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Impact of the breeding and non-breeding seasons on the semen quality parameters in Magra ram

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Abstract
The present study was undertaken to assess the effect of season on semen characteristics OF Ma**g**ra rams. The semen was collected from Magra rams (n=7) (1.5-3 years) during non- breeding (May-June) and breeding season (August-September) of year, 2018. Semen volume, pH, sperm concentration, individual sperm motility, mass motility, live sperm percentages, semen index, total sperm per ejaculate and abnormal spermatozoa were evaluated to assess semen quality. The results showed that overall mean value of semen pH, mass motility (0-5 grade), individual sperm motility (percent) and live sperm percentage were significantly (p<0.05) higher during breeding season while, sperm concentration ($\times 10^6$/ml), abnormal sperm percentage, sperm per ejaculate ($\times 10^6$) were lower during non-breeding season. Semen volume (ml) and semen index ($\times 10^9$) did not differ significantly between these two seasons. However, semen quality was within breed able limit and ram can be used for breeding purpose during both the season.

Keywords: Seasonality, Magra ram, semen quality parameters

Introduction
Sperm quality, which is an indicator of fertility in rams, is affected by several factors including season, temperature, humidity and day length. Season is one of these elements that has the greatest impact on changes in semen quality and fertility. Seasonal differences in ram reproduction may have direct or indirect impacts (Olah *et al.*, 2013) [16]. Ram is highly responsible for the reproductive performance and genetic improvement in a flock as 50% of genes are transmitted by male to the progeny (Lassoued *et al.*, 2013) [12]. In sheep, sexual behavior and semen quality are the main factors that limit male reproduction efficiency along the year and could vary from breed to breed (Aller *et al.*, 2012; Moghaddam *et al.*, 2012) [2, 13], season of the year (Schanbacher and Lunstra, 1976) [19]. As a result, screening the semen for quality enables the removal of low-quality semen and can shed light on the spermatozoa's ability to fertilize. The analysis of sperm production is of great importance because it is significantly correlated with sexual activity. Consequently, the goal of the current study was to determine how seasonality affected Magra ram seminal features.

Materials and Methods
Experimental location
The present study was carried out at ICAR-CSWRI, ARC, Bikaner, and Rajasthan. During non-breeding (May-June) and breeding season (August-September) of year, 2018.

Experimental animal
Magra rams (n=7), aged 1.5-3 years, weight 38±5 kg and having good libido and adaptability to artificial vagina semen collection technique were used 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 suggested by Indian Council of Agricultural Research (ICAR, 2014). Animals allowed for grazing at least for 7 hours per day in range and provide feed in shed as per standard practice of the institute.

Collection of samples
Semen samples were collected twice in a week for 3 consecutive weeks using sterilized artificial vagina during morning hours before feeding during the breeding and non-breeding season. A total of 84 ejaculates (6 ejaculates/ram/season) were collected from all the rams.

Soon after collection, the semen collection cups were labeled and transferred to the water bath at 34 °C.

Semen analysis

Ejaculatory volume (ml) was directly from the graduated semen collection cup. A small drop of neat semen was put on a pre-warmed grease free clean, dry glass side and examined under low power microscope at 10X for mass motility which was assessed on the basis of vigour of wave motion of the spermatozoa in mass. Individual sperm motility was assessed by placing a cover slip on a drop of diluted semen (5 µl fresh semen mixed with 495 µl normal saline) on a grease free clean glass slide under the microscope with bio-therm stage attached. The motility was observed under high power at 40X magnification and expressed in terms of percentage of progressively (0-100) motile sperms. The concentration of spermatozoa was estimated with the help of Neubaur's counting chamber (Haemocytometer) and expressed in millions/ml of semen (Evan and Maxwell, 1987). The percentage of live spermatozoa was determined with the help of eosin – nigrosin stain as advocated by Swanson and Bearden (1951). The compound stain was prepared by dissolving 1 gm of eosin (BDH water soluble) and 5 gms of nigrosin (BDH water soluble) in 100 ml of buffer solution (2.94% sodium citrate dihydrate solution in double glass distilled water). The solution was warmed in water bath for 30 minutes and filter after cooling through what man's filter paper No. 40. A small drop of semen was taken in a clean, dry watch glass and mixed with 8-10 drops of the compound stain. A thin smear was prepared on a clean grease free slide and dried in the air. The smear was examined under the high power of microscope (100x). The sperm taking partial stain with either anteriorly or posteriorly were considered as dead. At least 300 total sperms were counted in different fields and live sperm percentage was calculated. Sperm cells which appeared colourless were counted as live one and the pink sperms cells as dead one. Total number of spermatozoa per ejaculate was calculated by multiplying ejaculate volume to sperm concentration per ml. Semen index of each ejaculation was estimated, as an indicator of semen quality by (Moghaddam *et al.*, 2012) [13].

