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Effect of exogenous melatonin administration on testicular biometry of Sirohi buck during non-breeding season in Southern Rajasthan

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Abstract
This investigation was conducted to study the effect of exogenous melatonin administration on testicular biometry of twelve sexually mature adult male Sirohi bucks during non-breeding season. Treated (T) animals (n=06) were administered single subcutaneous injection of melatonin. The Scrotal circumference (cm) and testicular volume (cm^3) in control group [28.04±0.140, 167.90±4.439 (right) and 166.24±3.800 (left)] was comparable with that of treated group [28.91±0.193, 269.88±10.041 (right) and 267.53±9.568 (left)] for the first non-breeding season and for the second non-breeding season, control group [27.17±0.174, 120.05 ±5.843 (right) and 116.95 ±5.696 (left)] was comparable with that of treated group [32.50±0.302, 330.37 ±11.397 (right) and 329.22 ±10.846 (left)]the differences found significant for the both non-breeding seasons. Exogenous melatonin treatment significantly increased the scrotal circumference, testicular length, testicular width and testicular volume. In conclusion melatonin administration improved testicular biometry of Sirohi bucks during non-breeding season.

Keywords: Melatonin, Sirohi, buck, testicle, biometry, non-breeding

Introduction
The reproductive seasonality is a phenomenon influenced mainly by annual variations in the photoperiod, which increase proportionally to latitude such that reproductive and non-reproductive seasons are well defined among seasonal species [1]. Seasonal reproduction is an adaptive physiological process utilized by animals that live under natural environmental conditions to anticipate annual changes in day length, temperature and food availability [2]. Goats can be induced to breed outside the natural breeding season [3]. The various strategies to induce breeding outside the natural breeding season in goats include, manipulation of daylight length [2, 4] and the administration of gonadotrophins following progestogen priming with either intravaginal sponges or subcutaneous implants [5]. Problems in the manipulation of daylight length on most farms have created increased interest in the exogenous administration of melatonin (as an oral administration, or a subcutaneous implant) [6]. Treatments with exogenous melatonin, mimicking the effect of short days, generally stimulate the reproductive activity in short day breeders [7]. In the male, the objective is to cause recrudescence of spermatogenetic activity for a sufficient time to produce a large number of good-quality spermatozoa and store them in the epididymis for use in artificial insemination (AI) or in natural mating [8]. Exogenous administration of melatonin by slow-release implants may be a reliable method for controlling reproductive rhythm [9, 10]. Recent studies have demonstrated that melatonin treatment increased scrotal diameter in rams and improved reproductive performance of estrous synchronized ewes naturally mated with these melatonin-implanted rams during the non-breeding season [11]. Indeed, exposure of bucks or rams to 2 or 3 months of long days, followed by either natural photoperiod, artificial short days or a melatonin treatment– a hormone that mimics night –stimulates their endocrine and sexual activity during the non-breeding season for about 2 months [7, 12, 13]. Melatonin treatments during the non-breeding period can increase testicular growth [14]. Thus, the aim of this study was to determine the effect of exogenous melatonin administration on testicular biometry of Sirohi bucks during non-breeding season in southern Rajasthan.

Materials and methods

The present study was carried out at the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Navania, Vallbhnagar, Livestock Research Station, Navania, Vallbhnagar and Semen Laboratory, Gir Cattle Breeding Farm Livestock Research Station, Navania, Vallbhnagar, Rajasthan University of Veterinary and Animal Science, Bikaner.

Animal

Twelve sexually mature adult male Sirohi bucks aged between 2-3 years and managed under semi-intensive system under All India Coordinated Research Project (AICRP) unit of Sirohi Goat at Livestock Research Station, College of Veterinary and Animal Science, Vallbhnagar, Navania were included in the present study. All the bucks were reared under uniform conditions of feeding, management and housing. All the bucks were previously trained to ejaculate into the artificial vagina while using dummy in the crate. Their ejaculates having mass motility +5 and above 90% individual motility, sperm abnormality less than 10% were selected for primary study and more than 50% post thaw motility in breeding season were selected for further study.

