

## Original Research Article

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## Semen Characteristics of NARI Suwarna Rams during Breeding (Winter) and Non-breeding (Summer) Seasons

Venkanagouda Doddagoudar\*, M. K. Tandle, R. G. Bijurkar, N. A. Patil,  
Ashok Pawar, Shrikant Kulkarni and Vinay Tikare

Veterinary College, KVAFSU, Nandinagar, Bidar, Karnataka, India

\*Corresponding author

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The NARI Suwarna ram, a triple cross sheep strain known to carry the *FecB* gene was evaluated for seasonal variations in seminal characteristics during breeding (winter) and non-breeding (summer) seasons. In the study, semen samples from five sexually mature rams were evaluated for various seminal parameters, the live sperm percentage was significantly ( $p < 0.05$ ) higher during breeding (winter) season whereas other semen characteristics *viz.* semen volume, colour, wave motion, individual motility, sperm concentration, acrosome integrity, sperm abnormalities did not show any significant variation ( $p > 0.05$ ) between or within breeding (winter) and non-breeding (summer) seasons. Hence, it is concluded that NARI Suwarna rams can produce equally good quality semen in winter as well as summer seasons.

### Introduction

Many sheep breeds do have a fairly well documented gonadal activity cycle which is influenced mainly by photoperiod (Gómez-Brunet *et al.*, 2008) and high latitudes (Sarlós *et al.*, 2013). NARI-Suwarna is a strain of sheep developed by Nimbkar Agricultural Research Institute (NARI), Phaltan, Maharashtra with 90% Deccani or 60% Deccani + 30% Madgyal and 10% Garole breed proportions. It is known to inherit *FecB* gene from the Garole breed capable of producing and raising twin lambs, hence improving profitability in sheep farming.

Seasonal variability in different breeds is found to be influenced by the latitudes where animals are raised, higher the latitude in the northern hemisphere, greater was the seasonality (Abecia *et al.*, 2012). However, in tropical regions, other ambient factors such as ambient temperature, relative air humidity, rain distribution and nutrition seem to have effects on reproductive physiology in seasonal animals (Rosa and Bryant, 2003). Some studies have reported seasonal variability in tropical and subtropical breeds (Santos *et al.*, 2015; Belkadi *et al.*, 2017) whereas others have reported no seasonal variability (Benmoula *et al.*, 2017; Malejane *et al.*, 2014).

except for minor variations. Previous studies have reported some of the seminal characters in NARI Suwarna rams (Kadaganchi 2017; Jasrotia 2018; Muniyappanavar *et al.*, 2020) but did not study seasonal variability of these characters which may hold the key in the ability to preserve its semen for longer durations so that it can be used for up-gradation or crossbreeding of local sheep to improve their reproductive efficiency. The studies on seasonal changes in the seminal quality of Indian sheep breeds in general and NARI Suwarna rams in particular are scarce. Hence, the present study was conducted to evaluate the seasonal changes in NARI Suwarna ram semen.

## Materials and Methods

### Location and selection of animals

The study was carried out at Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bidar, Karnataka, India which lies between 17°35' and 18°29' North latitude and 76°41' to 77°39' East longitude. The study period extended over six months from December-2018 to May 2019 and split equally into two seasons, winter from December-2018 to Feb-2019 and summer from March to May-2019. Five sexually mature NARI Suwarna rams maintained under semi-intensive housing system with free grazing for 5-6 h daily and fed concentrate sheep feed at 200 g per day along with routine deworming and vaccinations were used for semen collection.

### Semen collection

Semen was collected twice in a week using ram as a dummy with the artificial vagina (AV) as per the standard procedure during early morning hours before feeding throughout the study period. Soon after collection, the semen collection tubes marked and transferred to a water bath at 36°C (Plate

1 and 2).

### Semen Evaluation

The semen volume was measured using a graduated collecting tube (0.1 ml accuracy) soon after collection and samples with foreign material or abnormal colour were rejected.

For wave motion, a drop of neat semen placed on a pre-warmed glass slide (37°C) without any coverslip, examined directly under the microscope (100x) and the wave motion was scored on a scale of 1-5 (Rahman 2014).

The individual motility of spermatozoa was assessed by placing a coverslip on a drop of diluted semen (10µl semen mixed with 200µl normal saline) on a clean glass slide under the microscope with biothermal stage attached. The motility was observed under high power at 400X magnification at 5 different fields and averaged and expressed in terms of percentage of progressively (0-100) motile sperm (Rahman, 2014).

