



Effect of Self Made Intravaginal Progesterone Sponge on Fertility in Goat

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ABSTRACT

Comparative studies of intravaginal sponges containing three different doses of progesterone on estrous synchronization; fertility, fecundity and prolificacy of synchronized does by natural mating were conducted during the breeding season at Central Sheep Breeding Farm, Hisar. A total of 60 Beetal does selected randomly and divided into group I, II and III with 20 does in each, and subsequently intravaginal sponges containing 50, 100 and 150 mg P4 were inserted respectively for 14 days and an i/m injection of PMSG (@200IU/doe) was injected just after sponge removal in each doe. Effective number of animals remained 55 as 5 does dropped their sponges. Forty eight to sixty hrs after sponge removal, 14, 16 and 15 does from group I, group II and group III respectively were synchronized to estrus and subsequently hand mated individually with bucks of proven fertility. The fertility, fecundity and prolificacy per cent after synchronization of oestrus in group I, group II and group III were 78.57, 87.50 and 86.66; 128.57, 156.25 and 166.66; 163.63, 178.57 and 192.30 respectively. Eighteen animals (6 does from each group) selected randomly out of 60 animals for progesterone estimation. Blood samples were collected immediately before insertion of intravaginal sponges (day 0) and then subsequently on day 7, 14 after sponge insertion and 48 and 72 hrs after sponge removal. Progesterone concentration was above basal level in most of animals on day 0 in all the groups. Increase in plasma progesterone concentration (with in group) was recorded on day 7 and decreased on day 14 & 48 hrs after sponge removal. These changes were significant in group II & III while non-significant in group I. Progesterone concentration decreased non-significantly 72 hours after sponge removal in all the three groups. Significant difference in plasma P4 concentration was reported in between group I and group II & III on day 7 & 14.

Key words: Synchronization, Progesterone, PMSG, Fertility, Fecundity and Prolificacy.

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INTRODUCTION

Oestrus synchronization is a highly useful reproductive managemental tool to augment fertility and fecundity in goats. Farmers can use this technique to complement other assisted reproductive technologies such as artificial insemination (AI) for reproductive management. Oestrus synchronization permits kidding to occur at suitable times in order to take advantage of feed and labour availabilities, markets and increasing price trends. In the past, synchronization of oestrus focused only in cattle, sheep and dairy goats for optimal timing of milk production. However, with the upcoming interest in meat goat production, attempts have been made to use synchronization programmes in meat goats.

The various methods of oestrus synchronization that have and can be used include daylight hour's alteration, timed hormonal treatment combined with light alteration and buck effect. Research have shown that timed hormonal treatment is the most convenient and successful method to synchronize oestrus in goats (Whitley and Jackson, 2004). Strategies can be employed to extend the luteal phase by supplying exogenous progesterone/progestagen (Corteel *et al.*, 1988) or to shorten this phase by prematurely regressing existing corpora lutea (CL) with PGF2 α or an analogue (Nutti *et al.*, 1992). The traditional product of choice for ES in goats is the intravaginal sponge impregnated with progesterone or progestagen (e.g. flurogestone acetate or methyl acetoxypogesterone) for 9 to 19 d (a period that is long enough for corpora lutea to undergo timely regression no matter at what stage of the cycle the animals were at the outset) followed by PMSG injected two days prior to sponge removal.

MATERIALS AND METHOD

Sixty Beetal goats aged 3 - 5 years old, weighing 34 - 44 kg and 9 healthy Beetal bucks aged 3 - 4 years, weighing 52 - 62 kg were used in present study. This study was carried out at Central Sheep Breeding Farm, Hisar. All goats had previously kidded and weaned their last kid. Estrous synchronization was conducted during the natural breeding season (September–October; autumn). The animals were kept indoor at night and had access to natural grazing area for most of the day. Water and mineral licks were available ad libitum.

Preparation of intravaginal sponges: A synthetic flat foam (about 4 cm thickness) was cut in similar shape (about 4 cm diameter) and tied with 16-20 cm long cotton thread, sterilized by autoclaving, loaded with a solution of natural progesterone (V900699 Progesterone Vetec™ reagent grade, 98%) in ethanol in 50, 100 and 150 mg of three different concentrations and dried in air.

