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Estrus induction using Intra-vaginal progesterone sponge with or without GnRH in Anestrus Tellicherry does

Venkatesh Sirivati, Murugavel K, Kantharaj S and Hemalatha H

Abstract
The research work was designed to assess the effectiveness of the inclusion of GnRH in intra-vaginal progesterone-releasing sponge-based estrus induction schedule on the reproductive efficiency of anestrus Tellicherry does. A total of 30 healthy does which were in postpartum anestrus even after 6 months of kidding were selected for this study. 15 does were placed in control group and other 15 does in treatment group. A progesterone sponge was inserted intravaginally into all the does for 12 days in both groups and all the does were inseminated at 48 h post-withdrawal of the sponge. When progesterone sponge was removed from the does in treatment group, GnRH at 4 mcg per goat was given intramuscularly. Ultrasonography was done for pregnancy diagnosis after 45 days of artificial insemination. The outcome of the present experiment indicate that the inclusion of GnRH injection at the time of removal of the intravaginal sponge improved the estrus induction and conception rates in anestrus does.

Keywords: Tellicherry, estrus induction, intravaginal progesterone releasing sponge, buserelin acetate, anestrus

1. Introduction
Goats are considered equivalent to cows for poor farmers in case of their excellent adaptability and acceptable production under limited resources (Dash *et al.*, 2013) [5]. Millions of rural households rely on goats as a source of income and employment, contributing 8.5% of the livestock GDP to the Indian economy (Kumar *et al.*, 2005) [8]. Goats are mated randomly and do not exhibit the sort of clear-cut signs of estrus that cattle do (Sunil *et al.*, 2018) [19]. Seasonality is one of the most unique aspects of caprine reproduction, the majority of goat breeds exhibit sexual activity with alternating periods of anestrum, owing to seasonal variations (Notter, 2002; Amoah *et al.*, 1996; Simões, 2015) [11, 1, 16]. In order to reduce the cost of reproduction management in goats, it became necessary to hunt for reproduction techniques that help in the synchronization of estrus in goats including non-breeding seasons using an array of pharmacological substances. In sheep and goats, intravaginal sponges added with progesterone analogs were utilized for prolongation of the luteal phase. After removal of the sponge, the animals will instantly come into estrus (Skliarov *et al.*, 2021) [17]. The inclusion of GnRH analog or eCG at the end of intravaginal progesterone sponge-based protocol improves the estrus induction, ovulation and pregnancy rates in dairy goats (Motlomelo *et al.*, 2002; Husein *et al.*, 2005) [10, 6]. But eCG has some undesirable effects like the development of antibodies against eCG in certain does, after frequent estrus induction treatments (Roy *et al.*, 1999) [14]. The inclusion of GnRH injection in estrus induction protocol has been proven for effective control of LH surge and ovulation in a synchronized manner, (Tamanini *et al.*, 1985; Cameron *et al.*, 1988) [21, 4] resulting in improved pregnancy rates in goats (Husein *et al.*, 2005) [6]. Moreover, administration of GnRH prior to insemination in ewes increased the yield of fertilized ova with the selection of a dominant follicle 1- 2 days after GnRH treatment (Twagiramungu *et al.*, 1995; Walker *et al.*, 1989) [23, 25]. Even though there is a lot of literature regarding estrus induction using intravaginal sponges and GnRH in anestrus ewes, reports on anestrus does are scanty (Skliarov *et al.*, 2021) [17]. Therefore, the present research work study was planned to assess the usefulness of the inclusion of GnRH in intra-vaginal progesterone-releasing sponge-based estrus induction protocol on estrus induction and pregnancy rate in anestrus Tellicherry does.