Statistical analysis

The values of parameters were subjected to one way ANOVA for statistical comparisons using SPSS software V 17.

Results

Table 1: Seminal characteristics (Mean ±SE) during non-breeding and breeding season in Magra ram

Season	Volume (ml)	pH	Mass motility (0-5 grade)	Individual sperm motility (percentage)	Sperm concentration (× 10^6/ ml)	Live sperm percentage	Abnormal sperm percentage	HOST	Sperm per ejaculate (× 10^6)	Semen index (× 10^9)
Non-breeding	0.91±0.5	6.99±0.02^a	3.38±0.10^a	66.14±2.0^a	4873.81±150.84 ^{b}	71.89±0.46^a	10.67±0.36^b	59.07±0.54 ^{a}	4467.5±291.49^b	20993.74±1708.90
Breeding	0.90±0.4	7.15±0.02^b	3.88±0.15^b	74.38±2.1^b	3490.48±168.39 ^{a}	79.85±0.61^b	9.24±0.30 ^{a}	66.94±0.48 ^{b}	3289.76±269.27^a	19153.76±1534.28

Different superscripts (a, b) in the same column indicate significant differences (p<0.05),

The overall mean value of semen volume (ml) was 0.91±0.05 and 0.90±0.04 during non-breeding and during breeding season, respectively. Semen volume didn't differ significantly (p<0.05), in both seasons. Semen pH was 6.99±0.02 during non-breeding season whereas, 7.15±0.02 during breeding season. Semen pH was significantly (p<0.05) higher during breeding season compared to non-breeding season. Mass motility (0-5 grade) was 3.38±0.10 during non-breeding season and 3.88±0.14 during breeding season and significantly (p<0.05) higher during breeding season compared to non-breeding season. Individual sperm motility (percent) was 66.14±2.02 during non-breeding season and 74.38±2.03 during breeding season which was significantly (p<0.05) higher during breeding season compared to non-breeding season. Sperm concentration (×10^6/ml) was 4873.81±150.84 during non-breeding season and3490.48±168.39 during breeding season which was significantly (p<0.05) higher during non-breeding compared to breeding season. Live sperm percentage was 71.89±0.46 during non-breeding season and 79.85±0.61 during breeding season which was significantly (p<0.05) higher during breeding season compared to non-breeding season. Abnormal sperm percentage was 10.67±0.36 during non-breeding season whereas, 9.24±0.30 during breeding season which was significantly (p<0.05) higher during non-breeding season compared to breeding season. Sperm per ejaculate (×10^6) was 4467.5±291.49 during non-breeding season whereas, 3289.76±269.27 during breeding season which was significantly (p<0.05) higher during non-breeding season. Semen index (×10^9) was 20993.74±1708.91 during non-breeding season whereas, 19153.76±1534.28 during breeding season which did not differ significantly between two seasons.

