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Original Article

Estrus Synchronization in Black Bengal Goat Using Synthetic Progesterone

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A B S T R A C T

Estrus synchronization is an effective technique that overcomes the bottlenecks associated with estrus detection failure in livestock. This study was directed to elucidate the beneficial outcomes of estrus synchronization in Black Bengal does by using intramuscular injection of synthetic progesterone. Two studies were carried out with a total of forty (40) adult cyclic fertile does weighing 10-14 kg and aged between 1-2 years. Among them, thirty (30) does were arbitrarily assigned into three (3) groups and injected with 12.5, 15.0, and 17.5 mg progesterone, separately for 14 days, while the other ten (10) does were kept untreated (control). Almost similar behaviors were observed for all does in estrus within 61-73 hours after withdrawal of progesterone and duration of estrus was 24-36 hours. Then the heated does were artificially inseminated with deep-frozen semen within 24 hours of estrus. It was observed that estrus response and conception rate were significantly higher ($p<0.05$) in 12.5 mg (90% and 87.50%) and 15.0 mg progesterone (90% and 88.89%) treated does than those of untreated (40% and 75%) followed by treated with 17.5 mg (60% and 66.67%). In all groups, gestation period was ranged from 153-158 days and parturition was normal. The litter sizes were similar (1.83 and 1.88) for 12.5 mg and 15.0 mg progesterone treated does but significantly lower ($p<0.05$) in untreated group (1.25) followed by 17.5 mg progesterone treated group (1.50). Therefore, it might be assumed that 12.5 and 15.0 mg progesterone could be used as an effective way for estrus synchronization in Black Bengal does.

Introduction

Black Bengal goats comprise over 90% of the total goat population in Bangladesh (Huque, 2021) and are legendary for their outstanding prolific profile, excellent meat and skin quality (Husain *et al*., 1996; Hoque *et al*., 2016). Goat rearing is an essential source of income for poor people and distressed women with little capital investment in Bangladesh (Hoque *et al*., 2011; Kabiraj *et al*., 2011). In addition, goat meat isn't just expensive consistently, yet additionally there is a colossal interest for goats at the time of Eid-ul-Azha in Bangladesh (Asad *et al*., 2018). Therefore, regularity in their breeding and reproduction is essential to obtain the highest return at the earliest time. Estrus detection is critical for successful artificial insemination (AI) and controlled breeding program. However, estrus detection of animals is laborious, time-consuming, and exposed to human error (Peaker, 1978; Khandoker *et al*., 2009). Moreover, low forces of estrus signs as well as lack of technical knowledge are major causes in the most cases estrus detection failure (Fakruzzaman *et al*., 2012). Furthermore, practices of the traditional management system make it difficult to implement an artificial insemination program based on naturally occurring estrus (Quan and Wei, 2015). Therefore, synchronization of estrus has been practiced as a labor saving tool for those producers who want to capitalize on superior genetic material available through the AI (Yang *et al*., 2016; Skliarov *et al*., 2021). Synchronization of estrus is a technique by which a significant number of females in a flock are brought into estrus by a predetermined time (De Ziegler *et al*., 2007). It reduces the time needed for detecting estrus. Related to a strategy for controlling the ovulation time, estrus synchronization limits the overall period of parturition in a flock (Cooper, 1974). Also, the synchronization of estrus technique may be an important strategy to cope with the seasonality in rainfall and availability of green grass in the fields (Chemineau *et al.*, 1999; Loutradis *et al.*, 2003). The majority of goat breeds perform different reproduction activities depending on seasonal changes, latitude/longitude, the length of the photoperiod, and other factors (Hassan *et al.*, 1970; Ishwar and Pandey, 1990). Therefore, estrous synchronization together with AI in does is essential for the improvement of reproductive efficiencies and management processes (Chemineau *et al.*, 1999). Controlled breeding of goats involves artificial control of estrous and ovulation with exogenous hormone treatments (Chao *et al.*, 2008). Estrus synchronization is done either by lengthening the luteal phase using progesterone analogs in conjunction with or without gonadotrophins or by shortening through using prostaglandin ($PGF_2\alpha$) or by the combination of both techniques (Rosado *et al.*, 1998; Greyling and van der Nest, 2000; Motlomelo *et al.*, 2002). However, several researchers have administered progestagens in cyclic female goats with satisfactory results and proposed that the utilization of progestagens for synchronization improves fertility, pregnancy, and kidding rates (Bongso *et al.*, 1982; Baril *et al.*, 1996; Cline *et al.*, 2001; Freitas *et al.*, 2004; Paula *et al.*, 2005). Therefore, use of progesterone in synchronization of goat estrus is becoming more popular in the upcoming days in contrast to the use of prostaglandin (Amarantidis *et al.*, 2004; Skliarov *et al.*, 2021). Although a successful attempt had been taken to synchronize the estrus in Black Bengal goat by using $PGF_2\alpha$ (Khandoker *et al.*, 2009), but no research has so far been initiated using progesterone. Therefore, the present study was set out to enhance the potential aspect of progesterone in estrus synchronization of Black Bengal goat using synthetic progesterone.

