The Pharma Innovation Journal 2022; SP-11(7): 2680-2683

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 2680-2683

www.thepharmajournal.com
Received: 07-04-2022
Accepted: 12-05-2022

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

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

JS Mehta
Professor & Head, Department of Veterinary Gynaecology & Obstetrics, College of Veterinary & Animal Sciences, Rajasthan University of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

Dinesh Saharan
MVSc, College of Veterinary & Animal Sciences, Rajasthan University of Veterinary & Animal Science, (RAJUVAS), Bikaner, Rajasthan, India

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

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

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

Seminal attributes of Magra ram during breeding season in arid region of Bikaner

Shivendra Kumar Bhalothia, Tapendra Kumar, JS Mehta, Dinesh Saharan, Nirmala Saini and Ashok Kumar

Abstract
This study was carried out to evaluate the seminal characters of fresh Magra ram semen. Eight (n=8) mature Magra rams having body weight 37.5±2.13kg and good libido were randomly selected for quality semen assessment. Semen was collected using artificial vagina method twice in a week for 3 consecutive weeks. Total 48 fresh seminal ejaculates were collected and evaluated for physico-morphic and functional attributes. The mean percentages of semen volume (ml), concentration (million/ml), mass activity (0-5 scale), individual progressive motility (percent), sperm abnormality (%), sperm viability (%) and pH were evaluated and recorded 0.915±0.04, 3927.08±38.38, 4.23±0.10, 76.396±0.53, 3.125±0.09, 79.938±0.51, 6.913±0.03, respectively. This study indicated that most of the seminal parameters evaluated for semen quality were within range of good quality semen and can be used for preservation for artificial insemination purpose in the field condition for faster genetic improvement.

Keywords: Sheep, artificial insemination, semen, breeding season

Introduction
Sheep (*Ovis Aries*), since they are used for farming, have been with humans throughout all of recorded history (Ryder, 2007) [23]. The majority of the world's countries now engage in sheep farming (Hatziminaoglou, 2006) [10], were Asia accounting for 44.9% of the world's sheep population and India coming in third. In India, approx 80% of landowners are small and marginal farmers and 15-20% of families lack access to land for such people, small ruminant farming is primary source of livelihood. Sheep is known as "5 star animal" with its multi-facet utility for wool, meat, milk, skin and manure form an important component of rural economy particularly in the arid, semi-arid and mountainous areas of the country. Magra sheep is one of the most significant carpet wool breeds, do well in the hot, dry regions of Rajasthan. Most field-raised sheep are non-descript and low productive. One of the most essential reproductive methods for accelerating genetic advancement is artificial insemination (AI).It facilitates the distribution of semen from superior sire to a large number of females, allowing the improvement of desirable characteristics, e.g. milk, meat, and wool production (Maxwell and Salamon, 1993) [13]. According to O'Hara *et al*. (2010) [18], AI using fresh, liquid-preserved, or frozen-thawed semen increases the lambing rate in sheep breeding. Fresh semen is successfully used in AI in ewes with a conception rate of 40–60% through the cervix (Anel *et al*., 2005) [2]. However fresh semen has a short shelf life, limited transport window, therefore limiting spread of AI in field condition (Najafi *et al*., 2014) [17]. Prior to AI, the evaluation of the flock's semen is crucial for determining how successfully it reproduces and how profitable it will be (Maclaren, 1988) [12]. Therefore, the goal of the current study was to analyse the fresh semen characteristics of Magra ram for AI purposes.

Materials and methods
Experimental location and management of animals
This study was conducted 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 during year 2019-20. Magra rams (n=8) having age from 1.5 to 3 years, body weight 37.5±2.13 kg with good libido and trained in semen donation using artificial vagina (AV). All the rams were fed on the standard diet, formulated according to the requirement for mature breeding ram suggested by Indian Council of Agricultural Research. According to the institute's regular

procedure, rams were given concentrate in the shed and permitted to graze on the range for at least 7 hours each day. All the rams were cared under the same managerial condition, and free access to clean drinking water. Rams were kept separated from ewes. A general health management program for deworming, vaccination for disease prevention was followed during the experiment as prescribed by the health calendar of the institute.

Collection of semen samples

Throughout the duration of the study, semen samples were collected twice a week for three consecutive weeks using sterile AV in the morning hours before feeding. Ewe in estrus was used as dummy. The semen collection cups were tagged and moved to the water bath at 37°C shortly after semen collection.

