NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 54, 2021 (4): 157–161
ISSN 1337-9984, E-ISSN 1338-0095

BASIC QUALITY PARAMETERS OF RAM SEMEN FROM SLOVAK NATIONAL SHEEP BREEDS

Elena KUBOVIČOVÁ[1]*, Andrej BALÁŽI[1], Andrea SVORADOVÁ[1], Jakub VOZAF[2], Alexander V. MAKAREVICH[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

Fertility is a very complex biological function that depends on several properties of spermatozoa. Male fertility may be defined as the ability of a sperm cell to fertilize an oocyte and support development to produce viable offspring. When insemination doses from outstanding rams are intended to long-term storage in the gene bank, it is necessary to determine their sperm quality prior to cryopreservation. The aim of our study was to check the quality of fresh semen collected from rams of the three national sheep breeds: Native Wallachian (NW), Improved Wallachian (IW) and Slovak Dairy Sheep (SDS). We evaluated basic parameters of spermatozoa, such as sperm concentration, motility and morphology (sperm abnormalites) using CASA assay. No significant differences in the studied parameters among the breeds were recorded. Our results indicate that rams of three national breeds are characterized by a proper sperm quality for storage in the gene bank. The evaluation of the concentration, motility and morphology of sperm could be used at selection of best insemination doses for cryopreservation.

Key words: Slovak sheep breeds; ram; sperm motility; morphology

INTRODUCTION

Male fertility may be defined as the ability of a sperm cell to fertilize an oocyte and support development to produce viable offspring. There are numerous factors that contribute to the overall fertility of a given male. In rams, such factors may include the breed, season of the year, age of the male, nutritional status of the animal, reproductive management, method and the frequency of semen collection (Talebi *et al.*, 2009), as well as environmental factors, such as a humidity and temperature changes (Kunavongkrit *et al.*, 2005). The variation within these factors determines what makes one male more fertile than another.

Sperm quality refers to a number of parameters, such as sperm volume, presence of abnormal components (i.e., urine or blood) and seminal plasma volume. More specific spermatozoa characteristics, which can further illustrate differences in fertility and performance, include morphology, motility, DNA integrity, acrosome integrity and membrane integrity (Aziz *et al.*, 2007; Zorn *et al.*, 2012). The question then arises which criteria of semen/sperm are potentially useful in insemination programs because are more likely to inform us about the functionality of the sperm and the likelihood of its ability to fertilize the oocyte. Several abnormalities have been associated with immaturity of sperm and reduced fertility (Sánchez-Pozo *et al.*, 2013).

***Correspondence:** E-mail: elena.kubovicova@nppc.sk
Elena Kubovičová, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

Received: November 10, 2021
Accepted: November 25, 2021

In our study we evaluated three national sheep breeds: Native Wallachian (NW), Improved Wallachian (IW) and Slovak Dairy Sheep (SDS) breeds. NW breed is original coarse-wool breed with milk, meat and wool production, adapted to the conditions of mountain farming in Slovakia. IW breed was generated by the intentional combined crossing of NW sheep with the rams of various imported semi-fine-wool and semi-coarse-wool breeds (Texel, Hampshire, Cheviot, Leicester and Lincoln). This breed is characterized by improved wool production and milk production as a secondary trait. SDS breed is a specialized dairy breed focused on increasing milk production and quality. It is a more recent national breed of sheep, which was created by the crossing of the original breeds –Tsigai and Improved Wallachian sheep (with a smaller share of Merino sheep) with specialized dairy breeds – Lacaune and East-Friesian sheep. All mentioned breeds belong to Slovakian animal gene resources (Chrenek *et al.*, 2019) and their biological samples have to be deposited in the gene bank.

When insemination doses from outstanding rams are intended to long-term storage in the gene bank, it is necessary to determine their sperm quality prior to cryopreservation. Therefore, the aim of our study was to examine the basic quality of fresh semen collected from rams of the three national sheep breeds.

MATERIAL AND METHODS

Semen collection

Clinically healthy rams of Native Wallachian (n = 2), Improved Wallachian (n = 2) and Slovak Dairy Sheep (n = 2) breeds aged 3-7 years were used in our experiments. 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 by an electro-ejaculation, as previously described by Kulíková *et al.* (2018) in the period from March to November. The ejaculates were diluted by a Triladyl extender (MiniTube, Tiefenbach, Germany) containing 20 % of egg yolk at a ratio of 1:10.

Sperm motility assay

Sperm motility and concentration were measured by CASA. Semen samples were diluted in a saline (0.9 % NaCl; Braun, Melsungen, Germany) at a ratio of 1:40 (v/v), 2.5 µl of the sample were placed 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 Sperm VisionTM software (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), WOB – wobble –VAP:VCL)) were analysed.