Materials

All the chemicals and consumables were procured from established and renowned suppliers maintaining AR grade (Grade of Analytical Reagents). Synthetic progesterone for veterinary use was purchased from the local distributor.

Selection of experimental animals and their management

Two studies were conducted with a total of forty (40) cyclic fertile adult does weighing 10-14 kg and aged between 1-2 years. Among them, thirty (30) does were arbitrarily assigned into three groups (group A, B and C) so that different age groups were represented in each group and injected with 12.5, 15.0, and 17.5 mg progesterone, respectively for 14 days. The other ten (10) does were kept untreated (control) to observe the estrus signs between synchronized and natural estrus. The feeding and management of the does were provided according to the previous studies (Khandoker *et al.*, 2009; Kabiraj *et al.*, 2011). Briefly, the does were raised in the stall feeding system and fed Napier and/or German grass twice a day at *ad-libitum* basis. The commercial concentrate feed was supplied (crude protein content: 120 kg/kg DM and energy content: 10.4 MJ ME/kg DM) once in the morning and another in the evening at the rate of 120 gm/doe. *Ad-libitum* clean and safe water was supplied all the time. Goats were allowed for grazing and exercise for at least 1 to 2 hours daily in a confinement area. The feeding regime was identical for all goats under experimentation. Routine vaccination and deworming were performed according to the standard prescribed schedule. The biosecurity was maintained according to the routine procedure.

Estrus Synchronization

Estrus Synchronization was done using synthetic progesterone analog. Firstly, the last date of showing estrus of all does was recorded. Then a date was fixed on which all the does were in between 4-18 days of the estrus cycle. Each animal in the selected groups was assigned to intramuscular progesterone injections at 12.5 mg, 15.0 mg and 17.5 mg for groups A, B, and C, respectively. These treatments were given for 14 days regularly. After removing the progesterone treatment, the does were noticed closely for 3 days. Heat detection was done twice daily for 1 hour at 9.00-10.00 am and 5.00-6.00 pm till the end of the behavioral estrus in all goats. The behavioral symptoms of estrus are shown in Fig. 1. The time from removal of the treatment to the beginning of estrus was also observed.

Figure 1. Photographs showing estrus behaviors of does, where, (A) Allowing to be mounted, (B) Mucous discharge and swelling of valva, (C) Tail flagging, (D) Licking and rubbing each other, (E) Mock fighting and (F) Sniffing the vulva

Insemination and conception of does

Synchronized does were artificially inseminated within 24 hours after onset of estrus by using deep-frozen semen for insemination. The motility of semen was at least 60% and the sperm concentration was 100 million sperm per dose. Inseminated does were kept watch on for a return to estrus by 20-65 days (up to 3 estrus cycle length) following AI. Goats that are not reversed to estrus were considered to be pregnant. Consequently, estrus behavior, time from withdrawal of treatment to showing estrus (days), duration of estrus (hrs), conception rate (%) after insemination, gestation, parturition, and litter size were also recorded.

Statistical Analysis

The data were scrutinized and a χ^2 (Chi-square) test (Steel and Torrie, 1986) or one-way ANOVA was performed to compare the effect of treatments.

Results and Discussion

Signs of estrus

It was noticed that the does of all treatment groups showed almost similar estrus behavior within 61-73 hours and the duration of estrus was 24-36 hours. The comprehensive differences of signs of estrus between normal to synchronized estrus were presented in Table 1. The most prominent symptom of estrus including mounting flock mates or allowing others to be mounted, sniffing, licking, and rubbing of each other, and mock fighting between does was observed to be almost similar in all progesterone treated or untreated does in estrus. Another vital sign of estrus, the mucus discharge from the vulva was observed to be normal, clear, stringy, thick, and white in the does treated with 12.5 and 15.0 mg but found to be thin and watery in the does treated with 17.5 mg progesterone. Swelling of the vulva is the most common sign of estrus was observed in 12.5 mg treated does but was minorly appeared in 15.0 and 17.5 mg progesterone treated does. Frequent urination is another marked symptom of estrus that was observed in 12.5 mg and 15.0 mg treated does but absent in the 17.5 mg treated group. The reason for the variation in these signs might be due to the quantity of synthetic progesterone that affects the responses on the stimuli to the hypothalamic-gonadal axis as described in different studies (Crosby *et al.*, 1991; Yu *et al.*, 2018). Other minor signs of estrus like nervousness, general attitude change, increased bleating, vocal activity, restlessness, interest in male or male pen, feed intake were also noticed to be similar in all treated and untreated groups. However, these results were comparable to the previous studies describing normal and synchronized estrus of Black Bengal goat by Khandoker *et al.* (2009) and Fakruzzaman *et al.* (2012).

