International Journal of Veterinary Sciences and Animal Husbandry 2024; 9(1): 721-724

ISSN: 2456-2912
VET 2024; 9(1): 721-724

www.veterinarypaper.com
Received: 01-10-2023
Accepted: 05-11-2023

Tapendra Kumar
Ph.D. Scholar, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Pramod Kumar
Assistant Professor, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Science, Bikaner, Rajasthan, India

Nirmala Saini
Principal Scientist & Head, ICAR-Central Sheep & Wool Research Institute, Arid Region Campus, Bikaner, Rajasthan, India

Ashok Kumar
Scientist, ICAR-Central Sheep & Wool Research Institute, Arid Region Campus, Bikaner, Rajasthan, India

Shivendra Kumar Bhalothia
Ph.D. Scholar, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Sandeep Dholpuria
Assistant Professor, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Science, Bikaner, Rajasthan, India

Maya Mehara
M.V.Sc. Scholar, Department of Veterinary Public Health, College of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Prakash
Research Associate AICRP on Goat, College of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Corresponding Author:
Tapendra Kumar
Ph.D. Scholar, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Fresh seminal characteristics of magra ram in breeding season

Tapendra Kumar, Pramod Kumar, Nirmala Saini, Ashok Kumar, Shivendra Kumar Bhalothia, Sandeep Dholpuria, Maya Mehara and Prakash

Abstract
The purpose of this study was to analyze the key characteristics of fresh Magra ram semen. A total of eight adult Magra rams, with an average body weight of 37.5±2.13kg and good libido, were randomly chosen for the assessment of semen quality. Over a period of three consecutive weeks, semen samples were collected twice a week using the artificial vagina method. In total, 48 seminal ejaculates were collected and examined for their physical and functional attributes. The average values for semen volume (ml), pH, mass activity (rated on a scale of 0-5), concentration (million/ml), individual progressive motility (%), sperm viability (%), and sperm abnormality (%) were recorded as 0.86±0.01, 7.02±0.01, 4.27±0.09, 3853.89±12.8, 79.22±0.20, 80.21±0.24, and 4.04±0.19, respectively. The findings of this study suggest that the majority of the evaluated seminal parameters indicate good quality semen, which can be effectively used for artificial insemination purposes in the field, leading to faster genetic improvement.

Keywords: Ram, artificial insemination, semen, breeding season

Introduction
Sheep (Ovisaries) domestication has been a constant companion to human beings throughout history, and their farming is practiced in almost every corner of the world (Hatziminaoglou, 2006 and Ryder, 2007) [13, 31]. These animals are highly valued for their exceptional ability to provide meat, wool, milk, manure, and skin, making them a five-star asset in harsh environmental conditions. In arid, semi-arid, and mountainous regions of India, where crop and dairy farming are not economically viable, sheep serve as a mobile ATM for small-scale farmers and landless laborers (Pampori *et al*., 2018) [27]. However, the availability of purebred superior germplasm is scarce compared to non-descript sheep in the field conditions of Rajasthan, India.

Artificial insemination (AI) is a highly effective method for genetic improvement when combined with accurate progeny testing schemes. It offers a significant increase in the rate of genetic progress compared to natural service. To achieve maximum fertility, fresh semen should be inseminated within 10-14 hours after collection. Cervical insemination with liquid stored semen has proven to be a satisfactory alternative to frozen semen. However, the limitation of this method is that it is challenging to implement in field conditions located far from semen production centers. In small ruminants, the success of AI depends on various factors, particularly those related to semen processing and preservation. Liquid-stored semen is preferred over cryopreserved semen due to its convenience, cost-effectiveness, and higher quality. Cryopreserved semen often suffers from poor quality and reduced fertilization ability due to the injuries caused by freezing and thawing. Chilling semen helps to reduce metabolism and maintain sperm viability for an extended period. Fresh semen is effectively utilized in artificial insemination (AI) in ewes, resulting in a conception rate ranging from 40% to 60% through the cervix (Anel *et al*., 2005) [3]. However, the short shelf life and limited transport window of fresh semen pose challenges in spreading AI in field conditions (Najafi *et al*., 2014) [25]. Prior to AI, it is crucial to evaluate the semen quality of the flock to determine its reproductive success and profitability (Mac Laren, 1988) [20].

Hence, the objective of the current study was to assess the characteristics of fresh semen from Magra rams for the purpose of preserving it for AI.

