Estrus and fertility responses in acyclic ewes treated with short, medium or long-term GnRH-PGF$_2$α-GnRH protocols supplemented with intra-vaginal progesterone therapy

HARIOM$^{1,5}$, HARPREET SINGH$^2$, RAVI DUTT$^1$ and L C RANGA$^1$

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125 004 India

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ABSTRACT

The present study was conducted with the aim to compare the effectiveness of the short-term (5 days), moderate-term (8 days) and long-term (12 days) progesterone therapy in the form of intravaginal AVIKESIL-S® progesterone sponge developed by CSWRI, Avikanagar (Rajasthan), during modified GnRH-PGF$_2$α-GnRH treatments during non-breeding season (May-July) in crossbred (Nali×Rambuillet) ewes. The seasonal anestrus ewes were enrolled into four groups. In Group 1 (n=50), ewes were treated with GnRH on day 0, PGF$_2$α on day 5, and second GnRH on day 7 plus progesterone supplementation with intravaginal progesterone sponge from day 0 to day 5 while in Group-2 (n=50), ewes were treated with GnRH on day 0, PGF$_2$α on day 8, and second GnRH on day 9 plus progesterone supplementation with intravaginal progesterone sponge from day 0 to day 8. Further, in Group 3 (n=50), ewes were treated with GnRH on day 0 and PGF$_2$α on day 12 and second GnRH on day 13 plus progesterone supplementation with intravaginal AVIKESIL-S® sponge from day 0 to day 12. Group 4 (n=50), comprised of untreated control animals. Results showed that estrus synchronization rate (%) was significantly higher in Group 2 and Group 3 in comparison to Group 4 (control). There was significant difference in the duration of estrus, pregnancy rate, lambing rate in Group 2 as compared to Group 4 (control) but prolificacy and fecundity were not statistically significant (p>0.01) among the groups during out of breeding season. Thus, modified Ovsynch protocol with 8-day progesterone therapy is better for augmenting fertility in crossbred ewes during non-breeding season.

Keywords: AVIKESIL-S®, Crossbred ewes, Ovsynch, Progesterone sponge, Seasonal anestrus
conventional Ovsynch protocol originally devised for cattle species may not be applicable for sheep attributable to species differences in the seasonality and duration of estrus cycles (21d vs 16–17d) for cattle and sheep, respectively (Ptaszynska 2011). Incorporating a single intravaginal insert to the timed AI program increases progesterone concentrations, but did not benefit fertility in cows that have CL at the initiation of the Ovsynch (0–7–9) protocol (Bisinotto et al. 2015). Apparently, the standard Ovsynch protocol should be modified for having different follicular and luteal dynamics (Kulaksiz et al. 2013). Considering these facts, we aimed to compare the effectiveness of the short-term (5 days), moderate-term (8 days) and long-term (12 days) progesterone therapy during modified GnRH-PGF$_{2\alpha}$-GnRH treatments (0,5,7; 0,8,9; 0,12,13) during non-breeding season.

**MATERIALS AND METHODS**

This study was conducted at Central Sheep Breeding Farm located at Hisar, Haryana, India at latitude 29°N and longitude 75°E with average elevation of 215 m from the sea level. The study was conducted during the non-breeding (May–July months; summer season) season. The continental climatic conditions were mainly present at the farm with a significant annual variation in the temperature (summer and winter). A total of 200 crossbred (Nali × Rambuillet) ewes aged between 3–5 years, weighing 34–45 kg and 20 healthy crossbred (Nali × Rambuillet) rams aged 3–4 years, weighing 50–60 kg, were selected on the basis of their previous breeding history with the absence of any reproductive illness. The animals were identified with ear tags. The animals had access to natural grazing area for most of the day with supplementary concentrate feeding, ad lib. drinking water and mineral licks available under iso-managerial conditions. All the ewes had previously lambed and weaned their last lambing at least 6 weeks earlier. The rams were used for breeding ewes at least two months prior to the experiment.

**Experimental design:** The experimental ewes were enrolled into four protocols during non-breeding season (3 treatment groups and 1 control group). Each treatment as well as control group comprised randomly (parity-wise) selected 50 ewes.

Group 1: All ewes were treated with GnRH (Buserelin acetate, 4 µg, i.m. Receptal®Vet MSD Animal Health India) on day 0, PGF$_{2\alpha}$ (cloprostenol, 125 µg, i.m. Estrumate™ MSD Animal Health India) on day 5, and second GnRH (Buserelin acetate, 4 µg, i.m.) on day 7 plus progesterone supplementation with intravaginal AVIKESIL-S® sponge from day 0 to day 8 (i.e. for 8 days) (Fig. 2).