Table 1. Behavioral signs of estrus between normal and synchronized estrus.

Characteristic	Behavioral signs of natural estrus (control)	Signs of synchronized estrus		
		12.5 mg	15.0 mg	17.5 mg
Mounting flock mates or willing to be mounted	This trait was observed in all the times during estrus.	As same as normal	As same as normal	As same as normal
Mucus discharge form vulva	Discharge was clear and stringy at the beginning of the cycle. The color and consistency were after that gradually changed to become thick and white throughout estrus.	Mucus was normal, clear, stringy, thick and white	Same as 12.5 mg treated does	Mucus was low, thin and watery
Swelling of vulva	Estrogen causes a generalized swelling in the vulva lips. The vulva may appear to be from pink to a reddish color due to an increased blood supply and the swelling	Same as normal	Swelling of vulva was minor	Swelling of vulva was minor
Tail flagging	Tail flagging back and forth while it is held at about a 45-degree angle	Same as normal	As same as normal	As same as normal
Licking and rubbing each other	Present	Present	Present	Present
Mock fighting	It is also a common characteristic	Present	Present	Present
Sniffing the vulva	The heated does sniff the vulva each other	Present	Present	Present
Frequent urination	Present	Present	Present	Absent
Restlessness	During estrus, the does are looked very excited and restless	Does were restless and nervous	Not so	Not so
Vocal activity	Increased	Increased	Increased	Increased
General attitude change	Positive	Positive	Positive	Positive
Feed intake	Gradually decreased	Not decreased	decreased	decreased

Response to estrus

The effect of progesterone on estrus synchronization of Black Bengal goat is summarized in Table 2. The estrus response was 90% for the does treated either with 12.5 mg and 15.0 mg but the does remain untreated showed significantly lower ($p<0.05$) response (40%) followed by treated with 17.5 mg progesterone (60%). The synchronization of estrus in livestock focuses on the manipulation of either the luteal or the follicular phase of the estrous cycle. In goats and sheep, the prospect for controlling the estrus cycle is better during the luteal phase than controlling the follicular phase, because this phase is longer and more responsive to the manipulation (Robinson, 1965). However, prostaglandin $PGF_{2\alpha}$ and its synthetic analogs are the luteolytic factors in females having a functional corpus luteum at the time of treatment (Cooper, 1974; Salvador *et al.*, 2001). In some studies, gonadotrophins such as PMSG administration have also been shown to stimulate follicular growth and increases ovulation rate and fertility (Hoque *et al.*, 2021) and induce tighter synchrony of ovulation in both anestrous and cycling goat (Chemineau *et al.*, 1999; Cline *et al.*, 2001). In this study, 12.5-15.0 mg progesterone was found efficient in suppressing heat and simultaneously triggering off physiological mechanisms for the regression of the corpus luteum; hence the withdrawal of the progesterone led to an increase in estrus manifestation. These findings of the present study were in agreement with the reports which indicated that removal of the progesterone by luteolysis of the corpus luteum results in increased estrogen secretions leading to the onset of estrus (Kojima *et al.*, 1992; Lucy *et al.*, 2001). Moreover, some studies reported that extending the luteal phase using exogenous progesterone might be better than shortening this phase by regressing the existing corpora lutea (Hoque *et al.*, 2019; Anggraeni *et al.*, 2021). Furthermore, several successful synchronization experiments in goats were conducted by different doses of synthetic progesterone (Hashemi *et al.*, 2006; Abu *et al.*, 2008; Sun *et al.*, 2019; Salim *et al.*, 2020), suggesting that progesterone treatment might be an effective method of estrus synchronization in goats. In the present study, the time from withdrawal of progesterone treatment to the onset of estrus ranged from 60-72 hrs (2.5-3.0 days). The time to onset of estrus in several studies was observed to be 1-4 days (3 days mean) following progestagen withdrawal in Saanen and Black Bengal goats (Alacam *et al.*, 1985; Ishwar and Pandey, 1990), were in accordance with the present study. However, the duration of estrus in this study was ranged from 24-36 hrs. A similar duration (28-36 hrs.) was also reported by Akusu and Egbunike (1990) in West African dwarf goats. Moreover, other studies by Medan *et al.* (2002) and Motlomelo *et al.* (2002) with progesterone-induced estrus synchronization in goats are also in accordance with the present study.