2. Materials and Methods

Healthy goats maintained in a commercial goat unit at Auroville near Puducherry were utilized for the study. A total of 30 healthy does that have not shown signs of estrus even after 6 months of kidding were utilized for the present study. All 30 goats were confirmed as non-pregnant based on ultrasonography. The selected does were placed in two groups i.e. 15 does in control and 15 does in treatment group. Most of the day, the animals were allowed freely to graze in their natural habitats, with supplemental concentrate feeding, *ad libitum* access to water along with mineral licks and staying indoors at night. Progesterone releasing intra-vaginal sponges (AVIKESIL-S ®) with 350 mg natural progesterone were obtained from Central Sheep and Wool Research Institute, Indian Council for Agricultural Research. The sponge was kept intravaginally in all 30 goats and maintained for 12 days. In the treatment group, injection of GnRH (Gynasure - Buserelin acetate) @ 4mcg per animal was given intramuscularly on the day of removal of the intravaginal sponge. Does from all the groups were monitored for signs of estrus from early morning to evening for every 120 min. interval, after the removal of intravaginal progesterone sponges. Estrus detection was done based on estrus signs like reddening of the vulva, vaginal discharges, flagging of the tail, increased vocalization and occasional "riding" by other does. 48 h after taking the progesterone sponges from the vaginal passage, all the 30 goats were inseminated with the frozen semen (Fig.1 & Fig.2). The does were subjected to pregnancy diagnosis 45 days post-insemination by the using Bright-mode veterinary ultrasound scanner (Honda Electronics, Japan) with 3.5 MHz abdominal probe. The effectiveness of the estrus induction hormonal protocol was determined by measuring the following parameters. The estrus induction rate, which is measured by the number of animals identified in estrus divided by total number of animals in each group into 100. The interval between the removal of sponge and the time animal showing the estrus signs (h) is recorded by the duration of time between the removal of sponge and onset of estrus. Estrus duration (h) for all the does in each group was also recorded. The conception rate was measured by number of pregnant does by total does inseminated following treatment in both the groups by 100. The prolificacy percentage for both the groups was measured as the total young ones born divided by number of does in both groups into 100. The fecundity rate for both the groups was measured as the number of kids born divided by number of does inseminated in each group into 100. the data obtained from the present study will be statistically analyzed.

3. Results and Discussion

The outcome of the present study on two different estrus induction schedules followed in control and treatment groups are given in Table 1. There was a 100% vaginal sponge retention rate seen in both control and treatment groups. Similar results have been reported in previous studies in which progesterone intra-vaginal sponges were used for 7 to 12 days period (Kumar *et al.*, 2016; Yadav *et al.*, 2020; Kumar *et al.*, 2005; Sunil *et al.*, 2018) [7, 27, 8, 19] in small ruminants. The 100% retention rate in the present study may be due to the good management system followed in the goat farm. It was reported in the previous studies that the sponge retention rate in small ruminants is based on the technique of sponge insertion in the vaginal passage, farm operation system (Omontese *et al.*, 2012) [12], intravaginal implant dimentions (Swelum *et al.*, 2018) [20], consistency and texture of the intravaginal progesterone sponge. A higher estrus induction rate in the treatment group (86.66%) when compared to the control group (53.33%) was recorded, which may be due to the intramuscular injection of GnRH). Injection of GnRH at the time of removal of the sponge was reported to increase the measurement of the diameter of the Graafian follicle and elevated concentrations of plasma estrogen concentration at the time of estrus (Wolfenson *et al.*, 1994) [26]. The outcomes of the research work also concurs with the outcome of previous woks of Suresh *et al.*, 2021 and Skliarov *et al.*, 2021 [18, 17] who have reported an 85-90% estrus induction rate using an intravaginal progesterone sponge in ewes. The time duration between the cessation of progesterone treatment and the time of showing signs of estrus is 32.34±2.21 h and 28.94±1.14 h in the control group and treatment group correspondingly. There is no significant difference (p>0.05) noted regarding the interval between the end of progesterone treatment and onset of estrus among the two groups. The outcome of the present research work is almost comparable to the findings of the other studies (Yadav *et al.*, 2020; Babu *et al.*, 2019) [27, 2]. However, some authors reported longer intervals between the cessation of progesterone treatment and the time does showing estrus signs (35h to 38h) (Vinay Yadav *et al.*, 2021; Dash *et al.*, 2017; Sanjeev *et al.*, 2023; Babu *et al.*, 2020) [24, 5, 15, 3]. Similarly, the duration of estrus in treatment and control groups were statistically similar. The present finding shows that GnRH has no influence on estrus duration and the same has been reported earlier in Ewes (Martinez *et al.*, 2019) [9] (Table 1). The present result concurs with the findings of Babu *et al.*, 2020; Tamanini *et al.*, 1985; Vinay Yadav *et al.*, 2021) [3, 21, 24]. However, dash *et al.*, 2017 [5] reported longer duration of estrum (36.20±0.63) when compared to the present findings. The conception rate is higher in the treatment group of does (40.00%) compared to the control group of does (13.33%) and it is statistically significant (p≤0.05) (Table 1). Higher conception rate in the treatment group may be due to GnRH injection at the time of taking out the intravaginal progesterone sponge from the vaginal passage of the does. The administration of GnRH was reported to synchronize the LH surge (Piersona *et al.*, 2003) [13]. In the treatment group, five does gave birth to twins and one doe gave triplets, whereas two does gave birth to twins and no triplets in the control group. The prolificacy was 200.00% and 216.66% in the control and treatment groups correspondingly. The difference between the both the groups are not significant statistically (Table 1). Our findings results are comparable to the findings of Teleb *et al.*, 2007 [22] who recorded that administration of GnRH along with progesterone sponge treatment increases the conception rate, and consequently increase the litter size in goats. In the present study, fecundity was 13.33% and 86.66% in the control group and the treatment group correspondingly. The difference in the fecundity rate among both groups is highly significant (p≤0.01). Increased fecundity % in the treatment group is due to a high number of young ones born to does present in the GnRH group when compared to the non-GnRH group. High fecundity in the treatment group may be attributed to the GnRH at the cessation of sponge treatment which might have increased the follicular development leading to multiple ovulations (Piersona *et al.*, 2003) [13].

