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Effects of eCG administration along with progesterone based vaginal sponge on fertility response in Sirohi goats

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Abstract
Fertility response following fixed time insemination was studied in 18 Sirohi goats, randomly divided into 3 groups**.** All animals except control group were implanted progesterone impregnated vaginal sponge for a period of 10 days. Animals of group-I Additionally, intramuscular injection of 300IU eCG on day 9 (one day before implant removal) and 125µg of the $PGF_2\alpha$ analogue cloprostenol at the time of implant removal (day 10). Oestrus detection and timed artificial insemination was performed at 48 hours after last injection of $PGF_2\alpha$ analogue cloprostenol. Animals of group-II were implanted progesterone impregnated vaginal sponge for a period of 10 days followed by intramuscular injection of 125µg $PGF_2\alpha$ analogue cloprostenol at time of sponge removal on day 10, followed by oestrus detection and timed artificial insemination at 48 hours. Animals of group–III were kept as control and injected 1 ml sterile normal saline intramuscularly on corresponding days of treatment groups and timed artificial insemination (TAI) was performed similar to treatment groups.
The fertility response using different progesterone based synchronization protocols revealed highest oestrus synchronization, induction and conception rate in group-I (100, 100 and 83.33%) respectively with interval to oestrus of 24-36 hours after removal of the sponge, whereas, lower oestrus synchronization, induction and conception rate in group-II (83.33, 66.67 and 66.67%), and group-III (16.67, 16.67 and 16.67%) was recorded interval to oestrus in group-I (24-36 hours after sponge removal) was lower as compared to group-II and group-III (36-48 hours after sponge removal).

Keywords: Oestrus synchronization, Sirohi, goat, progesterone implant

Introduction
Goat has a major contribution to economy of small and marginal farmers as they can be reared with limited resources and promises faster economic returns. Hence they are also designated as poor man's cow. They are one of the major meat producing animals and it constitutes 26.40 percent of total livestock population in India, ranking second in population after cattle. However, the goat population (135.17 million) has declined by 3.82 percent over the previous census in India. Madhya Pradesh ranks seventh in India with 8 million goats population (Livestock Census, 2012) [23].
Oestrus synchronization and fixed-time breeding is helpful especially when oestrus detection is not very efficient or due to lack of time to perform it. An additional bonus of fixed-time breeding is that it synchronizes ovulation and thus, ensuring the breeding activity throughout the year even in non breeding season and kidding. In goats, oestrus control is generally brought about by the use of progestagen-impregnated vaginal pessaries with limited success (Leboeuf *et al.*, 1998 and Holtz, 2005) [22, 14].
Different synchronization methods using different pharmaceutical agents such as gonadotropin releasing hormone progestagens and prostaglandins (Andrabi *et al.*, 2015) [4] and different durations of treatment like short term and long duration protocols have been used to synchronize oestrus in goats (Amoah *et al.*, 1996 and Karaca *et al.*, 2010) [3, 19].
Intravaginal sponges impregnated with progesterone in combination with prostaglandin and equine chorionic gonadotrophin (eCG) have been used for oestrus synchronization in goats (Karaca *et al.*, 2010) [19].
Various progesterone preparations like fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP) have been used with varying results (Whitley and Jackson, 2004) [35]. In a routinely used synchronization method intravaginal sponge is implanted for a period of 11-19 days, combined with injections of equine chorionic gonadotrophin and an analogue of $PGF_2\alpha$ 48h before or at sponge removal **(**Freitas *et al.*,1997 and Amarantidis *et al.*, 2004) [12, 2].

As the release of progesterone from the sponge decline over a period of time, therefore, a short-term treatment provides higher average concentration of progesterone during treatment period. Short periods of progestagen sponge's treatment for as short as 6-9 days have been reported to be successful in inducing/synchronization oestrus in goats (Ozturkle *et al.,* 2003 and Dogan *et al.,* 2008) [27, 8].

Materials and Methods

Animals: The Sirohi goats used for the present study were reared under semi intensive housing conditions. Doe's grazed on natural pastures during the day and were penned at night. When penned, all animals received 250 g/head of a concentrate containing 18% crude protein. All the Animals were fed commercially available concentrate mixture along with gram straw and seasonally available quality green fodder like berseem, mustard and oat etc. They were also given additional supplement of mineral mixture, gur/jaggery and mustard oil, time to time. Deworming was practiced 2 times in a year. Clean drinking water was made available to the animals *ad lib*. All the animals included in the present study were maintained in almost optimal and nearly identical conditions of feeding and management.

