



Effect of Ovsynch Estrus Synchronization Protocol on Fertility in Crossbred Ewes

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ABSTRACT

The present study was designed to test the comparative efficacy of Ovsynch estrus synchronization protocols during two different phases's i.e. breeding and out-of-breeding season in crossbred ewes. During both phases a total of 60 ewes were randomly divided into four groups (n=15 in each group) viz. Group I (Control out-of-breeding season), II (Treatment out-of-breeding season), III (Control breeding season) and IV (Treatment breeding season). The Ovsynch protocol (Group II and IV) consisted of buserelin acetate on day 0, cloprostenol sodium on day 5 and buserelin acetate on day 7 intramuscular (im). Blood sampling was carried out for estimation of plasma progesterone concentration in due course of study. Trans-rectal real-time B-mode ultrasonography (USG) 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 Ovsynch protocol was almost equally effective in induction of estrus resulting in better pregnancy rate and subsequent fertility during both seasons. The progesterone concentration did not vary significantly among all groups (P<0.05) on day 14, 25 and 45 post-mating. Thus, the Ovsynch protocol could be advised for improvement of reproductive efficiency during breeding as well as out-of-breeding season in crossbred ewes throughout the year.

HIGHLIGHTS

- Ovsynch protocol is equally effective for estrus induction in breeding and out-of-breeding season in ewes.
- Ovsynch protocol could improve reproductive efficiency in out-of-breeding season in ewes.

Keywords: Sheep, Estrus synchronization, Ovsynch, GPG, Ultrasonography

The reproductive seasonality is one of the constraints in achieving higher reproductive efficiency in ewes. Ovarian cyclical activity is a result of interaction and coordinated effect of multiple hormones in the biological system. 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 (Abecia *et al.*, 2011). The Ovsynch/GPG (GnRH-PGF₂α-GnRH) protocol of estrus synchronization in ewes consists of an injection of GnRH on the starting day of protocol (day

0) to initiate the ovulation of dominant follicles, followed by an injection of PGF₂α on day 5 or day 7 to induce luteolysis, followed by injection of GnRH on day 7 or day 9 and timed AI or mating of animals on observed estrus (Ashmawy, 2011). In a study by Maracek *et al.* (2009) 50 ewes were subjected to modified GPG protocol (0-5-7), 45 induced to estrus and 24 produced lambs. However,

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contrasting results has been observed by different workers with the use of GPG protocol on the synchrony of ovulation in ewes (Ali *et al.*, 2009; Hashem *et al.*, 2015). But, as per the available literature is concerned, the studies about the efficacy of Ovsynch estrus synchronization protocol in ewes are meager in the tropical and semi-tropical areas like India. The protocol seem to be cheaper and does not require the expertise to be performed so could be implicated in field conditions for improvement in the reproductive efficiency in sheep. The hypothesis of the present study was that the use of cheaper estrus synchronization protocols (Ovsynch) in crossbred ewes shall improve the reproductive performance in terms of fertility during breeding as well as out-of-breeding season to enable better economic returns throughout the year irrespective of seasonality.

MATERIAL AND METHODS

Site and time of study

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 for a period of one year in two phase's *i.e* out-of-breeding season (May-June; summer) and breeding season (September-October; autumn). The prior permission from the institutional animal ethics committee (IAEC) was taken for conducting the experiment.

Experimental animals and design of study

A total of sixty (30 during each season) crossbred (Nali×Rambuillet) ewes aged between 3-5 years, weighing 34-45 kg and fifteen 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 four groups' *viz.* one control and one treatment group during each season having 15 animals in each group. Group I and IINB were during out-of-breeding season while Group III and IV were during breeding season. Group II and IV received Ovsynch protocol {4 µg buserelin acetate (Gynarich®-Intas, India) im, 125 µg cloprostenol sodium im (Vetmate®-Cargill, India) and 4 µg buserelin acetate im (Gynarich®-Intas, India) on day 0 (start of treatment), 5 and 7, respectively} (Fig. 1). The animals under breeding season were pre-synchronized with double PG (Prostaglandin) protocol at interval of 10 days with 125 µg cloprostenol sodium im (Vetmate®-Cargill, India) to bring animals at same stage of estrous cycle and were selected 4th day post second PG injection. The animals under Group I and III were kept as control and were not subjected to any treatment. Thereafter, ram parading for estrus detection was carried out in all groups and the ewes were allowed for natural mating to occur until next 96 h.

