheep of medium size (Farid and Makarechian 1976). For genetic improvement program of Persian Karakul, superior rams from nucleus flock are transported to target farms for *in situ* AI of ewes with undiluted fresh semen, during the breeding season. Transportation of ram and frequent semen collection affect semen quantity and quality. Therefore, appropriate transportation of diluted semen is preferable to transportation of outstanding rams; however no data is available regarding the reproductive performance of Karakul ewes after fixed time artificial insemination with diluted fresh or cooled semen. The current study was designed to introduce the best method of fresh semen preservation for fixed time AI in Karakul sheep.

MATERIALS AND METHODS

Location and experimental animals: The experiment was carried out during the breeding season in a flock of Karakul

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sheep in the Aliabad-Kamin Sheep Genetic Improvement Center affiliated with Jihad-Agriculture Organization in Saadatshahr city. From sheep flock of station, ewes (180) lambed at least once, 3- to 5-year old, weighing between 40 and 45 kg, were selected. Body condition scores (BSC), scored by palpation of lumbar region (Yilmaz *et al.* 2011), ranged from 3 to 3.5 (scale 1–5). Estrus of ewes were synchronized by vaginal insertion a controlled internal drug release device containing 0.3 g progesterone (CIDR®, inter Ag, New Zealand) for 12 days and an intramuscular injection of 500 IU equine chorionic gonadotropin (eCG) (Folligon, Intervet, Holland) at CIDR removal (Hashemi *et al.* 2006).

The females grazed on natural pastures during the day (06.00–17.00 h) and were kept indoors at night throughout the experiment. Rams were fed a mixture of wheat straw, hay and barley grain according to the recommended requirements of the NRC 2 months before the commencement of the experiment in two equal quantities per day. Access to a mineral salts lick and water was *ad lib*.

Semen preparation: Semen samples were collected from 7 proven fertile Karakul rams using an artificial vagina fitted with a graduated collecting tube (Fernandez-Abella *et al.* 2003). Immediately after collection, all ejaculates were placed in a water-bath at 37°C. An aliquot from each ejaculate was taken for semen evaluation and all evaluations were performed by the same operator. Semen samples were evaluated for volume and mass motility on a scale of 0 (immotile) to 5 (vigorous motility) and only ejaculates over 0.5 ml, mass activity ≥3 and progressive motility ≥70% were pooled (Paulenz *et al.* 2002).

Pooled semen was split into 2 equal fractions. One fraction was used for undiluted semen and the rest was divided into 2 parts and diluted (a part with ultra heat treated (UHT) long life milk (1% fat) and the other part with (hydroxymethyl) amino-methane (TRIS)-egg yolk extender) (Paulenz *et al.* 2003). Milk extender was prepared from a commercial UHT skim milk (Ramak, Shiraz, Iran) adding antibiotics (100,000 IU of sodium penicillin and 100 mg of dihydrostreptomycin/100 ml). TRIS-egg yolk extender composed of TRIS (3.63 g), glucose (0.5 g), citric acid (1.99 g,), and supplemented with 15% (v/v) egg yolk. Sodium penicillin and dihydrostreptomycin were added (100,000 IU and 100 mg, respectively) and the volume was increased to 100 ml with double-distilled water (Salamon and Maxwell 2000). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Samples were loaded into 0.25 ml straw and sealed with polyvinyl alcohol powder. Final concentrations of spermatozoa were at least 900×10^6 and 400×10^6/straw in undiluted and diluted samples, respectively. The half of straws was placed in a refrigerator to use as cooled semen and others was used as fresh semen. The temperature of cooled semen was lowered slowly to 10°C and stored for 8 h.

Fixed time artificial insemination: All treated ewes were inseminated 48 h after the end of the treatments by cervical route. Ewes were sorted by age, BW and BSC, respectively, and allocated to 6 groups (30 ewes in each group) considering sorted list. The females in each group were inseminated with one of the following treatments, viz. fresh semen without extender; fresh semen diluted with Tris-egg yolk; fresh semen diluted with milk; cooled semen without extender; cooled semen diluted with Tris-egg yolk; and cooled semen diluted with milk.

A breeding rack was used for cervical insemination and the hindquarters of the ewe lifted over the top rail while the front legs remained standing on the ground. A dose of semen in 0.25 ml straw was deposited into the external os of the first cervical fold of each ewe, using speculum fitted with an internal light source and insemination pipette (Menchaca *et al.* 2005).

Variables and statistical analysis: The following parameters were calculated in each of the treatments:

Lambing rate: number of lambed females/number of females treated × 100

Fecundity rate: number of lambs at birth/ number of females treated × 100

Prolificacy rate: number of lambs at birth/ number of lambed females in expected time × 100

These parameters were analyzed using the chi-square test. The statistical software programme of SAS was used for the analyses.

RESULTS AND DISCUSSION

Most of the females had lambed during the expected time after AI with undiluted fresh semen (Table 1). Several ewes were excluded from the data due to no lambing or death in different treatments.

Table 1. Distribution of time of lambing of ewes and lamb number of Karakul ewes in a reproduction period

Treatment	Ewe No		Lambed ewe*					Multiple birth no		Lamb no
	Initial	Data	A	B	C	D	E	Single	Twin	
Fresh semen undiluted	30	28	20	3	5	1	1	16	4	24
Fresh semen with Tris	30	29	16	5	8	1	0	13	3	19
Fresh semen with milk	30	27	13	4	10	1	2	9	4	17
Cooled semen undiluted	30	28	11	8	9	1	1	11	0	11
Cooled semen with Tris	30	27	13	9	5	2	1	7	6	19
Cooled semen with milk	30	29	13	13	3	1	0	13	0	13

A, Lambing in expected time; B, lambing in 30–40 days after expected time; C, lambing in next time; D, without lambing; E, died ewe.