The sperm concentration was expressed as the total number of spermatozoa in millions (10<sup>6</sup>) per ml of neat semen. Sperm concentration in semen was determined by using an improved Neubauer counting chamber after dilution of semen with 1:1000 with diluting fluid (Perumal *et al.*, 2017). The sperm concentration was calculated using the formula

$$\text{Number of sperm/ml} = \frac{N \times D \times 4}{n} \text{ million sperm}$$

Where N = Number of spermatozoa counted; D = Dilution rate; n = number of tertiary squares counted.

To ascertain the percentage of live spermatozoa, semen mixed (1 drop) with one drop of Eosin (2 %) and one drop of Nigrosin (10 %) and smears were prepared within 30

seconds of mixing and examined under oil immersion objective (1000X) after air drying (Srivastava and Pande, 2017). All stained and partially stained spermatozoa were considered as dead and the unstained spermatozoa as live (Plate 3). The percentage of live spermatozoa was determined by counting at least 200 spermatozoa.

Acrosome integrity was evaluated by using Giemsa stain, a small drop (10 µl) of each sperm sample was placed on a grease-free slide and a drop (30 µl) of Sorenson's phosphate buffer was mixed with it and a smear was prepared. Then air-dried smears were placed in Hancock's fixative for 15–20 min in Coplin jar. Post-fixation, the slides were washed under slow-running tap water for another 15–20 min followed by washing with distilled water. Finally, the slides were placed in a Coplin jar containing Giemsa working stain and left overnight at 37 °C. On next morning, the slides were removed, washed with slow-running tap water and finally with distilled water, air-dried and were observed under oil immersion at a total magnification of 1000X. A total of two hundred spermatozoa were counted and the percentage of intact acrosome was calculated in the neat semen samples (Watson, 1975) (Plate 4).

Hypo-osmotic swelling of spermatozoa were evaluated by incubating semen samples with test (150 mOsm/L) and control (300 mOsm/L) solutions in a water bath at 37°C for 30 minutes followed by examining a small drop of sample under phase contrast microscope at 400x and curled sperms counted as positive HOST and others as negative (Plate 5 and 6). The proportion of swollen sperm in the control samples were subtracted from the proportion of swollen sperm in the HOST solution, the resultant figure was considered as a percentage of HOST reactive spermatozoa (Jeyendran *et al.*,

1992).

Rose Bengal stain (3%) was used for counting the percentage of normal and abnormal sperm (Pervage *et al.*, 2009). Two drops of sodium-citrate buffer were placed on a clean dry glass slide; one drop of mixed semen was added and spread by covering with another slide. The slide was dried in the air and stained with Rose Bengal stain for 15-20 minutes, excess stain rinsed by dipping the slide in distilled water. The slide with smear was dried in the air and observed under a microscope with oil immersion magnifications (1000X) for various sperm abnormalities by counting two hundred spermatozoa and the percentage was calculated (Plate 7).

## **Results and Discussion**

### **Weather data for breeding (winter) and non-breeding (summer) seasons during the study period**

The weather data, temperature (max & min), relative humidity (RH), bright sunshine hours (BSSH) and rainfall from December 2018 to May 2019 was collected from Agriculture Research Station, Halladkeri, Bidar, Karnataka, India and is presented in Figure 1. The minimum and maximum temperatures along with relative humidity showed appreciable variation between the seasons whereas, bright sunshine hours were slightly lower for winter season and both seasons had similar low total rainfall (Figure 2).

### **Volume, colour, wave motion and individual motility**

The semen volume, colour, wave motion and individual motility did not show any significant variation ( $p>0.05$ ) between or within breeding (winter) and non-breeding (summer) seasons in NARI Suwarna rams (Table 1). These findings are in line with

studies of Cárdenas-Gallegos *et al.*, (2012) and Benmoula *et al.*, (2017) reporting no seasonality for semen volume, wave motion and individual motility in different breeds of rams. Further, the observed colour of semen as creamy white was in agreement with earlier reports for NARI Suwarna ram semen by Kaimal (2015), Kadaganchi (2017), Jasrotia (2018) and Muniyappanavar (2019) as creamy to creamy white colour.