Sperm morphology

A drop (5 µl) 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

Data were expressed in percentage as the mean ± standard error of the mean (SEM) and the differences beetewen groups were analysed by a Chi-square test.

RESULTS AND DISCUSSION

Semen *in vitro* evaluation is an important aspect that must be accurately done to ensure the use of breeding rams with good fertility. Semen volume, sperm concentration, motility, viability, membrane integrity and morphology are the indicators of sperm functionality (Peňa *et al.*, 2005), which allow detection and elimination of cases of male infertility or subfertility (Verstegen *et al.*, 2002). The color indicates damage or infection in reproductive tract.

In our study, ejaculates from rams of our national breeds Native Wallachian, Improved Wallachian and Slovak Dairy Sheep were evaluated using the CASA system. It is known, that CASA measurements are more closely related to fertility than the subjective motility measurements (Joshi *et al.*, 2001). Comparison of the parameters of semen from three national sheep breeds is shown in the Table 1. No significant differences in the evaluated parameters (concentration, total and progressive motility) were observed.

The occurrence of morfological abnormalities in ram sperm is presented in the Table 2. The percentage of morfological abnormalities in all three breeds was shown to be about 5 %. The differences in percentages of the morphological abnormalities observed among rams of all three breed were slight and statistically insignificant.

More often sperm head abnormalities observed in all tree studied ram breeds were: enlarged heads or reduced heads. In the case of the sperm tail (flagellum), the most occurred abnormalities were: separated tail, knob twisted tail and rounded tail. The less occurred abnormalities were: cytoplasmic drop, broken tail and rounded tail.

According to the de Paz *et al.* (2011) the relationship between ram sperm head morphometry and fertility depends on the methodology of evaluation, with the best results obtained when a system based on light microscopy with a digital camera and convention anaylsis is used.

Differences in sperm head have been directly related to conception rates in some species. Spermatozoa with malformed heads (enlarged or reduced sperm head) are less motile, which may lead to reduced fertilization rate (Adeniji *et al.*, 2010).

When using for artificial insemination, up to 15 % morphologically abnormal sperm for fertility have been currently accepted (Fernandez *et al.*, 2004; Holt and Lloyd, 2010; Malama *et al.*, 2013).

It is well-known that damaged spermatozoa, the lost and changes of the acrosome, membrane damaged, higher occurence of the cytoplasmic droplets (CD) and uncapacitated sperm cells cannot fertilize oocytes (Xu *et al.*, 2013). The intact acrosome is crucially important for transit, penetration, acrosome reaction and fertilization (Juyena and Stelleta 2012; Partyka *et al.*, 2012). It was also shown that the cytoplasmic droplets,

Table 1. Motility parameters of ram sperm form three national sheep breeds

Rams	Concentr. (x 10^9)	TM (%)	PM (%)	STR (%)	LIN (%)	WOB (%)
NW	1.687 ± 0.20	77.050 ± 2.90	72.20 ± 2.40	0.851 ± 0.04	0.524 ± 0.03	0.586 ± 0.03
IW	1.55 ± 0.63	81.12 ± 2.72	77.91 ± 3.15	0.840 ± 0.04	0.503 ± 0.03	0.569 ± 0.03
SDS	3.89 ± 1.24	93.59 ± 2.24	89.16 ± 2.23	0.79 ± 0.30	0.44 ± 0.02	0.55 ± 0.00

NW – Native Wallachian; IW – Improved Wallachian; SDS – Slovak Dairy Sheep; TM – total motility; PM – progressive motility; STR – straightnes; LIN – linearity; WOB – wobble – departure of actual sperm track from average path.

Table 2. Morphological abnormalities in the ram sperm from three national sheep breeds

Breed	Overall damages (%)	Damage of the sperm flagellum (%)	Cytoplasmic droplets – CD (%)	Damage of the sperm head (%)
NW	5.288 ± 0.28	4.623 ± 0.09	0.269 ± 0.04	0.240 ± 0.08
IW	5.067 ± 0.20	3.521 ± 0.08	0.317 ± 0.06	0.192 ± 0.02
SDS	4.873 ± 0.19	3.953 ± 0.07	0.468 ± 0.08	0.452 ± 0.06

NW – Native Wallachian; IW – Improved Wallachian; SDS – Slovak Dairy Sheep

which represent excess residual cytoplasm, can impair overall sperm function and produce higher levels of reactive oxygen species (Aziz *et al.*, 2004), potentially leading to male infertility (Rengan *et al.*, 2012).

