Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.25 No.1 Ser. No.86 2024

# IRANIAN JOURNAL OF VETERINARY RESEARCH

**Short Paper**

# Evaluation of the efficiency of estrus synchronization protocols combined with natural service and ultrasonography on ewe reproductive performance during non-breeding season

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10.22099/IJVR.2024.48021.6981

(Received 3 Aug 2023; revised version 25 Mar 2024; accepted 14 Apr 2024)



## Abstract

**Background:** Estrus synchronization is an important assisted reproductive technology to improve the reproductive performance in ewes. Various protocols have been used with variable success rates, however; literature regarding field applicable estrus synchronization is meagre. **Aims:** The present study was designed with the aim to evaluate the estrus synchronization protocols on reproductive performance in ewes using different hormones. **Methods:** Experimental ewes were divided randomly into three groups (n=15). Ewes of all groups received intravaginal sponge for 12 days. Subsequently, $NP_4$-GnRH and $NP_4$-eCG groups received 8 µg of buserelin acetate or 200 IU of eCG intramuscularly, respectively on day 12 whereas $NP_4$-Insulin group received insulin 0.2 IU/kg body weight subcutaneously for three consecutive days started on the day of sponge removal. Estrus detection commenced 24 h after sponge removal in $NP_4$-GnRH and $NP_4$-eCG groups and 24 h following last injection of insulin in $NP_4$-Insulin group. The ewes in estrus were separated and pen mated. The conception rate was determined by ultrasonography. **Results:** The estrus response and conception rates were 71.43, 92.86 and 53.85%, and 70.00, 84.61, and 71.43%, respectively in $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin groups. The lambing rates were the same as the conception rates. The single and multiple birth rates were 71.41, 36.36 and 60.0%, and 28.57, 63.64, and 40.0% whereas prolificacy was 128.57, 190.91, and 140.00%, respectively in $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin groups. **Conclusion:** In conclusion, the estrus synchronization protocol including intravaginal progesterone sponge and eCG was found to be more effective under field conditions.

**Key words:** eCG, GnRH, Insulin, Natural service, Vaginal sponge

## Introduction

Ewes are one of the important livestock of Vindhyan region of India and considered as the most important means of livelihood of farmers. In this region, ewes are reared mainly by poor and marginal farmers through pastoral system. The reproduction and production of the ewes of Vindhyan region are affected due to hot climate, scarcity of feed and fodder and limited water availability. Furthermore, reproductive behaviour of ewes is influenced by many factors such as nutrition, season, temperature, photoperiod, latitude, and breeds (Ciornei *et al*., 2022). This necessitates manipulation of ovarian activity to improve the reproductive performances of ewes at farmer's doors. Estrus synchronization is a useful tool to reduce the labour cost, shortening of breeding season and controlled lambing at a suitable time to take the advantages of forage availability and market demands (Sen and Onder, 2016; Hameed *et al*., 2021). Several estrus synchronization strategies have been addressed to improve the reproductive performances in ewes, however; most of them are combined with artificial insemination (AI). In contrast to large animals, implementation of AI in ewes under field condition is impractical and results in low fertility rate (Vilarino *et al*., 2013) due to complex reproductive tract anatomy of the ewe and difficulty to determine the fertile period of the estrus cycle to inseminate the ewes (Alvarez *et al*., 2019).

Estrus synchronization strategies in ewes include manipulation of photoperiod, exposure of rams and use of exogenous hormones including progesterone, prostaglandins, gonadotropins and melatonin (Mura *et al*., 2019; Yu *et al*., 2019; Dias *et al*., 2020; Nakafeero *et al*., 2020), however; only strategies that provide tight synchrony, acceptable level of fertility, cost effectiveness, high feasibility and minimum handling are considered ideal under field conditions. Intravaginal sponges, impregnated with either natural or synthetic progesterone [most commonly flurogestone acetate