In contrast, studies have reported significant seasonal variation for volume, colour, wave motion and individual motility in Hamdani rams (Juma and Al-Kassab, 2009) and increased semen volume in summer, semen colour being milky to creamy during summer and spring while having a thin density in autumn and winter with higher mass motility and individual motility in the summer season. Similar seasonal variations were also reported for volume, wave motion and individual motility in Dorper (Malejane *et al.*, 2014), Naimi and Najdi rams (Al-Anazi *et al.*, 2017). Further, some studies have reported seasonal variations for volume but not for wave motion and individual motility (Milczewski *et al.*, 2015; Belkhiri *et al.*, 2017) whereas, other studies have reported seasonal variations for wave motion and individual motility but not for semen volume (Chella *et al.*, 2017).

The variations among the findings of different authors could be due to breed (Gündoğan 2007; Zamiri *et al.*, 2010) and seasonal variations (Hamidi *et al.*, 2012; Oláh *et al.*, 2013) or age, method of semen collection and frequency of semen collection (Foote, 1978; Salhab *et al.*, 2003; Malejane *et al.*, 2014). Further, wave motion is usually seen as a superficial indication of the motility and viability of the sperm (O'Hara *et al.*, 2010) and it is the function of sperm concentration along with motile sperm percentage (McGowan, 2019) and assessments are highly subjective and often have limited repeatability

(DeJarnette, 2005).

### **Sperm concentration, live sperm, plasma membrane integrity and acrosome integrity**

The overall average live sperm percentage was significantly ( $p < 0.05$ ) higher in breeding (winter) than non-breeding (summer) season and plasma membrane integrity showed significant ( $p < 0.05$ ) variation only within non-breeding (summer) season but not between breeding (winter) and non-breeding (summer) seasons. The sperm concentration and acrosome integrity did not vary significantly ( $p > 0.05$ ) between or within breeding (winter) and non-breeding (summer) seasons in NARI Suwarna rams (Table 2).

The present findings of seasonal variability in live sperm percentage are consistent with the findings of higher viability noticed for winter in Ouled Djellal (Belkadi *et al.*, 2017) and Zulu rams (Chella *et al.*, 2017) and contradictory with the findings of lower viability noticed in winter for Ghezel and Mehraban (Zamiri and Khodaei, 2005), Dorper (Malejane *et al.*, 2014) and Boujaad rams (Badi *et al.*, 2018). Some studies have reported no seasonality for sperm viability as in INRA180 rams (Benmoula *et al.*, 2017). Regarding HOST, the present findings are comparable with findings of Kaimal (2015) in NARI Suwarna rams with no seasonal variation but contradictory to Ntemka *et al.*, (2019) with reported seasonal variation for sperm viability in Chios rams. Regarding sperm concentration and acrosome integrity, the present study is consistent with Azawi *et al.*, (2012) in Awassi rams observing no variations between the seasons for both these parameters while for sperm concentration only with other workers (Kaimal 2015; Belkhiri *et al.*, 2017; Benmoula *et al.*, 2017; Ntemka *et al.*, 2019). Further, in complete contrast to the present study, Azawi and

Ismaeel (2012) reported seasonal variations with lower sperm concentration and viability with higher acrosomal damages during winter in Awassi rams. Similarly, Kumar *et al.*, (2016) in Jakhrana bucks observed similar post-thaw HOST percentages between the seasons but significantly higher post-thaw acrosome integrity during the winter season. Further, D'Alessandro and Martemucci (2003) also observed significantly lower acrosomal damage during the breeding season in boars.

The variations in the live sperm percentage, concentration and acrosome integrity have been reported to vary due to methodological errors, feeding variation, breeds of rams and their adaptability in varying agro-climatic conditions of the places of investigation, season, frequency and method of semen collection (Oláh *et al.*, 2013; Malejane *et al.*, 2014). In the present study, plasma membrane

integrity was varying only within the non-breeding (summer) season which can be attributed to lower viability as observed in the summer resulting in higher dead sperm count releasing more ROS and damaging the plasma membrane from the resultant lipid peroxidation due to its high PUFA composition (Sikka, 2004; Ayala *et al.*, 2014). However, this did result in the significant variation only within the summer season but not between the seasons, which could be due to variation in the adaptability of individual rams to higher temperatures during non-breeding (summer) season.

**Sperm abnormalities**

The sperm abnormalities did not vary significantly ( $p>0.05$ ) either between or within breeding (winter) and non-breeding (summer) seasons in NARI Suwarna rams.