Buck Semen: Semen was collected from breeding bucks of the Sirohi breed by Artificial Vagina method and evaluated using standard methods of macroscopic (colour, consistency and volume) and microscopic (mass motility, progressive spermatozoa motility, percent abnormal spermatozoa, percent live spermatozoa and sperm concentration) tests. The excellent to good quality semen was diluted ten times in egg yolk citrate extender and stored at 5ºC and used within 3 days for insemination. The minimum standards taken for the semen are as follows-

Macroscopic-
Colour and consistency - pale yellow to creamy white
Volume- 0.5 to 1.5 ml
Microscopic -
Mass motility- 80-90%
Progressive spermatozoa motility – 50-60%
Percent abnormal spermatozoa - <5%
Percent live spermatozoa - >90%
Sperm concentration- 1800-2000×10^6/ ejaculate

Selection of animals: A total of eighteen healthy adult non pregnant Sirohi goats reared in Goat Unit Livestock Farm Amanala, Co.V.Sc. & A.H., N.D.V.S.U., Jabalpur were selected for the study. The selection of animals was done on the basis of history of kidding at least 45-60 days before, with no apparent gynaecological disorders and non-pregnant on two consecutive ultrasonographical examinations.

The selected postpartum Sirohi goats were randomly divided into three groups, each comprising 6 animals (n=6). Animals of group-I were implanted progesterone impregnated vaginal sponge for a period of 10 days. Additionally, intramuscular injection of 300IU eCG on day 9 (one day before implant removal) and 125µg of the $PGF_2\alpha$ analogue cloprostenol at the time of implant removal (day 10). Oestrus detection and timed artificial insemination was performed at 48 hours after last injection of $PGF_2\alpha$ analogue cloprostenol. Group II animals were implanted progesterone impregnated vaginal sponge for a period of 10 days followed by intramuscular injection of 125µg $PGF_2\alpha$ analogue cloprostenol at time of sponge removal on day 10, followed by oestrus detection and timed artificial insemination at 48 hours. Animals of group-III were kept as control and injected 1 ml sterile normal saline intramuscularly on corresponding days of treatment groups and timed artificial insemination (TAI) was performed similar to treatment groups.

Monitoring of animals for fertility response

The goats were assessed after vaginal sponge removal until the onset and duration of oestrus to confirm the interval of oestrus and after expected oestrus does were individually teased with an aproned male at morning and evening and on basis of behavioral signs, oestrus induction was observed. Fertility response in terms of oestrus synchronization and conception rate was studied. The synchronization rate was assessed by visual observation for oestrus signs at the time of artificial insemination.

Oestrus induction were determined in goats by observing the signs in does including vaginal discharge, frequent micturition, stand to be mounted, mounted on other goats and buck showed sniffing of perineal region, flehmen reaction and mounting on goats.

Does of all the groups were inseminated at oestrus as mentioned in experimental design using liquid semen (at least 100 x 10^6 spermatozoa per dose). Pregnancy was confirmed by ultrasonography four to six weeks after insemination and conception rate was calculated.

Results & Discussion

Induction of estrus: In the present study oestrus induction in group-I (Progesterone impregnated vaginal sponge + eCG + $PGF_2\alpha$) was 100% however, in group–II (Progesterone impregnated vaginal sponge + $PGF_2\alpha$) and group-III (control) it was 66.67 and 16.67%, respectively.

Almost similar findings were also reported by Amarantidis *et al*. (2004) [2] who recorded ≥95% using FGA sponges plus intramuscular injection of $PGF_2\alpha$ and 400 IU of PMSG, 80% induction treated with 300 mg prepared progesterone sponges for 18 days followed by 300IU eCG on the day of implant withdrawal (Sharma and Purohit, 2009) [34], 100% using double $PGF_2\alpha$ (Dinoprost tromethamine) intramuscular injection (5 mg) at time interval of 11 days plus an intramuscular injection 400IU PMSG two days before second injection of $PGF_2\alpha$ (Juma *et al.,* 2009) [18], 72% induction treated with Chronogest sponges containing 30 mg FGA intravaginaly for 12 days followed by given 500IU PMSG I/M two days before sponge removal (Dzieciol *et al.,* 2011) [9] and 94.50% induction treated with progesterone impregnated vaginal sponges (60 mg MAP) for 11 days and 48 hrs prior to removal of the sponges, intramuscular injection of 400 IU eCG and cloprostenol 0.075 mg (Mehmood *et al.,* 2011) [26]. While the animal of control receiving no treatment showed 20% oestrus induction (Dzieciol *et al.,* 2011) [9].