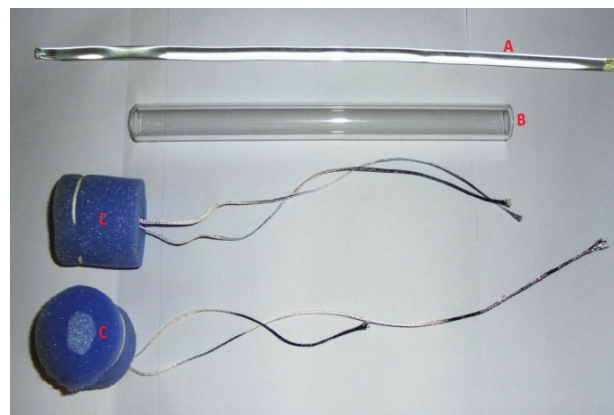


Fig. 1: Intra vaginal sponges with applicator assembly (A. glass rod as plunger, B. cylindrical glass pipe as speculum, C. intravaginal sponges).

Insertion of intravaginal sponges: The intravaginal sponge was inserted in one end of the sterilized speculum which was then lubricated with xylocaine jelly. The sterilized speculum with the sponge was inserted into the vagina. The sponge was then pushed in with the plunger as deep in the vagina as possible. The hanging string on the sponge was tucked into the lower vulvar commissure with help of the finger (Fig 2).

Removal of intravaginal sponges: Sponges were removed on day 14th by pulling the string gently and simultaneously 200 IU PMSG was injected intramuscularly just after sponge removal to each doe.



Fig. 2: Insertion of intravaginal sponge

Table 1: Experimental design for progesterone plus PMSG treatment in does

Treatment Groups	Progesterone Treatment (intravaginal sponges)	PMSG Treatment on sponge removal
Group I (n = 20)	50 mg P4 for 14 days	200 I.U PMSG
Group II (n = 20)	100 mg P4 for 14 days	200 I.U PMSG
Group III (n = 20)	150 mg p4 for 14 days	200 I.U PMSG

Blood sampling and hormonal assay: Blood samples were collected via jugular vein puncture (from 18 does; as 6 does selected randomly from each group) immediately

before insertion of intravaginal sponges (day 0) and then subsequently on day 7th, 14th after sponge insertion and 48 and 72 hrs after sponge removal, in sterilized, heparinized tubes and centrifuged at 3000 rpm for 20 minutes. Separated plasma was pipetted into plastic vials and these vials were stored at -20°C until estimation of progesterone by ELISA.

Mating of synchronized does: Does in estrus were detected using aproned bucks at evening and morning from sponge removal to 96 hrs after sponge removal. The does in all groups were hand mated at 48 and 60 hrs. after sponge removal at synchronized estrus. A total of 9 bucks were used, with buck doe ratio being 1: 5. The bucks used were not allowed any mating for a period of one week before being introduced to the does.

Clinical data calculations: The synchronized does were observed through the gestation till kidding and the fertility, prolificacy and fecundity were worked out.

Fertility: Fertility includes the number of females that gave birth. Breeding group refers to all females exposed to a buck but not necessarily bred does (i.e., females that did not exhibit estrus, and therefore not mated).

Fertility = (Number of does kidded/Breeding group) × 100

Prolificacy:- Prolificacy was given by the number of kids born alive divided by the number of females kidding. Number of kids born alive excludes abortions, stillbirths, pregnancies terminated and kids dead as a result of dystocia. Number of kidding females represents females with at least one kid delivered at term dead or alive.

Prolificacy = (Number of kids born/Number of does kidded) × 100

Fecundity:- Number of kids born alive as above and number of females mated in the breeding group includes all females placed in a breeding treatment that were inseminated or naturally serviced; hence, it excludes goats not coming in estrus in the groups.

