

ESTRUS SYNCHRONIZATION TO COMBAT REPRODUCTIVE SEASONALITY IN CROSSBRED EWES

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ABSTRACT

The present study was designed to test the efficacy of a cheaper intra-vaginal progesterone releasing sponge (AVIKESIL-S®) and eCG (Equine chorionic gonadotropin) estrus synchronization protocols in non-breeding season in crossbred ewes. A total of 30 ewes were randomly divided into two groups (n=15 in each group) viz. Group I (Control) and II (Treatment). 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 Group II. 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 effective in induction of estrus resulting in better pregnancy rate and subsequent fertility during non-breeding season. The progesterone concentration did not vary significantly between groups (P<0.05) on day 14, 25 and 45 post-mating. Thus, the protocol could be advised for improvement of fertility during non-breeding season in crossbred ewes.

Keywords: eCG, Estrus synchronization, Reproductive efficiency, Sheep, Ultrasonography

The reproductive seasonality is one of the constraints in achieving higher reproductive efficiency in ewes. The cyclical reproductive activities are affected by the hormones at various stages which enable the concept of using various exogenous hormones either alone or in combination for the estrus synchronization and improvement of reproductive efficiency in ewes. Intra-vaginal progesterone (P₄) with different active compounds like medroxyprogesterone acetate (MPA), fluorogestone acetate (FGA) etc., in various form with or without combination of different gonadotropins are mainly used for estrus synchronization in sheep (Abecia *et al.*, 2011; Singh *et al.*, 2018). eCG (equine chorionic gonadotropin) induces superovulation which results in increased pregnancy rate, prolificacy and ultimately improved fertility (Ghasemi-Panahi *et al.*, 2016). Das *et al.* (1999) found better estrus response, conception rate and lambing rate in the P₄ + eCG protocol as compared to P₄ sponge alone. Synchronization of estrus was carried out under field conditions in the semi-arid tropical region using AVIKESIL-S®+200 IU eCG in 471 ewes with fixed time intra-cervical insemination resulting in good estrus response and lambing rate (De *et al.*, 2015). The AVIKESIL-S® is an indigenously designed P₄ device which is cheaply available as compared to the other P₄ containing products which paves higher cost to the small and marginal farmers. 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 during non-breeding season to enable better economic returns throughout the year irrespective of seasonality.

MATERIAL AND METHODS

Experimental site

The study was conducted at Central Sheep Breeding Farm (latitude 29° N and longitude 75° 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 in non-breeding season (May-June; summer) in the crossbred ewes.

Experimental animals and management

A total of thirty crossbred (Nali×Rambouillet) ewes aged between 3-5 years, weighing 34-45 kg and six healthy crossbred (Nali×Rambouillet) 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.

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Experimental design and selection of animals

The animals were selected on the basis of their pregnancy status. Further, to confirm the non-pregnancy, the real-time B-mode ultrasonography (USG) and plasma P₄ estimation was done before selection. The experimental animals were randomly divided into two groups viz. one control and one treatment having 15 animals in each group. The animals under Group II were subjected with AVIKESIL-S®intra-vaginally (Indigenously designed intra-vaginal P₄ sponge made by Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan, India containing 350 mg natural P₄) for 12 days with an injection of 200 IU eCG (Folligon®- MSD Animal Health, India) IM on the day of sponge removal. The animals under Group I were kept as control and were not subjected to any treatment. Thereafter, ram parading for estrus detection was carried out in both the groups and the ewes were allowed for natural mating to occur until next 96 hours.

Data Collection

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 as estrus 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 by 100. 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 by 100. The ewes lambed during November-December 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 by 100. Furthermore, percent prolificacy was calculated as total number of lambs born in each group/number of ewes lambed in the group multiplied by 100. Moreover, the fecundity percentage was calculated as number of lambs born/number of ewes mated multiplied by 100.

Plasma Progesterone (P₄) estimation

For estimation of plasma P₄ (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°C till further P₄ analysis. Plasma P₄ concentration was assessed via ELISA (enzyme linked immune-sorbent assay) method with Plasma P₄ ELISA kit (CALBIOTECH, USA) using Read Well Touch

ELISA Plate Analyzer (Benchtop®, USA).

Real-time B-mode ultrasonography (USG)

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

Statistical analysis

The obtained data from the treatment as well as control group were statistically analyzed using GraphPad Prism version 8.0.1 (244) software. The values expressed as percentage were compared using chi square test between treatment and control 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 too much higher in animals subjected to estrus synchronization than those not subjected (control) (Table 1). 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. 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 (Oliveira *et al.*, 2016). There was 100% retention rate of the P₄ sponges in the ewes. The pregnancy detected through USG (5.0-6.0 MHz) on day 25 and 45 post-mating was significantly greater (P<0.001) in treatment group than control (Table 1). Embryonic losses were also noticed between day 25 to 45 post-mating in both control and treatment group (Table 1). The embryonic mortality observed was consistent with the findings of Schrick and Inskeep (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 gestation (Buckrell, 1988; Fowler and Wilkins, 1984). The findings of the present study indicated that early pregnancy diagnosis could also be a means for assessment of late embryonic deaths occurring post maternal recognition of pregnancy (MRP). The fertility (%) on the basis of the number of animals mated was significantly greater (P<0.001) in treatment group than