(FGA) or medroxyprogesterone acetate (MPA)] have been used worldwide in small ruminants. These vaginal sponges are generally used for a short (5-7 days) or long (12-14 days) periods (Karaca *et al*., 2009; Martinez-Ros *et al*., 2019) and have high retention rate (>90%). Although ewes exhibit signs of estrus within 24 to 48 h after removal of the vaginal sponge, high variation has been observed in estrus response as well as fertility (Wildeus, 2000). The estrus synchronization by using intravaginal sponges is generally combined with gonadotropins as latter stimulate the synchrony of follicular growth, maturation, and ovulation. Unfortunately, literatures regarding estrus response and fertility are inconsistent (Titi *et al*., 2010; Santos-Jimenez *et al*., 2020). In small ruminants, it is reported that insulin promotes the follicular growth by decreasing the follicular atresia and thus increasing the number of gonadotropin-dependent ovarian follicles (Gong *et al*., 1994; Majumdar *et al*., 1997). Knowledge on use of insulin for synchronization of estrus in sheep is meagre. Furthermore, implementation of pregnancy diagnosis using ultrasonography in synchronization strategy may improve the reproduction and production potential of ewes. The modern ewe husbandry has improved reproductive as well as productive efficiencies through manipulation of ovarian activity, however; such techniques are not available at farmer's door where mostly traditional systems of sheep farming exist. This is mainly due to the lack of field applicable strategy for the synchronization of estrus and ovulation. Hence, the present study was designed to evaluate the reproductive performances of ewes subsequent to field applicable estrus synchronization, ultrasonography and natural service using different protocols in ewes.

## Materials and Methods

### Study period and location of the study

The present study was conducted during non-breeding season (December to May) at livestock farm complex of Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, BHU, Varanasi, situated between 25.2677° N latitude and 82.9913° longitude. The average annual rain fall and humidity in this area are 1110 mm and 57% whereas mean annual minimum and maximum temperature are 20°C and 33°C, respectively.

### Selection of experimental ewes

A total of 45 crossbred maiden ewes, aged between 2 to 4 years, weighing 28-32 kg were selected for the study. All ewes were multiparous and had no history of dystocia, prolapse, still birth or other reproductive disorders. B-Mode transabdominal ultrasonography (USG) was used to examine the non-pregnancy status of the ewes. The experimental ewes were maintained under standard farm management practices. They grazed daily for 6-8 h and were additionally provided with concentrate ration (including maize, choker, mustard cake, and mineral mixture) @ 200 g/ewe/day. Drinking water was available *ad libitum*.

### Experimental procedure

After selection, the experimental ewes (n=45) were divided randomly into three equal groups (n=15) i.e. $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin. Intravaginal sponges containing natural progesterone (Avikesil-S procured from ICAR-CSWRI, Avikanagar) were used in all three groups for 12 days. The ewes of $NP_4$-GnRH group received 8 µg of buserelin acetate (2 ml of Receptal; MSD) whereas the ewes of $NP_4$-eCG group received 200 IU of eCG (Folligon, MSD) intramuscularly, on the day of sponge removal. The ewes of $NP_4$-Insulin group received insulin 0.2 IU/kg body weight subcutaneously, for three consecutive days started on the day of sponge removal (Fig. 1). The dose and frequency of insulin administration were chosen from a previous study conducted on goats (Suguna *et al*., 2009). Estrus detection was performed in 12 h intervals (morning and evening), commencing 24 h after sponge removal in $NP_4$-GnRH and $NP_4$-eCG groups and 24 h following last injection of insulin in $NP_4$-Insulin group and continued up to 96 h. The ewes were considered in estrus when stand to be mounted by the aproned ram. The ewes in estrus were separated and pen mated with fertile ram (1:5) to save the labour cost.

B-mode trans-abdominal ultrasonography (Sonosite vet, Portable USG machine, USA) using trans-abdominal probe of 5.0 MHz frequency was performed at the beginning of the experiment to select non-pregnant ewes. Further, USG was performed between 45-60 days post mating for the diagnosis of pregnancy in ewes. The reproductive performance of each group was evaluated in terms of estrus response (number of ewe showed estrus/number of ewe treated × 100), conception rate (number of ewe conceived/number of ewe showed estrus × 100), lambing rate (number of ewe lambed/number of ewe conceived × 100), single birth rate (number of ewes lambed single lamb/total number of ewes lambed × 100), multiple birth rate (number of ewes lambed twins or triplets/total number of ewes lambed × 100) and prolificacy rate (total number of lambs/total number of ewes lambed × 100). In addition, the percentage of twin and triplet lambs obtained in each experimental group was also evaluated. The data were analysed by Fisher's Exact Test at 95% level of confidence using SPSS software (version 27.0).

### Ethics statement

Ethical clearance for this study was granted by the Institutional Animal Ethics Committee (F.Sc/IAEC/ 2016-17/1129).

