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Seasonal response of spermatozoa to hypo-osmotic swelling test (HOST) and correlation with seminal characteristics of Magra ram

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Abstract

Study on the seasonal response to Hypo-osmotic Swelling Test (HOST) and correlation with seminal attributes are essential to predict quality and fertility of semen. A total of 7 Magra rams, aged 1.5-3 years, weight 38 ± 5 kg, were sampled twice in a week for 3 consecutive weeks during breeding and non breeding seasons. A total of 84 ejaculates (6ejaculates/ram/season) were collected and evaluated for HOST and seminal attributes. Results of the study indicated significantly ($p < 0.05$) higher mean value of HOST (percent) during breeding compared to non breeding season. Semen parameters were correlated to assess semen quality and observed that semen volume was negatively correlated with sperm concentration and was positively correlated with sperm per ejaculate and semen index. Semen pH was positively correlated with live sperm and HOST percentage. HOST was positively correlated with mass motility, individual sperm motility, live sperm percentage and was negatively correlated with abnormal sperm percentage. Further mass motility was positively correlated with individual sperm motility, semen index and live sperm percentage and similar to HOST, was negatively correlated with abnormal sperm percentage. Individual sperm motility was positive with live sperm percentage and semen index and was negative with abnormal sperm percentage. Therefore, the present study revealed that Magra rams are capable of breeding throughout year and HOST can be used as an important tool in assessing the seminal quality.

Keywords: HOST, seminal attributes, correlation, magra ram, Seasonal response

1. Introduction

Reproductive seasonality is mainly shaped by photoperiod, season and genetics (Malpoux, 2006, Sarlos *et al.* 2013) [22, 28] and clearly reflected by detectable changes in behaviour, testicular dimensions, gametogenesis and hormone secretion (Glover *et al.* 1990) [15]. There is a need to acquire more information concerning the seasonal variations in ram semen characteristics (Karagiannidis *et al.* 2000 [19], Talebi *et al.* 2009) [31] to bring revolution in the assisted reproductive technologies particularly artificial insemination (AI) in ovine species. Physical characteristics of semen alone are not completely satisfactory for semen appraisal (Barrios *et al.* 2000) [5]. Plasma membrane integrity is a fundamental requisite for sperm survival and successful conception (Gwathmey *et al.* 2006) [16]. Super-vital staining and HOST are two basic tools to evaluate the sperm membrane integrity where HOST proved to be a good and the most preferred due to simple, easy, economical and fast test (Tartagni *et al.* 2002, Padrik *et al.* 2012) [32, 26]. Yearlong comparative studies during breeding and non-breeding seasons with their correlation could be useful for completing the findings and reducing the reproductive challenges of the species. To the best of our knowledge, there is no study regarding seasonal variation of HOST with correlation of seminal attributes in Magra sheep. Therefore, the present study was undertaken to uncover the effect of season on HOST responsive spermatozoa and to assess its correlation with seminal characteristics in Magra ram.

2. Materials and Methods

2.1. Geographical location and climate

The trial was performed as per compliance of ethical standards at ICAR-CSWRI ARC, Bikaner during non-breeding (May-June) and breeding season (August-September) of year, 2018. Bikaner is situated in the arid region of the Western part of Rajasthan (India) which is

located at 73°28'2"E longitude and 28°01'N latitude at an altitude of 230 m above the mean sea level with annual rainfall 200-300 mm, the average annual minimum and maximum ambient temperature ranges from 4°C to 46°C. The summers are hot, while winters are very cold.

2.2. Experimental Animals

Magra rams (n=7), aged 1.5-3 years (Mean-2.3), weight 38±5 kg having good libido and adaptability to ejaculate semen in artificial vagina (AV), were enrolled in the present study during both the seasons. All the rams were fed on the standard diet, formulated according to the requirement for mature breeding ram and were allowed for grazing at least for 7 h per day in range as per standard ICAR (2013). All the rams were maintained in isomanagerial condition under proper hygienic conditions, had free access to water and were kept separated from ewes. A general health management program for deworming and vaccination for disease prevention was followed during the experiment as per yearly health calendar of the institute.

2.3. Semen collection and quality evaluation

Semen was collected from each ram twice in a week for 3 consecutive weeks using sterilized AV in a pre-warmed semen collection cup at 37°C during morning hours before feeding throughout the period of study. Before commencement of the study, initial 2 semen collections were thrown away to minimize epididymal reserve. Ewe was used as dummy and 2 false mountings were carried out for optimum semen production. Prior to collection, the prepuce was wiped clean to prevent contamination of semen. Soon after collection, the semen collection cups were labeled with ram number and transferred to the laboratory and immersed in a water bath at 37°C. A total of 84 ejaculates (6 ejaculates/ram/season) were collected from all the rams and were evaluated for volume (mL), sperm concentration ($\times 10^9$ sperm/mL), mass motility (scale of 1-5), progressive sperm motility, percentage live and normal sperm, total number of spermatozoa per ejaculate (Evans and Maxwell 1987) [14] and semen index (Moghaddam *et al.* 2012) [23]. HOST was performed as per method given by Sharma *et al.* (2012) [29]. Semen pH was recorded using digital multiparameter pH meter HI2020 (Hanna Instruments, Italy).

