Influence of Farming System on the Quality of Frozen Ram Semen Evaluated by the CASA System

Dorina Nadolu[1], Zoia Camelia Zamfir[1*], Andreea Hortanse Anghel[1,2*], Laura Marinică[2], Elena Ilișiu[1], Oana Corina Preșa[1], Ana Enciu[1]

[1] Research and Development Institute for Sheep and Goat Breeding, Palas, Constanța, Reproductive Biotechnologies Laboratory
[2] Ovidius University of Constanta, Faculty of Natural Sciences and Animal Sciences

Abstract
In the current context of the implementation of strategies to reduce greenhouse gas (GHG) emissions in the livestock sector, animal exploitation systems are analysed not only from the point of view of sustainability, but also of productive and reproductive performance. The objective of this study was to evaluate the quality of frozen semen from rams exploited in two different systems: permanent housing and grazing. Semen was collected from four rams (two in housing and two on pasture), and the quality of sperm after thawing was analysed using the CASA system. The results revealed significant differences between the frozen semen from rams maintained in the two exploitation systems, an important role, having environmental factors, nutrition and activity level that influence reproductive function. Statistical analysis of semen parameters revealed that rams exploited in stables presented significantly higher values of normal morphology compared to those raised on pastures ($p=0.0015$). Frozen sperm from rams exploited on pastures recorded significantly higher values of motility ($p=0.0046$) with an average of 10 ejaculates of 39.35% compared to 30.56% in the case of rams exploited in stables and increased nutrition, grazing system, characterized by a level of sperm motor variation, can favour the occurrence of certain morphological anomalies.

Keywords: motility, progressive motility, sperm abnormalities

1. Introduction

The system of maintenance of small ruminants, namely permanent housing (intensive, controlled system) and extensive maintenance on pasture (direct exposure to the natural environment) influence the reproductive performance of males. The two exploitation systems modulate spermatogenesis and semen quality through microclimate factors, physical activity level, diet and exposure to stress factors. Climate change represents one of the most important current challenges in all social and economic fields, with a particular impact on agriculture and animal husbandry as an important factor in ensuring a healthy and sufficient diet. In the field of animal husbandry, the increase in average temperatures and changes in the frequency of precipitation significantly influence the reproductive function of small ruminants, species with reproduction modulated by the length of day-light and temperature. Climate change can also directly affect the quality of semen in males (rams and goats), with consequences on reproductive efficiency. Studies show that prolonged exposure to high temperatures causes alterations in spermatogenesis, reduced sperm motility and an increase in the percentage of morphological abnormalities [1, 2].

The concept of carbon footprint in animal husbandry is important given that intensive sheep and goat farming systems contribute to

* Corresponding author: Camelia Zoia Zamfir, zamfirzoica@yahoo.com Andreea Hortanse Anghel, ahanghel@yahoo.com

greenhouse gas emissions, which are in turn influenced by environmental conditions.

There is a close link between small ruminant farming systems and carbon footprint, determined by the way feed resources are used and the conditions of maintenance. The permanent housing system involves higher energy consumption in the procurement and management of feed, which leads to increased greenhouse gas emissions at the farm level, but the emission values per production unit are reduced by increasing production efficiency [3].

The exploitation system based on rational grazing has a lower energy consumption, contributing to the reduction of the carbon footprint per surface, also ensuring the retention of carbon in the soil [4] but has the disadvantage of lower productive performances.

Therefore, the exploitation conditions have an influence on the environment but also on the physiological status of the animals, including the reproductive function. The microclimate in the shelter (temperature, humidity, ventilation), the density of the animals, physical activity and the type of food influence spermatogenesis in rams. In the stable, if the microclimate conditions, especially ventilation and temperature, are not correctly ensured, conditions favorable to the occurrence of testicular hyperthermia can be created, with negative effects on the production and quality of spermatozoa. When grazing, animals benefit from a higher level of physical activity but are exposed to climatic variations, including high temperatures in the warm season, which limit reproductive performance in rams and goats, affecting both quantitative and qualitative parameters of semen. Thus, the increase in ambient temperature is associated with a reduction in ejaculate volume, a decrease in total and progressive sperm motility, as well as an increase in the incidence of morphological abnormalities [5,6].

Environmental and management factors play an important role in regulating reproductive function in sheep, influencing both spermatogenesis and the fertilization capacity of sperm [7, 8]. Season and mainly temperature influence the main sperm parameters: ejaculate volume, sperm concentration, motility and morphology. Studies show a decrease in semen quality during periods of high temperatures, namely reduced sperm motility and increased incidence of morphological abnormalities [9, 10]. Temperature directly influences reproductive activity but also indirectly by changing the availability and quality of feed, which can lead to nutritional deficiencies and implicitly to a decrease in sperm quality.

