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

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Abstract
This study was conducted to evaluate the effect of exogenous melatonin administration on freezability of Sirohi buck semen during non-breeding seasons. Twelve sexually mature adult male Sirohi bucks aged between 2-3 years were selected for this study. Treated (T) animals (n=06) were administered single subcutaneous injection of melatonin @18mg/50kg body weight. During first non-breeding season, the post thaw microscopic seminal parameters progressive motility (37.50 ± 1.241 & 50.42 ± 0.948), live sperm count (51.21 ± 0.458 & 69.25 ± 0.382), dead sperm count (39.92 ± 0.611 & 21.75 ± 0.422), abnormal sperm count (8.88 ± 0.243 & 9.00 ± 0.170), curled tail sperm count (35.55 ± 0.759 & 51.00 ± 0.715) were found significant at $p<0.05$ in between control and treated group. During second non-breeding season, the post thaw microscopic seminal parameters progressive motility (29.59 ± 1.427 & 58.75 ±1.058), live sperm count (42.25 ± 0.789 & 66.90 ±0.779), dead sperm count (48.63 ± 0.844 & 25.55 ± 0.662), abnormal sperm count (9.13 ± 0.125 & 7.57 ± 0.204), curled tail sperm count (36.15 ± 1.114 & 61.36 ± 0.773) were found significant at $p<0.05$ in between control and treated group. Exogenous melatonin treatment significantly improved the post thaw motility, live sperm count, dead sperm count, abnormal sperm count and coiled sperm count. In conclusion, melatonin administration improved freezability of Sirohi bucks semen during non-breeding seasons.

Keywords: Melatonin, sirohi, buck, non-seasonal, freezability

Introduction
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 (Malpaux *et al.*, 1999) [1]. This allows them to make the necessary physiological adjustments in advance of the actual sexually quiescent interval or breeding period (Reiter *et al.*, 2010) [2]. According to Chemineau *et al*. (1995) [3] seasonality in small ruminants represents a major limitation to farmers and the milk and meat industries. Goats are generally described as seasonal polyestrus breeder in temperate countries but seasonality of breeding in goats of tropical countries yet remains to be established. Seasonal changes in plasma testosterone levels have reflected that Suffolk rams (Katongole *et al.*, 1974) [4] and Pygmy bucks (Howland *et al.*, 1985) [5] are seasonal breeders, although they showed sexual activity throughout the year. However, in tropical regions, other factors such as ambient temperature, relative air humidity, rain distribution, and nutrition also seem to have effects on reproductive physiology in seasonal animals (Rosa and Bryant, 2003) [6]. Goats can be induced to breed outside the natural breeding season (Amoah and Gelaye, 1989) [7]. 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) (Chemineau *et al.*, 1995) [3]. 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 (Chemineau *et al.*, 1992) [8]. In semen production centers, where sires of high genetic value produce semen for artificial insemination, seasonality of rams and bucks sexual activity is also a major drawback because the lowest activity occurs exactly when the need for liquid semen to inseminate at farms is the highest (sheep) and because it imposes a 6- month complete stop of semen collection in the production process of deep - frozen semen (goats)

(Gaunand *et al.,* 2014) [9]. Thus, in female and male goats and sheep, there is a strong demand for non invasive, sustainable, cheap and efficient techniques to control out - of - season breeding (Gaunand *et al.,* 2014) [9]. Although semen production continues throughout the year bucks, semen quality is lower in the non- breeding season (Chemineau *et al.,* 1992; Kaya *et al.,* 2000) [8, 10]. The best semen quality in bucks during the breeding season, that is, during the period of shorter daylight when testosterone release is increased (Al-Ghalban *et al.,* 2004; Delgadillo *et al.,* 2004) [11, 12]. However, in the subtropical climatic zone, the sexual activity of bucks in the non- breeding season has been induced using artificial daylight in the period from November to January in conjunction with melatonin treatment (Delgadillo *et al.,* 2001, 2002; Ramadan *et al.,* 2009; Zarazaga *et al.,* 2010) [13-16]. Overall, the melatonin has important effects on the regulation of testicular development, male reproduction and improving sperm quality by regulating the gonadotropins and testosterone hormones.