Group 3: Ewes were treated with GnRH (Buserelin acetate, 4 µg, i.m.) on day 0 and a dose of PGF$_{2\alpha}$ (cloprostenol, 125 µg, i.m.) on day 12 and second GnRH (Buserelin acetate, 4 µg, i.m.) on day 13 plus intravaginal progesterone supplementation through AVIKESIL-S® sponge from day 0 to day 12 (i.e. for 12 days) (Fig. 3).

Group 4 (Control): Ewes of control groups during non-breeding season were exposed to unsynchronized breeding for 27 days. Detection of estrus in all the ewes was done by ram parading every four-hour interval starting from 2nd GnRH injection in treatment groups till next 96 h and natural mating was allowed with proven rams. The rams used were not allowed to mate any sheep one week prior to their introduction in the experimental flock. A total of 5 rams were used in each group for mating with ewes exhibiting estrus.

**Ultrasoundographic scanning:** For the ultrasonographic study of reproductive status and early pregnancy diagnosis, a real time B-mode, portable Ultrasonography machine (SONPSCAPE S6/S6Pro/S6BW Portable Digital Color Doppler Ultrasound System) equipped with a linear array trans-rectal multi-frequency transducer using frequency of 5–7 MHz. Transrectal real-time B-mode ultrasonography (USG) was carried out on day 28 post mating and pregnancy was confirmed by the presence of amniotic vesicle, or fetal image and embryonic heart beat. Further, the USG was repeated on day 45 post-mating to estimate the overall pregnancy rate and embryonic mortalities between day 25 and 45 post-mating. Fertility variables recorded in all the groups were estrus synchronization rate, duration of estrus (h), pregnancy rate (%), embryonic mortality (%), prolificacy (%), fecundity and twining rate (%).

**RESULTS AND DISCUSSION**

All the progesterone impregnated intravaginal sponge remained in place until the time of withdrawal (no losses). Estrus synchronization rate was higher in Group 2 (26/50, 52%) and lowest in Group 4 (10/50, 20%) (Table 1). Estrus synchronization rate (%) was significantly higher in Group 2 (p<0.01) as well as Group 3 (p<0.05) compared to the Group 4 (control) (Fig. 4). The average duration of estrus was significantly higher in Group 2 (35.6±3.3 h) as compared to Group 4 (26.5±2.3 h). The duration of estrus was similar (p>0.05) in Group 1 and Group 3.

Progesterone has a ‘priming’ effect on the central nervous system, which enhance the response to gonadotropins administered after the end of progesterone treatment (Naslaij et al. 2001). The high blood progesterone concentrations after implant introduction lower the secretion of LH. Such a decrease in LH availability after implant introduction, being the hormone, which supports dominant follicles (Campbell et al. 1995), causes atresia of large follicles present in the ovaries and promotes follicular turnover, leading to the appearance of new preovulatory
Contrarily, Ali et al. (2009) and Khalilavi et al. (2016) recorded lower estrus duration in different estrus synchronization protocols during non-breeding season. The observation of almost similar duration of estrus in the ewes irrespective of the treatment groups might be due to 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 during non-breeding season.

The pregnancy rate was highest (48%) in Group 2 and the lowest (16%) in Group 4. Pregnancy rates was significantly (p<0.01) higher in Group 2 as compared to Group 4. However, the pregnancy rates were similar (p>0.05) between in Group 1 and Group 3 (Table 2). Further, at 45th day post-mating, the number of ewes reconfirmed pregnant were 23, 15, 15 and 8 in Groups 2, 3, 1 and 4, respectively. The pregnancy rates were highest (46%) in Group 2 and lowest (16%) in Group 4. Pregnancy rates in Group 2 were significantly (p<0.01) higher than in the Group 4.

Embryonic mortality during non-breeding season was 6.2% in Group 1 and Group 3, followed by 4.1% in Group 2. There was no embryonic mortality in Group 4 (control). The lambing rate was highest in Group 2 and lowest in Group 4 (46% versus 16%) with a significant (p<0.01) difference. Lambing rates in Group 1 and Group 3 were similar (p>0.05). Twins were produced in Group 2 and 3 only with similar (p>0.05) twinning rates of 4.4 and 6.6%, respectively. Prolificacy percent was highest in Group 3 but was not significantly (p>0.05) different from the other experimental groups during the same season. Fecundity was similar (p>0.05) among the groups and was highest (92%) in Group 2.