Therefore, reproductive performance in small ruminants is also influenced by the interaction between climatic factors, the type of exploitation and the carbon footprint.

2. Materials and methods

The study was carried out in the ICDCOC Palas farm, at the beginning of the breeding season (June) when the rams were checked for mating compatibility. From the total number of males checked, we selected 4 Merinos de Palas rams, aged 4 years, with a live weight between 83-90 kg, which had natural matings in previous seasons and obtained similar results at the age of 2 and 3 years, respectively a percentage of pregnant ewes of 89.78-92.12% and during the semen checks they had an ejaculate volume of 1.6-2.4 ml, with dense and very dense density and an initial motility of over 85%. Following the processing and freezing of these ejaculates collected in the previous 2 years, the thawed semen had a motility of 33.45-49.81%.

The 4 selected rams were part of two batches exploited under different conditions in the year of the study, respectively 2 were maintained in permanent housing (the usual way of maintaining males on the farm) and the other 2 were exploited traditionally on a cultivated, fenced pasture, provided with a shade, they were part of an experimental batch for carbon footprint monitoring. The animals maintained on the cultivated pasture with a floristic composition of Dactylis (70%) and Clover (30%) with an approximate consumption of 6-8 kg of green mass received an addition of concentrates consisting of barley (600 g) and oats (200 g) administered in the morning, before grazing and 500 g of alfalfa administered in the evening. Rams in the stable received a ration consisting of 2 kg of alfalfa hay, 1.5 kg of concentrates (75% barley and 25% oats), 2 kg of corn silage and oat straw ad libitum. Both groups of rams received 0.5 kg of carrots/day in the ration, one month before preparation for mating, respectively before semen collection for verification and freezing.

Semen collection was done with an artificial vagina, using a ewe detected in estrus as a dummy. During two days, before the collection to check the quality of the sperm and its freezing, the rams were collected to get used to the procedure and to the veterinary technician who performed the sperm collection, but especially to remove the residual seminal material deposit from the ampullae of the vas deferens where, after the sexual rest of 7-9 months, there is a very high concentration of dead, aged spermatozoa, with reduced motility or with numerous anomalies.

5 ejaculates were collected from the 4 rams, in 5 consecutive days and were processed using the same freezing technology, making three successive dilutions, the first two with TRIS diluent with the addition of 20% egg yolk and to the third dilution 7% glycerol was added as a cryoprotectant agent. The diluent used was prepared in the Reproductive Biotechnology Laboratory of the institute. After performing the dilutions and equilibration of the semen, the semen was packed into 0.25 ml straws, identified with the serial number of the rams and the date of processing, and the actual freezing was carried out with the Micro-Digitcool automatic freezer (IMV Technologies, L'Aigle, France) connected to the liquid nitrogen tank. After freezing, the semen straws were placed in the quarantine liquid nitrogen container for 1-3 days, when the qualitative verification of the frozen semen was performed by thawing a straw from each ejaculate. The qualitative analysis was performed with the CASA (Computer Assisted Sperm Analysis) system (CEROS II ZEISS, IMV Technologies, L'Aigle, France) using HTCASA II software (version 1.13.7; Hamilton Thorne Inc., Beverly, MA, USA). Thawed semen samples were diluted with 0.025% glutaraldehyde solution and spread by capillarity on Leja 20 micron slides with 4 3 µl chambers. Among the parameters analyzed by the CASA system, in the present study we followed and compared total motility and progressive motility, the percentage of total morphological anomalies.

Correlations were calculated between ejaculates from rams with the same maintenance mode but also between the two exploitation modes: pasture and stable.

The experimental data were entered into a column-type database and processed in Excel to obtain descriptive statistical values (mean, standard error, minimum and maximum values) and to compare and establish the differences between the spermatic performances of the four rams among themselves or on groups of ejaculates obtained according to the exploitation system we used t-Test with unequal variances (t-Test: Two-Sample Assuming Unequal Variances).

3. Results and discussion

From the rams selected from the group operated in the stable, 5 ejaculates were collected with a volume ranging from 1.4 to 1.9 ml from the first ram (A) and an average of 1.64±0.195 (ml) and 1.6-2.6 ml from the second ram (B) with an average of 2.14±0.422 (ml). In the rams operated on pasture, the volume was between 1.7-2.6 ml with an average of 2.1±0.381 (ml) in the third ram (C) and 1.6-2 ml in the fourth ram (D) with an average of 1.86±0.167 (ml).