## Results

Four ewes, one from each $NP_4$-GnRH and $NP_4$-eCG group and two from $NP_4$-Insulin group, were excluded from the experiment due to loss of vaginal sponge. The reproductive parameters i.e. estrus induction rate, conception rate, lambing rate, multiple birth rate,

numbers of lamb born (single, twins and triplet) and prolificacy in $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin group are shown in Table 1. The estrus response was significantly higher ($P<0.05$) in $NP_4$-eCG group than that of $NP_4$-Insulin group but did not differ significantly ($P>0.05$) with $NP_4$-GnRH group. Though, conception rate was higher in $NP_4$-eCG group but no significant difference in conception rate and lambing rate was recorded among the groups and all the conceived ewes were lambed. The single birth rate was recorded slightly higher in $NP_4$-GnRH group but did not differ significantly from other groups ($P>0.05$). The multiple birth rate was non-significantly ($P>0.05$) higher in $NP_4$-eCG group than those of $NP_4$-GnRH and $NP_4$-Insulin groups. The triplet birth was recorded only in $NP_4$-eCG group. The prolificacy rate was non-significantly ($P>0.05$) higher in $NP_4$-eCG group. In addition, 3, 2, and 2 non-pregnant ewes were identified at early stages in $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin groups, respectively using USG between 45-60 days after natural service.

**Table 1:** Estrus response, conception rate, lambing rate, multiple birth (single, twins and triplets) rate, and prolificacy

| Reproductive parameters | Groups | | |
|---|---|---|---|
| | $NP_4$-GnRH | $NP_4$-eCG | $NP_4$-Insulin |
| Estrus response (%) | $71.43^{ab}$ | $92.86^{a}$ | $53.85^{b}$ |
| Conception rate (%) | $70.00^{a}$ | $84.61^{a}$ | $71.43^{a}$ |
| Single birth rate (%) | $71.42^{a}$ | $36.36^{a}$ | $60.00^{a}$ |
| Multiple birth rates (%) | $28.57^{a}$ | $63.64^{a}$ | $40.00^{a}$ |
| Twins birth rate (%) | $28.57^{a}$ | $36.36^{a}$ | $40.00^{a}$ |
| Triplet birth rate (%) | 0.0 | 27.27 | 0.0 |
| Lambing rate (%) | $70.00^{a}$ | $84.61^{a}$ | $71.43^{a}$ |
| Prolificacy rate (%) | $128.57^{a}$ | $190.91^{a}$ | $140.00^{a}$ |

Values with different superscripts in rows differ significantly ($P<0.05$)

