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SEMEN QUALITY ASSESSMENT OF IMPROVED WALLACHIAN SHEEP BREED: A PRELIMINARY STUDY

Andrea SVORADOVÁ[1], Marián MACHÁČ[2], Andrej BALÁŽI[1], Jaromír VAŠÍČEK[1,2], Rastislav JURČÍK[1], Ján HUBA[1], Peter CHRENEK[1,2]*

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Nitra, Slovak Republic

ABSTRACT

Viability and sperm motion parameters are the most common indicators of sperm quality. Semen samples (n = 15) of Improved Wallachian (IW) sheep males with no evidence of genital tract infections were used to determine the quality of semen. We applied computer-assisted semen analysis (CASA) system to determine sperm motility parameters. Flow cytometry (FCM) was used to evaluate occurrence of live or apoptotic sperm. Our results showed quite good motility parameters: 81.12 ± 2.72 % of total motility (TM) and 77.91 ± 3.15 % of progressive motility (PM). Moreover, the ratio of viable sperm was 70.77 ± 4.81 % and of apoptotic sperm – 9.37 ± 3.37 %. These results suggest that semen can be collected from IW sheep and used for artificial insemination or cryopreservation for gene bank of animal genetic resources. However, evaluation of higher sample numbers using different methods are needed to analyse the semen quality in details.

Key words: Improved Wallachian sheep; ram sperm; flow cytometry; CASA

INTRODUCTION

The preservation of biological material in a stable state is a fundamental requirement in biological science, agriculture and biotechnology (Pegg, 2015). Quality evaluation of genetic resources of local species is necessary. Genetic material can be used for recovering the lost variation within breeds and restoring breeds, which will become endangered due to destruction of their natural habitats. Moreover, artificial insemination (AI) and also cryopreservation require good quality of insemination doses and samples (David *et al*., 2008).

Improved Wallachian (IW) sheep breed was generated by the crossbreeding of Native Wallachian sheep with rams of various imported semi-coarse-wool and semi-fine-wool breeds (Hamsphire, Cheviot, Texel, Lincoln and Leicester). In 1982, IW sheep was recognized as a new semi-coarse-wool breed suitable mainly for mountainous areas. Nowadays, 128 930 animals of this sheep breed are kept in Slovakia (Chrenek *et al*., 2019). Therefore, it is necessary to optimize appropriate methods of collection and evaluation of ram sperm for the purpose of their storage in the animal gene bank.

Semen assessment, essential for the study of ram quality, generally includes the evaluation of sperm motility and viability. There are various techniques to assess pre-storage semen quality. Standard technique, such as the Computer-assisted sperm analysis (CASA), has been used for determination of sperm motility parameters (Luna-Orozco *et al*., 2019). Flow cytometry (FCM) using fluorescent staining techniques has been

***Correspondence:** E-mail: peter.chrenek@nppc.sk
Peter Chrenek, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

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extensively used for the analysis of cell suspensions containing live and dead sperm because of its higher sensitivity (Bucak *et al*., 2019). FCM allows characterisation of sperm parameters, such as cell size (forward scatter, FSC-H), cell complexity (side scatter, SSC-H) and fluorescence.

Apoptosis, a programmed cell death, is a natural way to remove damaged and old cells to maintain cell and tissue homeostasis (Aitken and Baker, 2013). To distinguish among the apoptotic and live cells, a membrane-permeant nuclear stain that brightly stains the nuclei of living cells, SYBR-14, can be used (Garner and Johnson *et al*., 1995). On the other hand, there are many other fluorescent dyes, such as Yo-Pro-1, 7-AAD, Annexin V for detection of apoptotic and dead cells (Farah *et al*., 2013). This study was focused on the quality assessment (motility and viability) of IW sheep sperm.

MATERIAL AND METHODS

Semen collection

Clinically healthy rams (n = 2) of Improved Wallachian sheep breed aged 1-7 years were used in this experiment. The rams were housed under external conditions in individual stalls, fed with hay bale and oats; water and mineral salt were supplied *ad libitum.* The semen samples were collected twice a week by electro-ejaculation, as previously described by Kulíková *et al*. (2018).

CASA

Semen samples (n = 15) were diluted in a saline (0.9 % NaCl; Braun, Melsungen, Germany) at a ratio of 1:40 (v/v), placed (2.5 µl) into a pre-warmed Leja Standard Count Analysis Chamber (depth of 20 microns; MiniTüb, Tiefenbach, Germany) and evaluated under a Zeiss AxioScope A1 microscope using the CASA system (Sperm Vision™, MiniTüb, Tiefenbach, Germany). For each sample, six microscopic view fields were analysed and average concentration (10^9 per ml), percentage of total motile spermatozoa (motility > 5 µm.s^{-1}) and percentage of progressive motile spermatozoa ((motility > 20 µm.s^{-1}), VCL (velocity curved line, lm.s^{-1}), VSL (velocity straight line, µm.s^{-1}), STR (straightness – VSL:VAP, velocity average path), LIN (linearity – VSL:VCL), BCF (beat cross frequency, Hz)) were analysed.