Fecundity = (Number of kids born/Number of does mated) × 100

All these factors were calculated on the basis of conceptions on the synchronized estrus and effective number of does used for treatment.

RESULTS AND DISCUSSION

The clinical and fertility outcomes of synchronization of oestrus in does during breeding season are presented in Tables 2 to 3. Out of a total 60 does used, 5 does (one doe from group II; and two does from group I and group III each) had to be excluded from the study at the time of sponge removal, because in these animals the sponge had been expelled before scheduled 14 days. Therefore, the synchronization and fertility studies were restricted to remaining 55 animals. Some of the animals showed a slightly cloudy vaginal discharge at the time of sponge removal, but this was cleared in all the animals within 48 hours.

Forty eight to sixty hours after the sponge removal, 45 does (14, 16 and 15 does from group I, II and III respectively) were observed as exhibiting good behavioral oestrus (Table 2; Fig 3) and were subsequently hand mated individually using 9 beetal bucks (buck:doe ratio 1: 5). In remaining does, no doe was observed in estrus till 96 hrs after sponge removal.

Between 145 and 152 days of mating at the synchronized oestrus, the animals were observed for kidding and 38 does kidded (11, 14 and 13 does from group I, group II and group III respectively) out of 45 mated. From the 38 does which kidded, 68 kids (18, 25 and 25 kids from group I, group II and group III respectively) were obtained. Thirteen does had single kids whereas 20 does had twins and 5 does had triplet kids. Thus a total of 68 kids were born from 38 does. Seven does out of the 45 mated at synchronized oestrus failed to conceive (Table 3).

The fertility per cent after synchronization of oestrus based on number of does mated in group I, group II and group III was 78.57, 87.50 and 86.66 respectively; the fecundity per cent was 128.57, 156.25 and 166.66 respectively; and prolificacy per cent was 163.63, 178.57 and 192.30 respectively. The number of kids born per doe mated in group I, group II and group III were 1.28, 1.56 and 1.66 respectively and the number of kids born per doe kidded were 1.63, 1.78 and 1.92 respectively (Table 3; Fig 4).

Table 2: Synchronization of estrus observed after progesterone plus PMSG treatment

Sr. no.	Parameter	Group I (50 mg P ₄)	Group II (100 mg P ₄)	Group III (150 mg P ₄)
1	Number of does treated	20	20	20
2	Number of does excluded from the experiment	2	1	2
3	Effective number of does for clinical studies	18	19	18
4	Does synchronized	14	16	15
5	Synchronization of estrus (%)	77.77	84.21	83.33

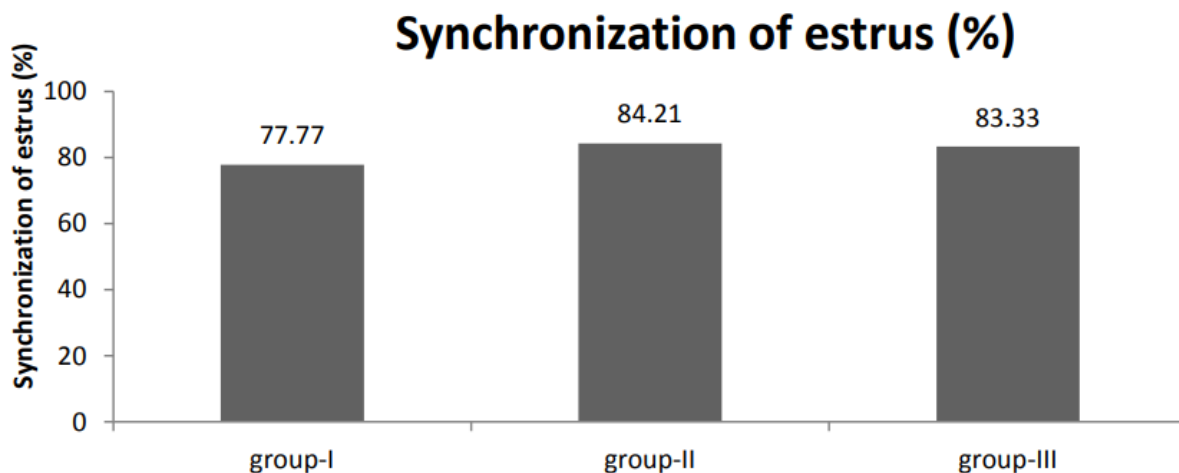


Fig. 3: Synchronization of estrus observed after progesterone plus PMSG treatment