Sheep are seasonal animals so the impact of the season must be also taken into account. The quantity and quality attributes of seminal characteristics in rams can be differed in breeding and non-breeding seasons. In our experiments we performed ram ejaculate collections since spring to autumn months of the year. There were not differences in the quality of the ejaculates depending on the month or season of the year (data not shown).

Semen volume, sperm concentration, percentage of live sperm, abnormal sperms and sperm motility varied within breeds with season change. In particular, seasonal variations in ram semen characteristics were observed by Zaher *et al.* (2020) in Assaff and Aewasi ram breeds, by Azawi and Ismaeel (2012) in Awassi breed and by Moghaddam *et al.* (2012) in Iranian cross-bred rams. Nevertheless, these authors concluded that the semen has the capability and quality to be used for AI in breeding programmes throughout the year. Moreover, other authors also showed that, despite seasonal variations, the semen of Zulu rams (Ngcobo *et al.*, 2020) or Assaf and Merino rams (Fernandez *et al.*, 2004; 2021) was acceptable for AI in both breeding and non-breeding season.

CONCLUSION

These results indicate that rams of three national breeds are characterized by a proper sperm quality for storage in the gene bank as no differences in the analysed parameters among the evaluated breeds were recorded. The basic sperm examination for the concentration, motility and morphology could be used for selection of best insemination doses for cryopreservation.

ACKNOWLEDGEMENTS

The study was financiaslly supported by the Slovak Research and Development Agency under the contract no. APVV-20-0006.

REFERENCES

Adeniji, D. A., Oyeyemi, M. O. & Olughemi, J. R. (2010). Sperm morphological characteristic and mating behaviour of Proviron treated West African Dwarf bucks with testicular degeneration. *International Journal of Morphology*, 28(2), 623−636. https://scielo.conicyt.cl/pdf/ijmorphol/ v28n2/art46.pdf.

Azawi, O.I. & Ismaeel, M.A. (2012). Effects of seasons on some semen parameters and bacterial contamination of Awassi ram semen. *Reproduction in Domestic Animals*, 47(3), 403−406. https://doi:10.1111/j.1439-0531.2011.01888.x

Aziz, N., Saleh, R. A., Sharma, R. K., Lewis-Jones, I., Esfandiari, N., Thomas, A. J., Jr. & Agarwal, A. (2004). Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. *Fertility and Sterility*, 81, 349−354. https:// doi: 10.1016/j.fertnstert.2003.06.026

Aziz, N., Said, T., Paasch, U. & Agarwal, A. (2007). The relationship between human sperm apoptosis, morphology and the sperm deformity index. *Human Reproduction*, 22, 1413−1419. https://doi: 10.1093/humrep/dem016

Chrenek, P., Makarevič, A. V., Kubovičová, E., Bulla, J. & Supuka P. (2019). *Slovak national animal breeds*. SPU in Nitra, 99 p., ISBN 978-80-552-1982-0

Fernandez, M., Giráldez, F. J., Frutos, P., Lavín, P. & Mantecon, A. R. (2004). Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behavior of mature Assaf rams. *Theriogenology*, 62(1-2), 299−310. https://doi.org.10.1016/j.theriogenology.2003.10.003

Fernandes, M., Hernández, P. R., Simões, J. & Barbas, J. P. (2021). Effects of tree semen extenders, breeding season month and Freezing-thawing cycle on spermatozoa preservation of portuguese Merino sheep. *Animals*, 11, 2619. https://doi.org/10.3390/ani110926.

Holt, W. V. & Lloyd, R. E. (2010). Sperm storage in the vertebrate female reproductive tract: How does it work so well? *Theriogenology*, 73, 713−722.

Joshi, A., Bag, S., Naqui, S. M. K., Sharma, R. C., Rawatt, P. M. & Mital, J. P. (2001). Effect of short-term and long-term preservation on motion characteristics of Carole ram spermatozoa: A prolific microsheep breed of India. *Asian Australasian Journal of Animal Sciences*, 14, 1527−1533. https://pdfs.semanticscholar.org/6756/fa2a31fbc91f599b1c8f6f88cca3e34b483a.pdf

Juyena, N. S. & Stelletta, C. (2012). Seminal plasma: an essential attribute to spermatozoa. *Journal of Andrology*, 33(4), 536−551. https://doi: 10.2164/jandrol.110.012583. Epub 2011 Oct 20. PMID: 22016346.

