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Comparative assessment on the effect of long *vis-à-vis* short-term progesterone priming on reproductive attributes in silent estrus goats

KRISHNAPPA BALAGANUR[1]✉, S S MISRA[2], AJIT SINGH MAHLA[2], KALYAN DE[3], RAJIV KUMAR[2], S K SANKHYAN[2] and MARAPPAN GOPI[1]

ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan 304 501 India

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ABSTRACT

Silent estrus results in spread-out kidding and extended inter-kidding interval in goats. Progesterone (P_4) priming for 12-14 days could be one of the approaches for estrus induction with optimum fertility. The present work was carried to comparatively assess the duration of P_4 priming on estrus and fertility in silent estrus goats. Sirohi does (n=18) with a history of anestrus and high basal P_4 in paired samples were equally divided into three groups. Does in P-0 d group received 200 IU of equine chorionic gonadotropin by intramuscular route. Does in group second and third were subjected to per-vaginal P_4 sponges for 7 (P-7 d) and 14 (P-14 d) days, respectively and were given 200 IU equine chorionic gonadotropin following sponge withdrawal. Estrus was monitored for 72 h at 12 h intervals using teaser buck to record estrus per cent, induction time and duration. Estrus induction was highest in P-14 d (6/6) than P-0 d and P-7 d (4/6 and 5/6), respectively. Estrus induction time was shorter in P-7 d and P-14 d than P-0 d. The estrus duration was shortest in P-14 d group. The conception and pregnancy rate did not differ significantly among the groups, though all the does in P-14 d conceived. The fertility was non-significantly higher in P-14 d (6/6) and P-7 d group (5/5) than P-0 d (3/4). It could be concluded that P_4 priming for 7-14 days in silent estrus does results in improved reproductive performances.

Keywords: Breeding season, Estrus induction, Goat, Progesterone sponge, Silent estrus

Reproductive efficiency has major influence on profitability and sustainability of goat farming. Despite of being seasonal breeder, its reproduction is also influenced by other environmental stimuli, such as availability of nutrients and social interactions (Mani *et al.* 1996). Prevalence of silent heat due to nutrition insufficiency in goats is about 17.6% (Zarazaga *et al.* 2005). The HPG axis is the key mediator between changes in external environment and internal responses, influencing reproductive anatomy and physiology, reproductive behaviour and efficiency (Meza-Herrera and Tena-Sempere 2012). During transition from anoestrus period to cyclicity, goats develop silent estrus where ovulation is not accompanied by oestrous behaviour (Fatet *et al.* 2011) and is because of incomplete progesterone (P_4) priming (Fabre-Nys and Martin 1991). Silent estrus preceded by anoestrus results in spread-out kidding and extended inter-kidding interval that makes the management difficult. Silent heats have been reported in various breeds of goat, mostly in the early breeding season (Thompson *et al.* 1983, Farshad *et al.* 2008).

Present address: [1]ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru, Karnataka. [2]ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan. [3]ICAR-National Research Centre on Pig, Rani, Assam. ✉Corresponding author email: drkittyb@rediffmail.com

Hormonal treatment is an essential approach to have control on ovulation, pregnancy (Husein *et al.* 2005) and timed kidding for obtaining uniform neonatal crop in terms of age and development (Husein and Kridli 2003). Progesterone, progestogens and gonadotropins are the hormones used for estrus induction during low or non-breeding seasons in the form of pessaries, sponges or slow releasing vaginal devices. High level of circulating P_4 modulates the hypothalamo-pituitary activity through its negative feedback mechanism on the release of gonadotrophins and is followed by a pre-ovulatory LH surge (Kim *et al.* 2003) on P_4 withdrawal. Concurrently, equine chorionic gonadotropin (eCG) is administered at the time of P_4 withdrawal to improve the development of follicles (Simões 2015). At the Sirohi Goat Research Station, 14-day estrus induction protocol is followed at the end of the major breeding season in apparently acyclic does to ensure maximum kidding. Low levels of subluteal P_4 delays the emergence of follicular wave (Johnson *et al.* 1996) and estrus. Intravaginal P_4 devices are being used to induce cyclicity. Attempts were made to shorten the P_4 exposure from 11 or more days to 5-7 days in goats (Menchaca *et al.* 2007) and sheep (Malik *et al.* 2021). The prime objectives were to cut down the duration of P_4 priming to reutilize the intravaginal devices, high practicability for usage under field conditions, reduce the oxidative stress due to the device contact with the vaginal mucosa and animal welfare concern with minimum economic expenses (Souza *et al.* 2011). Moreover, a short-term protocol in anestrus/silent estrus does have advantage to conventional one, due to more flexibility under field conditions, especially to induce estrus to overcome spread-out kidding. The effect of duration of progesterone priming in anestrus animals have been generated in small ruminants and was found as effective as traditional long-term priming with acceptable pregnancy rates (Rubianes *et al.* 1998, Knights *et al.* 2001, Hameed *et al.* 2020, Malik *et al.* 2021). However, there is little information about the duration of the P_4 priming in silent estrus does. The present study aimed to comparatively evaluate the short and long-term P_4 priming on estrus induction efficiency, fertility, fecundity and prolificacy in silent estrus Sirohi does.