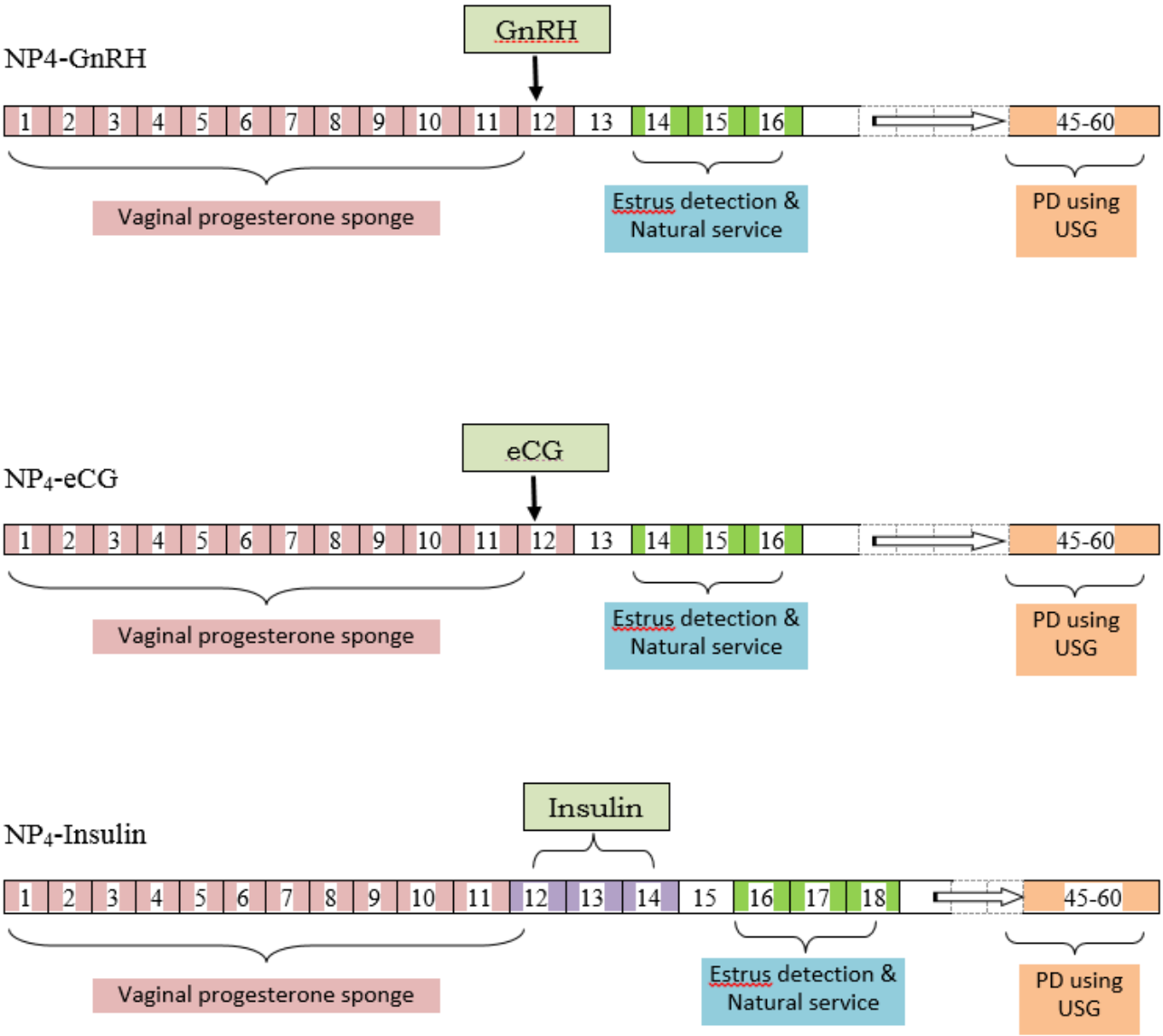


**Fig. 1:** Protocols of estrus synchronization and the duration of treatments in ewes. Day 1: Day of vaginal sponge insertion, day 12: Day of vaginal sponge removal and administration of different hormones in each protocol, Days 14, 15, and 16: Days of estrus detection and natural service in $NP_4$-GnRH, and $NP_4$-eCG groups, Days 16, 17, and 18: Days of estrus detection and natural service in $NP_4$-Insulin group, and Days 45-60: Days of pregnancy diagnosis using ultrasonography

## Discussion

Progesterone has been used successfully to synchronize the estrus in ewes but shows high variations in estrus response and fertility (Wildeus, 2000). It is administered as oral, injectable, implants or intravaginal (CIDR or intravaginal sponges). Of which, intravaginal sponges containing natural or synthetic progesterone are considered as the choice of synchronization method in ewe and doe. The use of intravaginal sponges for long duration (≥14 days) often leads to vaginitis and subsequent purulent discharges (Reinoso-Pelaez *et al*., 2023). However; in present study, none of the ewes showed vaginitis or purulent discharges following use of intravaginal sponge containing natural progesterone (Avikesil-S) for 12 days. Moreover; vaginal sponge was lost in 4 ewes out of 45. However, literatures regarding loss of vaginal sponge are inconsistent (Husein *et al*., 2007; Yu *et al*., 2022). The proper observation of ewes and re-insertion of vaginal sponge immediately after loss may further improve the reproductive efficiency of ewes in large flocks.

