Original Research

Effect of Estrus Synchronization using AVIKESIL-S® with eCG on the Reproductive Efficiency in Crossbred Ewes

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Abstract

The present study was designed to investigate the efficacy of the cheaper intra-vaginal progesterone releasing sponge (AVIKESIL-S®) and eCG (Equine chorionic gonadotropin) estrus synchronization protocol on the reproductive traits in crossbred ewes. A total of 30 ewes were randomly divided into two groups (n=15 in each group), control and treatment, after initial examination for non-pregnancy. Prior to selection of ewes for estrus synchronization, pre-synchronization was done using double prostaglandin (PG) protocol. AVIKESIL-S® (Intra-vaginal sponge with 350 mg natural progesterone designed by ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India) was inserted for 12 days with 200 IU eCG intramuscular (IM) on the day of sponge removal to the animals belonging to treatment group. Blood sampling was carried out for estimation of plasma progesterone concentration in due course of study. Trans-rectal real-time B-mode ultrasonography was done for early pregnancy diagnosis at day 25 post-mating with reconfirmation on day 45. The results of the present study showed that the protocol was highly effective in induction of estrus resulting in better pregnancy rate and subsequent fertility in treated animals. The fecundity (%) was significantly greater (P<0.05) in treated group than control. The progesterone concentration did not vary significantly between both groups (P>0.05) on day 14, 25 and 45 post-mating. Thus, the protocol could be advised for improvement of fertility and reproductive efficiency in crossbred ewes.

Key words: AVIKESIL-S®, eCG, Ultrasonography, Reproductive Efficiency, Sheep

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Introduction

Sheep is an important economic livestock species, contributing much to the Indian economy, especially in arid, semi-arid and mountain areas. The harsh environmental conditions in arid and semiarid areas, lack of pasture land for grazing, prolonged postpartum anestrus period (Wagenmaker *et al*., 2009), nutritional deficiencies (Kennedy *et al*., 1994), seasonality of reproduction and poor fecundity rate of Indian breeds are among some of the reasons for poor economic returns experienced by the rural sheep farmers depending solely on sheep farming for their livelihood. One of the most special characteristics of ovine reproduction is seasonality. Most breed of sheep breed during a restricted time period during the year. Therefore, their reproduction follows a seasonal pattern by alternating periods of anestrus and periods of sexual activity (Notter, 2002). The major problem that the farmer faces in sheep is reproductive management, this necessitates looking for reproductive techniques, which could decrease the cost of production. In order to achieve this goal, various pharmacological agents have been used to synchronize estrus in sheep during the breeding and non-breeding season. Estrus synchronization in sheep is a reproductive technology that allows insemination and lambing to occur at specific moments of the production systems (Evans and Maxwell, 1987). Estrus synchronization can be carried out by the conventional methods like alteration in the light exposure period, ram exposure and the use of hormonal treatments. Hormonal treatment to control ovulation and reproduction is a perquisite for successful breeding and increasing the number of pregnant females (Motlomelo *et al*., 2002, Husein *et al*. 2005) yielding more uniform newborn crop (Husein and Kridli, 2003).

The progesterone (P_4) impregnated intra-vaginal sponge, left in situ for 12-17 days in the breeding season, is a widely used method as it is comparatively cheaper and widely available (Gordon, 1997). AVIKESIL-S® is an indigenously designed cheaper and easily available intravaginal P_4 sponge made by ICAR-Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan, India containing 350 mg natural P_4.Naqvi *et al*. (1996) used indigenously prepared intravaginal P_4 sponges for 12 days with 600 IU eCG on the day of P4 sponge removal in native and crossbred ewes during summer season. Das *et al*. (1999) compared two estrus synchronization protocols during breeding and non-breeding season *viz*. indigenously made P_4 sponge (350 mg P_4) *vs* P_4 sponge along with 200 IU eCG and found better estrus response (100% *vs* 89%); conception rate (80% *vs* 67%) and lambing rate (70% *vs* 44%) in the P_4+eCG protocol as compared to P_4sponge alone.