Kulíková, B., Baláži, A., Tóthova, J., Jurčík, R., Huba, J. & Chrenek, P. (2018). Dilution factors affects the ability of ram sperm to survive cryopreservation: short communication. *Slovak Journal of Animal Sciences*, 51(1), 41−44. http://www.cvzv.sk/slju/18_1/6_kulikova.pdf

Kunavongkrit, A., Suriyasomboon, A., Lundeheim, N., Heard, T. W. & Einarsson, S. (2005). Management and sperm production of boars under differing environmental conditions. *Theriogenology*, 15, 63(2), 657−667. https://doi: 10.1016/j.theriogenology.2004. 09.039. PMID: 15626423.

Malama, E., Bollwein, H., Taitzoglou, I. A., Theodosiou, T., Boscos, C. M. & Kiossis, E. (2013). Chromatin integrity of ram spermatozoa. Relationships to annual fluctuations of scrotal surface temperature and temperature-humidity index. *Theriogenolog*y, 80(5), 533−541. https://doi: 10.1016/ j.theriogenology.2013.05.019

Moghadam, G. H., Pourseif, S. & Rafat, S. A. (2012). Seasonal variation in semen quantity and quality traits of Iranian ceossbred rams. *Slovak Journal of Animal Sciences*, 45 (3), 67−75.

Ngcobo, J. N., Nephawe, K. A., Maquehashu, A. & Nedambale, T. L. (2020). Seasonal variations in semen parameters of Zulu rams preserved at 10 °C for 72 h during breeding and non-breeding season. *American Journal of Animal and Veterinary Sciences*, 15(3), 226−239. https://doi: 10.3844/ ajavsp.2020.226.239

Partyka, A., Łukaszewicz, E. & Niżański, W. (2012). Effect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. *Theriogenology*, 77(8), 1497−1504. https://doi: 10.1016/j.theriogenology.2011.11.006. Epub 2012 Jan 5. PMID: 22225691.

De Paz, P., Mata-Campuzano, M., Tizado, E. J., Alvarez, M., Alvarez-Rodriguez, M., Herraez, P. & Anel, L. (2011). The relationship between ram sperm head morphometry and fertility depends on the procedures of acquisition and analysis used. *Theriogenology*, 76, 1313−1325.

Peňa, F. J., Saravia, F., Johannisson, A., Walgren, M. & Rodríguez-Martínez, H. (2005). A new and simple method to evaluate early membrane changes in frozen–thawed boar spermatozoa. *International Journal of Andrology*, 28(2), 107−114. https://doi.org/ 10.1111/j.1365-2605.2005. 00512.x

Rengan, A. K., Agarwal, A., van der Linde, M. & du Plessis, S. S. (2012). An investigation of excess residual cytoplasm in human spermatozoa and its distinction from the cytoplasmic droplet. *Reproductive Biology and Endocrinology*, 10, 92. https://doi.org/10.1186/ 1477-7827-10-92

Sánchez-Pozo, M. C., Mendiola, J., Serrano, M., Mozas, J., Björndahl, L., Menkveld, R. S., Lewis, S. E. M., Mortimer, D., Jørgensen, N., Barratt, C. R. L., Fernández, M. F. & Castilla, J. A. (2013). Proposal of guidelines for the appraisal of semen quality studies (SEMQUA). *Human Reproduction*, 28(1), 10−21. DOI: 10.1093/humrep/des355.

Talebi, J., Souria, M., Moghaddam, A., Karimi, I. & Mirmahmoodi, M. (2009). Characteristics and seasonal variation in the semen of Markhoz bucks in western Iran. *Small Ruminant Research*, 85, 18−22. https://doi.org.10.1016/jsmallrumres 2009.06017

Verstegen, J., Iguer-Ouada, M. & Onclin, K. (2002). Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology*, 57(1), 149−179. https://doi: 10.1016/ s0093-691x (01)00664-1

Xu, H., Yuan, S. Q., Zheng, Z. H. & Yan, W. (2013). The cytoplasmic droplet may be indicative of sperm motility and normal spermiogenesis. *Asian Journal of Andrology*, 15(6), 799−805. https://doi.org/10.1038/ aja.2013.69.

Zaher, H. A., Alawaash, S. A. & Swelum, A. A. (2020). Effects of season and breed on the reproductive performance of sheep. *Journal of Animal Reproduction and Biotechnology*, 35(2), 149−154. https://doi.org/ 10.12750/JARB.35.2.149

Zorn, B., Golob, B., Ihan, A., Kopitar, A. & Kolbezen, M. (2012). Apoptotic sperm biomarkers and their correlation with conventional sperm parameters and male fertility potential. *Reproduction Medicine*, 29, 357−364. https://doi: 10.1007/s10815-012-9718-x