Effect of Freezing on Spermatozoa from Tigaie Rams Belonging to the Mountain Ecotype

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Abstract
Our aim was to study the influence of freezing on the viability and frequency of abnormalities in frozen ram spermatozoa. Sperm was collected form 20 rams belonging to the mountain ecotype of the Tigaie breed using the artificial vagina technique and volume and motility were assessed. Afterward it was diluted with Tryladil (1:4) supplemented with 20% egg yolk and heated at 37°C. Subsequently the temperature decreased at a rate of 0.2°C/minute until reaching 4°C and an equilibration time of 2 hours followed. During this time the diluted sperm was packaged in 0.25 ml straws. After sealing these were kept 6 cm above liquid nitrogen level for 13 minutes (-120°C) and then plunged into nitrogen. Volume, motility and concentration were assessed before freezing. After thawing sperm morphology was assessed using Hancock's method and at the same time the endurance (at 10, 30 and 60 minutes) and HOST tests were performed. The highest motility (0.40) was graded at 30 minutes. It could be correlated with the increased percentage of HOST positive spermatozoa, 27.78%. The percentage of abnormal spermatozoa was also high (47.89%), 38.44% of them having acrosome flaws. Cryopreservation has a negative effect on the characteristics of sperm cells from Tigaie rams belonging to the mountain ecotype.

Keywords: freezing, mountain ecotype, ram semen, Tsigaie

1. Introduction

The protection of endangered breeds of domestic animals has become the goal of many conservation programs, which target both the in situ and ex situ possibility. The latter aims to bring about the creation of banks for the conservation of domestic animal genetic resources. These are essential to maintaining diversity and sustainable agriculture.
In order to achieve this goal endangered breeds must be characterized. [1]Previous research has studied the effects of freezing in the cryopreservation of Mangalita and Bazna semen [1]. Similar experiments have been performed by other authors [2]. In another paper we characterized Tigaie ewes from a reproductive point of view [3] while the present study aims to assess crucial sperm characteristics such as motility and abnormalities before and after freezing and thawing in rams belonging to the mountain ecotype of the Tigaie breed.

2. Materials and methods

Semen was collected form 16 rams belonging to the mountain ecotype of the Tigaie breed using the artificial vagina technique. Collected semen was placed in a warm water bath (35°C). Volume, concentration, motility and abnormal morphology of the head, midpiece and tail were assessed after collection.

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After initial evaluation, ejaculates with a motility superior to 0.7 were diluted with the extender at a concentration of 1:4. The extender was Tryladil supplemented with 20% egg yolk and heated at 35°C. After dilution semen the temperature decreased at a rate of 0.2°C/minute until reaching 4°C. The samples were equilibrated for 2 hours, during which the diluted semen was packaged in 0.25 ml straws, sealed with polyvinyl alcohol. These were kept 6 cm above liquid nitrogen (-120°C) level for 13 minutes and then plunged into the nitrogen (-196,8^{o}C).

After thawing the endurance (at 10, 30 and 60 minutes) and hypoosmotic swelling tests were performed. Spermatozoa from every sample were also coloured using the Hancock technique and examined using a phase contrast Karl-Zeiss microscope. It allows the assessment of abnormalities of the head, midpiece, tail and acrosome. Motility was graded form 0 to 5, while the number of abnormalities and HOST positive spermatozoa were expressed as percentages.

3. Results and discussion

The values of fresh semen characteristics were between the accepted limits for Tigaie rams. The average volume was 0.84 ml, while motility was graded at 0.91 (table 1).

When compared to the hill ecotype it became apparent that ejaculate volume in rams belonging to the mountain ecotype was lower. The concentration however, was higher.

It varied between 1.061 and 2.918 billion/ml, with an average of 1.98 billion/ml.

Table 1. Characteristics of fresh semen from Tigaie rams belonging to the mountain ecotype

Characteristics	Units	Țigaie, mountain ecotype Statistics			
		N	$\bar{X}\pm s\bar{X}$	s	V%
Volume	ml	16	0.84±0.06	0,241	28.53
Motility	grades	16	0.91±0.01	0,040	4.38
Concentration	10^9sp/ml	16	1.98±0.12	0,493	24.79
No.sp./ejac.	10^9sp/ejac	16	1.59±0.05	0,213	13.38
Abnormalities					
TOTAL	%	16	12.25±0.63	2,542	20.75
Head	%	16	4.00±0.25	1,032	25.81
Midpiece	%	16	3.62±0.32	1,310	36.14
Tail	%	16	4.37±0.20	0,806	18.42

The lowest values were associated with a decrease in ejaculate volume.

The most frequent abnormalities were of the head (4.00%), followed by the midpiece (3.62%) and tail (4.37%), making up a final percentage of 12.25%.

Following the initial evaluation semen was frozen and thawed. Sperm morphology was assessed using Hancock's method and at the same time the endurance and HOST tests were performed.

Because for certain ejaculates motility was below 0.3, semen collected from only 9 rams was analyzed after thawing.

Sperm motility was graded above 0.40 irrespective of the time when the analysis was performed: 10, 30 or 60 minutes (table 2). It could also be seen that motility increased from 0.41 at 10 minute to 0.49 at 30 minutes and then decreased after another 30 minutes.

Table 2. Characteristics of frozen semen from Tigaie rams belonging to the mountain ecotype

Characteristics	Units	Tigaie, mountain ecotype Statistics			
		N	$\bar{X}\pm s\bar{X}$	s	V%
Motility					
10 min	grades	9	0,41±0,038	0,114	27,72
30 min	grades	9	0,49±0,043	0,129	26,18
1 h	grades	9	0,40±0,045	0,136	33,78
HOST test	%	9	27,78±4,16	12,47	44,88
Abnormalities					
TOTAL	%	9	47,89±3,17	9,51	19,85
Acrosome	%	9	38,44±3,10	9,30	24,20

The percentage of HOST positive spermatozoa is 27.78%, this stresses the damage caused by freezing and thawing to the sperm membrane and the mitochondrial apparatus.

The total percentage of abnormal spermatozoa was not higher than 47.89%. The percentage of acrosome defects was as high as 34.20%, the effect of cryocapacitation, an event that occurs frequently in the ram [4].

4. Conclusions

The analysis of fresh semen indicated that values for the main semen characteristics are between the accepted values for this breed.

A low ejaculate volume was usually accompanied by reduced sperm motility.
If results are compared with those previously available for the hill ecotype, ejaculate volume was lower for the mountain ecotype but sperm motility was higher.
Low percentages of abnormal spermatozoa in freshly collected semen indicate that rams were well kept and collection frequency was optimal.
Following thawing sperm motility is drastically reduced. This decrease depends more on individual genetic variation than ecotype.
The endurance test affords a better idea of spermatozoa resistance than motility assessment performed immediately after thawing.
Low percentages of HOST positive spermatozoa are proof that cell membrane function has been damaged during freezing and thawing.
High percentages of abnormal spermatozoa and particularly acrosome defects indicate that sperm assessment based only on motility does not provide an accurate description of its quality.
Where cryopreservation is concerned there are no important differences between the hill and mountain ecotypes of the Tigaie breed.

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