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Short Communication

EFFICACY OF PROGESTERONE OR GNRH BASED ESTROUS SYNCHRONIZATION PROTOCOLS IN BEETAL GOATS DURING LOW BREEDING SEASON

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ABSTRACT

The present study was conducted to compare the effect of short-term and long-term progesterone and GnRH-based estrus synchronization protocols on fertility of Beetal goats during low breeding season. The goats (n=63) were divided into three groups to receive intravaginal progesterone sponges for short-term (STP; n = 20; 5days) or long-term (LTP; n = 22; 11days) and Ovsynch estrus synchronization protocols (OVS; n = 21). The STP and LTP treated goats were additionally injected with $PGF_2\alpha$ (75µg/doe) and PMSG (300IU/doe) according to schedule for LTP and STP protocol. The OVS goats were administered 1st injection of GnRH (25µg/doe) at day 0, $PGF_2\alpha$ at day 7 and 2nd GnRH injection at day 9. The goats were observed for estrus signs and bred at detected estrus using a fertile buck. The results indicated that estrus response, pregnancy rate, litter size, and twining rates were significantly higher ($p < 0.05$) in STP and LTP than OVS treated groups. In contrast, the variables like intervals from $PGF_2\alpha$ treatment to the onset of estrus or estrus to breeding, pregnancy loss and prolificacy rate were similar ($p > 0.05$) among groups. In conclusions, the short-term intravaginal progesterone-based sponges successfully synchronized the estrus before breeding and could be an effective alternative to conventional long-term progesterone-based or Ovsynch synchronization protocols in Beetal goats. Also, better pregnancy, and kidding rates, with minimal pregnancy loss, could be achieved by employing progesterone-based protocols in Beetal goats.

Key words: Estrus synchronization, Progesterone, GnRH, Fertility, Goats
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INTRODUCTION

Pakistan is amongst the top small ruminants rearing countries along with the great variety of sheep and goat breeds region wise. Tropical and subtropical environment favors the small ruminant production in Pakistan. Although, the sheep and goats are categorized as seasonal breeders, goats raised in this region exhibit lesser seasonality in breeding and kidding compared to the temperate zone. A higher breeding trend in goats is usually observed during September to December compared to other months of the year (Ahmad *et al*., 2014).

The economics and profitability of the goat farming business are linked with reproductive efficiency, kidding rate, and lifetime production of goats. (Fernandez-Moro *et al*. 2008; Saribay *et al*. 2020). In this regard, the estrus synchronization could be a useful tool for achieving higher profit targets by improving reproductive efficiency in goats.

Synchronization of the caprine estrous cycle using exogenous hormones provides the opportunity to minimize the financial costs by shortening breeding and kidding seasons; resulting in a minimum impact of seasonality on fecundity rates. Although the use of estrus synchronization has been available for decades, only a small percentage of farmers practice estrous synchronization technology for improving herd reproductive efficiency in developing countries. Moreover, the selection of suitable or alternative estrus synchronization protocols is essential to obtain the maximum estrus and fertility rates (Fernandez-Moro *et al*. 2008). As the occurrence of ovulation is observed at the mid of the estrus stage in goats, therefore, insemination or breeding after 12 hours of estrus is a prerequisite for a better pregnancy rate (Murtaza et. al. 2020). In this context, the selection of economical and handy protocol is essential to reduce the efforts of obtaining maximum estrus and fertility rates in anestrous goats (Alvarado-Espino *et al*. 2019).

In goats, the use of progesterone is a practical estrus synchronization protocol for cyclic and acyclic animals and results in a higher number of preovulatory-sized follicles (Fernandez-Moro *et al*. 2008). On the other hand, prolonged progesterone treatment often reduces fertility (Karaca *et al*. 2010) in part due to subluteal concentration of progesterone at the end of the treatment

(Rubianes *et al*. 1998), results in persistence of the dominant follicle (Flynn *et al*. 2000), and impairment of sperm transport in the genital tract (Martemucci and D'Alessandro 2011a). In this context, short-term treatment with progestogen or GnRH-based synchronization protocol is considered as a suitable alternative to long-term progestogens to overcome their adverse response or limitations (Riaz *et al*. 2012; Holtz *et al*. 2008). The induction of synchronized estrus and ovulation in dairy goats with different short-term (5-days) treatments using prostaglandins, eCG and GnRH favor the success of 5 days estrus synchronization protocol (Martemucci and D'Alessandro 2011b). However, there is no previous report about the comparative efficacy of progesterone (short or long term) and GnRH-based estrus synchronization protocols in Beetal goats. Therefore, the present study was designed to determine the effect of progesterone-based synchronization (short & long-term) and ovsynch protocols on estrus response, pregnancy, and kidding in Beetal goats during the low breeding season.