Semen quality assessment

Shortly after semen collection, each semen sample was examined for mass motility (scale 0-5). A small drop of fresh semen was put on a pre-warmed grease free clean, dry glass side and examined under low power microscope at 10X (Dewinter Binocular Microscope, Italy). The individual motility of spermatozoa was assessed by placing a cover slip on a drop of diluted semen (5µl semen mixed with 495µl normal saline) on a grease free clean glass slide under the microscope with At 40X magnification bio-therm stage attached and expressed in terms of percentage of progressively (0-100) motile sperms. The concentration of spermatozoa (millions/ml) was recorded with the help of Neubaur's counting chamber (Haemocytometer). Fresh semen (10 µl) diluted with spermicidal diluting fluid (9990 µl; 1:1000) as per the method described by Evan and Maxwell, 1987 [7]. pH of semen was recorded by using the digital multi parameter pH meter HI2020 (Hanna Instruments, Italy).

Sperm viability (live sperm percentage) and abnormalities were examined using the eosin–nigrosin staining technique (Swanson & Bearden, 1951) [27]. Briefly, for preparation of slide, small drop (30 µl) of semen was placed on clean, grease free slide and added equal amount of eosin–nigrosin dye mixed well with blunt end fine glass rod. After a min, a thin smear from the mixture was prepared on glass slide, air dried and sperm viability, and abnormality was assessed by counting 300 spermatozoa in different microscopic fields at 40X and 100X, respectively. The viability of spermatozoa was determined as appearance of complete exclusion of stain, while morphological deformity in head, body or tail was considered as abnormality in spermatozoa (Evans and Maxwell, 1987) [7].

Statistical analysis

The data were analysed using SPSS (Version 25.0, Chicago, IL, USA). The results were expressed as Mean ± SE using SPSS (Version 25.0, Chicago, IL, USA).

Result

Results of the present study are depicted in Table-1. The average semen ejaculate volume (ml) was 0.915±0.04 with a range of 0.74±0.09 to 1.10±.12 with no significant difference among the rams. Mass motility (0-5 scale) of semen was observed to vary from3.84±0.31 to 4.5±0.22 with an overall mean of 4.5±0.22withno significant difference among the animals. The mean individual progressive sperm motility percentage in the semen of different rams ranged between 73.84±1.1 to79.17±1.14 with the overall mean value of 76.84±1.38. Statistical analysis did not reveal any significant variation in progressive sperm motility among the rams.

The sperm concentration (million/ml) ranged from 3719.67±58.32 to 4018±106.76with an overall mean of 3927.08±38.38 with no significant difference among the animals. The overall mean pH of fresh semen was 6.913±0.03 with a range of 6.79±0.13 to 7.04±0.04 in Magra rams. There was no significant difference for the seminal pH among the animals. The average values of live sperm percentage in fresh ram semen was79.938±0.51, ranging from 77.34±1.59 to 82.67±1.20. The percentage of live sperm among rams showed no significant difference. In the semen of various rams, the mean sperm abnormality percentage ranged from 2.84±0.31 to 3.34±0.21, with a mean percentage of 3.125±0.09. A statistical examination of rams as a whole sperm showed no discernible difference between the individual rams.

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

Parameter	Mean ±SEM (range)	Parameter	Mean ±SEM (range)
Volume (ml)	0.915±0.04 (0.74±0.09to 1.10±.12)	Abnormal spermatozoa (%)	3.125±0.09 (2.84±0.31 to 3.34±0.21)
pH	6.913±0.03 (6.79±0.13to 7.04±0.04)	Mass Motility (0-5)	4.23±0.10 (3.84±0.31to 4.5±0.22)
Progressive motility (%)	76.396±0.53 (73.84±1.1to 79.17±1.14)	Sperm concentration (million/ml)	3927.08±38.38 (3719.67±58.32to 4018±106.76)
Sperm viability (%)	79.938±0.51 (77.34±1.59 to 82.67±1.20)		

Discussion

In the present study, semen ejaculate volume (ml) was 0.9150.04 ml, which is consistent with studies conducted on other indigenous sheep breeds by Pawar (2003) [20] in Patanwadi rams and Mahala (2019) in Magra rams. Although Bharti *et al*. (2007) [3], Kumar (2010), Toppo (2013) [28] and Rajasri (2016) [22] found lower values of semen ejaculates in various indigenous sheep breeders. In contrast, greater values in Patanwadi half-bred rams have been observed by Dabas (1991) [5]. These variations between different reports may be the result of several factors, including the season, the breed and surrounding environment of the rams with others. Individual differences in vigour, testicular size, age, body weight, activity of the accessory sex gland, functional disparity, and the bioavailability of associated hormones could all contribute to the variation in ejaculate volume (Moghaddam *et al*., 2012) [16].