The variation may be due to difference in dose of synchronization hormone like eCG, $PGF_2\alpha$ and sponge, frequency of observation of oestrus response (Khandoker *et al*., 2009) [21], number of bucks needed for the proper buck to doe ratio for detection of oestrus through mounting of other animals, frequent urination and searching for bucks and most inconsistent behavioral oestrus manifestations (Salami *et al*., 2009) [33].

Oestrus synchronization: In the present study, the oestrus synchronization was 100% in group-I however, 66.67 and 16.67% in group-II group-III, respectively.

Similar findings of 100% synchronization of oestrus treated with progesterone alone, progesterone with PMSG and hCG, and $PGF_2\alpha$ with PMSG and hCG were recorded by Ishwar and Pandey (1992) [17], 87.50% and 100% using 2ml and 1ml synthetic $PGF_2\alpha$ analogue dinoprost, respectively (Khandoker *et al.,* 2009) [21], 100 and 50% synchronization in experimental and control animal treated with MAP sponge implant for 17th day (Kausar *et al.,* 2009) [20], 100 and 93.30% synchronization using CIDR treatment for 14 days with 400 IU PMSG and 0.05 mg cloprostenol injection prior to CIDR removal and other CIDR treatment for 9 days with 0.05 mg cloprostenol injection 24 hours before CIDR removal, respectively (Abdul muin *et al.,* 2013) [1], 100% synchronization in Shami and Iraqi goats using vaginal sponge impregnated with 60 mg MAP followed by intramuscular injection of 400 IU of PMSG at the time of sponge withdrawal (Qayssar *et al*., 2014) [30].

The variation might be related to breed, nutrition, environment, oestrus induction protocol and effect of age and parity, breed, nutrition, location, management and climate (Mani *et al*., 1992; Romano, 2002 and Evans *et al.*, 2004) [25, 32, 10].

Interval to oestrus: In the present study interval to oestrus in group-I was 24-36 hours however, 36-48 hours in group-II and group-III. Similarly, interval to oestrus (22-30 hours) using sponge + eCG was recorded by Chemineau *et al*. (1992) [6], 41.5 ± 8.1 and 45.0 ±12.4 hours using MAP treatment for 12 and 14 days post withdrawal (Romano, 1996) [31], 34.40±4.16 hours using with progesterone-impregnated vaginal sponge plus PMSG and hCG (Machiya *et al*., 2012) [24].

Minimum interval to oestrus (12-24 hours) after sponge removal was reported by Mehmood *et al.* (2011) [26] using progesterone impregnated vaginal sponges (60 mg MAP) for 11 days and intramuscular injection of 400 IU eCG and cloprostenol 0.075 mg at 48 hours prior to sponge removal.

The minor differences in interval to oestrus may be due to age, breed, season, nutrition, the presence of a male and oestrus induction protocols with different dose of hormone (Battaglia, 2001; Kausar *et al.,* 2009 and Fatet *et al.*, 2011) [5, 20, 11].

Conception rate: In the present study conception rate in group-I was 83.33%. However, in group –II and group-III it was 66.67 and 16.67%, respectively.

Almost similar, conception rate (66.67%) using intramuscular injections of progesterone (12.5 mg daily for 7 days) followed by 400 IU of PMSG was recorded by Patil *et al.* (2000) [28], 68% and 54% by using intravaginal sponges containing 60 mg MAP and single dose 200 µg of delprostenate, respectively (Clariget *et al.,* 2012) [7], 79% using intravaginal progestagen sponges (45 mg cronolone) for 12 days with $PGF_2\alpha$ 0.5 ml on tenth day and at time of sponge removal 400IU pregnant mare serum gonadotrophin intramuscularly (Ince and Kokar, 2011) [16].

Lowest conception (44.40%) was reported by Mehmood *et al.* (2011) [26] using progesterone impregnated vaginal sponges (60 mg MAP) for 11 days. At 48 hrs prior to removal of the sponges, intramuscular injection of 400 IU eCG and cloprostenol (0.075 mg) was also given.

The variations in conception rate might be due to the effect of breed, season, endocrine profile and plane of nutrition (Patil *et al*., 2000) [28], geographic location detrimental effects of oestrus synchronization on sperm transport and survival in the female reproductive tract (Pearce and Robinson, 1985) [29] and following fixed-time AI in breed with dose of progestagens (Greyling and Van der Nest, 2000) [13].

Conclusions

The fertility response in terms of oestrus synchronization, induction and conception rate was improved by administration of eCG along with progesterone sponge in Sirohi goats.

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