**Table.1** Volume, colour, wave motion and individual motility (Mean±Standard error) in fresh semen of NARI Suwarna rams during breeding (winter) and non-breeding (summer) seasons

Parameters	Volume (mL)		Colour		Wave motion (1-5)		Individual motility (%)		
	Ram No.	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1		0.82±0.04	0.90±0.04	Creamy white	Creamy white	4.67±0.21	4.67±0.21	94.44±0.70	95.00±0.74
2		0.77±0.04	0.85±0.05	Creamy white	Creamy white	4.67±0.21	4.83±0.17	96.11±0.56	95.56±0.70
3		0.82±0.07	0.85±0.07	Creamy white	Creamy white	4.33±0.21	4.50±0.22	95.56±1.65	97.22±0.56
4		0.77±0.05	0.80±0.04	Creamy white	Creamy white	4.50±0.22	4.50±0.22	96.67±1.72	97.22±0.55
5		0.77±0.05	0.83±0.05	Creamy white	Creamy white	4.83±0.17	5.00±0.00	96.67±1.22	95.56±0.70
<b>Overall</b>		0.79±0.02	0.85±0.02	Creamy white	Creamy white	4.60±0.09	4.70±0.08	95.89±0.54	96.11±0.32

**Table.2** Sperm concentration (million/mL), live sperm (%), plasma membrane integrity (%) and acrosome integrity (%) (Mean ± Standard error) in fresh semen of NARI Suwarna rams during breeding (winter) and non-breeding (summer) seasons

Ram No.	Sperm concentration (million/mL)		Live sperm (%)		Plasma membrane integrity (%)		Acrosome integrity (%)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1	4041±154	3933±185	92.42±0.96	90.42±0.94	71.67±1.66	70.58±0.87 <sup>AB</sup>	96.17±0.42	95.42±0.47
2	3783±138	3725±602	91.67±1.10	90.83±1.16	74.75±1.45	73.75±1.38 <sup>B</sup>	96.75±0.53	96.83±0.40
3	3516±159	3758±164	92.67±1.17	91.25±1.02	73.33±1.41	73.25±1.22 <sup>AB</sup>	95.92±0.42	96.50±0.83
4	3425±216	3483±557	93.75±0.80	90.50±1.18	74.00±1.09	71.75±0.53 <sup>AB</sup>	95.67±0.64	96.42±0.49
5	3608±182	3658±123	91.50±0.92	91.50±0.89	69.83±0.25	69.42±0.96 <sup>A</sup>	95.00±0.52	94.92±0.70
<b>Overall</b>	3675±82	3711±60	92.40±0.44 <sup>a</sup>	90.90±0.44 <sup>b</sup>	72.72±0.62	71.75±0.52	95.90±0.24	96.02±0.28

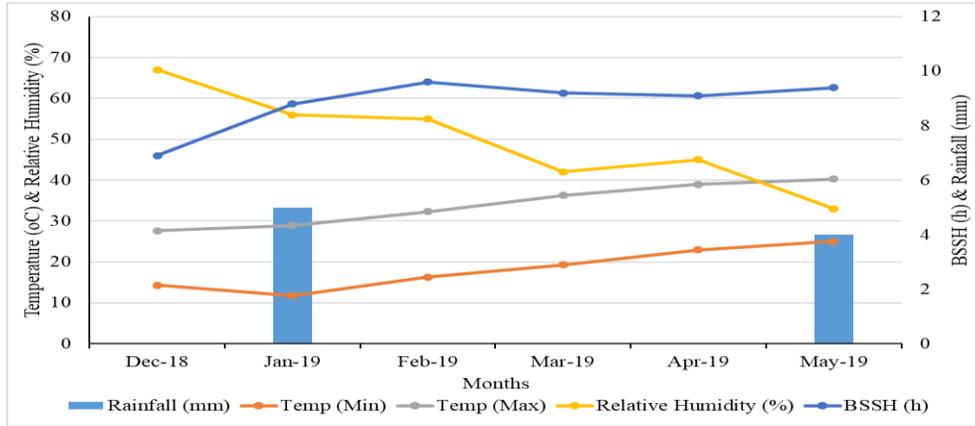
Note: <sup>ab</sup> Different superscript between the columns vary significantly (p<0.05)