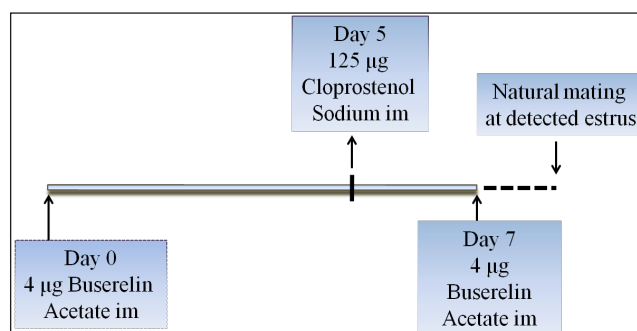


Fig. 1: Ovsynch estrus synchronization protocol consisting of GnRH-PGF₂α-GnRH injections on day 0 (start of treatment), 5 and 7, respectively

Data collection

The ewes were detected in estrus via ram parading and behavioral 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 in out-of-breeding and breeding season groups lambd during

November-December and March-April, respectively and the lambing data was recorded. The fertility or lambing rate was calculated as number of ewes lambled/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 lambled 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 analysis

For estimation of plasma progesterone (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 P4ELISA kit (CALBIOTECH, USA) using Read Well Touch ELISA Plate Analyzer (Benchtop®, USA).

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 all treatment groups (both in out-of-breeding and breeding season) 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 majority of data was found fairly symmetrical (Skewness 0.5 to -0.5) while, some data was moderately skewed (Skewness 1.0 to -1.0) which might be due to small sample size in some parameters. The data with high skewness (>1.0 and <-1.0) was not considered for the

statistical analysis. The values expressed as percentage were compared using chi square test among all groups in both seasons separately as well as between the seasons. The parameters which were expressed as mean \pm SEM were statistically compared via one way ANOVA test with Tukey adjustment among all groups. Unpaired Student's T-test was used to compare the embryonic/fetal heart rates on day 25 and 45 post-mating with the groups.

RESULTS AND DISCUSSION

The estrus induction rate (%) was observed to be non-significantly greater ($P>0.05$) during breeding season than out-of-breeding season (Table 1). Contrary to the present findings lesser estrus response rate had been reported previously using GPG/Ovsynch protocol during non-breeding season (Almadaly *et al.*, 2016; Lone *et al.*, 2016; Martemucci *et al.*, 2010). The poor response of ewes to GPG treatment during out-of-breeding season might be due to failure of the hormonal protocol and/or male effect to rebound from deep anestrus (Almadaly *et al.*, 2016). The use of GPG protocol during typical breeding season reported findings similar to current study but more scattered ovulations in Farfara ewes (Ali *et al.*, 2009). In contrast, lesser (Ashmawy, 2011; Hashem *et al.*, 2015) and greater (Abu El-Ella *et al.*, 2016; Oliveira *et al.*, 2009) estrus induction rate had been reported using GPG protocol during breeding season. The differential outcomes of the GPG protocol during breeding season could be attributed to types of hormones used, the depth of anestrus and breed differences. The response to GnRH is not always similar and it mainly relies on the stage of the estrous cycle at the time of its application (Kumar *et al.*, 2016). The inability of first GnRH injection to induce ovulation and formation of corpus luteum for action of $\text{PGF}_{2\alpha}$ might be a reason for the poor estrus induction rate in GPG protocol (Hashem *et al.*, 2015).

The interval (h) between end of treatment and onset of estrus was significantly greater ($P<0.001$) during out-of-breeding season than breeding season (Table 1). Husein and Kridli (2003) found similar interval to onset of estrus using P4 sponges with GP protocol in anestrus Awassi ewes during non-breeding season. However, inclusion of P4 sponges prior to GP protocol might be responsible for better estrus response due to P4 priming of CNS. The longer interval to onset of estrus using GPG protocol

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

Reproductive traits	Treatment groups			
	Out-of-breeding season		Breeding season	
	Group I (Control) (n=15)	Group II (Treatment) (n=15)	Group III (Control) (n=15)	Group IV (Treatment) (n=15)
Estrus induction rate (%)	—	53.33 (8/ 15)	60.00 (9/ 15)	66.67 (10/ 15)
Interval between end of treatment and onset of estrus (h)	—	44.75 ± 5.59 ^a	—	58.20 ± 2.80 ^b
Estrus duration (h)	—	23.25 ± 2.10	24.67 ± 2.33	26.67 ± 2.47
Pregnancy rate on day 25 post-mating (%)	13.33 ^a (2/ 15)	87.50 ^b (7/ 8)	88.89 ^b (8/ 9)	90.0 ^b (9/ 10)
Pregnancy rate on day 45 post-mating (%)	6.67 ^{aA} (1/ 15)	62.50 ^B (5/ 8)	66.67 ^B (6/ 9)	70.0 ^b (7/ 10)
Embryonic losses (between day 25-45 post-mating)	1 (50.0%)	2 (28.57%)	2 (25.0%)	2 (22.22%)
Lambing rate (%)	6.67 ^{aA} (1/ 15)	62.50 ^B (5/ 8)	66.67 ^B (6/ 9)	70.0 ^b (7/ 10)
Prolificacy (%)	100.0 (1/ 1)	100.0 (5/ 5)	100.0 (6/ 6)	100.0 (7/ 7)
Fecundity (%)	6.67 ^{aA} (1/ 15)	62.50 ^B (5/ 8)	66.67 ^B (6/ 9)	70.0 ^b (7/ 10)