In this light, the aim of this study was to determine the effect of exogenous melatonin administration on freezability of Sirohi buck semen during non-breeding season in southern Rajasthan.

Material and Methods

The present investigation was carried out at the department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Navania, Vallbhnagar, Gir Cattle Breeding Farm, Livestock Research Station, Navania, Vallbhnagar, RAJUVAS. A prior consent was obtained for the use of experimentation from ethical committee of college.

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 during breeding season were selected for further study.

Plan of work

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 freezability of Sirohi buck semen during non-breeding season 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 (Chemineau *et al*., 1995, Kumar and Purohit, 2009, Kumar, 2014) [3, 17, 18].The second group of bucks (n=6) were given only corn oil subcutaneously and considered as control group (C). The semen collections were done aseptically and hygienically on weekly basis during non-breeding seasons. The artificial vagina (Danish model) of size 20 cm in length and 4.5 cm in diameter with smooth lining (IMV Pvt. Ltd.) was used for semen collection. The semen collection cup with the freshly collected semen was immediately transferred to the laboratory and immersed in a water bath at 30 ºC. Total 48 semen samples non-breeding season-I and 96 semen samples non-breeding season-II during 4 weeks and 8 weeks were collected from the control (n=6) and treated (n=6) buck group per week in the semen collection area of semen laboratory, in a clean dust-free area.

Semen evaluation

All the semen samples from control and treated buck groups were subjected to macroscopic (physical parameters) and microscopic evaluation. Immediately after collection of semen from control and treated groups, the semen samples was pre-diluted to a low volume of extender and stored in water bath (30 °C). Dilution rate was decided according to its specific spermatozoa concentration as previously described by Purdy (2006) [19]. Optimum number of motile sperm per dose of french mini straw at the time of dilution was fixed at about 100 million sperm. As soon as the final volume of diluted semen had been determined the remainder of the diluent was added.

Filling, sealing and freezing of semen straw

Diluted semen was filled manually in French mini straws (0.25ml) with the help of bubbler comb setup and laboratory plug was applied using polyvinyl powder. The extended semen samples filled in straws from each buck were kept in a cold handling cabinet at +5 ^{0}C for 3 hours of equilibration period. These straws were placed horizontally on a rack situated 4 cm above the surface of a liquid nitrogen container for 15 min to achieve temperature of -140 ^{0}C. The straws on each rack were collected and quickly immersed in respective goblets containing liquid nitrogen and these goblets were transferred to liquid nitrogen container and then plunged into and stored in the liquid nitrogen (Kharche *et al.,* 2013) [20]. Thawing of the straws was carried out individually after 24 h of freezing at 40 ^{0}C for15 seconds in water bath for post thaw sperm evaluation (Kharche *et al.,* 2013) [20].

Post thaw microscopic seminal parameters

The post thaw microscopic seminal parameters evaluated included the progressive motility, live dead count, sperm abnormality, Hypo-osmotic swelling Test. All these were measured as described previously (Kharche *et al.,* 2013) [20].

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. Data represented as mean ± SEM and considered significant at $P<0.05$.

Result

Post thaw microscopic seminal parameters were carried out on the all semen samples collected and freezed from control and melatonin treated bucks during non-breeding season-I and II throughout the study. All results were 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 post thaw progressive motility during both non-breeding season-I and II. (Table no.1). During the non-breeding season-I, differences between the post thaw progressive motility of control group and treated group were seen (37.50 ± 1.241 and 50.42 ± 0.948).

Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (29.59 ± 1.427 and 58.75 ±1.058) (Table no.02).

Table 1: Post thaw Progressive motility (in percentage) two way ANOVA

Source of variation	D.F.	Sum of Square	Mean Sum of Square	F Value (calculat)	F Table Value		F(5%) S/N	F(1%) S/N
					F(5%)	F(1%)		
Between groups	3	22420.14	7473.38	123.6364	2.67	3.92	S*	S**
Total	143	30882.64						
Error	140	8462.5	60.44643	-	-			

Table 2: Comparative table of the mean ± S.E. of post thaw Progressive motility (in percentage) in control and Melatonin treated Sirohi bucks during first and second non-breeding season.

