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Comparative study of blood plasma testosterone of Magra rams during breeding and non-breeding season in Rajasthan

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Abstract
Sheep is considered to be short day breeder. Photoperiod and breed are two main elements that influence reproductive seasonality and are intimately linked to the seminal feature (Malpaux, 2006; Moghaddam *et al*., 2012; Sarlos *et al*., 2013). In the current study, seven grazing rams, weighing 35±5 kg, with good libido and aged between 1.5 and 3 years, were used in both seasons. Blood samples were collected in sterilized 10 ml tubes by jugular veni puncture and serum testosterone assay was done using sheep T (Testosterone) ELISA kit. Magra rams' serum testosterone levels were significantly (p<0.05) greater during breeding season than non breeding season.

Keywords: Blood plasma testosterone, Magra rams during breeding, non-breeding

Introduction
Sheep breeds from temperate regions exhibit more pronounced reproductive seasonality than those from tropical and desert regions. Rajasthan, India's tropically desert region is home to the Magra sheep breed which is characterized by white face with light brown patches around the eyes and well known for world best carpet wool production. In Indian condition particularly western part of Rajasthan, sheep are mostly aseasonal and polyestrous having cyclicity throughout the year. However, depending upon climatic condition, feed and fodder availability, sheep breeders have defined two breeding seasons i.e., Aug -Sept and Feb-March during the year for optimum productivity through better management. These defined breeding seasons can be followed at organized sheep sector, but unfortunately sheep sector is mostly unorganized in the region and sheep are reared by poor landless, small and marginal farmers. In the field condition, there is paucity of elite germplasm and most of animals are non descript with poor growth rate and reproduction efficiency. A flock's ability to reproduce and develop genetically is largely dependent on its ram. Therefore, it's critical to pay attention to and comprehend the factors that control and affect semen production, as well as the depth of seasonality in rams (Zamiri and Khodaei., 2005; Zamiri *et al*., 2010; Olah *et al*., 2013) [21, 22, 15]. Reproductive seasonality is represented in detectable changes in testicular size, spermatogenesis, hormone release, and sexual behavior at high latitudes (Land, 1973) [10], which roughly corresponds to the ewes' breeding activity (Thibault *et al*., 1966) [20]. However, in the tropical region sheep breeds, depth of seasonality is less marked. Further rams are more sensitive to high environmental temperatures and may suffer from testicular degeneration, lower semen quality and subsequent subfertility or infertility (Rasooli *et al*., 2010) [18]. It is a well-established fact that, in temperate sheep breeds, the fertilizing power of frozen semen is hampered during the non-breeding season and enhanced during the breeding season (Colas *et al*., 1976) [4]. AI makes faster dissemination of superior germplasm at field level. Therefore, in order to make the most use of the best breeding males available today, more knowledge regarding the depth of reproductive seasonality is needed (Holt *et al*., 2007) [6]. Therefore, yearlong comparative studies between breeding and non-breeding seasons in rams will be useful for completing the findings and reducing the reproductive challenges of the species. To the best of our knowledge, there is paucity of literature regarding the seasonal changes in

serum testosterone among rams. Consequently, the goal of the current study was to determine how the season affected the testosterone profile of rams.

Materials and Methods

1. **Geographical location and climate:** The trial was conducted in Bikaner during the months of August and September, which is breeding season, and May through June, when there was no breeding season. The region is known for its 200–300 mm of annual precipitation, extremely chilly winters (lows of 2 °C to 4 °C in January), and scorching, dry summers (highs of 42 °C to 46 °C in June and July).
2. **Experimental Animals:** In the current investigation, seven grazing rams with a mean age of 1.5-3 years, a mean weight of 35±5 kg, and a good libido were employed in both seasons. Every ram was fed the same standard diet, which was developed in accordance with the Indian Council of Agricultural Research's recommendations for mature breeding rams. The institute's usual procedure is for animals to be fed in a shed and let to graze for at least seven hours per day on their range. Every ram was kept in an isomanagerial setting with adequate sanitation and unrestricted access to water. The rams were still apart from the ewes.
3. **Blood sampling and hormonal assay:** Using a jugular veni puncture, blood samples were drawn into 10 ml vials that had been sterilized. After the samples were brought to the lab, the serum was separated using centrifugation for 15 minutes at 2500–3000 rpm. It was then kept at -20 °C in sterile plastic storage vials until it was subjected to additional biochemical–hormonal examination.
4. **Serum Testosterone Assay:** Serum testosterone assay was done using sheep T (Testosterone) ELISA kit (Wuhan Fine Biotech Co., Ltd. Wuhan, China). The lowest detectable limit of the assay was 0.313 ng/ml with <0.188 ng/ml sensitivity. The intra- and inter-assay coefficients of variation was <8 and <10%, respectively.