The ovine pregnancy could be diagnosed at an earliest day 17–19 of gestation based on presence of anechoic intrauterine fluid by trans-rectal USG (5 MHz) while the embryo proper could be imaged between days 21 to 34 of gestation with placentomes visualization on day 26–28 (Garcia et al. 1993). Higher estrus duration had been recorded by Abu El-Ella et al. (2016) and Yadav et al. (2020b) using Ovsynch (0, 5, 7) protocol. Contrarily, Ali et al. (2009) and Khalilavi et al. (2016) recorded lower estrus duration in different estrus synchronization protocols during non-breeding season. The observation of almost similar duration of estrus in the ewes irrespective of the treatment groups might be due to 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 during non-breeding season.

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### Table 1. Estrus induction response in different groups in ewes during non-breeding season

<table>
<thead>
<tr>
<th>Group (n=50/group)</th>
<th>No. of ewes induced to estrus</th>
<th>Estrus synchronization rate (%)</th>
<th>Duration of estrus (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>17</td>
<td>34A, B</td>
<td>32.9±2.8A, B</td>
</tr>
<tr>
<td>Group 2</td>
<td>26</td>
<td>52A</td>
<td>35.6±3.3A</td>
</tr>
<tr>
<td>Group 3</td>
<td>19</td>
<td>38A</td>
<td>34.2±2.6A, B</td>
</tr>
<tr>
<td>Group 4 (Control)</td>
<td>10</td>
<td>20B</td>
<td>26.5±2.3B</td>
</tr>
</tbody>
</table>

Values with the different superscripts within a column differ significantly (A, B; p<0.01), (a, b; p<0.05).

Supplementation of intravaginal progesterone sponge for 5 days in a GnRH (day 0)-PGF,α (day 5) protocol promotes growth of dominant follicle when CL is not present (Titi et al. 2010). Further, Abu El-Ella et al. (2016) in Rahmani ewes and Yadav et al. (2020b) in crossbred ewes recorded 93.33, 93.33 and 53.33% estrus synchronization rates using Ovsynch protocol (0,7,9) conjuncted with progesterone sponge for 7 days during non-breeding, Ovsynch (0, 9, 11) protocol during mating season and Ovsynch (0, 5, 7) protocol during non-breeding, respectively. Interestingly, Almadaly et al. (2016) did not find any effect of Ovsynch (0, 5, 7) protocol during non-breeding season in Rahmani Egyptian Ewes. Variations in estrus response rate and the discrepancies between the results in the literature could be attributed to the difference in the breed variations, nutrition, seasonality effect, climatic and environment factors (Dogan and Nur 2006).

Higher estrus duration had been recorded by Abu El-Ella et al. (2016) and Yadav et al. (2020b) using Ovsynch protocol (0, 5, 7). Contrarily, Ali et al. (2009) and Khalilavi et al. (2016) recorded lower estrus duration in different estrus synchronization protocols during non-breeding season. The observation of almost similar duration of estrus in the ewes irrespective of the treatment groups might be due to 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 during non-breeding season. The pregnancy rate was highest (48%) in Group 2 and the lowest (16%) in Group 4. Pregnancy rates was significantly (p<0.01) higher in Group 2 as compared to Group 4. However, the pregnancy rates were similar (p>0.05) between in Group 1 and Group 3 (Table 2). Further, at 45th day post-mating, the number of ewes reconfirmed pregnant were 23, 15, 15 and 8 in Groups 2, 3, 1 and 4, respectively. The pregnancy rates were highest (46%) in Group 2 and lowest (16%) in Group 4. Pregnancy rates in Group 2 were significantly (p<0.01) higher than in the Group 4.

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(2017) observed 70% pregnancy rate in does through Ovsynch protocol (0, 7, 9) plus prostaglandin for 7 days during non-breeding. On the other hand, lesser pregnancy rates as 33.3 and 46.6% after timed AI using GPG protocol comprising of different GnRH doses (Martínez et al. 2013). Prolonged progesterone treatment period generally has been associated with low conception rates (Vinoles et al. 2001) and this reduced fertility has been attributed to a sperm transport impairment in the genital tract of the progesterone treated ewes (Hawk and Cooper 1977). Lower pregnancy rates after long term progesterone treatment compared to medium duration progesterone supplementation may be related to slower follicular turnover that promote the ovulation of persistent dominant follicles (Vinoles et al. 2001).