2.4. Statistical Analysis

The values obtained from parameters were analyzed by ANOVA (Analysis of Variance) using Statistical Package for Social Science (SPSS, version 25). Duncan's test was used to compare the means at a probability level of 5%. The Pearson's (r) correlation test was used to calculate correlations between HOST and other sperm parameters using SPSS version 25.

3. Results and Discussion

The growing interest in semen biology particularly in its physical attributes and their correlation is largely to recognize the problems of infertility and to predict the status of sexual activity in rams so that expanding demands of AI can be fulfilled. The quality of semen assessed by various conventional parameters and their correlations are important as on the basis of one parameter, a fair idea of other parameters can be formulated. Therefore the correlation of sperm plasma membrane with other physical seminal attributes is more important as it is a continuous structure

covering the whole sperm (Karp 2009) [20].

Results of the study revealed that overall mean value of HOST (Mean±SE) was 59.07±0.54 (ranged from 56.38±2.43 to 61.05±0.93) and 66.94±0.48 (ranged from 63.59 ±1.48 to 68.72±1.05) with a significant ($p < 0.05$) difference during non breeding and breeding season, respectively in Magra rams. Seasonal effect was seen in Murciano-Granadina and Payoya bucks where mean percentage of HOST positive sperms were the highest in summer and the lowest during spring season (Arrebola and Abecia 2017) [3]. The present findings are comparable of HOST responsive spermatozoa in Garut (Nalley and Arifiantini 2013a) [24] and Decani rams (Rajashri *et al.* 2017) [27]. The higher values of sperm positive to HOST compared to the present study were recorded by Boediono *et al.* (2004) [8] in Garut ram, Valente *et al.* (2010) [33] in Portuguese Serra da Estrela and Saloia ram, Nalley and Arifiantini (2013b) [25] in local ram of Indonesia, Alcay *et al.* (2014) [1] in Kivircik and Awassi ram and Azizunnesa *et al.* (2014) [4] in Indigenous ram. The lower values of HOST positive spermatozoa were observed by Soylu *et al.* (2007) [30] and Bucak *et al.* (2008) [11] in local rams, Camara *et al.* (2011) [12] in Santa Ines rams and Nalley and Arifiantini (2013a) [24] in Garut rams. These variations in HOST positive spermatozoa may be due to animal species breed, sperm membrane biochemical constituents, osmolarity and time of incubation (Barrios *et al.* 2000, Amorim *et al.* 2009) [5, 2].

Results of the overall correlation coefficients of semen volume was negatively correlated with sperm concentration ($r = -.232$) and was positively correlated with sperm per ejaculate ($r = 0.817$) and semen index ($r = 0.834$) which is consonance with findings of Karagiannidis *et al.* (2000) [19] in Chios and Friesian rams and Moghaddam *et al.* (2012) [23] in Iranian crossbred rams. Semen pH was positively correlated with live sperm percentage ($r = 0.509$) and HOST percentage ($r = 0.375$) and was negatively correlated with sperm concentration ($r = -0.389$), abnormal sperm percentage ($r = -0.227$) and sperm per ejaculate ($r = 0.226$) which are in agreement with the finding of the Moghaddam *et al.* (2012) [23] and Belkhiri *et al.* (2017) [7]. Juma and Al-Kassab (2009) [18] inferred that pH was reduced during summer due to increase of sperm concentration in Hamdani breed indicating negative correlation.

In the present study, mass motility was positively correlated with individual sperm motility ($r = 0.928$), live sperm percentage ($r = 0.432$), HOST percentage ($r = 0.442$) and semen index ($r = 0.218$) whereas, it was negatively correlated with abnormal sperm percentage ($r = -0.430$) and sperm concentration ($r = -0.305$) which is similar with findings of Chella *et al.* (2017) [13] in Zulu rams and Belkadi *et al.* (2017) [6] in Ouled Djellal rams. Moghaddam *et al.* (2012) [23] reported that mass motility was negatively correlated with abnormal sperm percentage and positively correlated with individual sperm motility which also favour the present findings. Individual sperm motility was positively correlated with live sperm percentage ($r = 0.465$), HOST percentage ($r = 0.436$), semen index ($r = 0.257$) and was negatively correlated with abnormal sperm percentage ($r = -0.387$) and sperm concentration ($r = -0.314$) which has compatibility with earlier studies in different livestock species (Moghaddam *et al.* 2012, Kaur and Sandhey, 2014, Belkhiri *et al.* 2017) [23, 21, 7]. The correlation between individual sperm motility, live sperm percentage and HOST was expected since they all are related to plasma membrane integrity (Brito *et al.* 2003 [10], Rajashri *et al.* 2017) [10, 27]. The semen samples showing

higher individual sperm motility, were also showing high live sperm percentage, HOST and low abnormality in the semen which may be attributed to the fact that motility is a function of intracellular adenosine triphosphate (ATP) content, hence, rapid leakage of intracellular ATP through the damaged sperm membrane is certain to affect sperm motility (Januskauskas and Rodriguez-Martinez, 1995 [17], Bohlooli *et al.* 2012) [9].

