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Short communication

Morphological and biochemical characteristics of Afshari and Afshari × Booroola Merino cross bred rams (cross-continental cross breeding) semen before and after cryopreservation

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ABSTRACT

The aim of the present experiment was to study the effect of cross-continental cross breeding on morphological and biochemical characteristics of Afshari and Afshari × Booroola Merino cross bred rams semen before and after cryopreservation. Semen samples were collected from ten mature and fertile rams (n = 5/breed) once per week for 8 weeks during the breeding season. The results showed no significant differences in ejaculate volume, pH, total motility, membrane integrity and percentage of abnormal sperm before cryopreservation between the two breeds ($P > 0.05$). Sperm concentration and progressive motility ($3.98 \pm 0.22 \times 10^9$ sperm/mL and $64.84 \pm 1.64\%$, respectively) in Afshari rams were significantly higher than those of Afshari × Booroola Merino ($1.83 \pm 0.26 \times 10^9$ sperm/mL and $52.92 \pm 1.94\%$, respectively) rams ($P < 0.01$). There were no significant differences in the level of Na^+, K^+, Ca^{++} and Lactate dehydrogenase enzyme (LDH) of seminal plasma as well as testosterone levels of plasma between the two breeds ($P > 0.05$). Total motility in frozen-thawed sperm was greater in Afshari ($44.15 \pm 4.15\%$) than that of Afshari × Booroola Merino cross bred ($18.07 \pm 4.80\%$) rams ($P < 0.01$). In conclusion, the two breeds were similar in semen characteristics except in sperm concentration and progressive motility before cryopreservation and total motility after freezing- thawing. It is assumed that semen of Afshari ram, evolved in cold regions, is more resistant to the freezing process.

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1. Introduction

The necessity of fertilizing large numbers of ewes with the semen of outstanding rams requires transportation of semen from collection sites to the sites of insemination. The urgency to exploit the rams over the long duration or at different times of the year encouraged research on preservation of sperm under artificial conditions (Salamon and Maxwell, 2000). Semen cryopreservation has a major role in the development of reproductive techniques and breeding programs in domestic animals. Artificial insemination using frozen semen is greatly important for improvement of male genetic performance in many domestic animals including sheep (Matsuoka et al., 2006a). Among Iranian sheep breeds, Afshari is one of the meat producing breeds which has high potentials for growth and reproduction and considered as one of the profitable breeds of sheep in national breeding programs. This breed is scattered in the area of about 20,000 Km^2 in Zanjan province and in some parts of Eastern and Western Azerbaijan and Kurdistan in Iran (Mohammadi et al., 2011). There is no documented and reliable data regarding the reproductive characteristics of Iranian rams. In recent years, several stations have been established for the genetic improvement of local sheep breeds in Iran (Zamiri and Khodaei, 2005). One of the breeding programs which has been extensively supported by the government is the marker-assisted introgression of Fec B gene from Booroola Merino (originated in Australia and New Zealand) to Afshari (originated in Iran) sheep. It has been demonstrated that Fec B gene scheme leads to increase the fecundity of the Afshari sheep (Qanbari et al., 2007). But, it is not clear whether such cross-continental cross breeding program could affect semen characteristics of resulting offspring. The working hypothesis of this study was that the different climatic and geographic conditions between the two breeds could affect semen characteristics and freezability of sperm of resulting offspring. Therefore, this study is the first to compare the morphological

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and biochemical characteristics of Afshari and Afshari × Booroola Merino cross bred rams semen before and after cryopreservation. Moreover, possible effects of such cross-continental cross breeding on the morphological and biochemical characteristic of offspring rams semen were also studied.

2. Materials and methods

2.1. Location and animals

This experiment was conducted during the breeding season (September to October 2015) at the experimental farm of the University of Zanjan, Zanjan (latitude: 48°31′21″N; longitude 36° 40′13″E; altitude: 1663 m), Iran. Ten healthy, mature (5–6 years old), fertile rams of Iranian Afshari and Afshari × Booroola Merino cross bred rams (n = 5/breed) with a live weight of 101 ± 3.08 kg, were randomly selected from the breeding flock. Rams were fed a mixture of straw–hay supplemented with barley (NRC, 2001) throughout the experiment. Mineral blocks and water were offered to rams *ad libitum*.