The ovarian response upon estrus synchronization protocols may vary breed to breed (Boscos *et al*., 2002), age, season, kind of progestagen, nutritional status, stress, environmental influences, male effect (Amarantidis *et al*., 2004; Kleemann *et al*., 2006) and the body condition score (Doney *et al*., 1982; Koyuncu, 2010; Madani *et al*., 2009). Administration of progesterone along with eCG (200-400IU, IM) at the time of progesterone implant removal has been one of the efficient ways to manage the reproduction

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during non-breeding season through proper induction of estrus, ovulation and early onset of estrus (Kumar *et al*., 2016). Synchronization of estrus was done under field conditions in the semi-arid tropical region using AVIKESIL-S® for 12 days in combination with 200 IU eCG on the day of sponge removal in 471 ewes with fixed time intracervical insemination 48 and 56 h after sponge removal and 79.4% estrus response and 60.42% lambing rate was recorded (De *et al*., 2015).

Most of the domestic Indian sheep breeds are less prolific in terms of fecundity rate and have a prolonged postpartum anestrus interval (Perez-Hernandez *et al*., 2002; Rhodes *et al*., 2003). The Rambouillet and Nali crossbred sheep are being reared at Central Sheep Breeding Farm, Hisar and in its adjoining areas. The reproductive traits of this crossbred sheep are not established still as well as the information regarding their reproductive performance post estrus synchronization is meager. Thus, the hypothesis of the present study was that the use of cheaper estrus synchronization protocol (AVIKESIL-S®+eCG) in crossbred ewes shall improve the reproductive performance in terms of fertility even during the period of normal reproductive activity as well as improvement in the prolificacy and fecundity.

Materials and Methods

The study was conducted at Central Sheep Breeding Farm (latitude 29º N and longitude 75º E with average elevation of 215 m from the sea level), Hisar, (Haryana) India. The institute is located at the place where mainly sub-continental climatic conditions are present with a significant annual variation in the temperature (summers and winters). The study was conducted during autumn (September-October) which is considered as the period of normal reproductive activity for sheep. A total of thirty crossbred (Nali×Rambuillet) ewes aged between 3-5 years, weighing 34-45 kg and six healthy crossbred (Nali×Rambuillet) rams aged 3-4 years, weighing 50-60 kg, were selected on the basis of their previous breeding history with absence of any reproductive illness. The animals had access to natural grazing area for most of the day with supplementary concentrate feeding, *ad libitum* drinking water and mineral licks available under iso-managerial conditions at in-door during night.

Experimental animals were randomly divided into two groups *viz*. control and treatment having 15 animals in each group. The animals were pre-synchronized with double PG protocol at interval of 10 days (125 µg cloprostenol sodium i/m) to bring animals at same stage of estrous cycle and were selected 4th day post second PG injection. The animals under treatment group were subjected with AVIKESIL-S® intra-vaginally for 12 days with an injection of 200 IU eCG (Folligon®- MSD Animal Health, India) IM on the day of sponge removal (Fig. 1).

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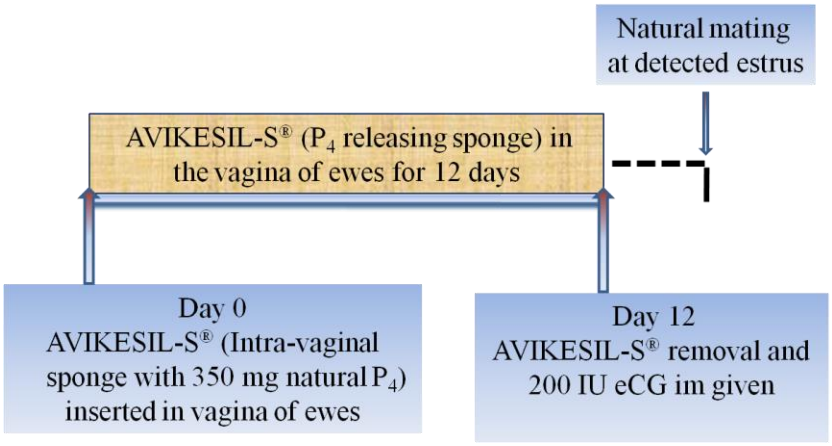

Fig. 1: Schedule of estrus synchronization protocol (AVIKESIL-S®+eCG)

The animals in control group were not subjected to any treatment after pre-synchronization. Thereafter, ram parading for estrus detection was carried out and the ewes were allowed for natural mating to occur until next 96 h. The animals of control group were also allowed to mate and show reproductive activity during similar period simultaneously. The ewes were detected in estrus via ram parading and behavioural signs. The time interval between the end of treatment and onset of estrus (h)as well asestrus duration (h) of all the ewes detected in estrus was recorded. The estrus induction rate was calculated by number of ewes detected in estrus/total number of ewes under treatment in each group multiplied by100. Also, pregnancy rate at day 25 and 45 post-mating was calculated by number of ewes detected pregnant/total number of ewes mated in each group multiplied by100. The ewes lambed during March-April, and the lambing data was recorded. The fertility or lambing rate was calculated as number of ewes lambed/total number of animals in breeding group multiplied by100. Furthermore, percent prolificacy was calculated as total number of lambs born in each group/number of ewes lambed in the group multiplied by100. Moreover, the fecundity percentage was calculated as number of lambs born/number of ewes mated multiplied by100.