Table 3: Fertility, Prolificacy and Fecundity observed in progesterone plus PMSG treated does

S. No.	Parameter	Group I (50 mg P ₄)	Group II (100 mg P ₄)	Group III (150 mg P ₄)
1	Does synchronized	14	16	15
2	Number of does mated	14	16	15
3	Number of does kidded	11	14	13
4	Number of kids born	18	25	25
5	Number of kids born/doe kidded	1.63	1.78	1.92
6	Number of kids born/doe mated	1.28	1.56	1.66
7	Fertility	78.57	87.5	86.66
8	Prolificacy	163	178	192
9	Fecundity	128.57	156.25	166.66

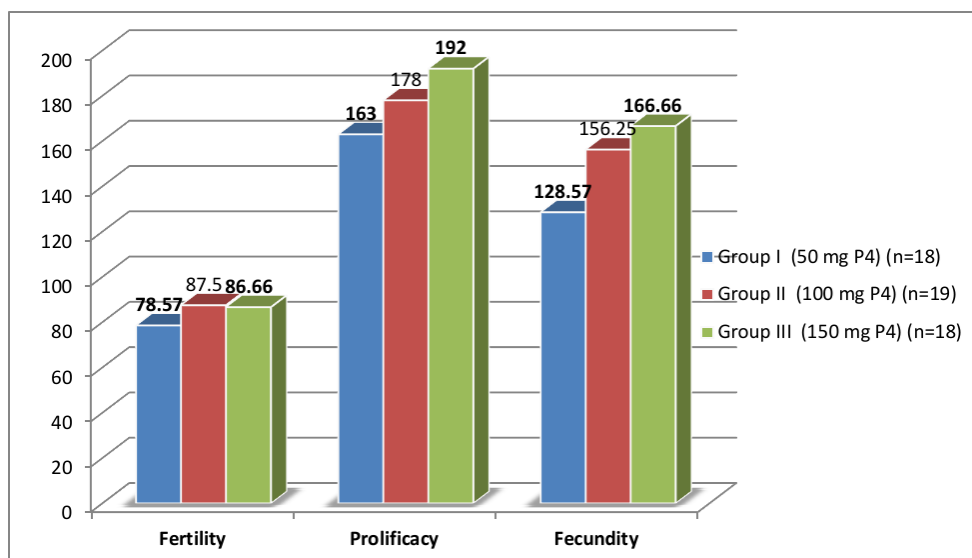


Fig. 4: Fertility, Prolificacy and Fecundity in progesterone plus PMSG treated does

In present study progesterone concentration was above the basal level in all the three groups on day 0 (except three animals one in each group in which plasma P4 concentration was found ≤ 0.5 ng/ml) and this concentration showed non significant difference between the three groups. Concentration of P4 increased in all the groups on day 7 after intravaginal sponge insertion and this concentration showed significant ($p < 0.05$) increase in group II (3.43 ± 0.18 ng/ml) and III (3.55 ± 0.14 ng/ml). However, P4 concentration showed non significant increase in plasma P4 concentration in group I (2.88 ± 0.21 ng/ml). Moreover, there was significant ($p < 0.05$) difference in plasma P4 concentration between group I and group II & group I and group III on day 7. However, non-significant difference was found in plasma P4 concentration between group II and group III.

In present study, progesterone concentration decreased significantly ($p < 0.05$) in all the three groups on day 14 in comparison with plasma progesterone concentration on day 7.