Embryonic mortality was recorded in treatments groups in the current investigation which is contrary to findings of Yadav et al. (2020a) during non-breeding season. In an another study, Yadav et al. (2020b) in crossbred ewes observed 50.0 and 28.7% embryonic mortality in control and Ovsynch (0, 5, 7) protocol, respectively between day 25 and 45 post mating during non-breeding season. This data seems to be higher due to small sample size of the animals. Embryonic losses noticed between day 28 to 45 post-mating were 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. Dixon et al. (2007) observed late embryonic mortality as 3.8% in non-breeding and transition season in ewes between 25 and 45 days of gestation. The factors causing early embryonic mortality in sheep are not well established, there is some evidence suggesting the involvement of luteal inadequacy, resulting from environmental factors such as heat stress or nutrition, has been shown to be a major cause of embryonic loss in sheep (Binelli et al. 2001). The lower lambing rate in control animals is due to less ovarian activity during non-breeding season in ewes which is in agreement with Yadav et al. (2020b). Higher lambing rate was recorded by Khalilavi et al. (2016) during anestrus season using intravaginal prostoglandin sponge+PMSG for short (66.66±0.16%) and long duration (80±0.17%). Likewise, higher lambing rate in treatment groups compared to control group is due to estrus induction and pregnancy by modified Ovsynch protocols.

The crossbred ewes under the study are usually known to be less prolific and rarely give birth to twins. Similar findings were recorded by Yadav et al. (2020b) non-breeding season. No twining was recorded in Group 1 and 4. This was due to comparatively less estrus response in these two groups. The estrus synchronization protocol did not improve prolificacy and fecundity which could be due to the less prolificacy of breed under study.

The fecundity (%) was statistically non-significant (p>0.05) among the treatment and control groups. Titi et al. (2010) reported that there was a trend for improved fecundity in the progesterone-supplemented Ovsynch group after natural mating in Damascus does. Yadav et al. (2020b) in crossbred ewes observed 66.7 and 62.50% fecundity in control and Ovsynch (0, 5, 7) treated ewes, respectively during non-breeding season. This difference can also be due to variation in protocols undertaken for the investigation. Short treatments result in a series of benefits like a better control of follicular response and ovulation, acceptable fertility rates (no lower than conventional progesterone treatments), shorter period for implementation of large scale FTAI programs, and in some cases allowing the reutilization of intravaginal devices (Menchaca et al. 2017). Notably, earlier tested treatment regimens of a shorter interval resulted in increased responses in naturally mated ewes (Vinoles et al. 2001) and goats (Fonseca et al. 2005). In the present study, it can be concluded that, medium duration of protocol yields better fertility response in terms of estrus synchronization rate, pregnancy rate, lambing rate, prolificacy and fecundity than short and long duration protocols comprising of GnRH-PGF2α GnRH plus progesterone supplementation during

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**Table 2. Fertility parameters in ewes of different groups during non-breeding season**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pregnancy rates on day 28 post mating (%)</th>
<th>Overall pregnancy rates on day 45 post mating (%)</th>
<th>Embryonic mortality (%)</th>
<th>Lambing rate (%)</th>
<th>Twins (% of lambing)</th>
<th>Prolificacy (%)</th>
<th>Fecundity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>16/50 A, B (32%)</td>
<td>15/50 A, B (30%)</td>
<td>6.2</td>
<td>14/50 A, B (28%)</td>
<td>–</td>
<td>14/14 (100%)</td>
<td>14/17 (82%)</td>
</tr>
<tr>
<td>Group 2</td>
<td>24/50 A (48%)</td>
<td>23/50 A (46%)</td>
<td>4.1</td>
<td>23/50 A (46%)</td>
<td>1/23 (4.3%)</td>
<td>24/23 (104%)</td>
<td>24/26 (92%)</td>
</tr>
<tr>
<td>Group 3</td>
<td>16/50 A, B (32%)</td>
<td>15/50 A, B (30%)</td>
<td>6.2</td>
<td>15/50 A, B (30%)</td>
<td>1/15 (6.6%)</td>
<td>16/15 (106%)</td>
<td>16/19 (84%)</td>
</tr>
<tr>
<td>Group 4</td>
<td>8/50 B (16%)</td>
<td>8/50 B (16%)</td>
<td>–</td>
<td>8/50 B (16%)</td>
<td>–</td>
<td>8/8 (100%)</td>
<td>8/10 (80%)</td>
</tr>
</tbody>
</table>

Values with the different superscripts within a column differ significantly (A, B; p<0.01), (a, b; p<0.05).

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**Fig. 4. Estrus synchronization rates in different groups in ewes during non-breeding season.** Bars with different uppercase letters differ significantly (A, B; p<0.01). Bars with different lowercase letters differ significantly (a, b; p<0.05).
non-breeding season. Further, studies are warranted with follicular dynamics along with endocrinological interrelationships with such protocols.

REFERENCES