	First non-breeding season	Second non-breeding season
Control	37.50±1.241^{b}	29.59±1.427^{a}
Melatonin Treated	50.42±0.948^{c}	58.75±1.058^{d}

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

The application of two-way ANOVA showed a significant effect on post thaw live sperm count were seen in between the control and treated groups during the non-breeding season-I and II (Table no.3). During the non-breeding season-I, differences between the post thaw live sperm count of control group and treated group were seen (51.21 ± 0.458 and 69.25 ± 0.382. Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (42.25 ± 0.789 versus 66.90 ±0.779) and were found significant (Table no.04).

Table 3: Post thaw live sperm count (in percentage) two way Anova Table

Source of variation	D.F.	Sum of Square	Mean Sum of Square	F Value (calculat)	F Table Value		F(5%) S/N	F(1%) S/N
					F(5%)	F(1%)		
Between groups	3	19507.81	6502.604	306.3202	2.67	3.92	S*	S**
Total	143	22479.75						
Error	140	2971.937	21.22812	-	-			

Table 4: Comparative table of the mean ± S.E. of post thaw live sperm count (in percentage) in control and Melatonin treated Sirohi bucks during first and second non-breeding season

	First non-breeding season	Second non-breeding season
Control	51.21±0.458b	42.25±0.789a
Melatonin Treated	69.25±0.382c	66.90±0.779c

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

Statistical analysis of the post thaw dead sperm during the period of investigation (non-breeding season-I and II) were found significant between the control and treated group (Table no. 5). During the non-breeding season-I, differences between the post thaw dead sperm of control group and treated group were seen (39.92 ± 0.611 and 21.75 ± 0.422). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (48.63 ± 0.844 and 25.55 ± 0.662) and were found significant (Table no.6).

Table 5: post thaw Dead sperm count (in percentage) two way Anova Table

Source of variation	D.F.	Sum of Square	Mean Sum of Square	F Value (calculat)	F Table Value		F(5%) S/N	F(1%) S/N
					F(5%)	F(1%)		
Between groups	3	17998.5	5999.5	289.8809	2.67	3.92	S*	S**
Total	143	20896						
Error	140	2897.5	20.69643	-	-			

Table 6: Comparative table of the mean ± S.E. of post thaw dead sperm count (in percentage) in control and Melatonin treated Sirohi bucks during first and second non-breeding season

	First non-breeding season	Second non-breeding season
Control	39.92±0.611^{c}	48.63±0.844^{d}
Melatonin Treated	21.75±0.422^{a}	25.55±0.662^{b}

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

Application of ANOVA analysis on the post thaw abnormal sperm count during the period of study (non-breeding season-I and II) were found significant between the control and treated group (Table no.7). During the non-breeding season-I, differences between post thaw abnormal sperm count of control group and treated group were seen (8.88 ± 0.243 and 9.00 ± 0.170). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (9.13 ± 0.125 and 7.57 ± 0.204) and were found significant (Table no 8).

Table 7: Post thaw abnormal sperm count (in percentage) two way ANOVA

Source of variation	D.F.	Sum of Square	Mean Sum of Square	F Value (calculat)	F Table Value		F(5%) S/N	F(1%) S/N
					F(5%)	F(1%)		
Between groups	3	70.0625	23.35417	18.40075	2.67	3.92	S*	S**
Total	143	247.75						
Error	140	177.6875	1.269196	-	-			

Table 8: Comparative table of the mean ± S.E. of post thaw abnormal sperm count (in percentage) in control and Melatonin treated Sirohi bucks during first and second non-breeding season

	First non-breeding season	Second non-breeding season
Control	8.88 ± 0.243^{b}	9.13 ± 0.125^{b}
Melatonin Treated	9.00 ± 0.170^{b}	7.57 ± 0.204^{a}

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

The application of two-way ANOVA showed a significant effect on post thaw curled tail sperm count were seen in between the control and treated groups during the non-breeding season-I and II (Table no.9). During the non-breeding season-I, differences between the post thaw curled tail count of control group and treated group were seen (35.55 ± 0.759 and 51.00 ± 0.715). Similarly, during the non-breeding season-II, differences between the control group and treated group was recorded (36.15 ± 1.114 and 61.36 ± 0.773) and were found significant (Table no.10).