There are no statistically significant differences in ejaculate volume between the average of the 10 ejaculates from the stall-raised rams (1.89±0.407 ml) and the average of the ejaculates (n=10) from the pasture-raised rams (1.98±0.305 ml), although the ejaculate volume from the pasture-raised rams is slightly higher (1.98 vs 1.89). All ejaculates were diluted to a concentration of 0.8x106 spermatozoa/ml, respectively 0.2x106 spermatozoa/processed straw. When checking the quality of the frozen semen with the CASA system, we followed the percentage of spermatozoa with normal morphology recorded in the 20 ejaculates (table 1).

Figure 1 shows the percentage of sperm with normal morphology as an average of the 5 ejaculates from each ram, as well as the average of the ejaculates collected from rams exploited in stables and on pastures.

The results obtained and presented in table 1 and figure 1 highlight the influence of the exploitation system on sperm morphology. Rams exploited in stables showed more constant values and a higher percentage of sperm with normal morphology compared to those raised on pastures. Rams exploited on pastures showed greater variability between ejaculates, including low values of normal morphology.

Table 1. Percentage of sperm with normal morphology depending on the exploitation system (%)

Stable			Pasture		
Ram	Ejaculate	Normal morphology	Normal morphology	Ejaculate	Ram
A	1	85.53	87.65	1	C
	2	87.08	70.92	2	
	3	87.46	74.00	3	
	4	87.27	75.16	4	
	5	87.15	74.33	5	
B	1	87.94	74.62	1	D
	2	87.71	47.85	2	
	3	86.97	74.83	3	
	4	87.69	75.63	4	
	5	88.34	77.23	5	

The average percentage of sperm with normal morphology was approximately 87.31% in rams in the stable, compared to 73.22% in those exploited on pasture. The difference between the two exploitation systems was statistically significant ($p < 0.05$), according to the t-test applied, the values of normal morphology being significantly higher in rams exploited in the stable compared to those on pasture ($p = 0.0015$).

The increased variability observed in rams raised on pasture, also highlighted by the large differences between the minimum and maximum values, indicates the influence of environmental factors on the spermatogenesis process. In contrast, controlled conditions in the stable seem to favor the stability and morphological quality of spermatozoa. These results can be explained by the influence of environmental factors and especially nutrition on reproductive function.

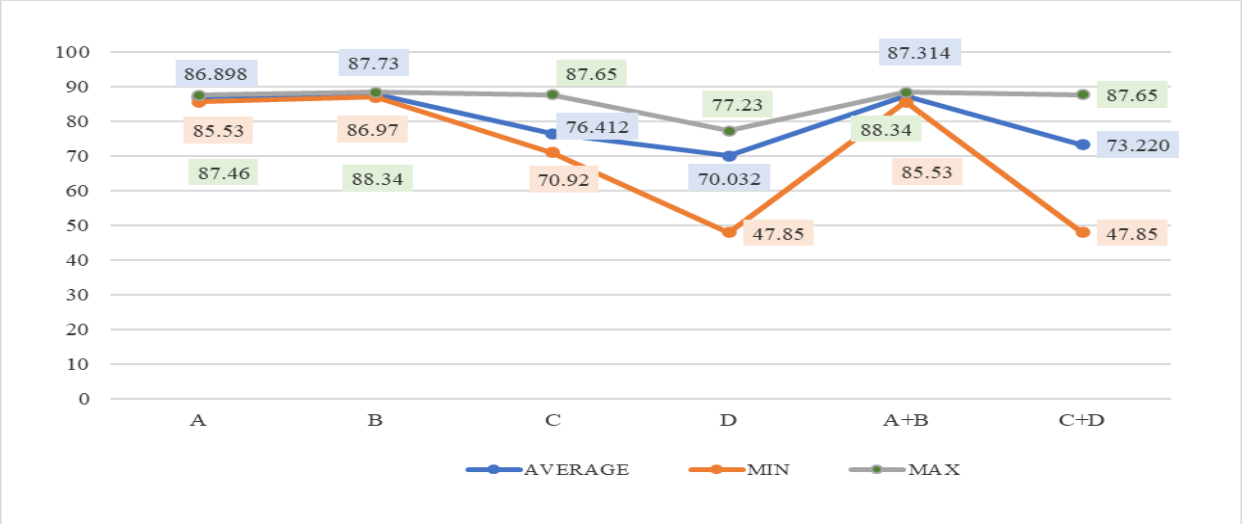

Figure 1. Evolution of the average percentage of sperm with normal morphology in relation to the average of minimum and maximum values from rams exploited in stables (A, B) and on pastures (C, D)

The stable system offers more controlled conditions, with constant nutrition and a reduced level of variation in environmental factors, which may contribute to a greater stability of sperm parameters. On the other hand, the grazing system involves exposure to variations in climatic and nutritional conditions, as well as a higher level of physical activity, factors that may negatively influence certain aspects of sperm quality.