As previously stated, in the present study estrus response was significantly ($P<0.05$) higher (92.86%) in the $NP_4$-eCG group as compared to the $NP_4$-Insulin but was not significantly different from $NP_4$-GnRH group. However, no significant difference in conception rate, multiple birth rates and prolificacy was recorded among groups. Lower estrus response (75%) following the use of vaginal sponge (Avikesil-S) alone containing of natural progesterone (350 mg) for 12 days (Das *et al*., 2000) indicates the role of eCG in growth and development of the ovarian follicles in ewes. In $NP_4$-eCG group, the estrus response was higher than those obtained by De *et al*. (2015) (79.4%) and De *et al*. (2016) (83.84%) using vaginal sponge containing natural progesterone for 12 days and 200 IU of eCG at the time of vaginal sponge removal. These differences might be due to variation in geographical region and/or breed of the ewes that were included in these studies. However, findings of the present study were similar to the findings of Quintero-Elisea *et al*. (2011) (95.8%) following the use of vaginal sponge containing synthetic progesterone (FGA 40 mg) for 10 days in combination with 200 IU of eCG at the time of vaginal sponge removal. The 200 IU of was found as most effective dose of eCG in synchronization protocol for ewes (Cosentino *et al*., 2019). In $NP_4$-eCG group, the conception rate was higher (84.61%) than the findings of De *et al*. (2015) (61.21%) and Quintero-Elisea *et al*. (2011) (69.6%) after artificial insemination but was similar to findings of Yadav *et al*. (2021) (92.32%) following natural service. In general, conception rate is always higher following natural service, compared to artificial insemination. In present study, the lambing rate was the same as the conception rate and no embryonic or fetal mortality was recorded. The multiple birth rates and prolificacy in $NP_4$-eCG group were 63.64% and 190.91%, respectively which were higher than the other groups. In addition, triplet birth was recorded only in $NP_4$-eCG (27.27%) and not in other groups indicating the role of eCG in ovulation. The lambing rate in $NP_4$-eCG group was similar to the findings of Yadav *et al*. (2021) (108.33%). The overall better reproductive performances in $NP_4$-eCG group might be due to the effect of eCG as it induces follicular growth, maturation and ovulation (Oliveira *et al*., 2016). The reproductive performances in $NP_4$-GnRH group were also satisfactory. Comparatively lower estrus response which was observed in this group might be due to the GnRH-induced follicular atresia in some animals as GnRH stimulates the release of luteinizing hormone (LH) that induces either ovulation or atresia of the dominant follicle followed by appearance of a new follicular wave (Webb *et al*., 1992; Peters *et al*., 1999). Comparatively higher twin birth rate in $NP_4$-Insulin group might be due to the effect of Insulin as administration of exogenous insulin stimulates the follicular development, oocyte maturation, steroidogenesis, ovulation rate and embryonic development in ruminants (Ramoun *et al*., 2007; Sarath *et al*., 2008) however, literatures regarding use of insulin in estrus synchronization protocol of ewes are meagre.

Implementation of pregnancy diagnosis following synchronization of estrus and breeding is important as it helps in early identification of non-pregnant ewes. In addition, about 20-30% pregnant ewes show gestational estrus that can be better managed in flocks. Diagnosis of pregnancy can be carried out easily and accurately using transrectal or transabdominal ultrasonography. In present study, diagnosis of pregnancy was included between 45-60 days after natural service and identified 3, 2, and 2 non pregnant ewes in $NP_4$-GnRH, $NP_4$-eCG, and $NP_4$-Insulin groups, respectively at early stage. The importance of this procedure is more prominent when large numbers of ewes are subjected to estrus synchronization. Thus, early identified non-pregnant ewes may be subjected to resynchronization to minimize the economic loss of the farmers. In present study, pregnancy was diagnosed using B-Mode transabdominal ultrasonography however, pregnancy may be diagnosed earlier (around 30 days of gestation) using B-Mode transrectal ultrasonography.

Many synchronization protocols of ewes have been combined with either timed artificial insemination (TAI) or artificial insemination at detected estrus (AIDE) however; these studies are conducted on low numbers of ewes under experimental conditions thus their conclusions are difficult to extrapolate to field conditions. Artificial insemination in ewe is not feasible under field conditions due to complex cervical anatomy which prevents the ideal procedure of AI (frozen-thawed semen/vaginal route), necessary to obtain acceptable fertility rate (reviewed by Alvarez *et al*., 2019). The low fertility rate is unacceptable for commercial sheep farmers. In addition, detection of estrus is difficult in large flocks of ewes and requires more labour. Inclusion of AI in synchronization protocol requires additional handling of ewes which is not desirable under field condition. Inclusion of natural service in the synchronization protocol of ewe minimizes the

additional handing of animals, reduces labour cost and results in high fertility rate.

In conclusion, considering the estrus response, conception rate, multiple birth rates, and ease of application, the $NP_4$-eCG strategy was found to be most advisable in large flocks under field condition. This protocol might be replaced with $NP_4$-GnRH strategy as satisfactory results was also recorded with $NP_4$-GnRH strategy and considering the fact that repeated use of eCG has been reported to be associated with humoral immune response (Roy *et al*., 1999) and development of ovarian follicular cysts (Vinoles *et al*., 2001), resulting in low pregnancy rates.

## Acknowledgements

Author is highly thankful to Banaras Hindu University for providing grant and necessary facilities to conduct the study. Author is also thankful to J. K. Chaudhary for his assistance in the data analysis.

## Conflict of interest

The author declares no conflict of interest.

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