Materials and Methods

Experimental location and management of animals

This research was carried out in collaboration with the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Bikaner, and ICAR-Central Sheep and Wool Research Institute (CSWRI), Arid Region Campus (ARC), Bikaner, in the year 2019-20. A total of 8 Magra rams, aged between 1.5 to 3 years and weighing 37.5±2.13 kg, were selected for the study. These rams exhibited good libido and were trained in semen donation using an artificial vagina (AV). They were all provided with a standard diet formulated according to the requirements for mature breeding rams as suggested by the Indian Council of Agricultural Research. As per the institute's regular procedure, the rams were given concentrate feed in the shed and allowed to graze on the range for a minimum of 7 hours each day. The rams were reared under identical management conditions and had free access to fresh drinking water. They were kept isolated from the ewes. Throughout the experiment, a general health checkup program was implemented, which included deworming and vaccination for disease prevention, following the health calendar of the institute.

Collection of semen samples

During the duration of the investigation, semen samples were collected twice a week for a continuous period of three weeks. This was done in the morning hours before feeding, utilizing a sterile AV. A dummy, in the form of an ewe in estrus, was employed. Following semen collection, the cups containing the samples were appropriately labeled and promptly transferred to a water bath set at a temperature of 37 °C.

Semen quality assessment

Following semen collection, each semen sample underwent examination for mass motility on a scale of 0-5. A small drop of fresh semen was placed on a pre-warmed, grease-free, clean, and dry glass slide. Subsequently, it was observed under a low-power microscope at 10X magnification using the Dewinter Binocular Microscope from Italy. To assess the individual motility of spermatozoa, a cover slip was placed on a drop of diluted semen (5µl semen mixed with 495µl normal saline) on a grease-free clean glass slide. This slide was then observed under the microscope with an attached bio-therm stage at 40X magnification. The motility of sperms was expressed as a percentage of progressively motile sperms on a scale of 0-100. The concentration of spermatozoa (millions/ml) was determined using Neubaur's counting chamber (Haemocytometer). Fresh semen (10 µl) was diluted with spermicidal diluting fluid (9990 µl; 1:1000) following the method described by Evan and Maxwell in 1987 [9]. The pH of semen was measured using the digital multi-parameter pH meter HI2020 from Hanna Instruments, Italy. To assess sperm viability (percentage of live sperm) and abnormalities, the eosin-nigrosin staining technique (Evan and Maxwell, 1987) [9] was employed. In brief, a small drop (30 µl) of semen was placed on a clean, grease-free slide and an equal amount of eosin-nigrosin dye was added. The mixture was thoroughly mixed using a blunt-end fine glass rod. After one minute, a thin smear was prepared on a glass slide, allowed to air dry, and then 300 spermatozoa were counted in different microscopic fields at 40X and 100X magnification to assess sperm viability and abnormality, respectively. The viability of spermatozoa was determined by the complete exclusion of stain, while any morphological deformity in the head, body, or tail was considered an abnormality in spermatozoa (Evans and Maxwell, 1987) [9].

Statistical analysis

The data were analyzed utilizing SPSS (Version 25.0, Chicago, IL, USA). The outcomes were presented as Mean ± SE employing SPSS (Version 25.0, Chicago, IL, USA).

Result and Discussion

Table 1: Mean values of physical properties of fresh ram semen throughout the period of the study (Mean±SEM)

Parameter	Mean±SE(Range)	Parameter	Mean±SE(Range)
Volume(ml)	0.86±0.01 (0.77±0.02 to 0.94±0.03)	Progressive Motility (%)	79.22±0.20 (77.92±0.49 to 80.50±0.56)
pH	7.02±0.01 (6.95±0.02 to 7.10±0.03)	Sperm Viability (%)	80.21±0.24 (79.33±0.61 to 81.66±0.80)
Mass Motility (0-5)	4.27±0.09 (3.40±0.30 to 4.67±0.21)	Sperm Concentration (million/ml)	3853.89±12.8 (3723.34±8.33 to 4002.67±4.88)
Sperm morphological abnormality (%)	4.34±0.50 (2.67±0.33 to 5.00±0.57)		