Design of experiment

The bucks were randomly divided into 2 groups with 6 animals in each group for the present experiment in order to study effect of exogenous melatonin administration on testicular biometry of Sirohi buck semen during non-breeding seasons in Southern Rajasthan. The first group treated (T) animals (n=06) were administered single subcutaneous injection of melatonin (Melatonin powder Sigma, USA dissolved in sterile corn oil) @18mg/50kg body weight as per previously described methods [6, 15, 16]. The second group of bucks (n=6) were given only corn oil subcutaneously and considered as control group (C).

Testicular biometry

The testicular biometry was performed during the two non-breeding seasons (I and II). The testicles of the bucks in control (C) and treated (T) group were measured every week during the experimental period. The testicles were gently pulled by the distal portion of the scrotum and, with a flexible tape, the scrotum circumference (cm) was measured at the largest longitudinal region as per method described previously [17]. The width (medium-side; cm) and the length (dorsoventral; cm) of each testicle was measured with a caliper.

The testicular volumes were obtained using the following formula:

$TV = 0.5236\ (TL)\ (TW)^2$ based on the equation by Bailey *et al.* (1998) [18], in which TV = testicular volume, TL = testicular length, and TW = testicular width.

Statistical analysis

Data were analyzed using the SPSS computerized program to calculate the analysis of variance (ANOVA). F- Test was used to evaluate the significant difference between means.

Results

Testicular biometry measurement carried out on the Melatonin treated and control group bucks during non-breeding season-I and II throughout the investigation. All results are expressed as mean ± standard error of the mean (SEM). The application of two-way ANOVA showed a statistical significant effect of melatonin administration on the scrotal circumference during both non-breeding season-I and II. (Table no.1). During the non-breeding season-I, differences between the scrotal circumference of control group and treated group were seen (28.04±0.140 cm and 28.91±0.193 cm). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (27.17±0.174 cm and 32.50±0.302 cm).

A significant effect of melatonin treatment on testicular length of right and left testicles were seen in between the control and treated groups during the non-breeding season-I and II [Table no.2 (A) & (B)]. During the non-breeding season-I, differences between the right and left testicular length of control group and treated group were seen (11.19±0.058 versus 12.61 ±0.095 and 11.18±0.064 versus 12.60±0.099). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (10.29±0.125 versus 13.50±0.146 and 10.24±0.125 versus 13.37 ±0.147) and were found significant.

Statistical analysis of the testicular width of the right and left testicle during the period of investigation (non-breeding season-I and II) were found significant between the control and treated group [Table no.3 (A) & (B)]. During the non-breeding season-I, differences between the right and left testicular width of control group and treated group were seen (5.35±0.064 versus 6.37 ±0.106 and 5.33±0.053 versus 6.35±0.096). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (4.64 ±0.091 versus 6.78 ± 0.089 and 4.59 ±0.092 versus 6.81 ± 0.086) and were found significant.

Application of ANOVA analysis on the testicular volume of the right and left testicle during the period of study (non-breeding season-I and II) were found significant between the control and treated group [Table no.4 (A) & (B)]. During the non-breeding season-I, differences between the right and left testicular volume of control group and treated group were seen (167.90±4.439 versus 269.88±10.041 and 166.24±3.800 versus 267.53±9.568). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (120.05 ±5.843 versus 330.37 ±11.397 and 116.95 ±5.696 versus 329.22 ±10.846) and were found significant.

There was a non-significant yet sequential increase in the testicular biometry (scrotal circumference, testicular length, testicular width and testicular volume) of treated bucks during the four weeks of the study in non-breeding season I and eight weeks of the study in season II. Comparison of the testicular biometry between melatonin treated and control bucks by two way ANOVA revealed that treated bucks had significantly higher ($P<0.05$) testicular biometry during both non-breeding season I and II compared untreated control.

Table 1: Comparative table of the mean ± S.E. of Scrotal circumference (cm) in control and Melatonin treated Sirohi bucks during non-breeding season-I and II.