For estimation of plasma P_4 (ng/mL) of ewes that underwent treatment, blood sampling was carried out in the morning (9-11 a.m.) via jugular venipuncture: at the start of treatment (time one), 14, 25- and 45-days post-mating (time two, three and four, respectively). The blood samples were collected in EDTA vials and subjected to centrifugation (3000 rpm for 15 min at 4 °C). The plasma was separated and stored at -20°Ctill further P_4 analysis. Plasma P_4 concentration was assessed via ELISA (enzyme linked immune-sorbent assay) method with Plasma P_4 ELISA kit (CALBIOTECH, USA) using Read Well Touch ELISA Plate Analyzer (Benchtop®, USA).

Real-time B-mode USG (Sonoscape S6, Portable USG machine, China) using trans-rectal probe at 5-6 MHz frequency was carried out at the start of the experiment for selection of non-pregnant animals. Further, USG

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was done on day 25 and 45 post-mating for early pregnancy diagnosis and assessment of embryonic mortality, respectively.

The obtained data from the both groups was statistically analyzed using GraphPad Prism version 8.0.1 (244) software. The normal distribution of obtained data was evaluated via Microsoft Excel (2013) software and the data which was found only fairly symmetrical (Skewness 0.5 to -0.5)was considered for further analysis while, moderately (Skewness 1.0 to -1.0) and highly skewed (skewness >1.0 and <-1.0) data was not considered for the statistical analysis. The values expressed as percentage were compared using chi square test between both groups. The parameters which were expressed as mean ± SEM were statistically compared via Unpaired Student's T-test.

Results and Discussion

The estrus induction rate (%) was observed to be significantly greater (P<0.01) in animals subjected to estrus synchronization than those not subjected to treatment (Table 1). All ewes underwent treatment showed estrus while only 60% showed estrus in the control group. There was 100% retention rate of the P_4 sponges in the ewes which could be attributed to the size and sponge texture where sponges absorb vaginal secretions and become heavier in texture and remained stuck in the vagina (Swelum *et al*., 2015). The findings of AVIKESIL-S®+eCG protocol were similar to previous study of Das *et al*. (1999). However, Kumar *et al*. (2016) found slightly lesser estrus induction rate using 200 IU eCG instead of 300 IU. De *et al*. (2015) also found a lesser estrus response rate using AVIKESIL-S®+200 IU eCG protocol in semi-tropical areas under field conditions. A lesser estrus response rate had been observed in previous studies during breeding season using P_4 sponge and 200 IU eCG in postpartum ewes (Das *et al*., 2001) and under field conditions (De *et al*., 2016). The use of eCG resulted in the greater percentage of ewes in the estrus during both seasons as a moderate dose of eCG could effectively result in the final growth and maturation of ovulatory follicles irrespective of the time of year (Oliviera *et al*., 2016).

The interval (h) between end of treatment and onset of estrus was 28.73±1.00 h in treatment group (Table 1). Husein and Kridli (2003) found similar interval to onset of estrus using P_4 sponges with GP protocol in anestrus Awassi ewes during non-breeding season. However, inclusion of P_4 sponges prior to GP protocol might be responsible for better estrus response due to P_4 priming of CNS. The use of eCG after sponge removal results in the increased size of antral follicles with resultant increase in estradiol concentration which might be precursor for the early onset of estrus (Barrett *et al*., 2004) and the use of eCG on the day of removal of sponges have more beneficial effect than 24 h later of sponge removal (Koyuncu and Alticekic, 2010). The mean duration of estrus in the ewes varied non-significantly (P>0.05) between both groups (Table 1). The study was supported by findings of Kumar *et al*. (2016) and Wani *et al*. (2017) with similar estrus duration during non-breeding season using AVIKESIL-S®+eCG.