Table 1 presents the Mean values (Mean±SEM) of physical properties of fresh ram semen over the course of the study. The range of semen ejaculate volume (ml) varied from 0.77±0.02 to 0.94±0.03, with an average volume (ml) of 0.86±0.01, showing no significant difference among the rams. Similar values were also observed in other indigenous sheep breeds, as reported by Pawar (2003) [28] for Patanwadi rams and Mahala *et al*. (2021) [21] for Magrarams. However, Bharti *et al*. (2009) [5], Toppo (2013) [36], and Rajasri (2016) [30] reported lower semen ejaculate values in various indigenous sheep breeds, while Dabas (1991) [7] estimated higher values for Patanwadi half-bred rams. These variations in semen volume among different authors' findings could be attributed to genotype differences, seasonal variations (Olah *et al*., 2013) [26], nutritional and health status, breed (Donavan *et al*., 2001; Alfaris *et al*., 2012; Moghaddam *et al*., 2012) [8, 2, 24], activation of primary and secondary genital organs, functional abnormalities, and the bioavailability of associated endocrinal hormones (Moghaddam *et al*., 2012) [24], as well as the number of semen collections per day (Jennings and McWeeny, 1976) [14]. The fresh semen pH exhibited an average of 7.02±0.01, ranging from 6.95±0.02 to 7.10±0.03, and no significant variation was observed among the rams. The findings of Kumar (2019) [17] and Mahala *et al*., (2021) [21] in Magra rams align with the current findings in the Magra ram. Khalifa (2017) [16] documented a slightly acidic pH in the

semen of different ram breeds. The disparity in pH values can be attributed to various factors such as the season of the year, frequency of ejaculation, and sperm concentration (McKinnon *et al*., 2011) [23]. A higher pH level may indicate urospermia or infection in the reproductive system, and a negative correlation has been reported between seminal pH and sperm concentration or the number of sperm (Griggers *et al*., 2001) [11]. The semen mass motility was observed to be 4.27±0.09 on a scale of 0-5, with a range of 3.40±0.30 to 4.67±0.21. Bharti *et al*. (2009) [5] and Toppo (2013) [36] also made similar observations in Chhotanagpuri rams, while Mahala *et al*. (2021) [21] observed similar results in Magra rams. Kayali *et al*. (2014) reported higher scores of mass motility in indigenous sheep breeds, whereas lower values were reported by Pawar (2003) [28], Alcay *et al*. (2014), Rahman *et al*. (2015) [29], and Rajasri (2016) [30] for different indigenous ram breeds. No significant difference was found among individuals in terms of mass motility score, which aligns with the findings of Bharti *et al*. (2009) [5] and Kumar (2010) [18]. The variations in mass activity may be attributed to differences in genetic makeup (breed), biochemical constituents of semen, and pre-sexual stimulation (Salisbury *et al*., 1978) [32]. The calculated average Sperm Concentration (million/ml) was 3853.89±12.8, ranging from 3723.34±8.33 to 4002.67±4.88, with a significant difference ($p<0.05$) observed among the rams. These values align with the findings of Kumar (2019) [17] in Magra rams and are consistent with other Indian ram breeds reported by Karagiannidis *et al*. (2000) [15], Gundogan *et al*. (2006) [12], and Bharti *et al*. (2009) [5]. However, the present result is higher than the results of Cox *et al*. (2015) [6] and Al-Anazi *et al*. (2017) [1], but lower than the findings of Sarlos *et al*. (2013) [33]. The variations in sperm concentration values may be attributed to factors such as climatic or geographic conditions, breeding season, individual nutritional status, and semen collection frequency (Gundogan *et al*., 2007) [12].

The mean of individual progressive sperm motility (%) ranged from 77.92±0.49 to 80.50±0.56, with an overall mean value of 79.22±0.20. There was no significant difference observed in the progressive sperm motility among the rams, which is consistent with a previous study on Magra rams by Kumar (2019) [17] and Mahala *et al*. (2021) [21]. These results are also in line with the findings of Suthar *et al*. (1999) [35]. However, Kumar *et al*. (2010) [18] in Malpura, Kumar (2019) [17], and Mahala *et al*. (2021) [21] in Magra rams reported lower individual sperm motility, while Farshad *et al*. (2010) [10] recorded higher motility in different breeds of rams compared to the present study. These findings suggest that factors such as breed, method of collection, interval between collections, ram's age at collection, and number of semen collections per day (Jennings and Mcweeny, 1976) [14] can influence sperm motility. The fresh ram semen had an average live sperm percentage of 80.21±0.24, ranging from 79.33±0.61 to 81.66±0.80. This finding aligns with Pawar's (2003) [28] observation in Patanwadi rams and Bharti *et al*.'s (2009) [5] observation in Chhotanagpuri ram. However, the results were higher than Kumar's (2019) [17] and Mahala *et al*.'s (2021) [21] findings in Magra rams. The variations in these results may be attributed to environmental and management conditions, including feeding variation, methodological errors, ram breeds, adaptability to agro-climatic conditions, season, age, and frequency of semen collection (Bhalothia *et al*., 2022) [4].