Table 2. Effect of extender type and storage time in 10°C on reproductive parameters of Karakul ewes (%)

Treatment	Lambing rate	Fecundity rate	Prolificacy rate
Fresh semen undiluted	71.42[a]	85.71[a]	120.00[b]
Fresh semen with Tris	55.17[b]	65.52[b]	118.80[b]
Fresh semen with milk	48.14[bc]	62.96[bc]	130.77[b]
Cooled semen undiluted	39.28[c]	39.25[c]	100.00[c]
Cooled semen with Tris	48.14[bc]	70.37[b]	146.20[a]
Cooled semen with milk	44.82[bc]	44.83[c]	100.00[c]

[a-c]Different superscripts in each column differ significantly ($P<0.05$).

The highest lambing and fecundity rates (Table 2) were obtained when ewe was inseminated with undiluted fresh semen. These reproductive indices in inseminated ewes with diluted fresh semen or undiluted cooled semen were significantly ($P<0.05$) lower than those recorded in inseminated ewes with undiluted fresh semen. The type of extender and storage of diluted semen at 10°C for 8 h did not significantly affect lambing rate of Karakul ewe. Prolificacy rate was not affected by diluting and type of extender of fresh semen and it was the highest ($P<0.05$) when ewes were inseminated with diluted cooled semen in Tris.

Although the use of freshly undiluted semen gave the best lambing and fecundity rates but the use of undiluted semen has not been used commonly. The most advantageous of AI is to use semen from 1 male of high genetic merit for many females and substantially increase the rate of genetic progress in herd (Eppleston and Maxwell 1993). On the other hand, a large number of females can be inseminated by diluted semen of a few genetically superior males, which can decrease the costs of keeping additional rams in herd. For these reasons, researches with undiluted semen are rare. Lapwood *et al.* (1972) reported 65% lambing rate by using undiluted semen in AI.

Structural alterations, particularly in the acrosomal region, a rapid and irreversible loss of motility, a drastic change in metabolism and a high rate of leakage of intracellular sperm constituents can be the consequences of lipid peroxidation of sperm. Semen cooling can delay aging and cellular death of sperm but cold shock can cause lower fertility in ewe (Lopez-Saez *et al.* 2000). Although semen fertility is decreased when semen is cooled due to a decrease in motility and morphological integrity, accompanied by a decline in spermatozoa surviving in the female reproductive tract (Maxwell and Salamon 1993), but our finding showed that storage of diluted semen of Karakul ram for 8 h at 10°C did not affect lambing and fecundity rates of Karakul ewe after fixed time AI. Fernandez-Abella *et al.* (2003) showed that following insemination with cooled semen (diluted in TRIS and stored for 6 h at 15°C), the lambing rate was significantly better than when chilled semen (diluted in TRIS and stored for 24 h at 5°C) was used. In India, fixed time AI with diluted semen in Tris egg yolk and stored at 5°C following estrus synchronization of ewes resulted in 60.42% lambing rate that was higher than 48.18% lambing rate in present study (De *et al.* 2015). This difference might be ascribed to differences in the number of inseminations for a ewe in these two studies (twice in 48 and 72 h vs once in 48 h after pessary removal). It has also been reported that breeding season can affect the lambing rate (De *et al.* 2016).

The decrease in lambing rate after dilution of semen in our study is similar to the finding of Menchaca *et al.* (2005) who reported the conception (pregnant ewes/inseminated ewes) rate of 54.0% using fresh semen diluted with TRIS-based extender without refrigeration (at 30°C). They did not report the difference between conception rates of ewes inseminated with fresh or stored diluted semen for 12 h (42.7%) that it is also concomitant with our finding using diluted semen stored for 8 h at 10°C. Paulenz *et al.* (2003) reported a 51.5 and 61.9% lambing rate using semen stored at 5°C diluted in a commercial TRIS-based or milk extender, respectively, in Norwegian Crossbred ewes. The overall conception rate of ewes inseminated with diluted (UHT-base) chilled semen (after 24 h of preservation at 5°C) was significantly lower than the control group inseminated with diluted fresh semen after 1 h of preservation at room temperature (56% vs 74% respectively, $P<0.05$) (Olivera-Muzante *et al.* 2011).

The type of extender and storage of diluted semen at 10°C for 8 h did not significantly affect lambing rate of Karakul ewe. It is similar to the finding in Persian Moghani ewe after insemination with fresh semen diluted in Tris or milk extender that was reported 65 and 55% lambing rates respectively (Ahangari *et al.* 1998). In Norway, AI with cooled liquid semen diluted in the milk-based extender resulted in a statistically significant ($P<0.01$) better lambing rates of about 10% units compared with semen diluted in the TRIS-based extender (Paulenz *et al.* 2003). This difference with our finding might be due to dissimilarities in breed and the method of insemination that was via vaginal insemination in the Norway study. Donovan *et al.* (2004) suggested that ewe breed can be a critical determinant of the potential for the exploitation of cervical insemination of frozen–thawed semen in the sheep breeding programs.

It can be concluded that the use of fresh undiluted semen and fresh diluted semen in milk or Tris extender are preferable for fixed time AI in ewes of genetic improvement center and Karakul sheep flocks located near to this center respectively. Cooled diluted semen can be used under 8 h storage times at 10°C in flocks that are placed in farther.

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