<sup>AB</sup> Different superscript between the rows vary significantly (p<0.05)

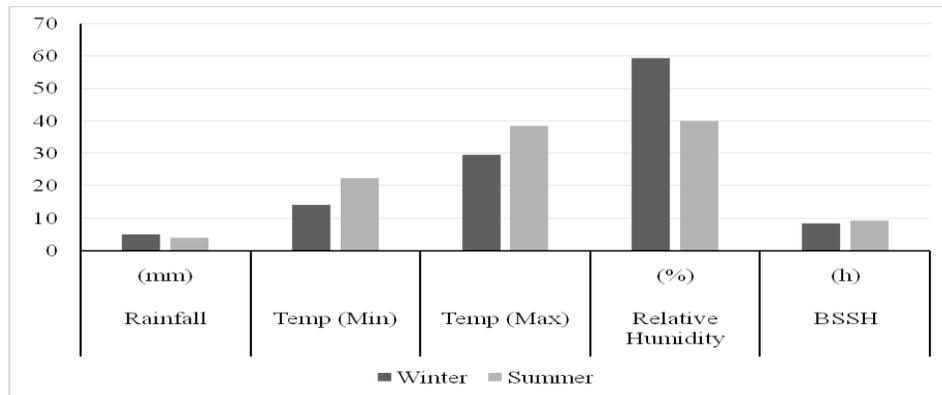
**Table.3** Percent of sperm abnormalities (Mean ± Standard error) in fresh semen of NARI Suwarna rams during breeding (winter) and non-breeding (summer) seasons

Ram No.	Sperm abnormalities (%)							
	Head		Midpiece		Tail		Overall	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
1	0.50±0.22	0.67±0.21	0.50±0.22	0.50±0.22	3.50±0.81	3.83±0.54	4.50±1.02	5.00±0.58
2	0.50±0.22	0.50±0.22	0.33±0.21	0.17±0.17	3.33±0.49	3.83±0.31	4.17±0.54	4.50±0.22
3	0.33±0.21	0.50±0.22	0.67±0.21	0.17±0.17	3.67±0.67	4.00±0.37	4.67±0.61	4.67±0.49
4	0.67±0.21	0.50±0.22	0.50±0.22	0.33±0.21	3.67±0.61	2.83±0.40	4.83±0.65	3.67±0.42
5	0.17±0.17	0.50±0.22	0.67±0.21	0.50±0.22	2.83±0.75	3.17±0.48	3.67±0.84	4.17±0.48
<b>Overall</b>	0.43±0.09	0.53±0.09	0.53±0.09	0.33±0.08	3.40±0.29	3.50±0.2	4.37±0.32	4.40±0.21

**Fig.1** Weather data for breeding (winter) and non-breeding (summer) seasons during the study period



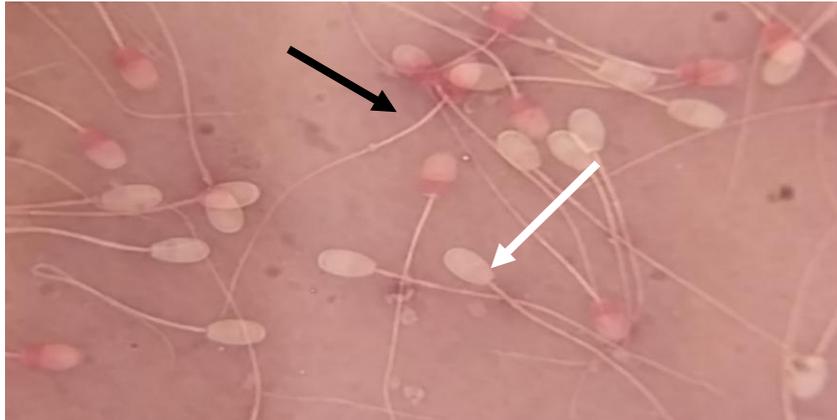
**Fig.2** Comparative weather data of breeding (winter) and non-breeding (summer) seasons during the study period