MATERIALS AND METHODS

All the procedures carried out in the present experiment were as per the approved guidelines of the Institute Animal Ethics Committee (IAEC-CSWRI/2017/IXX13455).

Site of the study: The experiment was conducted at the Sirohi goat unit at ICAR-Central Sheep and Wool Research Institute (CSWRI), Avikanagar (75°25' E, 26°18' N, at an altitude of 320 m above MSL). The experiment was conducted during peak summer (May-June), the ambient temperature ranged from 25 to 46.5°C, average photoperiod was 13.38 h and relative humidity was 80 to 93%.

Experimental animals and management: Animals were reared under semi-intensive system and allowed browse for 8-10 h (9:00 – 17:00 h) and during night they were offered with 0.3 kg concentrate feed (CP-12%; TDN – 67%) per day/head.

Experimental procedure: Multiparous Sirohi does (N = 265) were included in the breeding program. Towards the end of the breeding season, 245 animals were covered and the remaining 20 animals were found as apparently acyclic and were subjected to paired P_4 analysis (10 days apart) for confirming their acyclicity. Following P_4 confirmation (levels >1.0 ng/ml) (Rivera *et al.* 2003), 18 silent estrus animals were exposed to P_4 priming at different durations. The does in P-0 d group (n = 6) received only 200 IU of eCG (Folligon, Intervet) intramuscularly on day 0, while the does in P-7 d (n = 6) and P-14 d group (n = 6) were inserted with Avikesil-S® sponge in the vagina and kept *in situ* for 7 and 14 days, respectively. The does were injected 200 IU of eCG upon sponge withdrawal. The estrus was detected at 12 h intervals (6.00 and 18.00 h) for 72 h from the time of eCG injection using two aproned teaser bucks of proven libido (Abecia *et al.* 2011). Estrus does were mated with an elite buck and repeated after 24 h until the end of estrus. Blood samples were collected on day 21

