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Relative efficacy of short term progestagen and PGF2α with PMSG or GnRH or both on estrus synchronization in Hassan breed of ewes

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Abstract
A study was conducted to compare the efficacy of short-term progestagen and PGF2α with PMSG or GnRH analogue or both for estrus synchronization in Hassan ewes. Thirty nonpregnant ewes received Avikesil-S intravaginal sponge for 7 days followed by 125 µg cloprostenol on day of sponge retrieval. On the day of sponge removal, Group I (n=10) ewes received 300 IU PMSG; Group II (n=10) ewes received 4 µg buserelin actetate, 36 h thereafter, whereas Group III (n=10) ewes received both 300 IU PMSG and 4 µg buserelin acetate 36 h as in other groups and proven rams were used to detect estrus and for mating. The ewes were monitored and pregnancy was diagnosed using ultrasonography on day 30 post mating. Cent per cent sponge retention with 90 per cent estrus response was obtained in all groups. The interval to estrus in Group II was significantly higher ($p< 0.05$) followed by Group I (32.00 ± 2.00 h) and Group III (30.67 ± 2.11 h). The conception rate was higher (77.77%) in Group I compared to Group II (55.55%) and Group III (66.66%) with no significant difference. It can be concluded that short term Avikesil-S sponge treatment was efficient in Hassan breed of ewes for estrus synchronization. Avikesil-S+PGF2α+PMSG protocol was efficient estrus synchronization with better conception rate in Hassan breed of sheep than other treatment protocols studied.

Keywords: Hassan ewes, Estrus synchronization, Avikesil-S, Conception rate

Introduction
Estrus synchronization is induction of estrus in group of female animals, irrespective of the stage of estrous cycle by administration of exogenous hormones. Several methods have been employed since few decades to control the reproduction in ewes (Keisler, 2007) [19].

Estrus synchronization in ewes is achieved by exogenous progestagens or using $PGF_2α$, respectively to extend or reduce the length of luteal phase of estrous cycle (Kusina *et al*., 2000) [21]. Other hormonal treatments include use of gonadotropins like pregnant mare serum gonadotropin (PMSG) or human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH). The estrous cycle in ewes can also be controlled by non-hormonal methods like manipulation of light and ram effect (Iida *et al*., 2004) [15]. Shortened day length can be mimicked by administration of exogenous melatonin in ewes to estrus synchronization in anestrus (Jordan, 2005) [17].

The widely used and comparatively cheaper method of estrus synchronization is progesterone impregnated intra-vaginal sponge which is left in place for 12-17 days (Gordon, 1997) [14]. Ungerfeld and Rubianes (1999) [36] suggested to reduce the insertion period to 6-7 days to minimize the occurrence of vaginitis and reduced sponge retention rate following vaginal sponge insertion for long term. Long term progestagen treatment also causes reduced progestagen release from the sponge during terminal insertion period that causes inadequate LH release which in turn successive abnormal follicular development resulting in persistent follicles with affected ovulation quality (Gonzalez-Bulnes *et al.*, 2005) [13].

This duration is shorter than the half-life of a corpus luteum during natural estrous cycle and hence, the corpus luteum lysis should be included in cycling animals at sponge insertion (Letelier *et al*., 2009) [22] or at removal (Cox *et al*., 2012) [8] by injecting a single dose of $PGF_2α$ or its analogue. To induce ovulation during the non-breeding season and to obtain multiple birth rates, during sponge withdrawal an intramuscular injeection of equine chorionic gonadotrophin (eCG) is followed (Abecia *et al*., 2012) [2].

Short-term progestagen protocols resulted in either similar or better, but not lower fertility rates compared to classical long term progestagen treatment (Menchaca *et al*., 2018) [30].

Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan, developed an indigenously designed intra-vaginal sponge, AVIKESIL-S containing 350 mg of natural progestagen which has been successfully used for estrus synchronization in ewes by several researchers (Manvi, 2014; De *et al*., 2016; Mahendra, 2016; Kumar *et al*., 2016 and Gangadharaiah, 2017) [25, 9, 24, 20, 12].

Information about estrus synchronisation in Hassan sheep is lacking. Therefore, the study was planned to compare the efficacy of use of GnRH instead eCG in Hassan sheep breed with short term progesterone treatment.