2.2. Semen collection and fresh semen analysis

Semen samples were collected using artificial vagina, once per week for 8 weeks. Semen samples were maintained at 37 °C and immediately transferred to the laboratory for evaluation. The volume (mL), pH, sperm concentration (×10^9 sperm/mL), percentage of viable sperm, abnormal sperm, and membrane integrity, as well as percentage of total and progressive motility, were evaluated in all ejaculates. Semen volume was recorded using calibrated semen collection tube, and sperm concentration was recorded by Neubaur hemocytometer as well as pH was measured using a digital pH meter (TPS pH Cube®). Computer-assisted-semen-analysis (CASA) system was used to determine the sperm total and progressive motility. Percentage of viable and abnormal sperm was evaluated using eosin–nigrosin staining (evaluating 200 sperm in each sample using a light microscopy with × 400) (Evans and Maxwell, 1987). Any colorizations of spermatozoa cells were judged as signs of dead sperms and only sperms without any colorization were assessed as viable cells. Membrane integrity of the sperm was measured using hypo-osmotic swelling test (HOST). For this purpose, 5 μL of semen with 500 mL of a 100 mOsm hypo-osmotic solution (fructose and sodium citrate dihydrate) was incubated at 37 °C for 30 min. One drop of the incubated sample was placed on a pre-warmed slide, covered with a cover slip, and evaluated using phase-contrast microscopy with × 400. Five different microscopic fields (total of 200 spermatozoa) were assessed to record the percentages of intact sperm (Jeyendran et al., 1992).

2.3. Semen freezing and thawing

All ejaculates were diluted 1/2 (semen/extender) with a Tris-based cryoprotective extender (osmolality = 320 mOsm/L and pH = 7.2). The extended semen were slowly cooled to 4 °C for 2 h and subsequently loaded into 0.25 mL plastic straws (Matsuoka et al., 2006b). Afterward, semen straws were held at 5 cm above the level of liquid nitrogen in liquid nitrogen vapor for 15 min and subsequently, straws were plunged into liquid nitrogen. One week after freezing, three straws of each ram were thawed in a water bath (37 °C) for 30 s and evaluated for total motility, viability, functional integrity and morphology as well.

2.4. Seminal fluid and plasma testosterone assay

Semen and blood samples were collected at beginning (week 1) and the end (week 8) of the experiment. Semen samples were centrifuged (8000*g* for 30 min) and seminal fluid was harvested. The concentration of lactate dehydrogenase (LDH) and calcium in the seminal fluid was determined using a commercial kit (Pars-Azmoon Co., Tehran, Iran). In addition, the concentration of sodium and potassium was determined with Flame photometer (Jenway®, UK). The blood samples were centrifuged (1000*g* for 10 min) and plasma was recovered and stored at −20 °C, until plasma testosterone assay using a commercial RIA kit (Radim Co., Italy). The minimum detectable plasma testosterone concentration for the assay was 0.58 ng/mL. The intra- and inter-assay coefficients of variations were 8.26 and 11.76%, respectively.

2.5. Statistical analyses

Data were expressed as mean ± standard error and differences were considered statistically significant at $p < 0.05$. The Proc Mixed of the SAS was used for analysis of the repeated measures data (SAS, 2002).

3. Results

3.1. Fresh semen parameters

Fresh semen characteristics of Afshari and Afshari × Booroola Merino cross bred rams are shown in Table 1. There were no significant differences in semen volume and pH as well as the percentage of sperm total motility, viability, membrane integrity and morphology between breeds. However, the concentration of sperm and percentage of progressive motile sperm of Afshari rams ($3.98 \times 10^9 \pm 0.22$ sperm/mL and 64.84 ± 1.64%, respectively) was significantly higher than those of Afshari × Booroola Merino cross bred ($1.83 \times 10^9 \pm 0.26$ sperm/mL and 52.92 ± 1.94%, respectively) rams ($P < 0.01$).

3.2. Biochemical characteristics of seminal plasma and plasma testosterone concentrations

Biochemical constituents of seminal plasma in experimental rams are shown in Table 1. The results showed that there were no significant differences in the level of Na^+, K^+, Ca^{++} and LDH of seminal plasma between Afshari and Afshari × Booroola Merino cross bred rams. Moreover, there were no significant differences in the plasma testosterone concentrations of Afshari and Afshari × Booroola Merino cross bred rams.

3.3. Frozen – thawed sperm characteristics

The data of post-thaw sperm parameters are shown in Table 2. There were no significant differences in the percentage of sperm viability, membrane integrity and morphology after thawing. However, sperm total motility in Afshari (44.15 ± 4.15%) was significantly higher than that of Afshari × Boorola Merino cross bred (18.07 ± 4.80%) rams ($P < 0.01$).

4. Discussion

4.1. Fresh semen

In the present study, the sperm concentration of Afshari was higher than that of Afshari × Booroola Merino rams and was lower than those reported for Ghezel × Merino and Moghani × Merino rams (Asadpour, 2012). It has already been reported that Fec B gene does not affect the sperm concentration in rams (Kumar et al., 2007, 2012). Semen productions in rams are influenced by several factors such as breed, age, environment (photoperiod

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