Table 9: Post thaw Host Curled tail sperm count (in percentage) two way ANOVA

Source of variation	D.F.	Sum of Square	Mean Sum of Square	F Value (calculat)	F Table Value		F(5%) S/N	F(1%) S/N
					F(5%)	F(1%)		
Between groups	3	19079.24	6359.748	187.409	2.67	3.92	S*	S**
Total	143	23830.16						
Error	140	4750.917	33.93512	-	-			

Table 10: Comparative table of the mean ± S.E. of post thaw Host Curled tail sperm count (in percentage) in control and Melatonin treated Sirohi bucks during first and second non-breeding season.

	First non-breeding season	Second non-breeding season
Control	35.55 ± 0.759^{a}	36.15 ± 1.114^{a}
Melatonin Treated	51.00 ± 0.715^{b}	61.36 ± 0.773^{c}

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

Discussion

In the present investigation, a significant improvement in the post thaw microscopic seminal parameters was confirmed in the melatonin treated bucks after freezability during the non-breeding season. According to the Kupferschmied and Muther (1977) [21] reported a decrease in the semen characteristics of bucks during the non-breeding season, based on laboratory tests, and recommended the use of semen frozen during the preceding breeding season which was also observed in the seminal parameters of the control bucks. The variability in between the control and melatonin treated post thaw seminal parameters was in accordance with the study conducted by the Langford *et al.* (1987) [22] that melatonin administration stimulates the spermatogenic activity of ram testes by increasing the sensitivity of Leydig cells to luteinizing hormone. In one another study carried out by Van Vuuren *et al.* (1992) [23] also supported the effect of melatonin on the sperm quality, since there are melatonin binding sites in spermatozoa. The findings of this study of melatonin treatment during non-breeding season is also in accordance with the study of Kumar and Purohit (2009) [17] concluded that a single subcutaneous injection of melatonin can initiate the breeding season (irrespective of the season of the year). Further, similar finding is also reported by Casao *et al.* (2010a) [24] that Melatonin is reported to improve sperm quality in rams during the non-breeding season and in Mexican Creole bucks (Delgadillo *et al.*, 2001) [13]. In another species ram, the Melatonin implants administered in the non-breeding season can improve progressive motility and morphologically normal sperm rates (Kaya *et al.*, 2000) [10] which is also observed in this study. However, the literature on the effect of melatonin on freezability of buck semen is scanty, so other species findings were incorporated in this study. According to the Guerin *et al*. (1992) [25] influence of season has been reported on the fertilizing ability of spermatozoa after cryopreservation which is an important limiting factor for the large scale application of AI with frozen semen. In this study, an attempt was carried out with significant result that melatonin treatment was very effective in improving the post thaw seminal parameter hence freezability in treated buck semen. Karatzas st al. (1997) [26] were also reported that low conception rate found while using frozen-thawed semen collected and processed during non-breeding season which also reflects the effect of season as in this present study. The findings of the current study are also in accordance with the findings of Kaya *et al*. (2001) [27] in rams that melatonin administration to sperm donors improved freezability during cryopreservation. Post thaw seminal parameters viz increase in progressive motility, increase in live sperm count, decrease in dead and abnormal sperm count, increase in curled tail sperm count are in accordance with the findings of Murase *et al*. (2007) [28] that all these changes were attributes to change in melatonin and testosterone secretions.

Conclusion

The Melatonin implants administered during the non-breeding season had improved freezability i.e post thaw progressive motility, live sperm count, dead and abnormal sperm count, curled tail sperm count seminal parameters in Sirohi bucks which is an important limiting factor for the large scale application of AI with frozen semen especially not considered for cryopreservation during non-breeding season. Thus, the expansion of knowledge on use of melatonin during non-breeding season may contribute to the under-standing of the mechanism regulating melatonin and post thaw seminal parameters.

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