Flow cytometry

The sperm samples were analysed by a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA). At least 10,000 events (sperm) were analysed in each sample. Aliquots of fresh semen were subdivided into prepared tubes intended for flow cytometric assessment of sperm viability. Briefly, apoptotic cells were stained with Annexin V-FITC Apoptosis Detection Kit (AnV; Canvax, Cordoba, Spain) and live cells were detected using a SYBR-14 specific dye included in the LIVE/DEAD Sperm Viability Kit (Molecular Probes, Eugene, Oregon). For the detection of dead sperm, a DRAQ7 fluorescent dye (Biolegend, Koblenz, Germany) was used. Samples for Annexin V detection were incubated at the room temperature (RT) and samples for SYBR-14 staining were incubated at 37 °C for 15 min. After incubation samples were washed and afterwards analysed.

Statistical analysis

Obtained results were evaluated by an Excel software. Data were expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The purpose of the current experiment was to evaluate the quality of IW sheep breed semen using CASA system and flow cytometry. Sperm motility has been the most common examined parameter of spermatozoa quality (Fonseca *et al*., 2005).

Concentration, motility and kinematic parameters recorded for the IW sheep samples are presented in Table 1.

The percentage of total motile sperm recorded in this study was similar to Boshoff *et al*. (2018) and Špaleková *et al*. (2011), who reported total motile sperm in the range from 84 % to 89 %. However, our results showed higher percentage (78 %) of progressively moving sperm compared to 44 % reported by Boshoff *et al*. (2018). Similarly, sperm motility in our study was within the range of Khalifa *et al*. (2013) and Azizun *et al*. (2014), who reported 60-85 % of motile sperm, and Garner and Hafez (1982), who presented 60 % of sperm motility for the ram as a minimal value.

Table 1. Descriptive statistics of the kinematic parameters of fresh IW sheep sperm

Parameters	IW
C (x 10^9)	
Total motility (%)	1.55 ± 0.63
81.12 ± 2.72	
Progressive (%)	77.91 ± 3.15
VCL (µm.s^{-1})	132.16 ± 5.89
VSL (µm.s^{-1})	78.96 ± 8.55
LIN (VSL/VCL)	0.57 ± 0.03
STR (VSL:VAP)	0.87 ± 0.02
BCF (Hz)	36.55 ± 2.19

Values are presented as means ± SD.

For cytometric determination of the proportion of viable sperm, SYBR-14 staining was applied. The incidence of spermatozoa populations measured using flow cytometry is presented in Figure 1. The overall mean percentage of live sperm was 70.77 ± 4.81 % and the proportion of apoptotic cells was 9.47 ± 3.37 %.

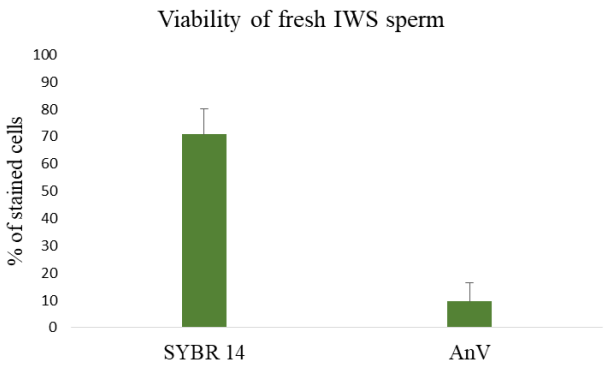

Figure 1. Viability of fresh IW sperm determined by a flow cytometry

The mean value of live sperm percentage observed in the fresh semen was in agreement with the observations of Alsamarrae (2009) and within the range that were published by Fernandez *et al*. (2004), Malama *et al*. (2013) and Kulíková *et al*. (2018). However, Buša *et al*. (2019) recorded lower percentage of motile and progressively moving sperm compared to our study.

Differences among the studies may be attributed to many factors that can affect the semen quality, such as genetic and environmental changes (Abdel-Rahman *et al*., 2000; Rege *et al*., 2000; Gundogan et. al., 2004), nutritional, physical (Colas, 1981; Toe *et al*., 1994) and seasonal (Rege *et al*., 2000) variations. This information is important for the timing of insemination or cryopreservation.

CONCLUSION

We conclude that the quality of IW sheep semen is suitable for subsequent usage for AI or cryopreservation. However, more detailed characteristics, such as acrosome status, production of reactive oxygen species (ROS), mitochondrial membrane potential etc. are needed to monitor in order to assess comprehensively the quality of ram sperm.

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