NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 52, 2019 (4): 178–180

ISSN 1337-9984
E-ISSN 1338-0095

SPERM MOTILITY OF RAMS FROM TWO SLOVAK SHEEP BREEDS: SHORT COMMUNICATION

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ABSTRACT

In the present study, fresh ram sperm samples (n = 52) from the Native Wallachian (NW; n = 26) and Improved Wallachian (IW; n = 26) Slovak sheep breeds were collected from males of each breed by electro-ejaculation and analysed for motility using CASA and morphology using light microscopy assessment. Our results showed no significant differences between the breeds (P ≤ 0.05) in the sperm concentration and motility traits (Table 1). Also both these ram groups did not differ (P ≤ 0.05) in sperm morphology parameters (Table 2). Since, this is only preliminary research, additional experiments using higher number of samples (rams) as well as other evaluation approaches are required in order to compare sperm characteristics for different breeds.

Key words: Slovak rams; sperm; quality; motility; morphology

INTRODUCTION

The success of artificial insemination (AI) depends on factors related to male and female fertility, the oestrous synchronization and insemination practices (David *et al.*, 2008).

Fertility is a very complex biological function that depends on several properties of the spermatozoa, including sperm motility and morphology, which could be some of the indicators of the spermatozoa quality. CASA (computer-assisted sperm analysis) technology has been used for more objective and reproducible evaluation of sperm motility in different mammalian species (Ax *et al.*, 2000; Kubovičová *et al.*, 2011).

Native Wallachian sheep is a typical seasonally poly-oestrous breed, which were brought to the territory of Slovakia in the 13th and 14th centuries. This breed is registered as a national genetic resource since 1992. At present, 2 554 animals of this breed are kept in Slovakia.

Improved Wallachian Sheep was generated by the intentional combined crossing of NW sheep with rams of various imported semi-coarse-wool and semi-fine-wool breeds (Hamsphire, Cheviot, Texel, Lincoln and Leicester). This breed is perspective mainly for mountainous areas; in 1982 it was recognized as a new semi-coarse-wool breed. At present, 128 930 animals of this sheep breed are kept in Slovakia (Chrenek *et al.*, 2019).

Therefore, in order to gain biological material from valuable Slovak sheep breeds it is necessary to optimize appropriate methods of collection and evaluation of ram sperm for the purpose of their cryopreservation and storage in the animal gene bank. The aim of our preliminary experiments was to compare several sperm characteristics (motility, progressive motility, sperm concentration and morphology)

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Received: November 13, 2019
Accepted: November 20, 2019

of rams from two different sheep breeds – Native Wallachian (NW) and Improved Wallachian (IW), determined by CASA assay.

MATERIAL AND METHODS

Semen collection and evaluation

Clinically healthy rams of NW and IW sheep breeds aged from 1 to 7 years were used in this experiment. All rams were maintained in the flock and fed oats; water was supplied *ad libitum*. The semen samples (n = 52) were collected from four rams by electroejaculation. After collection, the semen was transported to the laboratory in a thermos flask with pre-warmed water at 37 °C.

Sperm motility and concentration

Semen was diluted in a saline (0,9 % NaCl; Braun, Germany) at a ratio of 1:40, immediately placed (2 µl) into a Leja Standard Count Analysis Chamber (depth of 20 microns; MiniTübe, Tiefenbach, Germany) and evaluated under a Zeiss AxioScope A1 microscope using the CASA software (Sperm VisionTM, MiniTübe, Tiefenbach, Germany). For each sample, six microscopic view fields were analysed for the linearity, straightness and cross wobble (LIN, STR, WOB), sperm concentration (CON; 1 x 10^9), and percentage of total motility (TM; > 5 µm.s^{-1}) and progressively moving spermatozoa (PM; > 20 µm.s^{-1}), as previously described by Kulíková *et al.* (2018).