In terms of sperm viability, there was no significant difference among the Magra rams, which is consistent with Kumar's (2019) [17] and Mahala *et al*.'s (2021) [21] previous study in Magrarams. Regarding sperm abnormality percentage, it ranged from 2.67±0.33 to 5.00±0.57, with a mean percentage of 4.34±0.50 in various rams' semen. This finding is consistent with Gundogan's (2007) [12] observation. However, Kumar (2019) [17] and Mahala *et al*. (2021) [21] reported higher total sperm abnormality values compared to the present study, while Pawar (2003) [28] reported lower values (3.79±0.13). The variation in these results may be attributed to different agro-climatic conditions, methodologies, genetic makeup (breed and individual effect), age factor, and managemental factors such as feeds and feeding schedule (Saxena and Tripathi, 1987) [34]. There was a significant difference ($p<0.05$) in total sperm abnormalities among the rams, whereas Kumar (2019) [17] and Mahala *et al*. (2021) [21] found no significant difference in Magra rams.

Conclusion

The findings of the current investigation demonstrated that there were no significant variations in the spermiogram of Magra rams, except for their sperm concentration. This indicates that their semen can be utilized for both preservation and artificial insemination in sheep, thereby contributing to genetic enhancement at the field level.

Acknowledgement

The authors express their gratitude to Dean, CVAS, Bikaner. They are also appreciative of the support received from the Director, ICAR-CSWRI, Avikanagar, Project Coordinator, NWPSI, and PI on Magra Field Unit. Additionally, they acknowledge the Head, ICAR-CSWRI, ARC, Bikaner for their generous financial and technical assistance, as well as the provision of animals and lab facilities throughout the entire research period.

References

1. Al-Anazi Y, Al-Mutary MG, Al-Ghadi M, Alfuraiji MM, Al-himaidi AR, Ammari A. Seasonal variations in scrotal circumference and semen characteristics of Naimi and Najdi rams in Saudi Arabia. South African Journal of Animal Science. 2017;47(4):454-459.
2. Alfaris A, Abdalbari AF, Tahir, Jafer B, Hassan A. Laparoscopic intrauterine artificial insemination and ultrasonic pregnancy diagnosis in Arabi ewes. Journal of Evolutionary Biology Research. 2012;4(1):1-12.
3. Anel L, Kaabi M, Abroug B, Alvarez M, Anel E, Boixo JC, *et al*. Factors influencing the success of vaginal and laparoscopic artificial insemination in Churra ewes: a field assay. Theriogenology. 2005;63(4):1235-1247.
4. Bhalothia SK, Kumar T, Mehta JS, Saharan D, Saini N, Kumar A. Seminal attributes of Magra ram during the breeding season in the arid region of Bikaner. The Pharma Innovation Journal. 2022;SP-11(7):2680-2683.
5. Bharti MK, Sinha MP, Singh B, MAHTO D. Study on semen characteristics of Chottanagpuri rams. Indian Journal of Animal Reproduction. 2009;30(2):42-45.
6. Cox JF, Jeria E, Bocic A, Vera N, Soto-Saravia R, Dorado J. Characterization of the productive performance of highlander sheep in southern Chile. II. Male productive traits. Small Ruminant Research. 2015;130:189-192.
7. Dabas SK. Studies on seminal characteristics and biochemical constituents in Patanwadi rams and their crosses. M.V.Sc. Thesis, Gujarat Agricultural University, Sardar Krushi Nagar, Gujarat, India; c1991.
8. Donovan A, Hanrahan JP, Lilly T, Boland MP, Byrne GP, Duffy P. AI for Sheep Using Frozen-thawed semen.