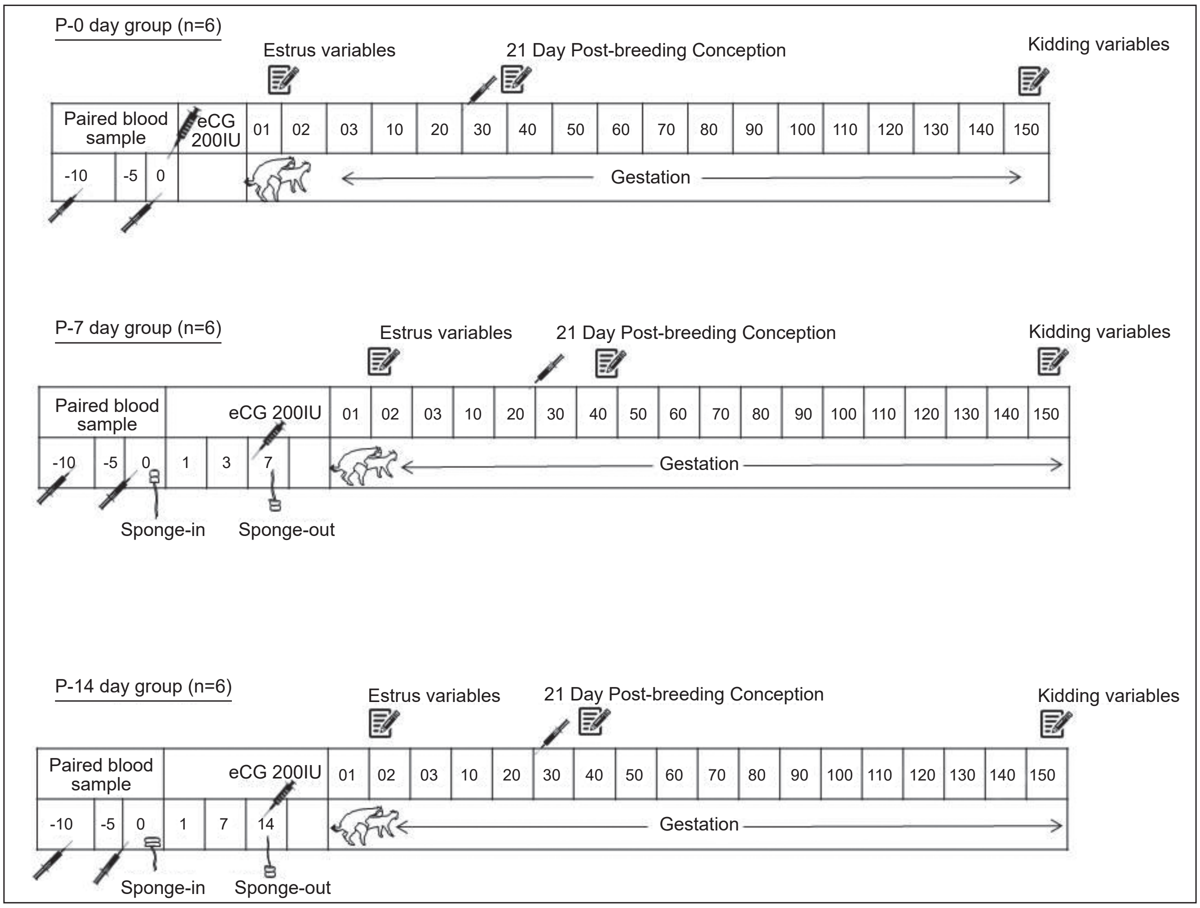

Fig. 1. Schematic representation of the paired sampling of blood, progesterone priming, gonadotropin injection, breeding at detected estrus, conception, fertility and kidding.

post breeding to estimate P_4 for confirmation of pregnancy (Fig. 1).

Progesterone assay: Paired blood samples at an interval of 10 days before the sponge insertion and 21 days post-breeding were collected aseptically. The plasma samples were analysed for P_4 concentration using radioimmunoassay-based kit (Immunotech, France) (Ranilla *et al.* 1994). The goats were considered cyclic/ silent heat if the plasma P_4 is greater than 1 ng/ml in any of the paired sample. Similarly, the goats were considered positive for pregnancy when the plasmatic P_4 concentrations on day 21 were greater than 1 ng/ml (Gonzalez *et al.* 2004).

Reproductive performance parameters: The following parameters were recorded for the reproductive performance:

$$\text{Estrus response (induction percent)} = \frac{\text{Number of females exhibiting overt estrus}}{\text{Number of treated females}} \times 100$$

Time to onset of estrus = Time elapsed from sponge removal to the occurrence of estrus

Estrus duration = Time elapsed from onset to end of estrus

$$\text{Conception rate} = \frac{\text{Number of does detected pregnant}}{\text{Number of does mated}} \times 100$$

$$\text{Kidding percentage} = \frac{\text{Number of does kidded}}{\text{Number of does bred}} \times 100$$

$$\text{Fertility rate} = \frac{\text{Number of does kidded}}{\text{Number of does exposed to the buck}} \times 100$$

$$\text{Prolificacy} = \frac{\text{Number of kids at birth}}{\text{Number of females kidded}} \times 100$$

$$\text{Fecundity} = \frac{\text{Number of kids at birth}}{\text{Number of does bred}} \times 100$$

Statistical analysis: One way ANOVA was used to compare the difference between groups for the continuous variables, i.e. estrus onset and estrus duration. The categorical variables like estrus induction, conception rate, pregnancy rate and fertility rate were analysed using Chi-square test.