The overall mean mass motility of was observed to be 4.23±0.10 which was similar to the findings of Bharti (2007) [3]; Toppo (2013) [28] and Kurmi (2014) in Chhotanagpuri rams. Though higher scores of mass motility have been reported by Mehta *et al.* (1972) [14]; Sharma *et al.* (1973) [26];

Kayali *et al.* (2014) in indigenous sheep breeds. Lower scores of mass motilityhave also been reported by (Pawar, 2003; Alcay *et al*. 2014; Rahman *et al.* 2015; Rajasri 2016; Manga 2017) [20, 1, 21, 22] for different indigenous breeds of ram. Bharti (2007) [3] and Kumar (2010) observed no significant difference in mass motility score between individual rams, which is in consonance with the present finding. The mass motility of sperms in different breeds of rams has been studied by various workers and variable reports have been made. Mass activity has been observed to vary with breed, biochemical constituents of semen and pre sexual stimulation (Salisbury *et al*., 1978) [25].

The overall mean live sperm percentage was found to be 79.94±0.51in Magra ram semen during the present study. The results of the present study are in accordance with finding of Pawar (2003) [20]; Bharti (2007) [3] and Mahala (2019) in Indegenous rams. Higher value of live sperm recorded by Durga *et al.* (1977) [6] in Mandhaya rams, Ingale (1996) in Nali rams, Kurmi (2014) in Chhotanagpuri rams and Khalifa (2017) [11] in Barki ram semen. Live sperm percentage may vary due to methodological errors, feeding variations, environment, breeds of rams and their adaptability in varying agro climatic conditions of the places of investigations, climate, season and frequency of semen collection (Pandey *et al*., 1985) [19].

The overall mean value of individual sperm motility percentage in fresh semen was 76.84±1.38. These results are in accordance with the results of Soltanpour *et al.* (2014) in Ghezel×Baluchi (GH×BL) and Ghezel×Arkhar merino (GH ×AM) crossbred ramsand Mahala (2019) in Magra rams. Higher scores of individual sperm motility than the present findings have been reported by Mehta *et al.* (1972) [14] in Russian Merino, Malpura and their crossbreeds.

The overall mean sperm concentration million/ml of semen in Magra ram was calculated to be 3927.08±38.38 million during the present study which is in corroboration with the reports of Yadav and Sattar (2001) [30] and Mahala (2019) in Magra rams. Whereas, higher values have been reported by Kumar (2010); kurmi (2014) and Toppo (2013) [28] and Rajasri (2016) [22] in different indigenous sheep breed. Lower values were recorded by Pawar (2003) [20] in Patanwadi ram, George *et al.*(2003) [8] in Garole rams, Alcay *et al.*(2014) [1] in Kivircik and Awassi rams, Manga (2017) in Deccani rams and khalifa (2017) [11] in Barki rams. The difference in sperm concentration might be due to climate, nutritional statusand frequency of ejaculation and method of semen collection of the rams under different experiments. The sperm concentration may also be influenced by frequency of collection, nutrition, genetic variation (Verma, 1999) [29] and breeding season (Dabas *et al.*, 1997; Gundogan *et al.*, 2007) [4, 9].

The overall mean pH was found to be 6.91±0.03 in Magra ram semen in the present study. The results of the present study are in accordance with Al-Samarrae (2009) in Arrabi sheep breeds and Mahala (2019) in Magra rams. Though higher scores of pH than the present findings have been reported by Khalifa (2017) [11] in Barki rams. Lower values were recorded by Moghaddam *et al.* (2012) [16] in Baluchi×Moghani (BL×MG) and Ghezel ×Arkharmerino (GH×AM) crossbred rams.

The overall mean value of abnormal sperm percentage in fresh semen was 3.13±0.09. Statistical analysis of variance did not reveal any significant variation in abnormal percentage among rams**.** These results are in accordance with the results of Kakadiya *et al*. (1995) and Pawar (2003) [20] in Patanwadi rams. Whereas, higher values have been reported byYadav and Sattar (2001) [30] in crossbred rams, Bharti *et al.* (2007) [3]; Kumar (2010) in Chhotanagpuri rams, Manga (2017) in Deccani rams and Mahala (2019) in Magra rams. Lower value recorded by Durga *et al*. (1977) [6] in Mandya rams and Mittal and Ghosh (1979) [15] in Marwari and Jaisalmeri ram.

In conclusion, the results of the present study revealed that Magra ram semen quality is better for preservation and can be used for artificial insemination for faster genetic improvement at field level.

Acknowledgement

Authors are thankful to Dean and Dean PGS, CVAS, Bikaner; Head, VGO, CVAS, Bikaner for providing financial and technical support. Authors are also thankful to the Director, ICAR-CSWRI, Avikanagar, Project Coordinator, NWPSI and PI on Magra Field Unit; Head, ICAR-CSWRI, ARC, Bikaner for providing animals and lab facilities during the research period.

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