MATERIALS AND METHODS

Location: The study was conducted at a private small ruminant farm located in Dunyapur, District Lodhran, Punjab, Pakistan. The area is located in east-central part of Pakistan, which is a semi-arid sub-tropical zone (29°48′06″N 71°44′27″E). Generally, the ambient temperature elevates up to 47 °C during summer and remains above 10 °C during the winter season. The study was conducted during March to May 2019.

Animal Management: A total of sixty-three (n = 63), non-pregnant, multiparous lactating Beetal goats of age (2–5 years) with body weight 40-45 Kg, were selected. The goats were kept in open shaded areas with free loafing land and had access to grazing for at morning time. The provision of concentrates was according to the physiological status of goats. All the goats had free access to drinking water and rock salts. The vaccination and deworming of each animal was ensured. Transabdominal ultrasonography (3.5 MHz transducer, Honda, HS-2200 Tokyo, Japan) of all goats was performed to ensure the normal status of reproductive tract before the start of the study.

Estrous synchronization protocol: The selected goats were randomly categorized into three groups for receiving one of the following estrous synchronization protocols; a) Short-term intravaginal sponge protocol (STP; n = 20), b) long-term intravaginal sponge protocol (LTP; n = 22) and c) Ovsynch protocol (OVS; n = 21). The timeline of each protocol has been depicted in Figure 1.

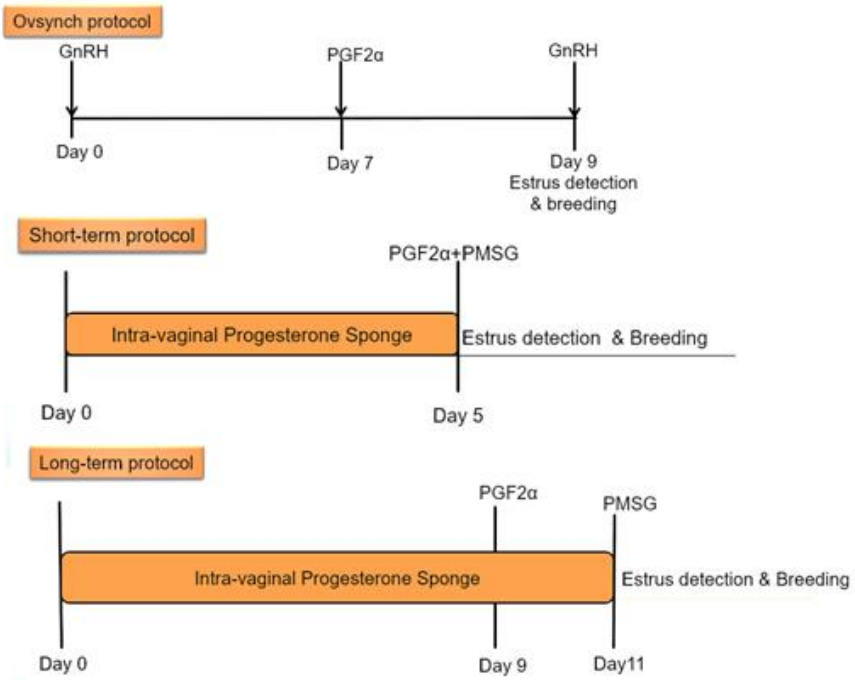

Fig. 1. The schematic timeline of Ovsynch (1st GnRH on day 0, $PGF_2\alpha$ on day 7 and 2nd GnRH on day 9), short- (Intravaginal progesterone sponges for 5 days, injection of $PGF_2\alpha$ and PMSG on day of sponge removal) and long-term (Intravaginal progesterone sponges for 11 days, $PGF_2\alpha$ on day 9 and PMSG on the day of sponge removal) progesterone-based synchronization protocols applied in Beetal goats during low breeding season.

Briefly, the OVS group was treated with GnRH injection (Dalmarelin®, FATRO, Italy: Lecirelin acetate; 25µg/doe IM) at day 0 followed by $PGF_2\alpha$ (Dalmazin®, FATRO, Italy: d-cloprostenol 75µg/ml) at day 7 and then GnRH (25µg/doe) at day 9. The STP group received intravaginal progesterone sponges (60mg Medroxyprogesterone acetate; Esponjavet®, Laboratorios Hipra, Spain) for 5 days. On day 5 sponges were removed followed by injection of $PGF_2\alpha$ (75µg/doe; IM) along with the injection of PMSG (OVISER®, LABORATORIOS HIPRA, Spain; 300IU/doe). The LTP group received intravaginal progesterone sponges for 11 days. On day 9 before sponge removal, an injection of $PGF_2\alpha$ (75µg /doe) was administered. The sponges were removed on day 11 and PMSG (300IU/doe) was administered to each doe in LTP group.