In present study, plasma P4 concentration decreased significantly ($p < 0.05$) in all the three groups at 48 hrs after sponge removal in comparison with day 14th of sponge insertion. However, non significant difference was found in plasma P4 concentration between the groups at 48 hours after sponge removal. But plasma P4 concentration increased non significantly in all the three groups at 72 hrs after sponge removal in comparison with 48 hrs after sponge removal. Furthermore, non significant difference was also seen in plasma P4 concentration between the groups at 72 hours after sponge removal.

The natural progesterone is known to be absorbed quickly from the site of administration in comparison to the synthetic progestogens. However, its biological half-life is much shorter because of its faster degradation in the body as compared to synthetic progestogens (Levy *et al.*, 1999). This quality probably could help in the removal of progesterone much faster from the circulation once the device is withdrawal from the body and synchronization could be more precise.

To test this hypothesis, sterile sponges were impregnated with 50, 100 and 150 mg of three different concentrations of progesterone and placed intra-vaginally in three groups with 20 does in each group for 14 days during breeding season. Forty five does were synchronized to estrus in 48 to 60 hrs after sponge removal, showing good estrus behavior. However Estrus Synchronization was seen 77.77%, 84.21% and 83.33% in group I, II and III respectively. So synchronization of estrus was highest in group receiving 100 mg P4 sponges and it was comparable with group receiving 150 mg P4 sponges. ES in group I with 50mg P4 was in agreement with Ahmed *et al.* (1998) using 40 mg P4 sponge with 300 IU PMSG injection two days before sponge removal with 77.70% ES in Nubian goats.

However, no work has been done with 100 and 150 mg P4 treatment but different authors have used different doses of P4 and PMSG. Furthermore, Das *et al.* (1998) used 350 mg P4 sponges with 200 IU PMSG injection at the time of sponge removal and 90% ewes were in estrus within 48 hrs of sponge removal. Similarly, Selvaraju *et al.* (2002) synchronized 100% Malabari goats using 332 mg P4 containing CIDR with 600 IU PMSG injection at the time

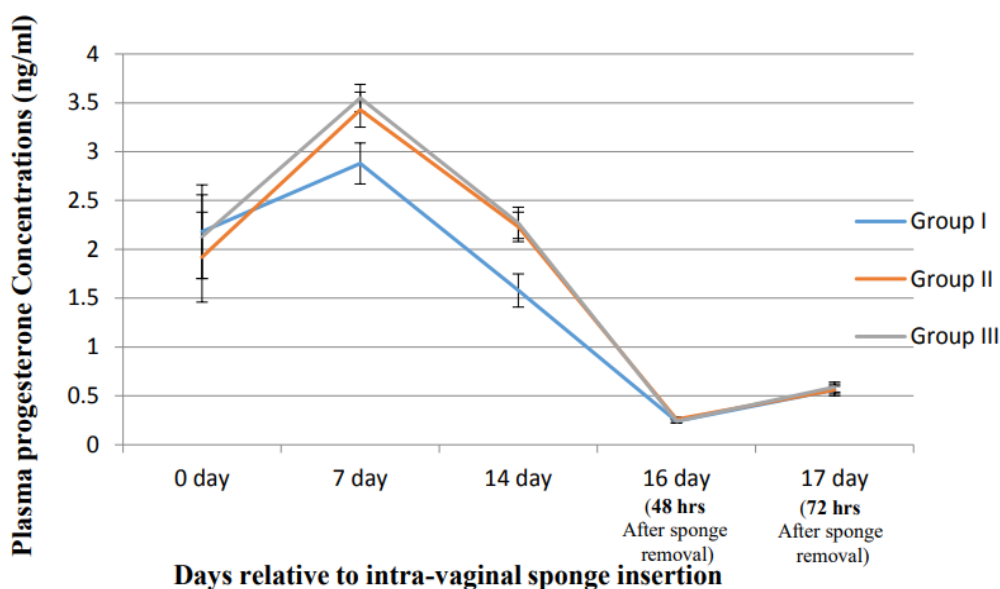


Fig. 5: Plasma progesterone concentrations (ng/ml) in three treatment groups.

of CIDR removal (18th day) and Ritar *et al.* (1989) found 55% ES using CIDR-G with 200 IU PMSG. However, we have found 84.21 and 83.33% Estrus Synchronization with 100 and 150 mg P4 sponges treatment, which is better than Ritar *et al.* (1989) but less than Das *et al.* (1998) and Selvaraju *et al.* (2002). This variation may be due to variation in dose of P4. Different authors have reported that estrus response and fertility vary greatly when intravaginal sponges are applied, dependent on species, breed, co-treatment, management, and mating system (Wildeus, 2000).