Materials and Methods

The study was conducted in the Hassan breed of sheep maintained in University farm at Animal Husbandary Polytechnic, Hassan, India during March- August, 2020. Thirty apparently healthy non pregnant ewes were randomly divided into three groups with ten each and were subjected to different estrus synchronization protocols using intravaginal sponge (Avikesil-S, CSWRI, Avikanagar, India) inserted for seven days followed by intramuscular administration of 125 µg cloprostenol (Estrumate®, MSD India Pvt. Ltd, Pune, India) at removal. On day of sponge withdrawal, ewes in Group I, received 300 IU eCG (Folligon® 1000 IU, MSD India Pvt. Ltd, Pune, India) intramuscularly, in Group II, 4 µg buserelin actetate (Receptal® 10 mL, 4 µg/mL, MSD India Pvt. Ltd, Pune, India) administered intramuscularly 36 h thereafter. While in Group III at the time of sponge removal 300 IU eCG administered and followed by 4 µg buserelin acetate administered intramuscularly 36 h of sponge withdrawal.

Estrus signs were monitored twice daily for 30 minutes each time (06:00 - 06:30 and 18:00 – 18:30) after sponge withdrawal with the help of teaser ram equipped with color marking on the brisket. The ewes detected in estrus by paint marking on rump and stands to be mounted was considered to be in estrus were separated immediately from the flock and placed with proven one ram to five ewes for mating.

On day 30 post breeding ewes were subjected for pregnancy diagnosis using real time, B-mode ultrasound scanner equipped with linear array rectal transducer of 4.0 to 8.5 MHz (Easi-scan, BCF Technology Ltd., UK) and again pregnancy diagnosis on 60 day post breeding either by trans-rectal or transabdominal ultrasonography to know embryonic mortality, if any.

The various reproductive parameters were recorded and used to derive the parameters as per the method described by Abdalla *et al*. (2014) [1].

Statistical analysis

The GraphPad prism software (version 8.4.3) used for analysis. The parametric reproductive traits like interval to estrus and duration of estrus were subjected to one way ANOVA with post hoc Tukey's test at 0.05 level of significance using and expressed as Mean ± SE. While, other non-parametric data like sponge retention rate, estrus response rate and conception rate were analysed with Chi-square test and expressed in percentage.

Results and Discussion

In all the treated groups, cent percent sponge retention rate obtained. Further, none of the ewes exhibited estrus while intravaginal sponges were *in situ*. Similar result of 100.00 per cent sponge retention has been reported using Avikesil-S for a period of 7 to 12 days (Mahendra, 2016; Kumar *et al*., 2016; Gangadharaiah, 2017; Yadav *et al*., 2020) [24, 20, 12, 39]. The factors affecting sponge retention rate in the vagina are sponge insertion techniques employed, management system (Omontese *et al*., 2012) [31] and size of intravaginal implant (Swelum *et al*., 2018) [35], texture and consistency of intravaginal sponge (Martinez-Ros *et al*., 2018) [28].

The estrus response rate of 90 percent obtained in all three study groups (Table 1). Similar results of 89.50-91.00 per cent were reported (Martinez-Ros and Gonzalez-Bulnes, 2019; Martinez-Ros *et al*., 2019) [27, 29]. Roshani *et al*. (2018) [33] and Yadav *et al*. (2020) [39] have reported higher estrus response rate of 95.00-100.00 per cent. The difference in estrus response rate could be due to variation in season and breed of ewe during/in which study was performed (Atalla, 2018) [5]. The success of controlling the estrous cycle using intravaginal progesterone based protocols depends on the absorption of effective dose of progesterone and the density of sponge (Robinson *et al*., 1968) [32]. The estrus response rate obtained using Avikesil-S for 7 days indicated that Avikesil-S had sufficient progesterone absorption for induction of estrus in Hassan breed of ewes.

The interval to estrus in Group II (45.33 ± 1.76 h) was significantly higher ($p < 0.05$) followed by Group I (32.00 ± 2.00 h) and Group III (30.67 ± 2.11 h). Martinez-Ros and Gonzales-Bulnes (2019) [27] and Lombardo *et al*. (2020) [23] reported similar interval to estrus of 33-34.1 h following almost similar protocols comparable to the Group I of the present study. Yadav *et al*. (2020) [39] obtained shorter interval to estrus of 28.73 ± 1.00 h than Group I using Avikesil-S for 12 days with administration of 200 IU eCG on sponge removal. A longer time of estrus onset of 41.60 ± 2.73 h compared to the results of Group I in the present study has been reported by Martinez-Ros *et al*. (2019) [29] but using CIDR for 7 days with 400 IU eCG on withdrawal. The eCG treatment advances onset of estrus in the long-term progestagen treatment than short-term (Martinez-Ros *et al*. 2019) [29].

In Altamurana ewes, Martemucci and D'Alessandro (2011) [26] reported shorter interval to estrus time of 37.30 ± 1.41 h compared to the present study results of Group II, by using 40 mg FGA intravaginal sponges for 5 days with 100 µg $PGF_2\alpha$ on sponge insertion followed by GnRH analogue 30 h post withdrawal.