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Table 1: Effect of estrus synchronization protocol on the reproductive traits of crossbred ewes during atypical and typical breeding seasons

Reproductive Traits	Control (n=15)	Treatment (n=15)
Estrus induction rate (%)	60.00^{A} (9/15)	100.0^{B} (15/15)
Interval between end of treatment and onset of estrus (h)	-	28.73±1.00
Estrus duration (h)	24.67±2.33	26.40±1.64
Pregnancy rate on day 25 post-mating (%)	88.89 (8/9)	86.67 (13/15)
Pregnancy rate on day 45 post-mating (%)	66.67 (6/9)	86.67 (13/15)
Embryonic losses (between day 25-45 post-mating)	2 (25.0%)	0
Lambing rate (%)	66.67 (6/9)	86.67 (13/15)
Prolificacy (%)	100.0 (6/6)	115.38 (15/13)
Fecundity (%)	66.67^{x} (6/9)	100.0^{y} (15/15)

Values bearing superscripts x and y differ significantly between groups within rows (P<0.05); Values bearing superscripts A and B differ significantly between groups within rows (P<0.01); Values expressed as percentage and mean±SEM not bearing superscript vary non-significantly within rows (P>0.05)

Das *et al*. (2000)found almost similar estrus duration during breeding season in crossbred ewes using 350 mg P_4 impregnated sponges(24.0±5.20 h) than 300 mg P_4 impregnated sponges (18.8±2.67 h).The observation of almost similar duration of estrus in the ewes irrespective of the treatment groups might be due the poor prolificacy of the breed and almost similar number of ova present at the induced estrus; however, slightly longer estrus duration in treatment group might be due to increased ovarian activity. The pregnancy detected through USG (5.0-6.0 MHz) on day 25 and 45 post-mating varied non-significantly (P>0.05) between treatment group control (Table 1). However, as the number of ewes to be pregnant were greater in treatment group than control due to better estrus induction rate. Das *et al*. (1999) and Das *et al*. (2001) observed conception rate of 80% and 100%, respectively using similar protocol. The pregnancy of ewes underwent treatment during typical breeding season were found almost similar to previous observations (Das *et al*., 2000; Das *et al*., 2001). The greater pregnancy rate observed in animals subjected to estrus synchronization protocol could be attributed to the more number of ewes expressing estrus with successful mating.

Embryonic losses were also noticed between day 25 to 45 post-mating in control group but not in animals underwent treatment (Table 1) which was consistent with the findings of Schrick and Indeep (1993) with the absence of heart beat and embryonic vesicle on day 45 indicating the embryonic wastage. The reason for occurrence of more false positives through trans-rectal B-mode USG at earlier might be due to early diagnosis of pregnancy or embryonic death at later stages of geatation (Fowler and Wilkins, 1984; Buckrell, 1988). The findings of the present study indicated that early pregnancy diagnosis could also be a mean for assessment of late embryonic deaths occurring post maternal recognition of pregnancy (MRP). The embryonic mortality in the control group could be due to poor luteal activity in the animals not subjected

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to estrus synchronization. This finding showed that the estrus synchronization of ewes resulted in better luteal activity to maintain early pregnancy.

The fertility (%) on the basis of the number of animals mated was non-significantly (P>0.05) higher in treated animals than control (Table 1). The fertility (%) of the ewes treated with AVIKESIL-S®+eCG protocol during atypical breeding season was found to be in agreement with Wani *et al*. (2017) who recorded 100% (3/3) lambing rate in anestrus ewes. A lesser fertility (%) has been observed by various previous studies using similar protocol *viz*.60.42% (De *et al*., 2015); 47.97% (De *et al*., 2016) and 50% (Kumar *et al*., 2016). The crossbred ewes under the study are usually known to be less prolific and rarely give birth to twins. Only two ewes gave birth to twins after use of AVIKESIL-S®+eCG protocol while, no twinning was found in ewes not treated with estrus synchronization protocol resulting in non-significantly (P<0.05) greater prolificacy in treatment group (Table 1). The use of eCG in the AVIKESIL-S®+eCG protocol at the time of sponge removal might be a reason for double ovulations and resulting twinning. The fecundity (%) was significantly (P<0.05) greater in animals underwent treatment that control (Table 1). The average plasma P_4 concentrations (ng/mL) on day 0 varied non-significantly (P>0.05) between both groups (Fig. 2).