Results and Discussion

The overall mean value of serum testosterone (ng/ml) was 2.93±0.09 during non breeding season which varied between 2.38±0.25 and 3.30±0.20 (Table1) whereas, the overall mean value of serum testosterone (ng/ml) was 3.75±0.12 during breeding season which was ranged from 3.07±0.29 to 4.42±0.19 among rams (Table1). Magra rams' serum testosterone levels were much (p<0.05) greater during breeding season than they were outside of it. (Table1). When the breeding season in Karakul rams began, there was a notable increase in the mean serum testosterone concentration (Kafi *et al*., 2004) [7]. This result is consistent with earlier studies that shown a noteworthy increase in plasma testosterone concentrations in various ram breeds during the breeding season (Zamiri and Khodaei, 2005; Zamiri *et al*., 2010) [21, 22] which is in the support to the present study. Similar to our study, low serum testosterone levels during summer in Malpura rams was obtained by Maurya *et al*. (2016) [12]. The hormone testosterone primarily acts as a mediator in the expression of several morphological and behavioral features in rams, however, little is known about the variables that contribute to individual differences in circulating testosterone levels (Preston *et al*., 2012) [17]. Rams' blood circulation levels of testosterone vary depending on their breed, age, diet, season, photoperiod, and many stressors, particularly those that include heat and transportation (Kridli *et al*., 2006) [9]. The hormone that regulates the activity of the testicular, epididymal, and accessory sex glands is testosterone. Numerous academics contend that testosteronemia is a reliable measure of both the quantity and quality of semen produced (Kishk, 2008) [8]. It is essential for the regulation of spermatogenesis, the reduction of germ cell death, and the maintenance of ram sexual behavior (Perkins and Roselli, 2007) [16]. In Argentine Pampinta and Corriedale rams, the effect of season on blood testosterone concentrations was highly significant (p<0.01), peaking in the summer (Aller *et al*., 2012) [1], Arrebola and Abecia (2017) [2] reported lowest plasma testosterone concentrations during winter and autumn, highest during summer in Murciano-Granadina and Payoya bucks similarly reported by Milczewski *et al*. (2015) [13] in Suffolk rams. Contrast to this, higher testosterone during autumn season was reported in Daglic and Chios rams (Gundogan, 2007) [5]. Rams' plasma testosterone levels peaked in the winter, with summer, fall, and spring showing little variation from one another (Belkhiri *et al*., 2017) [3]. Whereas Sarlos *et al*. (2013) [19] reported lowest testosterone concentration during winter and highest level was measured during autumn season in Racka rams.

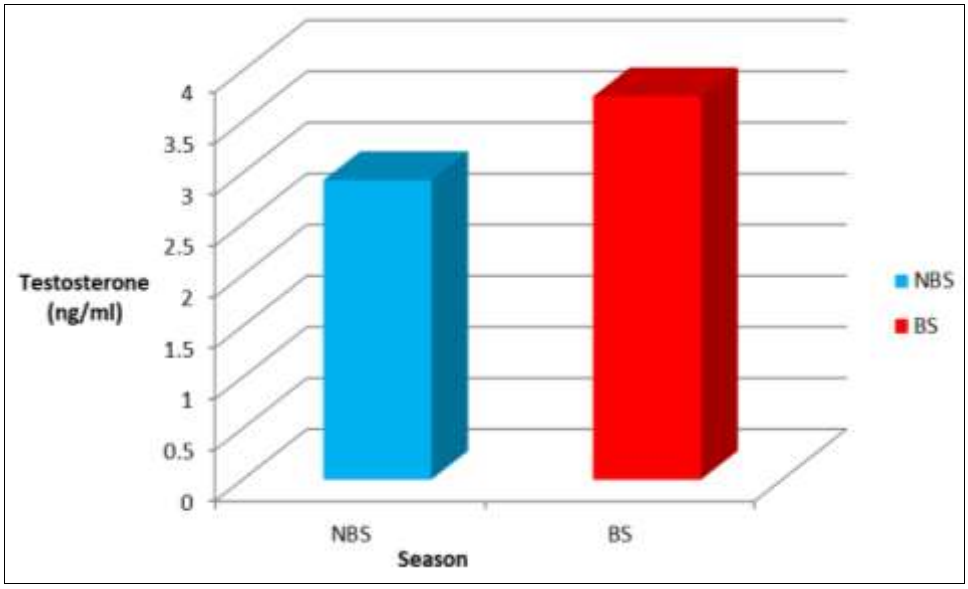

Fig 1: Mean serum testosterone level of Magra rams in different seasons

Table 1: Serum testosterone level (Mean ± SE) during different seasons in Magra rams

	1	2	3	4	5	6	7	Mean
non breeding season	2.73±0.21	3.05±0.17	3.11±0.15	3.21±0.31	3.30±0.20	2.70±0.21	2.38±0.25	2.93±0.09
breeding season	3.17±0.41	3.65±0.21	3.75±0.23	4.02±0.30	4.17±0.32	4.42±0.19	3.07±0.29	3.75±0.124

Conclusion

The results of this study showed that during breeding season, serum testosterone levels were considerably (p<0.05) greater than during non-breeding season. Even though seasonal changes impact testosterone levels, they remain within the range considered adequate for normal fertility. Rams can therefore be utilized for breeding all year round.

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