Values bearing superscripts A and B differ significantly between groups within rows ($P < 0.01$); Values bearing superscripts a, b, c and d differ significantly between/among groups within rows ($P < 0.001$); Values expressed as percentage and mean \pm SEM not bearing superscript vary non-significantly within rows ($P > 0.05$).

during out-of-breeding season was suggestive that second GnRH injection could be given later near the onset of estrus for better ovulation synchrony to improve the fertility (Martemucci *et al.*, 2010). A lesser (Hashem *et al.*, 2015; Kulaksiz *et al.*, 2013) and greater (Abu El-Ella *et al.*, 2016) interval to onset of estrus than present findings had been observed previously using GPG protocol during breeding season. The variability in response of first GnRH injection and occurrence of luteolysis at the time when largest follicle is in the regressing stage might be cause of subsequent late onset of estrus and delayed ovulation (Menchaca and Rubianes, 2004).

The mean duration of estrus in the ewes varied non-significantly ($P > 0.05$) among different groups (Table 1). The mean estrus duration of ewes using GPG protocol for estrus synchronization during typical breeding season was similar to previous findings (Hashem *et al.*, 2015; Kulaksiz *et al.*, 2013). However, longer estrus duration (40.00 ± 16.80 h) was observed in Rahmani ewes (Abu El-Ella *et al.*, 2016) using GPG protocol in breeding season. In contrast, Ali *et al.* (2009) observed estrus duration of 19.0 ± 2.1 h in Farfara ewes using GPG protocol which was quite lesser than present study. 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 during breeding season might be due to increased ovarian activity.

The pregnancy detected through USG (5.0-6.0 MHz) on day 25 and 45 post-mating was significantly ($P < 0.001$) lesser in control group during out-of-breeding season than treatment groups as well as control group of breeding season. The pregnancy rate on day 45 in Group I also followed the same pattern and differed significantly than Group II ($P < 0.01$), III ($P < 0.01$) and IV ($P < 0.001$). The pregnancy rate on day 25 and 45 post-mating varied non-significantly ($P > 0.05$) among Group II, III and IV (Table 1). The pregnancy rate of ewes underwent GPG protocol during typical breeding season was in concurrence with Abu El-Ella *et al.* (2016). On the other hand, Hashem *et al.* (2015) revealed 100% conception rate using GPG protocol in Barki and Rahmani crossbred ewes. However, lesser pregnancy rates were observed in other studies viz. 50% and 35.7% post timed AI and natural mating in Karagouniko ewes (Deligiannis *et al.*, 2005); 50% in Farfara ewes (Ali *et al.*, 2009) and 33.3% and 46.6% after timed AI using GPG protocol (Martínez *et al.*, 2013).

The embryonic losses *i.e.* 1 (50.0%), 2 (28.57%), 2 (25.0%) and 2 (22.22%) were also noticed in Group I, II, III and IV, respectively between day 25 to day 45 post-mating evidenced by absence of embryonic vesicle, amniotic fluid and absence of detectable heart rate in fetuses on day 45 post-mating. Embryonic losses noticed between day 25 to 45 post-mating were consistent with the findings of Schrick and Inskip (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 (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 fertility (%) on the basis of the number of animals mated varied significantly in Group I than Group II ($P<0.01$), III ($P<0.01$) and IV ($P<0.001$) but there was no significant ($P>0.05$) difference among Group II, III and IV (Table 1). The crossbred ewes under the study are usually known to be less prolific and rarely give birth to twins and thus no twinning was observed in any ewe. While, no twinning was found in ewes treated and kept as control so the prolificacy in each group was 100% (Table 1). However, the fecundity (%) was also significantly lesser in Group I than Group II ($P<0.01$), III ($P<0.01$) and IV ($P<0.001$) which varied non-significantly ($P>0.05$) among Group II, III and IV (Table 1). Martemucci and Alessandro (2010) recorded 80% fertility following GPG protocol during non-breeding season. However, the fertility (%) was lesser in other studies *viz.* 50% in Farfara ewes (Ali *et al.*, 2009); 20% in Awassi ewes (Titi *et al.*, 2010) and 35.7% in fat-tailed ewes (Kulaksiz *et al.*, 2013) during breeding season using GPG protocol. Hashem *et al.* (2015) observed 100% lambing rate in Barki and Rahmani crossbred ewes using GPG protocol in breeding season. The study was also supported by findings of Ali *et al.* (2009) and Hashem *et al.* (2015) with no twinning in Farfara and crossbred ewes respectively, subjected to GPG protocol in breeding season.