Table 1: Effect of hormonal treatment on the reproductive parameters of Tellicherry does

Reproductive Traits	Control (n=15)	Treatment (n=15)	Significance
Sponge retention rate (%)	100	100	NS
Estrus induction percentage (%)	53.33 (8/15)	86.66 (13/15)	*
Interval between cessation of treatment and onset of signs of estrus (h) (Mean ± SE)	32.34±2.21	28.94±1.14	NS
Estrus duration (h) (Mean ± SE)	24.75±0.31	26.31±0.43	NS
Conception rate (%)	13.33 (2/15)	40.00 (7/15)	*
Prolificacy (%)	200.00 (4/2)	216.66 (15/7)	NS
Fecundity (%)	13.33 (2/15)	86.66 (13/15)	**

NS: non-significant; *: Significant at 5%; **: Significant at 1%

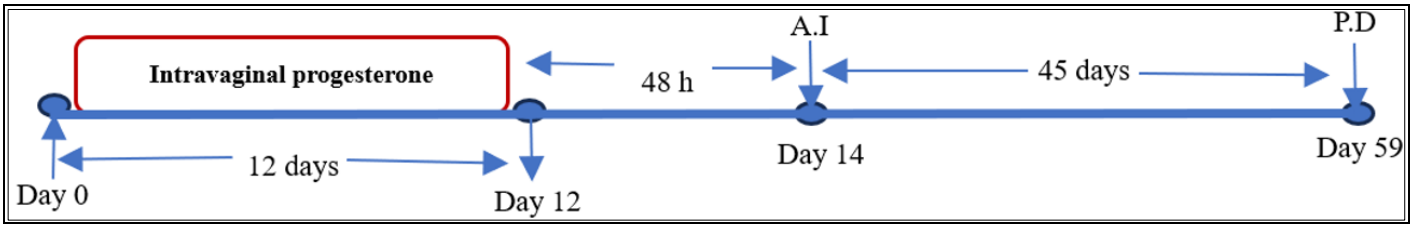

Fig 1: Control Group (n-15)

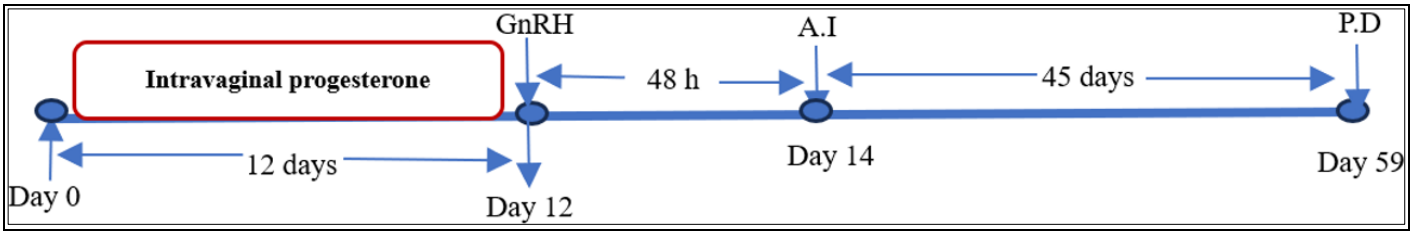

Fig 2: Treatment Group (n-15)

4. Conclusion

From the research work, it can be decided that GnRH at the time of withdrawal of intravaginal progesterone sponge improved the estrus induction and conception rates in anestrus goats.

5. Acknowledgment

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6. Conflict of Interest

There is no conflict of interest among the authors.

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