The increased variability observed in rams raised on pasture, also highlighted by the large differences between the minimum and maximum values, indicates the influence of environmental factors on the spermatogenesis process. In contrast, controlled housing conditions seem to favour the stability and morphological quality of

spermatozoa. These results can be explained by the influence of environmental factors and especially nutrition on reproductive function. The housing system offers more controlled conditions, with constant nutrition and a reduced level of variation in environmental factors, which may contribute to a greater stability of sperm parameters. On the other hand, the grazing system involves exposure to variations in climatic and nutritional conditions, as well as a higher level of physical activity, factors that may negatively influence certain aspects of sperm quality.

Similar results highlighting the fact that environmental factors and management can significantly influence semen parameters have been reported in the specialized literature, [11, 12].

An important parameter that defines the quality and fertilizing capacity of frozen semen is represented by motility, namely the percentage of live sperm. Checking the thawed sperm with the CASA computerized system allowed the determination of both total motility and progressive motility represented only by sperm that have forward movements.

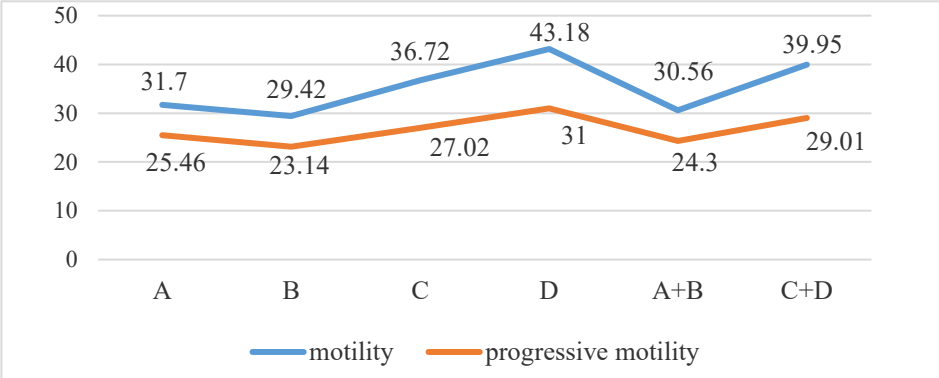

Figure 2. Evolution of the average motility and progressive motility of frozen sperm from rams exploited in stables (A, B) and on pastures (C, D))

Figure 2 shows the evolution of total motility and progressive motility of frozen sperm in rams raised in stables (A and B) and on pastures (C and D). It is observed that rams raised on pastures (C and D) recorded higher values for both total motility and progressive motility, compared to rams kept in stables (A and B). The average total motility was 39.95% in pastures compared to 30.56% in stables, and for progressive motility the values were 29.01% in pastures and 24.3% in stables. Statistical analysis by t-test for independent samples revealed statistically significant differences between the two farming systems, both for total motility ($p=0.004563$) and for progressive motility ($p=0.010626$), the values being lower than the significance threshold of 0.05.

These results indicate that the pasture farming system favors higher sperm motility, probably due to a higher level of physical activity and the influence of environmental factors. However, the higher variability observed in pasture-raised rams suggests a more pronounced influence of external conditions on the stability of these parameters.

Unlike motility, which was higher in pasture-raised rams, normal morphology was superior in animals kept in stables, which highlights different effects of the farming system on sperm parameters.

The total and progressive motility values obtained in this study fall within the ranges considered normal for frozen ram semen, namely total motility of 30–60% and progressive motility between 20–40% after thawing [13, 14]. The values of the two motilities are variable in specialized works depending on numerous factors related to the type of processing, namely the diluents used, the type and concentration of the cryoprotectant used, the rate of temperature decrease during freezing [15, 16, 17].

In conclusion, the exploitation system influences differently the morphological and functional parameters of ram semen, and an integrated evaluation of them is necessary for a correct assessment of the reproductive potential.

4. Conclusions

The morphological and functional parameters of frozen semen from rams are influenced by the management system. Normal morphology values were significantly higher in rams farmed in stables compared to those raised on pasture ($p<0.05$), indicating a favorable influence of controlled conditions on morphological quality. In contrast, motility and progressive motility values were significantly higher in rams farmed on pasture ($p<0.05$), suggesting a positive influence of environmental conditions and physical activity on sperm functional parameters.
These results highlight different effects of the management system on sperm quality, highlighting the importance of management in optimizing reproductive performance.