Discussion

In the present study, non-significantly higher semen volume was recorded during non-breeding season (summer) which is in accordance to the finding of Juma and Al-Kassab (2009) in Hamdani rams. The ejaculate volume showed slight variations during the seasons, a decrease being the most evident in winter and observations suggest that ram semen volume is lower in winter, with higher volumes being recorded in autumn and summer (Oberst *et al*., 2011) [15] which are in favour to the present finding in Magra ram. However the high ejaculate volumes were recorded in autumn and lowest in summer in Chios, Daglic and Akkaraman rams (Gundogan, 2007) [5]. Kafi *et al.* (2004) recorded significantly higher mean semen volume of Karakul rams during autumn and lower during summer. Many studies revealed that lower mass motility was observed during summer season compared to the other seasons of the year (Kafi *et al*., 2004; Aller *et al*., 2012; Moghaddam *et al*., 2012) [2, 13] which support the present findings. Nivsarkar *et al.* (1971) recorded no significant seasonal variation of mass motility of semen of Magra rams during spring and winter seasons. The progressive motility of sperm is significant in determining the fertility potential of sperms. The sperm motility in both Najdi and Naimi rams was lower in summer (Al-Anazi *et al*., 2017). Sperm progressive motility was low during winter and spring with a minimum being recorded in spring season (Aller *et al*., 2012) [2].

Similarly lowest progressive motility of sperms in GH×BL and AM×GH rams was reported in spring than winter season (Moghddam *et al*., 2012). Higher individual sperm motility in rams was probably due to higher concentration of sperms (Javed *et al*., 2000). Semen pH was higher during breeding seasonwhich is consonance with findings of Nivasarkar *et al*. (1971) and Aller *et al*. (2012) [2]. However, Juma and Al-Kassab (2009) in a study with Hamdani breed found that pH was reduced during summer due to increase of sperm concentration. Al-Anazi *et al*. (2017) recorded the lowest pH during summer season in Naimi and Najdi rams which support the present findings. Sperm concentration of Magra rams was significantly higher during non-breeding season which was similar to the finding in in Hamdani rams (Jumma and Al-Kassab, 2009). Contrary to this, the highest ($p<0.05$) sperm concentration was recorded in spring and autumn in Naimi and Najdi rams, whereas lower concentrations were recorded in winter and summer (Al-Anazi *et al*., 2017). This finding coincides with that of Hamidi *et al*. (2011) where spermatozoa concentration was highest in breeding season. However, many researchers observed no seasonal variations in sperm concentration of the ejaculate in rams (Oberst *et al*., 2011) [15], in Pampinta and Corriedale rams (Aller *et al*., 2012) [2] and in Magra ram (Nivasarkar *et al*., 1971) which is in the contradict to the present study.Live sperm percentage was significantly higher during breeding season in Magra rams which is accordance to finding of Kafi *et al*., 2004; Moghaddam *et al*., 2012; Pourseif *et al*., 2013 [13]. The percentage of live sperm was significantly increased in summer and decreased in winter seasons (Jumma and Al-Kassab, 2009) which are in contrary to the present investigation. In a similar study with Dorper rams, Malejane *et al*. (2014) found that the percentage of live spermatozoa (or viability) was similar but high in spring and summer and significantly different from autumn and winter. The higher occurrence of abnormal spermatozoa was reported in summer in different breeds of ram (Gundogan, 2006) [6]. The high percentage of sperm abnormalities during the winter and spring seasons can possibly be attributed to the low level of circulating plasma testosterone and a reduction in the thickness of the seminiferous tubules and spermatogenic activity (Barkawi *et al*., 2006). The highest values for total spermatozoa output were recorded during summer in Lacaune rams (Oberst *et al*., 2011) [15] and Argentine Pampinta and Corriedale rams (Aller *et al*., 2012) [2] which are in favour to the present study. Contrary to the current experiment, Kafi *et al*. (2004) and Belkhiri *et al*. (2017) reported the non-significant effect of season on total sperm output in Karakul and Ouled Djellal rams, respectively. The highest value of semen index was observed during autumn and lowest in spring season (Moghaddam *et al*., 2012) [13].

Conclusion

Results of study revealed that seminal quality parameters were within breed able limit during both breeding and non-breeding season. Therefore, ram can be used for breeding purpose throughout the year.

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