RESULTS AND DISCUSSION

The prevalence of silent estrus on the basis of absence of apparent estrus behaviour was 7.5% (20 / 265) in does. Mean P_4 concentration in paired samples of apparent anestrus does were 1.69 and 6.28 ng/ml.

Estrus response: The effect of different P_4 regimens for estrus attributes have been presented in Fig. 2. The number of does that responded to estrus induction were 4, 5 and 6 in P-0 d, P-7 d and P-14 d group, respectively. There were no significant differences in estrus induction response between the groups ($\chi^2_{df2} = 2.29$; $P = 0.31$) and is similar to the earlier reports by Malik *et al.* (2021).

Short-term P_4 priming protocols have an advantage as they result in tight kidding and would aid in proper healthcare of the kids uniformly and in turn reduce mortality (Whitley and Jackson 2004). Majority of estrus responses recorded in the study were within 48 h following

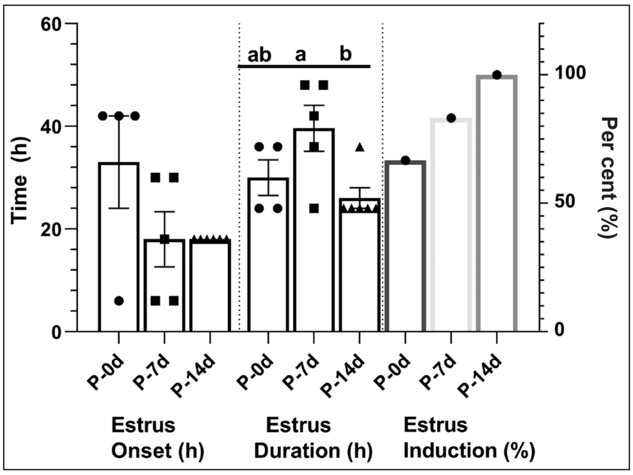

Fig. 2. Whisker bar plot representing effect of progesterone exposure duration on estrus onset, estrus duration and estrus induction per cent. [ab]Means bearing different superscripts differ significantly ($P < 0.05$).

P_4 removal (100%). The observed estrus response following P_4 priming were similar to the earlier studies where it ranged from 87–100% with FGA- or MAP-sponges (Amarantidis *et al.* 2004, Romano 2004). Priming of P_4 sponge-eCG for 7 (83%) and 14 days (100%) resulted in higher ($P > 0.05$) estrus response than treating with eCG alone (66.66%). Similarly, estrus response was higher (93%) in ewes treated with CIDR for 14 days than for 7 days (89%) (Harl 2014). Higher estrus response in primed animals might be due to additive effect of P_4 and spontaneous regression/aging of corpus luteum.

Estrus onset: Mean duration of estrus onset following the end of estrus induction protocol was earlier ($P = 0.09$) in does of both the groups treated with P_4 (18 h) than the untreated P-0 d does (33 h).

The estrus onset was 15 h earlier in P_4 exposed groups and similar positive response was observed in anestrus goats primed with P_4 (7 days) than non-progesterone primed ones (Amarantidis *et al.* 2004, Husein *et al.* 2005, Harl 2014). Even though, an increase in priming duration from 7 to 14 days had similar mean estrus onset (h), the hours of onset were more concentrated in long-term priming while it was dispersed in short-term. In concurrence with findings of present study, other studies in ewes reported short estrus onset in long-term P_4 priming than in short-term (Viñoles *et al.* 2001, Navanukraw *et al.* 2014). The low synchronization of estrus observed in the does primed with P_4 for short-term or without priming could be due to the persistence of functional corpus luteum of previous ovulation of the silent heat and might have hindered the gonadotropin release after sponge withdrawal (Viñoles *et al.* 2001).

Estrus duration: Estrus duration of P-14 d group was shorter ($P < 0.05$) than other groups (26 vs. 40 and 30 h). It is apparent from the Whisker bar plot (Fig. 2) that the estrus onset, duration and synchrony were short and tight in P-14 d group.

