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

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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$_2\alpha$-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$_2\alpha$ 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$_2\alpha$ 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$_2\alpha$ 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

Seasonality of reproduction, prolonged postpartum anestrus periods and poor fecundity rate in Indian breeds of sheep along with the prevailing nutritional deficiencies, harsh environmental conditions of arid and semiarid areas and lack of pasture land for grazing are impediments that causes great economic loss to the sheep farmers (Mohan 2017). Sheep in temperate regions are spontaneous ovulators and seasonally polyestrous with breeding season from fall to early winter (Duarte *et al*. 2010) and subsequent lambing during spring, while in the remaining part of year sheep remain acyclic. In ewes, follicular waves have been reported during the estrous cycle, seasonal anestrus, transition period and gestation (Bartlewski *et al.* 2000). Estrus induction and synchronization with progestagens is widely used for out-of-season breeding and for controlling reproduction in sheep. Earlier regimens of estrus synchronization in the ewes comprised long-term progesterone therapy for 12–14 days with intravaginal devices (Abecia *et al*. 2011), resulting in a high estrus induction response but a variable fertility (Menchaca and Rubianes 2004). During such long-term treatment, a sub-luteal progesterone concentration persists during the last days of treatment (Greyling *et al*. 1994) leading to an increase in LH pulse frequency and a prolonged persistence and aging of the ovulatory follicles (Menchaca and Rubianes 2004) that negatively affects the fertility. Several studies reported the use of Ovsynch (GnRH-PGF$_2\alpha$-GnRH) and other synchronization protocols to control ovarian functions for synchronization of estrus or ovulation in small ruminants, but with variable results (Senthilkumar *et al.* 2017).

A shorter period of treatment with the intravaginal device should be related to a lower incidence of vaginal inflammation and infection, presumptively leading to better fertility yields after natural breeding or cervical insemination. The short term administration of a progesterone source between the GnRH and PGF$_2\alpha$-treatment may be effective in delaying estrus and ovulation resulting in better estrus synchrony (Lamb *et al.* 2001). The GnRH-PGF$_2\alpha$ protocol was found to be effective in estrus synchronization in ewes but the effectiveness of short-term progesterone treatment with GnRH-PGF$_2\alpha$-GnRH regimen has not been evaluated. We hypothesized that the

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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 the 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 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 atleast 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 developed by Central Sheep and Wool Research Institute, Avikanagar (Rajasthan), India from day 0 to 5 (Fig. 1). The intravaginal AVIKESIL-S® sponge had 350 mg of natural progesterone.

Group 2: Ewes were treated with GnRH (Buserelin acetate, 4 µg, i.m.) on day 0 and a dose of $PGF_{2\alpha}$ (cloprostenol, 125 µg, α i.m.) on day 8 and second GnRH (Buserelin acetate, 4 µg, i.m.) on day 9 plus intravaginal progesterone supplementation through 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 ever 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.

Ultrasonographic 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 ($p<0.05$) 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 (Naslaji *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

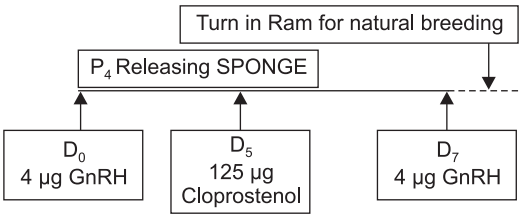

Fig. 1. Schematic diagram representing experimental design in Group 1.

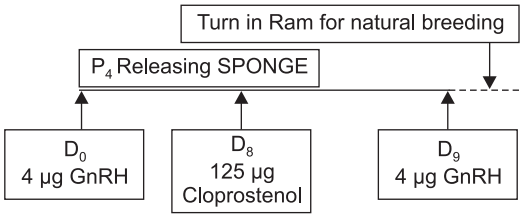

Fig. 2. Schematic diagram representing experimental design in Group 2.

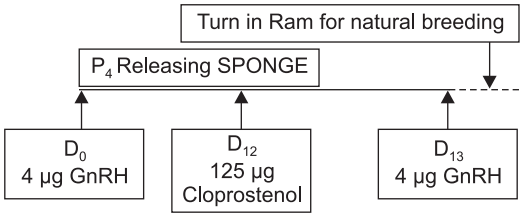

Fig. 3. Schematic diagram representing experimental design in Group 3.

follicles. Hence, removal of the progesterone sponge followed by administration of $PGF_2\alpha$ and GnRH analogue 2 days apart would assure the presence of a healthy growing preovulatory follicle able to induce estrus and reach fertile ovulation. Slightly longer estrus duration in Group 2 might be due to more ovarian activity than other groups.

