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Sustained delivery of exogenous melatonin restores ovarian cyclicity in postpartum Marwari ewes

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Abstract
Sheep husbandry is the major component among the agriculture enterprises which serves as a reliable and sustainable source of livelihood of small and landless farmers in India. Marwari ewes (n=30), aged 2-4 years were used in experiment. Melatonin was dissolved in corn oil and ewes (n=10) received corn oil (1 ml) MLT injection were grouped as control whereas ewes (n=20) received single subcutaneous injection of MLT @ 18 mg/sheep were grouped as treatment. In conclusion, results of present study revealed that single subcutaneous injection of melatonin at the rate of 18 mg per sheep is effective in ovarian rebound and inducing estrus in postpartum anestrus with higher lambing rate.

Keywords: Anestrus, Marwari sheep, melatonin, ovarian cyclicity

Introduction
Small ruminants are considered as short-day breeder, however, reproductive activity varies from superficial to deep anestrus which mainly depend on the breed, latitude, day light variation and season (Rosa and Bryant, 2003: Delgadillo, 2011) [9, 5]. This dependency of reproductive activity produces seasonal lamb and other sheep products, therefore affect farmers, sheep industry and ultimate consumers. Therefore, there is an urgent need of the day for physiological intervention to manipulate ovarian rebound in anestrus sheep so that constant supply of lamb and sheep products can be made available throughout the year. The most successful estrus induction and synchronization protocol for sheep is a combination of the hormone progesterone either in the form of a vaginal sponge or controlled internal drug release (CIDR) device for 12-14 days in situ the vagina along with Pregnant Mare Serum Gonadotropin (PMSG) on withdrawal of progesterone support. Though the vaginal sponge and CIDR are effective in inducing estrus with achievement of approximately 90% synchronization, it causes irritation to the vaginal epithelium and may result into the vaginal and uterine infection leading to decrease in conception rate following AI and natural mating (Xiaojie *et al.* 2022) [22]. Thus, it would be beneficial to have an alternative non-invasive method that is effective for estrus induction, easy to use, and painless to the animal and causes no complications in the reproductive tract of the ewe. Melatonin (N-acetyl-5-methoxytryptamine) is an indole derivative, synthesized and secreted during darkness by pineal gland and helps in advancement of ovarian rebound in sheep (Arendt *et al.* 1983) [3]. Melatonin is one of the best alternatives to induce estrus and reduce lambing interval in anestrus sheep (Luther *et al.* 2005) [15]. Melatonin as implant is used widely to advance the breeding season, thereby, improve reproductive performance in anestrus ewes (Forcada *et al.* 2002) [7]. However, there is paucity of reports regarding single subcutaneous injection of melatonin to advance the resumption of ovarian cyclicity in Indian sheep. Therefore, the preset study was designed to manipulate ovarian rebound and induce estrus in postpartum Marwari ewes to advance the breeding season and reduce lambing interval.

Materials and Methods
The present study was conducted at ICAR-CSWRI- Arid Region Campus, Bikaner. Marwari ewes (n=30), aged 2-4 years were weaned at 75 days post-lambing instead of regular practice

of weaning at 90 days post-lambing. At the commencement of the experiment, ewes were allocated into two groups according to their age, live weight and body condition score. Animals were daily offered roughage and commercial concentrate (300 g per animal having around 14% crude protein). All ewes had free access to fresh drinking water ad libitum. Ovarian acyclicity was confirmed by lack of mounting behaviour by ram (continuously for 10 days) and absence of estrus sign (mucus discharge, frequent urination, standing heat, bleating etc.) and serum progesterone profile < 1 ng/mL based on collection of blood sample of each ewe at 10 days before melatonin treatment. To avoid the male effect, male and female animals were housed in separate barn throughout the study period. Melatonin (Sigma Aldrich St. Louis, MO, USA) was dissolved in corn oil and ewes (n=10) received corn oil (1 ml) sans MLT injection were grouped as control whereas ewes (n=20) received single subcutaneous injection of MLT @ 18 mg/sheep (Singh *et al.* 2010) [9] were grouped as melatonin treated group. Corn oil was used as vehicle for sustained release of the melatonin. In all experimental ewes, estrus activity was tested twice daily in the morning and evening hours using whole apronized rams along with the observation of the behavioural signs of estrus. Ewes were observed for exhibiting signs of estrus up to 28 days post-treatment and responded ewes were mated with rams of proven fertility. Sheep standing at mounting by the ram was considered in heat. The number of sheep induced in estrus (estrus induction rate) was recorded for each group. Estrus induction interval was defined as the time elapsed between melatonin treatment and first accepted mount of the ram. Lambing rate was calculated as the number of ewes lambed in relation to the number of ewes bred. Litter size or fecundity was recorded as number of lambs bornon actual basis in relation to ewes lambed.

Results and Discussion

The resumption of ovarian rebound and reproductive performance is shown in Table-1. Estrus induction rate was higher in melatonin treated (95%) as compared to control group (70%). Time interval to initiate estrus activity was earlier in melatonin treated ewes (9 days) compared to control (12.4 days). Lambing rate at first induced estrus was higher in melatonin treated group (100%) as compared to control (71%) (Table 1). In melatonin treated group, two twin births were recorded sans control.The male effect from the apronised ram might also played role to induce estrus in control group.