Acknowledgements

The research was conducted on rams from the monitored batches to determine the carbon footprint project(ADER 8.1.3/2023), funded by the MADR.

References

1. Ben Moula A., Kchikich A, Chentouf M, Hamdache A, Bouraada K, Essafi M, Ezziyyani M, Climate change impacts on sheep and goat production and reproduction, Journal of Central European Agriculture, 2024, doi: https://doi.org/10.5513/JCEA01/25.4.4335
2. Rodriguez JND, Guimarães JD, Fonseca JF, Penitente-Filho JM, Rangel PSC, López CJR, Freitas RS, de Castilho EF, Rodrigues LFS, Oliveira MEF, Garcia AR, Climatic seasons and time of the day influence thermoregulation and testicular hemodynamics in Santa Inês rams raised under humid tropical conditions, Journal of Thermal Biology, 2023, doi: https://doi.org/10.1016/j.jtherbio.2023.103546
3. Gerber, P., Vellinga, T., Opio, C., & Steinfeld, H. Productivity gains and greenhouse gas emissions intensity in dairy systems, Livestock science, 2011, 139(1-2), 100-108.
4. Food and Agriculture Organization, Tackling Climate Change Through Livestock, 2013, https://www.fao.org/4/i3437e/i3437e.pdf
5. Peter J. Hansen. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences, 2009, 364(1534), 3341–3350. https://doi.org/10.1098/rstb.2009.0131
6.. Aitken RJ, Baker MA, Oxidative stress and male reproductive biology. Reproduction, Fertility and Development, 2004, 16(5), 581–588. https://doi.org/10.1071/RD03094
7. Delgadillo JA, Ungerfeld R, Flores JA, Véliz F G, The reproductive physiology of male sheep and goats: Environmental and management effects. Animal Reproduction Science, 2015, 162, 1–10 https://doi.org/10.1016/j.anireprosci.2015.09.001
8. Chemineau P, Malpaux B, Brillard JP, Fostier A, Seasonality of reproduction and production in farm fishes, birds and mammals. Animal, 2008, 2(3), 419–432, https://doi.org/10.1017/S1751731107001444
9. Marai MRA, El-Darawany AA, Fadiel A, Abdel-Hafez MAM, Reproductive performance traits as affected by heat stress and its alleviation in sheep. Tropical and Subtropical Agroecosystems, 2008, 8(3), 209–234.
10. Ben Moula A, Feki M, Rekik M, Hammami H, Seasonal variations in semen quality of Sicilo-Sarde rams. Animal Reproduction Science, 2013, 139(1–4), 99–106, https://doi.org/10.1016/j.anireprosci.2013.03.005
11. Martí JI, Aparicio IM, Leal CLV, García-Herreros M, Seasonal dynamics of sperm morphometric subpopulations and its association with sperm quality parameters in ram ejaculates, Theriogenology, 2012, 78(3), 528-541, https://doi.org/10.1016/j.theriogenology.2012.02.035
12. Maquivar MG., Smith SM, Busboom JR, Reproductive Management of Rams and Ram Lambs during the Pre-Breeding Season in US Sheep Farms. Animals, 2021, 11(9), 2503, https://doi.org/10.3390/ani11092503
13. Salamon S, Maxwell WMC, Storage of ram semen. Animal Reproduction Science, 2000, 62, 77–111, https://doi.org/10.1016/S0378-4320(00)00155-X
14. Watson PF, The causes of reduced fertility with cryopreserved semen. Animal Reproduction Science, 2000,60–61,481–492, https://doi.org/10.1016/S0378-4320(00)00099-3
15. Soylu MK, Nur Z, Ustuner B, Dogan I, Sagirkaya H, Gunay U, Ak, K, Effects of various cryoprotective agents and extender osmolality on post-thawed ram semen. Bulletin-Veterinary Institute In Pulawy, 2007, 51(2), 241,
16. Kubovičová E, Makarevich AV, Špaleková E, Hegedušová Z, Motility and fertilizing ability of frozen-thawed ram sperm from two sheep breeds, Slovak Journal of Animal Science, 2011, 44 (4):134-139,
17. Van de Hoek M, Rickard JP, de Graaf SP. Motility Assessment of Ram Spermatozoa. Biology. 2022,11(12):1715,https://doi.org/10.3390/biology11121715