Supplementation of intravaginal progesterone sponge for 5 days in a GnRH (day 0)-$PGF_2\alpha$ (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 (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 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.

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

Group (n=50/group)	No. of ewes induced to estrus	Estrus synchronization rate (%)	Duration of estrus (h)
Group 1	17	$34^{A, B}$	$32.9\pm2.8^{a, b}$
Group 2	26	52^{A}	35.6 ± 3.3^{a}
Group 3	19	38^{a}	$34.2\pm2.6^{a, b}$
Group 4 (Control)	10	$20^{B, b}$	26.5 ± 2.3^{b}

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

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 pregnancy rates had been reported by Hashem *et al.* (2015) and Yadav *et al.* (2020b) with Ovsynch protocol (0, 5, 7). Under Indian conditions, Senthilkumar *et al.*

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

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

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

(2017) observed 70% pregnancy rate in does through Ovsynch protocol (0, 7, 9) plus progesterone sponge 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 turn over 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-during breeding season. In an another study, Yadav *et al.* (2020b) in crossbred ewes recorded 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

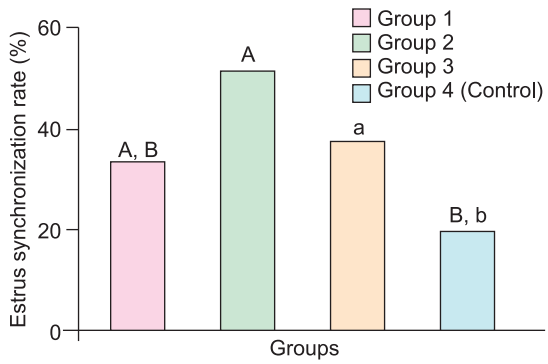

Fig. 4. Estrus synchronization rates in different groups in ewes during non-breeding season. Bars with different uppercase letters differ significantly (A, B; $p<0.01$). Bars with different lowercase letters differ significantly (a, b; $p<0.05$).

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 progesterone 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-during 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-$PGF_2\alpha$ GnRH plus progesterone supplementation during

non-breeding season. Further, studies are warranted with follicular dynamics along with endocrinological interrelationships with such protocols.

REFERENCES

Abecia J A, Forcada F and González-Bulnes A. 2011. Pharmaceutical control of reproduction in sheep and goats. *Veterinary Clinics of North America: Food Animal Practice* **27**: 67–79.

Abu E E, Teleb D, Abdel-Hafez M A M and Deghedy A M. 2016. Appraisal of different protocols for estrus synchronization in local Rahmani sheep. *Egyptian Journal of Sheep and Goats Sciences* **11**: 1–16.

Ali A, Hayder M and Saifelnaser E. 2009. Ultrasonographic and endocrine evaluation of three regimes for oestrus and ovulation synchronization for sheep in the subtropics. *Reproduction in Domestic Animals* **44**: 873–78.

Almadaly E, Ashour M, El-Kon I, Heleil B and Fattouh E S. 2016. Efficacy of various synchronization protocols on the estrus behavior, lambing rate and prolificacy in Rahmani egyptian ewes during the non-breeding season. *Asian Journal of Animal and Veterinary Advances* **11**: 34–43.

Bartlewski P M, Vanderpol J, Beard A P, Cook S J and Rawlings N C. 2000. Ovarian antral follicular dynamics and their associations with peripheral concentrations of gonadotropins and ovarian steroids in anoestrous Finnish Landrace ewes. *Animal Reproduction Science* **58:** 273–29.

Binelli M, Thatcher W W, Mattos R and Baruselli P S. 2001. Antiluteolytic strategies to improve fertility in cattle. *Theriogenology* **56**: 1451–63.

Bisinotto R S, Castro L O, Pansani M B, Narciso C D, Martinez N, Sinedino L D P and Thatcher W W. 2015. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol. *Journal of Dairy Science* **98**: 2515–28.

Campbell B K, Scaramuzzi R J and Webb R. 1995. Control of antral follicle development and selection in sheep and cattle. *Journal of Reproduction and Fertility Supplement* **49**: 335–50.

Dixon A B, Knights M, Winkler J L, Marsh D J, Pate J L, Wilson M E and Inskeep E K. 2007. Patterns of late embryonic and fetal mortality and association with several factors in sheep. *Journal of Animal Science* **85**: 1274–84.

Dogan I and Nur Z. 2006. Different estrous induction methods during the non-breeding season in Kivircik ewes. *Veterinarni Medicina* **51**: 133–38.

Duarte G, Hernandez M P N, Malpaux B and Delgadillo J A. 2010. Ovulatory activity of female goat adapted to the subtropics is responsive to photoperiod. *Animal Reproduction Science* **120**: 65–70.