The duration of estrus in P-14 d group was significantly

Table 1. Effect of progesterone exposure duration on conception and kidding per cent, fertility, twining per cent, fecundity and prolificacy in silent estrus (n=6) does

Parameter	P- 0 D	P- 7 D	P- 14 D
Conception (%)	100	100	100
Kidding (%)	75	100	100
Fertility (%)	75	100	100
Twinning (%)	0	40	50
Fecundity	0.75	1.4	1.5
Prolificacy	1.0	1.4	1.5

Means within row bearing similar superscript did not differ significantly ($P > 0.05$).

shorter than short-term P_4 exposed does. Moreover, progestagen priming can synchronize ovulatory wave and apparent in does primed with P_4 for 14 days (Rubianes and Menchaca 2003). P_4 priming (14 days) together with eCG could be ideal approach for early estrus onset and tight synchrony of ovulation in silent estrus goats. This approach will be more helpful for goat-keepers for tailoring timed artificial insemination for maximizing kid crop.

Fertility and fecundity: The mean of P_4 concentration of pregnant does was 8.88 ng/ml. The conception rate based on P_4 level was same in all groups (100%). However, there was no significant difference among the groups (Table 1) for kidding per cent (3/4, 5/5 and 6/6). The fertility/kidding per cent was highest ($P = 0.14$; $\chi^2_{df2} = 3.93$) in P-14 d (6/6) and P-7 d (5/5) than P-0 d (3/4). The fecundity and prolificacy recorded in P-0 d, P-7 d and P-14 d groups were 0.75, 1.4, 1.5 (3/4; 7/5; 9/6) and 1.0, 1.4, 1.5 (3/3; 7/5; 9/6), respectively with twinning rate of 0, 40 and 50%, respectively.

The fertility and kidding per cent were similar in each group, all the does bred to buck following with P_4 priming for 7 days and 14 days were fertile and kidded successfully. Similar to current study, Malik *et al.* (2021) reported non-significant difference in pregnancy rates ($P>0.05$) between short, medium and long term P_4 treatment groups. Khudhair *et al.* (2014) reported a significantly higher ($P < 0.01$) pregnancy rate (75 vs 50%) in long-term (12 days) than short-term (9 days) estrus synchronized goats during the breeding season. Long-term exposure to P_4 improved the overall fertility in goats. Progesterone priming during follicular growth phase improved the competence of the oocyte for cleavage and embryo development (Menchaca *et al.* 2018). The high fertility of induced estrus following P_4 priming for 14 days could be linked with early onset and tight synchrony of estrus. Romano (2004) reported that goats which exhibited estrus within 30 h after progestogen treatment had a higher fertility rate than those exhibited late. The importance of P_4 priming in anestrus goats was demonstrated by Husein *et al.* (2005), who performed estrus induction protocol (GnRH-$PGF_{2\alpha}$ protocol alone or with eCG) without P_4 priming and failed to produce fertile cycles. Further, the authors found success when they included 5-day P_4 priming in their synchronization protocol in anestrus goats. It signifies that P_4 priming is indispensable before gonadotropin treatment for high fertility of estrus in silent does. It is apparent from the study that the classic method of long-term P_4 plus eCG protocol (14 days) has more advantages in yielding more synchronous heat in treating the silent estrus goats at the end of the breeding season. However, the non-significant difference between the groups in estrus induction, estrus onset, kidding per cent, fertility and other reproductive variables in this study may be attributed to a small experimental population. In the present study, an increase in prolificacy and fecundity following long-term P_4 priming could largely be due to increased number of twin births. Moreover, Leyva *et al.* (1998) observed that the progestagen priming increased the number of follicles stimulated by eCG and the ovulation rate in anestrus ewes and henceforth the multiple births and similar effect was recorded in the present study.

It may be concluded from the study that short term exposure of progesterone for 7 days would give similar result in terms of fertility. However, long-term exposure to progesterone for 14 days together with equine chorionic gonadotrophin (eCG) induces highest estrus with tight synchrony with highest twinning among the silent estrus does.

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