The present study has revealed that use of slow-release melatonin in form of single subcutaneous injection could be an effective tool to commence early ovarian rebound in postpartum Marwari ewes. It is established fact that melatonin gives trigger for pulsatile secretion of GnRH in hypothalamaus which ultimately leads to ovarian rebound by stimulating secretion of FSH and LH in anestrus sheep (Malpaux *et al.* 2002) [16]. In summer anestrus sheep, melatonin implants is responsible for increases and maintains the circulating melatonin concentrations of 1200% increase in the daytime and 12% increase in the night-time plasma melatonin concentrations (Lincoln 2000; Gomez *et al*. 2006) [11, 10] and causes at least 10-fold increase in plasma concentrations of GnRH and gonadotrophins through its action both on hypothalamus and on pituitary. Thereby ultimately gives trigger for follicular growth and ovulation in sheep (Misztal *et al.* 2002) [17]. Therefore, it can be speculated that similar stimulus is given by subcutaneous melatonin injection and restore ovarian activity in postpartum ewes. The melatonin treated postpartum ewes had earlier initiation of ovarian activity than postpartum control ewes. Similar observations have been reported in postpartum ewes fed on melatonin (Kusakari and Ohara 1997) [13]. It is well known that lactation has profound effect on ovarian rebound, however, in the present study no effect of lactation was observed as weaning was done 15 days before initiation of the experiment. An early response to estrus during the present study could probably be ascribed to a low inherent level of melatonin in the Marwari ewes during the non-breeding season. It may probably be further explained on the basis that at this latitude, the seasonal anoestrus is not as deep as it is found in temperate latitude (Amoah *et al.* 1988; Delgadillo, 2011, Sexena *et al.* 2015) [2, 5, 20]. The shorter estrus induction interval in melatonin treated group could be speculated on the basis that the melatonin administered at the rate of 18 mg per sheep was enough for its maximum effect as it could have raised the serum melatonin concentration to the tune of the breeding season and initiate secretion of gonadotropin hormone.

Ghuman *et al.* (2010) [9] reported that melatonin treated summer anestrus buffalo heifers showing early induction of estrus might have dominant ovulatory follicles present or emerging on the day of treatment, whereas late responder animals have dominant non-ovulatory follicles, implying an animal-to-animal variation for attaining the threshold level of melatonin necessary to activate hypothalamus- pituitary-ovarian axis. This explanation found support from investigation in ewes where the interval between the insertion of melatonin implants and the onset of the estrus was positively correlated with pre-treatment concentrations of plasma melatonin (Chemineau *et al.* 1993) [4] and a very high eweto- ewe variability was observed in the night-time plasma melatonin concentrations (Zarazaga *et al.* 1998) [23]. Therefore, in the present study also, it can be speculated that response to treatment varied from sheep to sheep which is mainly affected by presence or absence of ovulatory dominant follicle on the ovary at the time of treatment.

Higher lambing rate was recorded in melatonin treated group as compared to control. The finding in current study is in concurrence with the previous reports (Papachristoforou *et al.* 2007; Kumar and Purohit, 2009; Hameed *et al.* 2019) [18, 12, 11]. The luteotrophic effect of the pineal hormone observed *in vivo* (Durotoye *et al.* 1997) [6] might be involved in the melatonin induced improvement in embryo viability along with improvement in uterine expression of progesterone receptors or their binding capacity. Luteotrophic role of melatonin was also reported by Vazquez *et al.* (2010) [21] in sheep with higher progesterone concentrations, greater embryo viability and pregnancy ratesin than those in nonimplanted animals. In several other studies involving sheep and goats (Forcada *et al.* 2006; Abecia *et al*. 2008) [8, 1] conception rate following melatonin treatment was higher which was attributed to increased embryonic viability. Future studies can be exploited for commercial purposes as the lower dose of melatonin along with ease of administration may be economically viable and useful for out of season breeding in sheep as well as to see the effect of dose of melatonin on ovulation rate and prolificacy during seasonal anestrus in this breed at this latitude.

Table 1: Effect of single S/C MLT Injection on ovarian rebound and reproductive performance in postpartum Marwari ewes

S. No	Treatment	Ewes exposed	Estrous response (%)	Estrous induction interval (days)	Lambing rate (%)	Twinning (%)
1	Control	10	7/10 (70%)	12.4 days (ranged 5-18)	5/7 (71%)	0/5 (0%)
2	Melatonin @ 18 mg/sheep	20	19/20 (95%)	9 days (ranged 2-22)	19/19 (100%)	2/19 (10.5%)

Conclusion

With an objective to evaluate the effect of exogenous melatonin on restoration of ovarian cyclicity in postpartum Marwari ewes, thirty Marwari ewes were weaned at 75 days post-lambing and were allocated into two groups according to their age, live weight and body condition score. Ovarian acyclicity was confirmed by lack of mounting behaviour by ram and absence of estrus sign continuously for 10 days and serum progesterone profile < 1 ng/mL of each ewe at 10 days interval before melatonin treatment. To avoid the male effect, male and female animals were housed in separate barn throughout the study period. Melatonin was dissolved in corn oil and control ewes (n=10) received corn oil (1 ml) sans MLT injection and melatonin treated ewes (n=20) received single subcutaneous injection of MLT@18mg/sheep. In all experimental ewes, estrus activity was tested twice daily in the morning and evening hours using whole apronized rams along with the observation of the behavioural signs of estrus. Melatonin treated group showed 95% estrus induction rate whereas control group exhibited only 70%. Time taken from treatment to onset of estrus was shorter in melatonin treated ewes (9 days) compared to control ewes (12.4days). Lambing rate was also higher in melatonin treated group (100%) as compared to control (71%). In melatonin treated group, two twin births were recorded sans control. In conclusion, results of present study revealed that single subcutaneous injection of melatonin at the rate of 18 mg per sheep is effective in ovarian rebound and inducing estrus in postpartum anestrus Marwari ewes with higher lambing rate.

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