Estrus detection and natural breeding: The STP and LTP groups after sponge removal and OVS group after $PGF_2\alpha$ shot, were monitored for estrus signs by introducing an aproned buck at every 6 h interval daily. The goats showing estrus signs such as Tail wagging, bleating, estrus mucus, stand to be mounted, vulvar hyperaemia, and circling in the presence of a buck (Panicker *et al*. 2015), were exposed twice daily to sexually active breeding bucks of previously known fertility.

Pregnancy diagnosis, pregnancy loss, prolificacy, and twinning rate: Pregnancy diagnosis was performed using transabdominal ultrasonography (3.5 MHz transducer, Honda HS-2200 Tokyo, Japan) after 40 days of breeding. The pregnant goats were further observed for live single or multiple fetuses with amniotic fluid. The pregnancy loss was calculated by considering a doe pregnant at Day 40 but not delivered any kid at the time of kidding. The prolificacy rate was determined using the formula: [Number of kids born/number of does kidding] × 100. The twinning rate was calculated by applying the formula: [Number of twin kidding /total number of does kidding] × 100. The litter size was calculated as average number of kids delivered by the total number of does.

Statistical analysis: The data were analyzed using SPSS (IBM SPSS for Windows, Ver.23) statistical software. The samples were analyzed by selecting the values 0.80 and Type 1 Error 0.05. Normality distribution was calculated by using Shapiro-Wilk ($n < 50$) test. The data were analyzed using chi-square test for estrus response, pregnancy rate, pregnancy loss, prolificacy, and twining rate. ANOVA one-way was used to compare litter size, interval from $PGF_2\alpha$ injection to the onset of estrus, and interval from onset of estrus to breeding between the groups.

RESULTS

The effect of short-term (STP) or long-term (LTP) progesterone and Ovsynch synchronization protocols on estrus response (%), interval from $PGF_2\alpha$ injection to onset of estrus and interval from onset of estrus to breeding (h), pregnancy rate (%), pregnancy loss (%), litter size (n), prolificacy (%) and twining rate (%) in Beetal goats during the low breeding season has been presented in Table 1.

The estrus response was significantly higher ($P < 0.05$) in STP and LTP than OVS treated group. In contrast, the interval from $PGF_2\alpha$ treatment to the onset of estrus and interval from onset of estrus to breeding were similar ($P > 0.05$) among groups.

The pregnancy rate and litter size were significantly higher ($P < 0.05$) in STP or LTP-treated Beetal goats than OVS treated group. There was no significant difference ($P > 0.05$) in prolificacy among the treatment groups; however, the twinning incidence improved significantly ($P < 0.05$) in STP and LTP compared to OVS treated goats.

DISCUSSION

In the current experiment, short- and long-term progesterone estrus synchronization protocols were compared with GnRH-based protocol in multiparous Beetal goats during the low breeding season. Previously, different progesterone (Hameed *et al*. 2020; Kausar *et al*. 2009) and GnRH (Ahmad *et al*. 2014, Riaz *et al*. 2012) or $PGF_2\alpha$ (Murtaza *et al*. 2020; 2019) based protocols were compared in cyclic and anestrous Beetal goats. In those studies, the classical protocols were used in view of the physiological status of animals, and variable outcomes were obtained which are comparable with the current data. The current data showed that LTP and STP regimens are equally effective to synchronize the estrus in goats during the low breeding season. In contrast, the GnRH based protocol did not efficiently synchronize the estrus as observed in the case of STP and LTP protocols. A similar pattern was observed in pregnancy or litter size in Beetal goats after LTP or STP treatments.

The results of an earlier study where short-term FGA (fluorogestone acetate intravaginal sponges) based synchronization treatment resulted in a pregnancy rate of 86.7% after natural mating (Martemucci and D'Alessandro 2011a), similar to the pregnancy rate of this study (85%). However, long-term FGA based protocol treated goat when bred through exocervical or intrauterine artificial inseminations, lower pregnancy rates of 62% or 42% were observed (Martemucci and D'Alessandro 2011b). In the present study, artificial insemination was not performed, and only natural breeding was applied that might have resulted in higher