The average plasma P4 concentrations (ng/mL) on day 0 varied non-significantly ($P>0.05$) among all groups (Fig. 2). The luteal function of the ewes underwent treatment was maintained equally as evidenced by Plasma P4

concentration on day 14, 25 and 45 post-mating which varied non-significantly ($P>0.05$) among all groups (Fig. 2). The values of plasma P4 were comparable with Almadaly *et al.* (2016) on day 0 while lower than Husein and Kridli (2003). The serum P4 concentration (ng/mL) on day 21 and 24 post insemination were comparable to the present study in Rahmani ewes subjected to different modalities of GPG protocol during breeding season (Ashmawy, 2011). P4 concentration in Rahmani ewes underwent GPG protocol during breeding season were almost similar to the present study on day of start of treatment and day 18 and 30 post-mating (Ashmawy, 2011).

The embryonic/fetal heart rates done to assess the embryonic/fetal viability on day 25 and 45 post-mating vary non-significantly ($P>0.05$) among all groups (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.

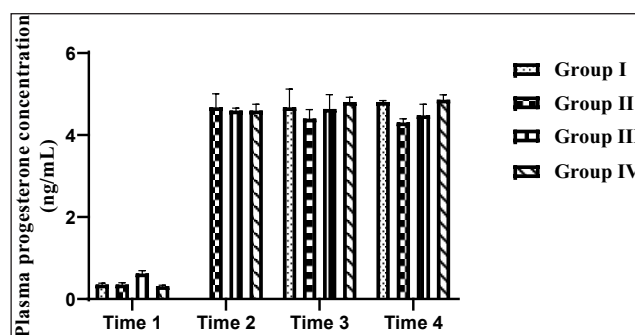


Fig. 2: The effect of estrus synchronization protocol during out-of-breeding and breeding season on plasma progesterone concentration (ng/ mL) in crossbred ewes at different time intervals which included: time 1 (at the start of experiment *i.e.* day 0), time 2 (day 14 post-mating), time 3 (day 25 post-mating) and time 4 (day 45 post-mating). Group I and III served as control during out-of-breeding and breeding season, respectively. Group II and IV stands for Ovsynch/ GPG protocol during out-of-breeding and breeding season, respectively.

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

Table 2: Embryonic/fetal heart rate per min (Mean \pm SEM) on day 25 and 45 post-mating in crossbred ewes subjected to estrus synchronization during atypical and typical breeding season

Season		Embryonic heart rate on day 25 post-mating (beats per min)	Fetal heart rate on day 45 post-mating (beats per min)
Out-of-breeding season	Group I (Control) (n=15)	127.00 \pm 1.00	123.50 \pm 0.50
	Group II (Treatment) (n=15)	129.29 \pm 1.55	126.80 \pm 1.24
Breeding season	Group III (Control) (n=15)	128.38 \pm 1.00	126.00 \pm 1.06
	Group IV (Treatment) (n=15)	132.44 \pm 1.89	129.71 \pm 2.25

Mean \pm SEM not bearing superscripts did not vary significantly ($P > 0.05$) among groups on day 25 and 45 post-mating, respectively as well within group on day 25 and 45 post-mating.

(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; Kähn, 1992).

CONCLUSION

The findings of the present study showed that Ovsynch/GPG protocol was successful in estrus induction during breeding as well as out-of-breeding season in crossbred ewes. The results in terms of estrus induction rate (%), pregnancy and subsequent fertility were pronounced in out-of-breeding season in animals treated than those not underwent estrus synchronization. However, the estrus synchronization protocol did not improve prolificacy and fecundity which could be due to the less prolificacy of breed under study. However, the use of protocol during out-of-breeding season showed promising results but not in breeding season, so the cheaper estrus synchronization protocol requiring no expertise in application could be advised for the improvement of reproductive outcomes in crossbred ewes during out-of-breeding season also to

ascertain increased economic returns of poor farmers. 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.

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