Fonseca J F, Bruschi J H, Santos I C, Viana J H M and Magalhaes A C M. 2005. Induction of estrus in non-lactating dairy goats with different estrous synchrony protocols. *Animal Reproduction Science* **85**: 117–24.

Garcia A, Neary M K, Kelly G R and Pierson R A. 1993. Accuracy of ultrasonography in early pregnancy diagnosis in the ewe. *Theriogenology* **39**: 847–61.

Greyling J P C, Kotze W F, Taylor G J, Hagendijk W J and Cloete F. 1994. Synchronization of oestrus in sheep: Use of different doses of progestogen outside the normal breeding season. *South African Journal of Animal Science* **24**: 33–37.

Hashem N M, El-Zarkounyb S Z, Tahaa T A and Abo-Elezzaa Z R. 2015. Oestrous response and characterization of the ovulatory wave following oestrous synchronization using $PGF_2\alpha$ alone or combined with GnRH in ewes. *Small Ruminant Research* **129**: 84–87.

Hawk H W and Cooper B S. 1977. Sperm transport into the cervix of the ewe after regulation of estrus with prostaglandin or progestogen. *Journal of Animal Science* **44**: 638–44.

Khalilavi F, Mamouei M, Tabatabaei S and Chaji M. 2016. Effect of different progesterone protocol and low doses of equine chorionic gonadotropin (eCG) on oestrus synchronization in Arabian ewes. *Iranian Journal of Applied Animal Science* **6**: 855–61.

Kulaksiz R, Ucar O and Daskin A. 2013. Effects of FGA sponge and ovsynch based protocols on reproductive performance of fat-tailed ewes during the breeding season. *Kafkas Univ Vet Fak Derg* **19**: 629–33.

Lamb G C, Stevenson J S, Kesler D J, Garverick H A, Brown D R and Salfen B E. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_2\alpha$ for ovulation control in postpartum suckled beef cows. *Journal of Animal Science* **79**: 2253–59.

Martinez T, Montanez V, De C A, Izaguirre F and Velazco Z. 2013. Effect of GnRH and D-chloprostenol application on pregnancy and prolificacy rates on Pelibuey ewes. *Revista MVZ Cordoba* **18**: 3612–17.

Menchaca A, Neto P C S and Cuadro F. 2017. Estrous synchronization treatments in sheep: brief update. *Rev Bras Reprod Anim* **41**: 340–440.

Menchaca A and Rubianes E. 2004. New treatments associated with timed artificial insemination in small ruminants. *Reproduction Fertility and Development* **16**: 403–13.

Mohan K M. 2017. Comparative study of reproductive efficiency in EWES synchronized with vaginal sponges and CIDR during breeding and non-breeding seasons. *Pharma Innovation Journal* **6**: 75–79.

Naslaji A, Hosseini S M, Sarhaddi F, Bolourchi M and Birjandi M R. 2001. Steroid priming shortens prostaglandin-based estrus synchronization program from 14 to 7 days in cattle. *Theriogenology* **56**: 735–43.

Ptaszynska M. 2011. Ovine reproduction. Compendium of Animal Reproduction. 6th Revised Edition. *Intervet Int. bv.* (*The Netherlands*) 125–147.

Schrick F N and Inskeep E K. 1993. Determination of early pregnancy in ewes utilizing transrectal ultrasonography. *Theriogenology* **40**: 295–306.

Senthilkumar K, Selvaraju M, Napolean R, Doraisamy K A and Mohan B. 2017. Pattern of oestrus and fertility rate following synchronization of ovulation in Tellicherry goats. *International Journal of Science Environment and Technology* **5**: 3289–96.

Titi H H, Kridli R T and Alnimer M A. 2010. Estrus synchronization in sheep and goats using combinations of GnRH, progestagen and prostaglandin $F_2\alpha$. *Reproduction in Domestic Animals* **45**: 594–99.

Vinoles C, Forsberg M, Banchero G and Rubianes E. 2001. Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. *Theriogenology* **55**: 993–1004.

Yadav V, Chandolia R K, Dutt R, Bisla A, Saini G, Singh G and Ranga L C. 2020b. Effect of ovsynch estrus synchronization protocol on fertility in crossbred ewes. *Journal of Animal Research* **10**: 543–49.

Yadav V, Chandolia R K, Dutt R, Bisla A, Saini G and Singh G. 2020a. Effect of estrus synchronization using AVIKESIL-S® with eCG on the reproductive efficiency in crossbred ewes. *International Journal of Livestock Research* **10**: 49–59.