Sperm morphology

A drop (5µl) of the ejaculate diluted with distilled water at the ratio 1:40 was placed onto the slide, covered with a coverslip and observed under a microscope with a 100x magnification objective under an immersion oil. We evaluated the morphology malformations of the sperm, such as separated tail, knob-twisted tail, torso tail, rounded tail, broken tail, retention of the cytoplasm drop, enlarged or reduced sperm head and other acrosomal sperm changes. For the determination of occurrence of morphological changes in sperm totally 400 sperm cells were examined.

Statistical analysis

The results were statistically processed by a t-test using a SigmaPlot software (Systat Software Inc., Germany). Data were expressed as the mean ± standard error of the mean (SEM). Values at $P \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

In our study 52 sperm samples from rams of NW and IW sheep breeds were analysed. There were no significant differences in the basic motility parameters such as CON, TM, PM, STR, LIN and WOB of fresh sperm samples between the tested rams (Table 1).

Common *in vitro* evaluation of sperm quality involves the subjective assessment of motility and the percentage of sperm with normal morphology (O'Hara *et al.*, 2010). The main changes that occur during semen storage include the reduction in motility, alterations in morphology and others which may result in declined fertility. However, most of these results are ambiguous.

Our results showed no difference in evaluated individual morphologic features such as separated tail, knob-twisted tail, rounded tail, etc. (Table 2).

Table 1. Concentration and motility parameters of ram sperm from two sheep breeds

RAM	CON (x10^9)	TM (%)	PM (%)	STR (%)	LIN (%)	WOB (%)
NW	1.095 ± 0.22	41.564 ± 5.4	37.642 ± 5.39	0.851 ± 0.04	0.524 ± 0.03	0.586 ± 0.03
IW	0.996 ± 0.2.	41.372 ± 5.72	36.564 ± 5.74	0.840 ± 0.04	0.503 ± 0.03	0.569 ± 0.03

The results are expressed as average ± SEM; $P \leq 0.05$.
NW – Native Wallachian sheep; IW – Improved Wallachian sheep; CON – concentration; TM – total motility; PM – progressive motility, STR – straightness; LIN – linearity; WOB – wobble.

Table 2. Occurrence of morphology malformations in ram sperm from two sheep breeds

RAM	TOTAL (%)	ST (%)	KT (%)	TT (%)	RT (%)
NW	5.288 ± 0.28	1.019 ± 0.09	0.827 ± 0.09	1.087 ± 0.12	0.702 ± 0.06
IW	5.067 ± 0.2	1.115 ± 0.08	0.760 ± 0.04	0.04 ± 0.07	0.731 ± 0.05

RAM	BT (%)	RCD (%)	SH (%)	LH (%)	ACH (%)
NW	0.983 ± 0.07	0.269 ± 0.04	0.115 ± 0.04	0.125 ± 0.04	0.154 ± 0.04
IW	0.875 ± 0.08	0.317 ± 0.06	0.067 ± 0.02	0.125 ± 0.03	0.144 ± 0.05

The results are expressed as average value ± SEM.
ST – separated tail; KT – knob-twisted tail; TT – torso tail; RT – rounded tail; BT – broken tail; RCD – retention of the cytoplasm drop; SH – small head; LH – large head; NW – Native Wallachian sheep; IW – Improved Wallachian sheep; ACH – acrosomal changes.

Kulaksiz *et al.* (2011) reported the increased percentage of abnormal spermatozoa with the increase in number of days in storage for all of the extenders tested in their study. Sperm morphology is extremely variable between even close species (Maroto-Morales *et al.*, 2010), however our results showed opposite trend, which could be due to fewer tested rams.

In conclusion, the results of this study are of preliminary character. Therefore, experiments involving higher number of samples (individuals), as well as other analytical approaches are needed in order to find the best assessment tool for evaluation of ram sperm to be stored in the national gene bank.

ACKNOWLEDGEMENTS

The study was financially supported by the Slovak Research and Development Agency (no. APVV-17-0124), VEGA 1/0049/19, and KEGA 026 SPU-4/2018.

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