

# Freezing of Spermatozoa from Tigaie Rams Belonging to The Hill Ecotype

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## Abstract

Our purpose was to study the influence of freezing on the viability and frequency of abnormalities in frozen ram spermatozoa. Sperm was collected from 20 rams belonging to the hill 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 straws. After sealing these were kept 6 cm above liquid nitrogen level for 13 minutes (-120°C) and then plunged into nitrogen (-196,8°C). Volume, motility and concentration were assessed before freezing. After thawing the Hancock, endurance (at 10, 30 and 60 minutes) and hypoosmotic tests were performed. Abnormalities of the head, midpiece and tail were also investigated using the Hancock method. After thawing motility sperm decreased to 0.32-0.42, while only 25% of the spermatozoa were HOST positive. The percentage of abnormal spermatozoa was as high as 50%, the acrosome being flawed in most cases (34.20%). Cryopreservation has a negative effect on sperm cells from Tigaie rams belonging to the hill ecotype.

**Keywords:** freezing, hill ecotype, ram semen, Tsigaie

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## 1. Introduction

Animal breeds native to Romania and other countries are the result of selection and adaptation. Although they are essential to sustainable animal husbandry and food safety, they are currently at risk of disappearance.

Their protection 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.<sup>1</sup>

In order to achieve this goal endangered breeds must be characterized. 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 hill ecotype of the Tigaie breed.

## 2. Materials and methods

Semen was collected from 20 rams belonging to the hill 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

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of the head, midpiece and tail were assessed after collection.

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 Tryladiol 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°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 from 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 of 0.98 ml, while motility was graded at 0.90 (table1).

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

Semen characteristics	Units	Tigaie, hill ecotype			
		Statistics			
		N	$\bar{X} \pm s\bar{X}$	s	V%
Volume	ml	20	0.98±0.03	0.176	18.01
Motility	grades	20	0.90±0.01	0.075	8.38
Concentration	10 <sup>9</sup> sp/ml	20	1.47±0.10	0.482	32.81
No.sp./ejac.	10 <sup>9</sup> sp/ejac	20	1.38±0.08	0.361	25.97
Abnormalities					
TOTAL	%	20	13.65±0.50	2.277	16.68
Head	%	20	5.20±0.33	1.507	28.99
Midpiece	%	20	4.35±0.36	1.631	37.49
Tail	%	20	4.10±0.31	1.410	34.40

Sperm concentration had an average value of 1.47 billion/ml. Together with individual traits, the difference between ages in the rams has a major influence on concentration variability.

The percentage of abnormal spermatozoa is not unusual, 13.65%. Most abnormalities pertain to the head followed by the midpiece and tail.

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 10 rams was processed after thawing.

As seen in table 2, sperm motility ranges from 0.42 when measured at 10 and 30 minutes to 0.32 at 60 minutes.

The decrease from 0.90 in freshly collected semen to 0.42 is to be blamed on the freezing process and the damage it causes to the sperm membrane and the mitochondrial apparatus.

Further evidence to sustain this hypothesis was provided by the hypoosmotic test. It revealed that approximately 25% of the spermatozoa had an undamaged plasmatic membrane.

Half of the analyzed spermatozoa have an abnormal morphology. The percentage of acrosome defects was as high as 34.20%, the majority being represented by acrosomes that were either detached or in detachment. These are the effects of cryocapacitation [4], an event that occurs frequently in the ram. In other instances the acrosome was swollen (1-5 %) or deformed (1-6%). This explains why the number of lambs from cryopreserved semen has been low irrespective of the insemination technique used (laparoscopy or intracervical insemination) [5].

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

Semen characteristics	Units	Tigaie, hill ecotype			
		Statistics			
		N	$\bar{X} \pm s\bar{X}$	s	V%
Motility					
10 min	grades	10	0.42±0.033	0.105	25.03
30 min	grades	10	0.42±0.033	0.104	24.82
1 h	grades	10	0.32±0.026	0.081	25.70
HOST test	%	10	25.00±4.64	14.67	58.70
Abnormalities					
TOTAL	%	10	50.10±3.35	10.59	21.13
Acrosome	%	10	34.20±2.59	8.19	23.95

#### 4. Conclusions

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

Low percentages of abnormalities in freshly collected semen indicate that rams were well kept and collection frequency was optimal.

After having assessed the quality of fresh and cryopreserved semen and comparing the results with those that are available for rams belonging to the mountain ecotype it becomes apparent that following thawing spermatozoa motility is drastically reduced. This decrease is less dependent on the ecotype and more on individual genetic variation.

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 is insufficient and 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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