Live sperm percentage was positively correlated with HOST percentage ($r = 0.769$) and was negatively correlated with abnormal sperm percentage ($r = -0.459$) and sperm per ejaculate ($r = -0.173$) in the current study which are similar with findings of Moghaddam *et al.* (2012) [23]; Rajashri *et al.* (2017) [27] and Belkhiri *et al.* (2017) in rams. Abnormal sperm percentage was positively correlated with sperm per ejaculate ($r = 0.241$) and was negatively correlated with HOST percentage ($r = -0.359$) and are favored by reports of Bohlooli

et al. (2012) [9]. Sperm per ejaculate was positively correlated with semen index ($r = 0.882$). A significant negative correlation between HOST and sperm abnormalities indicates impaired plasma membrane activity in the defective or abnormal spermatozoa. Thus, the correlation of HOST with other seminal parameters indicates its efficacy as screening test for routine semen evaluation.

In conclusion, the present investigation revealed that Magra ram semen maintains HOST range for normal fertility during both the seasons indicating that breed can be used for breeding purpose during both the seasons. Results of correlation of HOST and seminal attributes indicates the importance of the HOST in assessing the functionality of spermatozoa and could be a helpful indicator in assessing the semen quality as a simple, frugal, easy tool and plays a pivotal role to rule out seminal quality which is yet to be explored for better utilization of this unique breed.

Table 1: Overall correlation matrix of physical attributes of semen in Magra rams

Parameters	Volume	pH	MM	IM	SC	Live sperm	Abnormal sperm	HOST	SPE	SI
Volume	1	-.049	.063	.076	-.232 *	-.022	.143	.071	.817 **	.834 **
pH		1	.117	.115	-.389 **	.509 **	-.227 *	.375 **	-.226 *	-.120
MM			1	.928 **	-.305 **	.432 **	-.430 **	.442 **	-.121	.218 *
IM				1	-.314 **	.465 **	-.387 **	.436 **	-.118	.257 *
SC					1	-.469 **	.260 *	-.414 **	.721 **	.510 **
Live sperm						1	-.459 **	.769 **	-.173	-.012
Abnormal sperm							1	-.359 **	.241 *	.081
HOST								1	-.184	.038
SPE									1	.882 **
SI										1

*: Significance at $p < 0.05$, **: Significance at $p < 0.01$ in above matrix, N=84

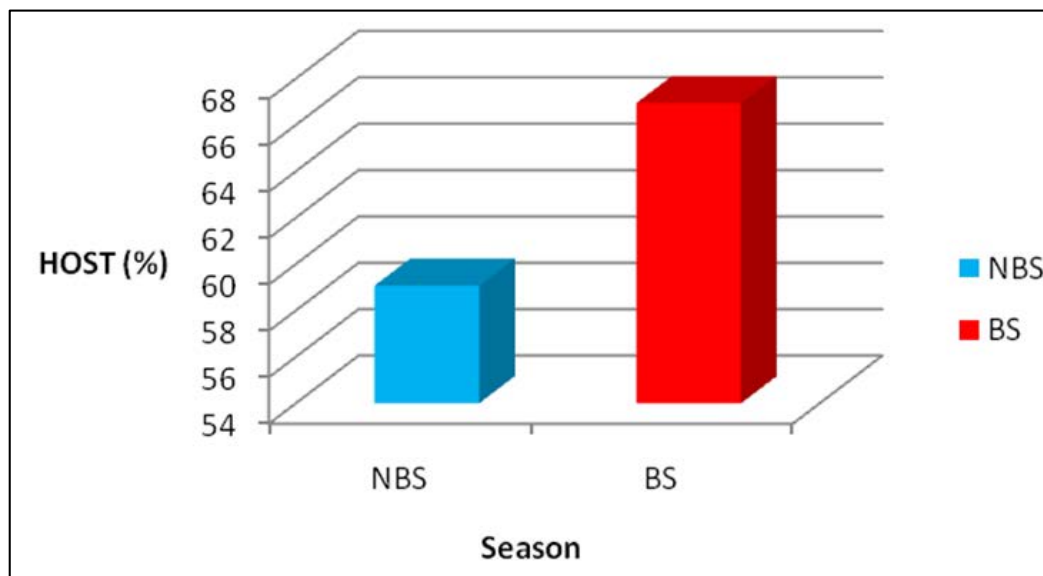


Fig 1: Mean percentage of HOST responsive sperms during breeding (BS) and non-breeding seasons (NBS) in Magra rams

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