End of project Report: Sheep Series No.11 Teagasc, Agriculture and Food Development Authority; c2001.
9. Evan G, Maxwell WMC. Salamon's artificial insemination of sheep and goats. Butterworths, London, 1987;123-194.
10. Farshad A, Khalili B, Jafaroghli M. Effect of butylatehydroxytoluene on the freezability of ram spermatozoa. Asian-Australasian Journal of Animal Science. 2010;23(10):1276-1281.
11. Griggers S, Paccamonti DL, Thompson RA, Eilts BE. The effects of pH, osmolarity, and urine contamination on equine spermatozoal motility. Theriogenology. 2001;56(4):613-622.
12. Gundogan M. Seasonal variation in serum testosterone, T3 and andrological parameters of two Turkish sheep breeds. Small Ruminant Research. 2007;67:312-316.
13. Hatziminaoglou I. Sheep and goats in Greece and in the world. Ed. Jahoudi, Thessaloniki Greece, 2006.
14. Jennings JJ, Mcweeny. Effect of frequent ejaculation on semen characteristics in rams. Veterinary Record. 1976;98:230-233.
15. Karagiannidis A, Varsakeli S, Alexopoulos C, Amarantidid I. Seasonal variation in semen characteristics of Chios and Friesian rams in Greece. Small Ruminants Research. 2000;37(1-2):125-130.
16. Khalifa MA. Effect of supplementing ram semen extender with melatonin on oxidative stress indices and physical properties of chilled spermatozoa. International Journal of Animal Research. 2017;1-14.
17. Kumar A. Effects of administration of antioxidants on biochemical, hormonal, antioxidant profiles, and seminal characteristics of Magra rams in the arid region of Rajasthan. 2019; Ph.D. Thesis, College of Veterinary and Animal Science, Bikaner, Rajasthan.
18. Kumar D, Joshi A, Naqvi SMK. Comparative semen evaluation of Malpura and Bharat Merino rams by computer-aided sperm analysis technique under semi-arid tropical environment. International Journal of Animal and Veterinary Advance. 2010;2(1):26-30.
19. Lopez Saez A, Ortiz N, Gallego L, Garde J.J. Liquid storage (5 °C) of ram semen in different diluents, Archives of Andrology. 2000;44(2):155-164.
20. Mac Laren APC. Ram fertility in southwest Scotland. British Veterinary Journal. 1988;144(1):45-54.
21. Mahala SK, Kumar A, Mehta JS, Kumar A, Kumar P, Bhalotia SK, Saini N, Chopra A. Seasonal response of spermatozoa to the hypo-osmotic swelling test (HOST) and correlation with seminal characteristics of Magra ram. The Pharma Innovation Journal. 2021;10(10):1992-1995.
22. Maxwell SSW. Storage of ram semen. Animal Reproduction Science. 2000;62:77–111.
23. Mckinnon AO, Squires EL, Vaala WE, Varner DD. Equine reproduction. Wiley-Blackwell. 2nd Edition. 2011.
24. Moghaddam G, Pourseif MM, Rahimi AA. Study of correlation between reaction time and refractory period (as indices of libido) with semen characteristics in Arkhar Merino× Moghani and Baluchi×Moghani rams. Italian Journal of Animal Science. 2012;11(4):e66.
25. Najafi A, Najafi MH, Zanganeh Z, Sharafi M, Martinez-Pastor F, Adeldust H. Cryopreservation of ram semen in extenders containing soybean lecithin as a cryoprotectant and hyaluronic acid as an antioxidant. Reproduction in Domestic Animals. 2014;49(6):934-940.
26. Olah J, Kusza S, Harangi S, Posta J, Kovacs A, Pecsi A, Csilla B, Javor A. Seasonal changes in scrotal circumference, the quantity and quality of ram semen in Hungary. Archiv Tierzucht. 2013;56(10):102-108.
27. Pampori ZA, Sheikh AA, OvAarif, Hasin D, Bhat IA. Physiology of reproductive seasonality in sheep. Biological Rhythm Research. 2018;51(4):1-13.
28. Pawar KJ. Studies on semen qualities and the effect of certain semen diluents additives on the preservability of Patanwadi ram semen at ultra-low temperature. Ph.D. thesis, submitted to Gujarat Agricultural University, Sardar Krushi Nagar, Gujarat; c2003.
29. Rahman HMR, Paul AK, Maruf AA, Rahman MM, Islam MT, Bari FY. Characterization of native ram semen in Bangladesh. Wayamba Journal of Animal Science. 2015;7:1076-1083.
30. Rajasri. M.V.Sc. thesis submitted to P.V.N.R Telangana veterinary University, Hyderabad, 2016. 23.
31. Ryder LM. Sheep and man. Duckworth and Co Ltd. UK, 2007;24.
32. Salisbury GW, Van Demark NL, Lodge JR. Physiology of reproduction and artificial Insemination of cattle. 2nd edition. WH. Freeman and Co., San Francisco; c1978.
33. Sarlos P, Egerszegi I, Balogh O, Molnar A, Cesh S, Ratky J. Seasonal changes of scrotal circumference, blood plasma testosterone concentration and semen characteristic in Racka rams. Small Ruminants Research. 2013;111(1-3):90-95.
34. Saxena VB, Tripathi SS. Seasonal effect on sperm morphology of Nali rams. The Indian Journal of Animal Sciences. 1987;57(4):294-296.
35. Suthar BN, Sharma VK, Kavani FS. Seminal characters and their interrelationships on Patanwadi and Halberd Merino rams. Indian Journal of Veterinary Medicine. 1999;23:307-309.
36. Toppo TP. Studies on cryopreservation and post-thawed assessment of Chhotanagpuri ram semen (Doctoral dissertation, Birsa Agricultural University, Kanke, Ranchi, Jharkhand); c2013.