The fertility results obtained in group I, II and III were 78.57, 87.50 and 86.66 per cent respectively based on number of does mated. Different authors have reported fertility based on number of does mated, between 33 to 70 per cent using intravaginal sponges containing P4 (Fukui *et al.*, 1999; Ahmed *et al.*, 1998; Kusina *et al.*, 2000 and Das *et al.*, 1998); between 64 to 100 per cent using CIDR containing 300 mg P4 in does (Waldron *et al.*, 1999) and in sheep (Carlson *et al.*, 1989; Hamra *et al.*, 1989 and Godfrey *et al.*, 1997); between 37 to 50 per cent using progestagen containing sponges in does (Ritar *et al.*, 1990 and Menegatos *et al.*, 1995) and in ewes (Baril *et al.*, 1992; Freitas *et al.*, 1996 and Robin *et al.*, 1994) under different mating systems.

In present study we have found better fertility (78.57%) in group I (50 mg P4) compared to 33% observed by Ahmed *et al.*, (1998) while using 40 mg P4 sponge with 300 IU PMSG injection two days before sponge removal. This difference indicates that besides dose of P4 concentration and PMSG, other managerial factors also affect synchronization and fertility.

No studies have reported fertility using 100 and 150mg P4 treatments. However, different authors have reported variable results while using different exogenous P4 with or without PMSG. Fukui *et al.* (1999) reported 33.3 and 41.4% fertility at two different farms using 500 mg P4 sponge (12 d) plus 500 IU PMSG. Similarly, Selvaraju *et al.* (2002) reported 83.33% fertility in Malabari goats (332 mg P4, CIDR, 18 d plus 600 IU PMSG) and Das *et al.* (1998) found 50% fertility (350 mg P4 sponges, 12 d with 200 IU PMSG in sheep). All the above mentioned results have fertility percentage less than the present study.

In present study highest fertility was recorded in 100 mg P4 treatment. This is in accordance with findings of Lamond (1964) and Gordon (1971) that reported optimal fertility was likely to be associated with minimal doses of 30-100 mg progesterone.

In present study, 78.57 to 87.50 per cent fertility was obtained when P4 along with PMSG was used, which was in consistence with Das *et al.* (1999) that reported 44% fertility with 350 mg P4 alone, while along with 200 IU PMSG

70% fertility was obtained. However, Das *et al.* (1999) again in sheep found increased fertility while P4 treatment with PMSG injection applied. This increased fertility was due to super ovulatory effect of PMSG.

The fecundity per cent after synchronization of oestrus in group I, group II and group III was 128.57, 156.25 and 166.66 respectively; and prolificacy per cent was 163.63, 178.57 and 192.30 respectively. The number of kids born per doe mated in group I, group II and group III were 1.28, 1.56 and 1.66 respectively and the number of kids born per doe kidded were 1.63, 1.78 and 1.92 respectively.

In present study, prolificacy was highest (192.30%) in 150 mg P4 treated group, it was comparable with results obtained in group I and II. However, in our study results with 50 mg P4 (group I) are comparable with Ahmed *et al.* (1998) who reported 160 per cent prolificacy after using 40 mg P4 plus 300 IU PMSG and 150 per cent prolificacy while using P4 alone. But in present study, comparatively higher fecundity (128%) was obtained than Ahmed *et al.* (1998) who reported 55.5% fecundity. This difference in results may be due to breed difference. Similar prolificacy results as found in group I were comparable with different concentration of P4 (Waldron *et al.*, 1999) and progestagens (Ahmed *et al.*, 1998; Freitas *et al.*, 1996 and Ritar *et al.*, 1990). Kusina *et al.* (2000) reported 171% prolificacy with 500 mg P4 and Fukui *et al.* (1999) reported 200% prolificacy with 500mg P4 along with 500 IU PMSG. These reports are comparable with group II and group III. However, many studies have been conducted with comparable prolificacy as group II and III with P4 treatment (Ritar *et al.*, 1990) and progestagens (Baril *et al.*, 1993 and Ritar *et al.*, 1990).