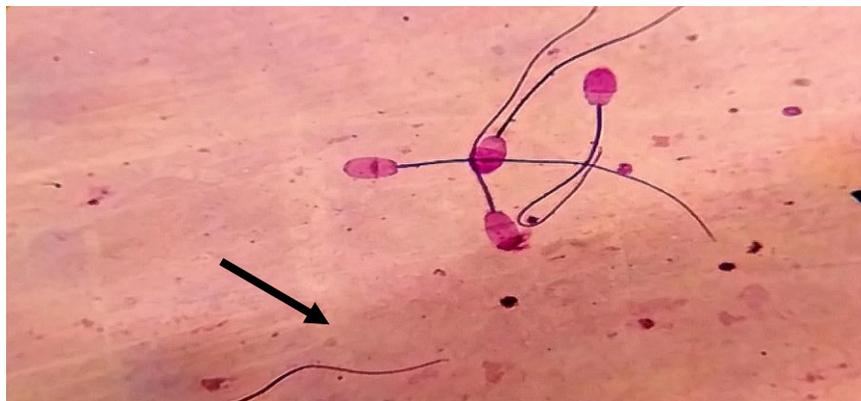
**Plate.1** Semen collection from ram



**Plate.2** EosinNigrosin stain for live (white arrow) and dead (black arrow) sperm count



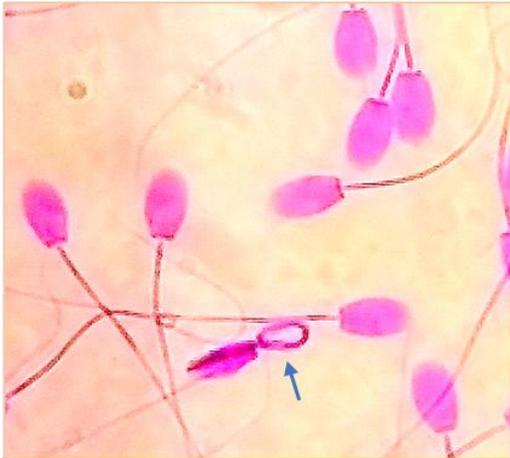
**Plate.3** Giemsa staining to determine acrosome integrity: Intact acrosome (white arrow) and damaged acrosome (black arrow)



**Plate.4** Hypo-osmotic swelling test: Swollen/ coiled sperm tails under phase contrast microscope (wet smear)



**Plate.5** Rose Bengal staining for sperm abnormalities



a. Coiled mid piece



b. Double head with tightly coiled tail



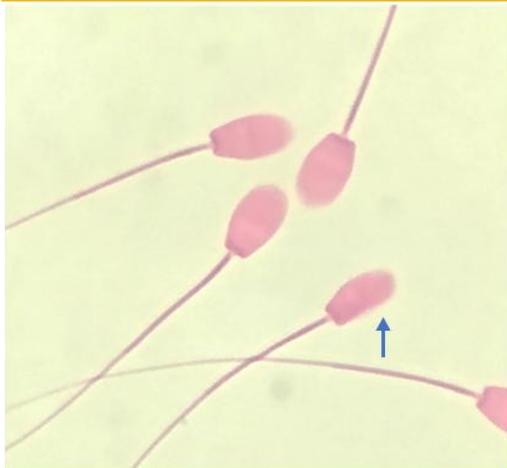
c. Abaxial attachment



d. Tail less head and coiled tail



e. Abnormal head



f. Abnormal head

The present findings are in agreement with no seasonal effect but with lower abnormalities, as reported by Kaimal (2015) in NARI Suwarna rams. Similarly, this is also in agreement with Milczewski *et al.*, (2015), Benmoula *et al.*, (2017), and Chella *et al.*, (2017) in Suffolk, INRA180 and Zulu breed respectively. Whereas, contradictory to the finding in Akkaramann and Awassi (Gundogan, 2006), Ghezel × Baluchi and Arkhar Merino×Ghezel (Moghaddam *et al.*, 2012), Racka (Sarlós *et al.*, 2013), Dorper (Malejane *et al.*, 2014), Saint Croix (Santos *et al.*, 2015), Ouled Djellal (Belkadi *et al.*, 2017) and Chios rams (Ntemka *et al.*, 2019). Findings of all these studies suggestive of significant variation in sperm abnormalities due to breed and season. As the present study did not show seasonal variation for sperm abnormalities, suggestive of equally good quality semen production both during breeding (winter) and non-breeding (summer) season in NARI Suwarna rams.

To conclude, despite significantly lower live sperm percentage during summer season NARI Suwarna rams can produce equally good quality semen in winter as well as summer season. Hence, can be used for semen collection during both the summer and winter seasons.

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