Group	First non-breeding season	Second Non-breeding season
Control group	28.04 ± 0.140^{b}	27.17 ± 0.174^{a}
Melatonin Treated group	28.91 ± 0.193^{c}	32.50 ± 0.302^{d}

Mean having different superscripts in a column small letter (a,b,c,d) significantly differ ($P<0.05$).

Table 2A: Comparative table of the mean ± S.E. of right testicular length (cm) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second Non-breeding season
Control	11.19±0.058[b]	10.29±0.125[a]
Melatonin Treated	12.61 ±0.095[c]	13.50 ±0.146[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Table 2B: Comparative table of the mean ± S.E. of left testicular length (cm) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second non breeding season
Control	11.18±0.064[b]	10.24±0.125[a]
Melatonin Treated	12.60±0.099[c]	13.37 ±0.147[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Table 3A: Comparative table of the mean ± S.E. of right testicular width (cm) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second non-breeding season
Control	5.35±0.064[b]	4.64 ±0.091[a]
Melatonin Treated	6.37 ±0.106[c]	6.78 ± 0.089[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Table 3B: Comparative table of the mean ± S.E. of left testicular width (cm) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second non-breeding season
Control	5.33±0.053[b]	4.59 ±0.092[a]
Melatonin Treated	6.35±0.096[c]	6.81 ± 0.086[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Table 4A: Comparative table of the mean ± S.E. of right testicular volume (cm^3) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second non-breeding season
Control	167.90±4.439[b]	120.05 ±5.843[a]
Melatonin Treated	269.88±10.041[c]	330.37 ±11.397[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Table 4B: Comparative table of the mean ± S.E. of left testicular volume (cm^3) in control and Melatonin treated groups during non-breeding season-I & II.

Groups	First non-breeding season	Second non-breeding season
Control	166.24±3.800[b]	116.95 ±5.696[a]
Melatonin Treated	267.53±9.568[c]	329.22 ±10.846[d]

Mean having different superscripts in a column small letter (a, b, c, d) significantly differ (p<0.05).

Discussion

In the present study, we confirmed increase in the testicular biometry (scrotal circumference, testicular length, testicular width and testicular volume). The significant increase in testicular biometry was comparable with those previously reports by Fitzgerald and Stellflug (1991) [14] who also found that melatonin treatments during the non-breeding period can increase testicular growth in rams. The effect of melatonin on testes is also reported by the Chemineau *et al.* (1992) [8], Asher *et al.* (1993) [19], Daramola *et al. (*2006) [20]. Melatonin implants can have positive effects on sperm quality and increase the scrotal circumference and testes volume of normospermic and pathospermic rams in anoestrous seasons [21] which is in accordance with the findings of the present study. The administration of melatonin in treated bucks showed its effect on the testicular growth which is in agreement with the findings of Langford and coworkers, 1987 [22] that receptors for melatonin are located near the hypothalamus and adenohypophsis and have been found in Leydig cells indicating that melatonin might also have a direct effect on the testes[10]. Further, Langford *et al.* (1987) [22] also reported that melatonin stimulates spermatogenic activity of ram testes by increasing the sensitivity of Leydig cells to luteinizing hormone. Presumably, each breed has its own characteristics of pineal hormone secretion. In goat's production, buck fertility influences flock performance and reproductive efficiency since numerous does are generally bred to a single buck compared to the fertility of individual doe [23]; thus, selection of highly fertile bucks is vital for improved goat production [24, 25, 26]. Therefore, to improve goat production in the tropics, the reproductive efficiency and fertility of the bucks require attention [27] in terms of growth rate, body weight, sexual characteristics and soundness of sexual organ of the buck [28].

Conclusion

The present study provides, for the first time, data on the relationship between testicular biometry and exogenous administration of melatonin in Sirohi bucks during non-breeding seasons, highlighting increase in scrotal circumference and testicular volume post administration of melatonin implants. Therefore, this research lays foundation for investigating the effects on the increase of testicular mass which may result from exogenous melatonin during non-breeding seasons in Sirohi bucks.

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Conflict of Interest

All authors declare no conflicts of interest. All authors participated and approved the article for publication.

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