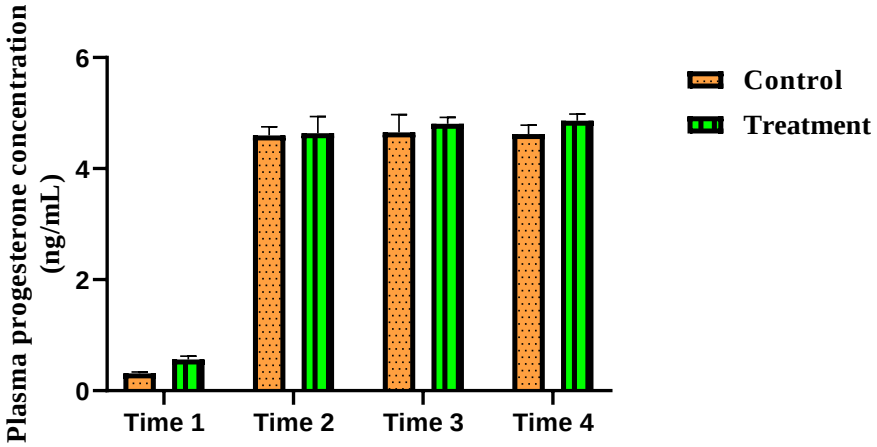

Fig. 2: The effect of estrus synchronization protocol during typical breeding season on plasma progesterone concentration (ng/mL) in crossbred ewes at different time intervals which included: time 1 (at the start of experiment *i.e* day 0), time 2 (day 14 post-mating), time 3 (day 25 post-mating) and time 4 (day 45 post-mating).

The luteal function of the ewes underwent treatment was maintained equally as evidenced by Plasma P_4 concentration on day 14, 25 and 45 post-mating which varied non-significantly (P>0.05) between both groups (Fig. 2). The values of plasma P_4 were comparable with Almadaly *et al*.(2016)on day 0 while lower

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than Husein and Kridli (2003). The presence of anechoic fluid followed by hypoechoic embryo proper in the lumen of uterus was considered to be the first and foremost reliable indication of pregnancy (Gearhart *et al*., 1988). The findings of present study were in accordance with Buckrell (1988) and Gonzalez-Bulnes *et al*. (2010) who found that imaging of embryo proper, heartbeat of embryo and placentomes might be possible from day 18, 18-23 and 26-28 days of gestation onwards in sheep, respectively. The variation in the time of the detection of embryo proper, embryonic heartbeat, placentomes might be due to differences in frequency of scanning, breed, litter size, and skill of the operator (Fridlund *et al*., 2013). The multiple assessment of pregnancy in ewes using USG could be a mean of detection of embryonic viability, fetal number count, late embryonic or fetal wastage and impending dystocia (Buckrell, 1988; Kahn, 1992). The embryonic/fetal heart rates done to assess the embryonic/fetal viability on day 25 and 45 post-mating vary non-significantly (P>0.05) between both groups (Table 2).

Table 2: Embryonic/fetal heart rate per min (Mean ± SEM) on day 25 and 45 post-mating in crossbred ewes subjected to estrus synchronization

Groups	Embryonic heart rate on day 25 post-mating (beats per min)	Embryonic heart rate on day 45 post-mating (beats per min)
Control (n=15)	128.38±1.00	126.00±1.06
Treatment (n=15)	129.31±1.28	126.15±1.43

Mean ± SEM not bearing superscripts did not vary significantly (P>0.05) between groups.

The findings of the present study regarding embryonic/fetal heart rate were similar to Yadav *et al*. (2019) but in contrast to other studies where higher ovine fetal heart rates were observed by other workers (Aiumlamai *et al*., 1992; Godfrey *et al*., 2010). The decrease in fetal heart rate with increase in gestational age was consistent with other reports in sheep (Aiumlamai *et al*., 1992; Godfrey *et al*., 2010). However, the difference in embryonic/fetal heart rate could be attributed to breed difference and other climatic factors.

Conclusion

The findings of the present study showed that AVIKESIL-S®+eCG protocol was highly effective in estrus induction during typical breeding season in crossbred ewes. The use of eCG in this protocol resulted in twinning in some of ewes which are not known for their better prolificacy resulting in better fecundity. The luteal function was well maintained in ewes subjected to AVIKESIL-S®+eCG protocol as no embryonic mortality were found in treated ewes. The multiple assessments of ewes during the first half of the gestation using USG could be a mean of detection of embryonic viability, fetal number count, late embryonic or fetal wastage and impending dystocia. Therefore, AVIKESIL-S®+eCG protocol should preferably be used for estrus synchronization and improving reproductive efficiency in sheep during even during typical breeding season to improve economic returns to poor small ruminant farmers.

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Conflict of Interest

The authors declare no conflict of interest among themselves.

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