The serum progesterone levels remained high as long as sponge was in situ and then declined subsequently after sponge removal. This was quite expected because the natural progesterone absorbed from the sponge in the circulation would cross-react with the progesterone antibodies in ELISA system in the same way as the endogenous progesterone and hence the measured value would be sum of these two. However, this did not happen in earlier experiment when FGA and MAP sponges were used, as these (progestagens) does not cross-react with progesterone in ELISA. After the withdrawal of progesterone impregnated intra-vaginal sponges on day 14, however, the levels of progesterone declined. Earlier studies reporting the effect of progesterone (Das *et al.*, 2001; Pierson *et al.*, 2001 and Boscos *et al.*, 2002) have not measured serum progesterone levels simultaneously and hence the comparison of progesterone levels with the present study is restricted with few studies (Naderipour *et al.*, 2012; Kusina *et al.*, 2000; Husein and Ababneh, 2007).

In present study significant increase in plasma progesterone concentration was reported on day 7 after intravaginal sponge insertion than the level observed at the time of sponge insertion in group II and III. Similar results were reported by Naderipour *et al.* (2012); Kusina *et al.* (2000); Husein and Abadneh (2007), while non-significant increase was reported in group I on day 7. Furthermore, non significant difference was observed between the groups on day 7. Possible reason could be lowered P4 dose (50 mg) in sponge in group I compared to other two sponges. No comparable study has been reported similar to this (50 mg) dose level. However, above authors have reported variable results of plasma progesterone on day 7 after intravaginal sponge insertion. This difference may be due to variable doses of exogenous progesterone intravaginal pessaries, management and season.

Plasma progesterone concentration at the time of sponge removal (14th day after sponge insertion) was found to be significantly decreased with in all groups. Similar results were reported by Naderipour *et al.* (2012) and Husein and Ababneh (2007). Significant decrease in P4 concentration was reported between groups on the day of sponge removal, due to comparatively lowered dose of P4 containing sponges being used. Fall in plasma progesterone concentration in present study at sponge removal was not associated with immediate return of estrus because still negative feedback inhibition of remaining plasma progesterone on GnRH or hypophysial axis, which remained until the P4 was cleared from blood.

In present study, the mean P4 level 48 hrs after sponge removal was less than 0.5 ng/ml in all treatment groups, that was in accordance with the studies of Alacam *et al.* (1985); Pathiraja *et al.* (1991); Husein and Ababneh, (2007) and Souza *et al.* (2011). This decrease in concentration was due to withdrawal of negative effect on GnRH which in turn initiates folliculogenesis and animals exhibit estrus within stipulated period.

Slight increase in progesterone concentration at 72 hrs in comparison to 48 hrs after intravaginal sponge removal in all treatment groups was recorded, it reveals that luteal activity has been started after increased ovulatory rate by PMSG injected at the time of sponge removal as reported by Souza *et al.* (2011).

At 48hrs and 72hrs after sponge removal non-significant difference in progesterone concentration was found between treatment groups, which may be due to increased GnRH activity immediately after sponge removal. The natural progesterone has much smaller half life than synthetic progesterone so quickly removed from the body. This results in abrupt withdrawal of negative feedback

on GnRH irrespective of progesterone concentration in sponge and animals show estrus behavior.

CONCLUSIONS

Low dose of natural progesterone along with PMSG can be successfully used for synchronization of estrus and conception in Beetal goats during breeding season. By using intravaginal sponges containing 100 mg as well as 150 mg P4 along with 200 IU PMSG provides a good synchrony of estrus with acceptable level of fertility.

CONFLICT OF INTEREST

None

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