fertility rates compared to those documented in earlier reports (Fonseca *et al*. 2017; Panicker *et al*. 2015). These data suggest that the progesterone-based protocol is an effective method for the resumption of estrus activity in goats than GnRH based ovsynch protocol. Though, this is not consistent with previous report where estrus intensity and conception rate were similar between progesterone and ovsynch treated goats (Panicker *et al*. 2015). The higher estrus response in the present study might be related to better support to follicular development following eCG administration or initial priming of brain with progesterone which could not be achieved following Ovsynch protocol. However, LTP protocol is associated with low estrus and fertility in goats because of inadequate follicular development or altered uterine milieu (El Kadili *et al*. 2019; Bukar *et al*. 2012; Martemucci and D'Alessandro 2011b). Although, the estimation of hormonal profile and follicular dynamics could have enhanced our understandings to compare the efficacy of LTP, STP or OVS protocol in Beetal goats, unfortunately said attributes were not recorded in this study. In future, comparison of LTP and STP regimens needs to be further explored in term of hormones and follicular dynamics during low and peak breeding seasons.

The profit of goat production is directly related to kidding frequency and litter size. The use of PMSG in progesterone-based protocol provides a way to enhance the follicles number, ovulation rate, subsequently higher pregnancy rate with litter size (Hameed *et al*. 2020). Increased pregnancy rate and litter size in STP and LTP groups might be linked with the inclusion of PMSG in submitted protocols. Previously, the PMSG dose was varying between 200 to 500IU at the time of sponge removal (Hameed *et al*. 2020; Sen and Onder 2016; Fonseca *et al*. 2017); however, selected a medium (300IU) dose rate of PMSG was a better choice to enhance the litter size without deteriorating the health of dam and neonates. The use of GnRH or FSH in progesterone-based protocols could have been an alternate to omit the associated disadvantages to PMSG treatment.

The suitability of these protocols needs careful consideration keeping in view the advantages and disadvantages of each. Like short-term progesterone-based (5-day) protocol offer a disctict advantage over conventional long-term (11-day) protocol due to short duration of application and it provide acceptable estrus response and pregnancy rate. On the other hand ovsynch is less labourous and cost effective, however it require the cyclic goats for better conception rates (Riaz *et al*. 2012). Based on current data and earlier observations, it has been observed that both progesterone-based protocols have the potential to synchronize the estrus in cyclic goats or present in anestrous conditions, effectively. Moreover, the short-term protocol provides a similar efficacy as long-term protocols in situations accompanied by long kidding intervals.

Table 1. Effect of short-term (STP) or long-term (LTP) progesterone and Ovsynch synchronization protocols on estrus response (%), interval from PGF2α injection to onset of estrus and interval from onset of estrus to breeding (h), pregnancy rate (%), pregnancy loss (%), litter size (n), prolificacy (%) and twining rate (%) in Beetal goats during low breeding season.

Variables	**STP** (n = 20)	**LTP** (n = 22)	**OVS** (n = 21)	***p*-value**
Estrus response (%)	20/20 (100)	22/22 (100)	17/21 (80)	0.040
Interval from $PGF_2\alpha$ injection to onset of estrus (hrs)	22.7 ± 2.3	19.3 ± 3.4	17.6 ± 2.2	0.120
Interval from onset of estrus to breeding (hrs)	51.5 ± 3.03	56.7 ± 4.5	62.8 ± 4.2	0.380
Pregnancy rate at day 40 of breeding (%)	17/20 (85)	17/22 (77.3)	9/21 (42.8)	0.005
Pregnancy loss (%)	02/17 (11.7)	02/17 (11.7)	01/09 (11.1)	0.909
Litter size (n)	1.35 ± 0.19	1.18 ± 0.17	0.52 ± 0.17	0.005
Prolificacy (%)	180 (27/15)	173 (26/15)	137 (11/08)	0.552
Twinning rate (%)	66 (10/15)	73 (11/15)	37.5 (03/08)	0.023

The p-value less than 0.05 indicate the significant difference along the rows.
Pregnancy rate: Number of does pregnant on ultrasonography/total number of does bred.
Pregnancy loss: Goats observed pregnant at day 40 of breeding by ultrasound scan but no kid delivered at the time kidding.
Litter size: Average number of kids delivered by the total number of does.
Prolificacy (%): [Number of kids born/number of does kidding] x 100.
Twinning rate (%): [Number of twin kidding/total number of does kidding] x 100.

Conclusions: In conclusion, the short-term intravaginal progesterone-based sponges successfully synchronize the estrus before breeding and could be an effective alternative to conventional long-term progesterone-based or Ovsynch synchronization protocols in Beetal goats. Also, better pregnancy and kidding rates, with minimal pregnancy loss, could be achieved by employing progesterone-based protocols in Beetal goats.

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Conflict of interest: The authors declare that they have no conflict of interest.

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