Table 1

Effect of estrus synchronization protocol on the reproductive traits of crossbred ewes during non-breeding season

Reproductive traits	Group I (Control) (n=15)	Group II (Treatment) (n=15)
Estrus induction rate (%)	-	86.67 (13/15)
Interval between end of treatment and onset of estrus (h)	-	35.77±1.46
Estrus duration (h)	-	24.46±1.59
Pregnancy rate on day 25 post-mating (%)	13.33 ^a (2/15)	100.0 ^b (13/13)
Pregnancy rate on day 45 post-mating (%)	6.67 ^a (1/15)	92.31 ^b (12/13)
Embryonic losses (between day 25-45 post-mating)	1 (50.0%)	1 (7.70%)
Lambing rate (%)	6.67 ^a (1/15)	92.31 ^b (12/13)
Prolificacy (%)	100.0 (1/1)	108.33 (13/12)
Fecundity (%)	6.67 ^a (1/15)	100.0 ^b (13/13)

Values bearing superscripts a and b differ significantly between/among groups within rows (P<0.001). Values expressed as percentage and mean±SEM not bearing superscript vary non-significantly within rows (P>0.05)

Table 2

Embryonic/fetal heart rate per min (Mean ± SEM) on day 25 and 45 post-mating in crossbred ewes

Groups	Embryonic heart rate on day 25 post-mating (beats per min)	Fetal heart rate on day 45 post-mating (beats per min)
Group I (Control) (n=15)	127.00±1.00	123.50±0.50
Group II (Treatment) (n=15)	132.31±1.90 ^x	127.33±1.39 ^y

Mean ± SEM not bearing superscripts did not vary significantly (P>0.05). Values bearing superscripts x and y differ significantly within row (P<0.05).

control (Table 1). A lesser fertility (%) has been observed by various previous studies using similar protocol during non-breeding season viz. 60.42% (De *et al.*, 2015); 47.97% (De *et al.*, 2016) and 50% (Oliveira *et al.*, 2016). Only one ewe gave birth to twins after use of AVIKESIL-S®+eCG protocol, while no twinning was found in ewes not treated with estrus synchronization protocol. The prolificacy was non-significantly (P<0.05) greater in treatment group (Table 1). The fecundity (%) was also significantly (P<0.001) greater in treatment group than control (Table 1). The average plasma P₄ concentrations (ng/mL) on day 0 varied non-significantly (P>0.05) between both groups (Fig. 1). The luteal function of the ewes underwent treatment was maintained equally as evidenced by plasma P₄ concentration on day 14, 25 and 45

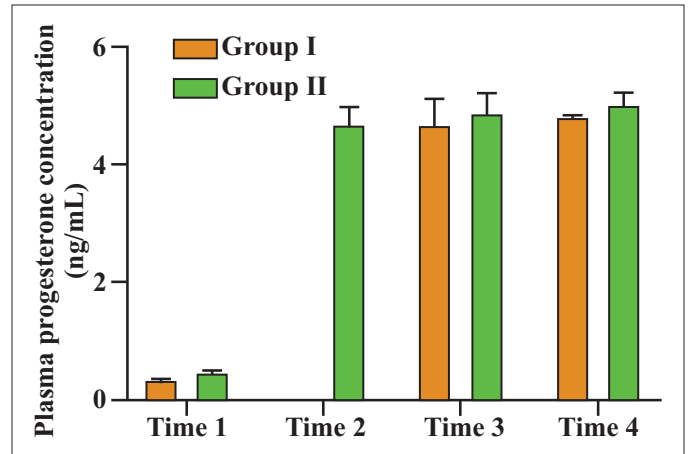


Fig. 1. The effect of estrus synchronization protocol during non-breeding season on plasma progesterone concentration (ng/mL) in crossbred ewes at different time intervals which included: time 1 (at the start of experiment i.e day 0), time 2 (day 14 post-mating), time 3 (day 25 post-mating) and time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

post-mating which varied non-significantly (P>0.05) between both groups (Fig. 1). The values of plasma P₄ were comparable with Almadaly *et al.* (2016) on day 0 while lower than Husein and Kridli (2003). 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 on day 25 than on day 45 post-mating (Table 2). The findings of present study were in accordance with Buckrell (1998) and Gonzalez-Bulnes *et al.* (2010) who found that imaging of embryo proper, heart beat 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.*, 2003). 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). However, the embryonic/fetal heart rate was significantly greater (P<0.05) on day 25 than 45 post-mating in the treatment group (Table 2). The findings of the present study regarding embryonic/fetal heart rate were 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.

The findings of the present study showed that the AVIKESIL-S®+eCG protocol was successful in estrus

induction during non-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. The luteal function was well maintained in ewes subjected to AVIKESIL-S®+eCG protocol. 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 non-breeding season also so that availability of lamb crop throughout the year could be ascertained with better economic returns to poor small ruminant farmers.

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