Table 1: Effect of synchronization protocols on non-parametric reproductive traits in Hassan ewes

Parameter	Group I (n=10)	Group II (n=10)	Group III (n=10)
Estrus response rate (%)	90.00	90.00	90.00
Conception rate (%)	77.77	55.55	66.66

Table 2: Effect of synchronization protocols on parametric reproductive traits in Hassan ewes (Mean ± SE)

Parameter	Group I (n=10)	Group II (n=10)	Group III (n=10)
Interval to estrus from sponge removal (h)	32.00 ± 2.00^{a}	45.33 ± 1.76^{b}	30.67 ± 2.11^{a}
Duration of estrus (h)	32.00 ± 2.00	28.00 ± 2.00	30.67 ± 2.11

Note: Values bearing different superscripts differ significantly ($P<0.05$)

Santos-Jimenez *et al*. (2020) [34] obtained 30 ± 2.5 h in Segurena ewes using CIDR for 5 days with 400 IU eCG and 5 mg dinoprost tromethamine on removal followed by GnRH analogue with propylene-glycol administered by subcutaneous route, 24 h post CIDR withdrawal which is similar to the results of Group III.

Shorter intervals to estrus in eCG treated group than non-eCG treated group attributed to the action of eCG on follicular growth by mediating faster pituitary endocrine responses and estradiol secretion (Amer and Hazzaa, 2009) [4]. Variation in interval to estrus among the treated groups is due to the status of CL and stage of follicular development at the time of $PGF_2\alpha$ administration (Martemucci and D'Alessandro, 2011) [26]. However, GnRH analogue administration has no effect on the onset of estrus (Martinez-Ros and Gonzales-Bulnes, 2019) [27].

In the present study, estrus duration of 32.00 ± 2.00, 28.00 ± 2.00 and 30.67 ± 2.11 h obtained in Group I, Group II and Group III, respectively with no significant (p>0.05) difference among treated groups. This is compatible with physiological range of estrus duration in ewes 24 – 36 h (Jainudeen *et al*., 2000) [16]. Yadav *et al*. (2020) [39] used Avikesil-S for 12 days with 200 IU PMSG on withdrawal obtained lower estrus duration of 26.40 ± 1.64 h in crossbred (Nali×Rambuillet) ewes in comparison to results of the study. This variation is attributed to estrus detection methods and frequency of detection influence the duration of the estrus (Martinez-Ros and Gonzales-Bulnes, 2019) [27].

The difference in the estrus duration attributed to breed, nutrition, presence of the male after sponge removal (Ungerfeld and Rubianes, 1999) [36], variation in the dosage of gonadotropin, duration of treatment along with the difference in age and reproductive status of the ewes (Wildeus, 2000) [38]. GnRH did not influence estrus duration (Cavalcanti *et al*., 2012) [7] whereas PMSG is more important in determining estrus duration by enhancing the recruitment of small follicles (Evans, 2003) [10].

The conception rate of in Group I, Group II and Group III were 77.77, 55.55 and 66.66 percent, respectively with no significant (p>0.05) difference among the treatment groups.

Martinez-Ros and Gonzales-Bulnes (2019) [27] and Kumar *et al*. (2016) [20] reported similar conception rate of 76.50 and 75.00 percent, respectively following almost similar protocols as of group I. Manvi (2014) [25] and Yadav *et al*. (2020) [39] reported higher conception rate of 100.00 and 86.67 percent, respectively than Group-I using Avikesil-S.

The higher conception rate in Group I and Group III than Group II could be attributed to exogenous gonadotropin (PMSG) administration which resulted in efficient follicles development and advances ovulation, further promotes proper luteinization to form CL (Valentim *et al*., 2016) [37]. The induction of ovulation by administration of GnRH is not always effective since it depends upon stage of cycle at which it is administered (Alminer *et al*., 2005) [3].

Variations in conception rate in current study and different studies might be due to difference in population size, the breed of ewe (Karagiannidis *et al*., 2001) [18], hormonal protocol used, body condition, effect of ram and breeding season (Ataman *et al*., 2006) [6]. Further, the conception rate depends on method and time of pregnancy diagnosis which in turn affects conception rate (Fridlund *et al*., 2013) [11].

Conclusion

It was concluded that Avikesil-S sponge was efficient in Hassan breed of ewes for successful estrus synchronization. Following Avikesil-S+PGF2α+PMSG protocol, efficient estrus synchronization and better conception rate was obtained in Hassan breed of sheep than other treatment protocols studied.

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Conflict of interests

The authors have no conflict of interest.

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