Table 2. Estrus response of does either in natural estrus or progesterone-induced synchronized estrus.

Parameters	Response				Level of Significance
	Natural estrus	Synchronized estrus			
Dose of progesterone (mg)	-	12.5	15.0	17.5	-
No. of treated does	10	10	10	10	-
No. of does in estrus	4	9	9	6	-
Percentage of estrus goats	60[c]	90[a]	90[a]	60[b]	*
Duration of estrus (hrs)	24.75±0.48	35.63±0.94	33.22 ±0.70	28.17±0.50	NS
Time from withdrawal of treatment to estrus (hrs)	-	61.63±0.78	61.78 ±1.28	72.91±1.19	NS

*Significant at p<0.05 level, NS-Non significant

Conception rate, gestation, and litter size

In the present study, animals were observed for reversal towards estrus after 65 days (3 cycle length), although all animals under study did not reverse to the estrus after 1st insemination. Therefore, service per conception in this study was 1.0. The conception rate of the does after estrus synchronization is presented in Table 3. The conception rate for the group 12.5 and 15.0 mg progesterone was found to be 87.50% and 88.89%, respectively but a significantly (p<0.05) lower rate (50%) was observed in the 17.5 mg treated group followed by the control group (75%). In all treatment groups, the gestation period ranged from 153-158 days and parturition was normal. The litter sizes were similar (1.83 and 1.88) in 12.5 mg and 15.0 mg but significantly lower (p<0.05) in the untreated group (1.25) followed by 17.5 mg progesterone (1.50). Baril *et al.* (1996) reported a 59% pregnancy rate in goats treated for 11 days with FGA intra-vaginal sponges (50 mg) which were superior to 17.5 mg progesterone injection in our study but inferior to the results of our 12.5 mg and 15.0 mg progesterone injection. Similarly, Motlomelo *et al.* (2002) reported a 49% pregnancy rate in goats treated with MAP sponges (60 mg) for 16 days which was inferior to the overall pregnancy rate rewarded in this study. But the conception rate of the does treated with 12.5 mg and 15.0 mg was higher than that of Zarkawi *et al.* (1999) reported (65.8%) in Damascus goats with MAP.

Table 3. Conception rate, gestation and litter size of inseminated does either in natural estrus and synchronized estrus.

Parameter	Response				Level of Significance
	Natural estrus	Synchronized estrus			
No. of does inseminated	4	8	9	6	-
No. conceived	3	7	8	4	-
Conception rate (%)	75.0[b]	87.50[a]	88.89[a]	66.67[b]	*
Gestation period (days)	153.33±0.88	157.25±0.67	157.88±1.44	154.43±0.72	NS
Parturition	Normal	Normal	Normal	Normal	-
Litter size	1.25[c]±0.25	1.83[a]±0.18	1.88[a]±0.23	1.50[b]±0.22	*

*Significant at p<0.05 level, NS-Non significant

Although a decline in fertility was reported in progesterone treated does as a detrimental effect of synchronization on sperm transport and survival in the female reproductive tract (Baumgarther *et al.*, 1974; Pearce and Robinson, 1985), no such decline in fertility was observed in this current study. Moreover, estrus synchronization using progesterone injection in our study not only created tight synchrony but also provided an acceptable level of fertility upon artificial insemination. Numerous researcher reported that the progesterone treatment increased the estrus response without altering the overall fertility, improving pregnancy, kidding rates, and suggesting that intramuscular injection of progesterone is a good option for estrus synchronization (Bongoso *et al.*, 1982; Niswender *et al.*, 2000; Al-Merestani *et al.*, 2003; Chao *et al.*, 2008; Chemineau *et al.*, 1999; Suresh *et al.*, 2021) which is accordance with the current study. The national economy of Bangladesh is mainly based on agriculture (Livestock, Crop and Fisheries). (Khandoker *et al.*, 2012; Hoque *et al.*, 2017; Selim *et al.*, 2017; Islam *et al.*, 2018) where Black Bengal goat is numerically and economically important and promising animal genetic resource among all farm animal genetic resources in Bangladesh. Moreover, Black Bengal goats are not only famous for their adaptability, fertility, prolificacy, meat and skin quality but also have an important role in generating employment, income, capital storage and improving household nutrition (Husain, 1999; Hoque *et al.*, 2011). Therefore, this novel technique of estrus synchronization is believed to increase the reproductive potential and will contribute new insights to conduct controlled breeding programs successfully.

Conclusions

From the study, it can be concluded that intramuscular injection of synthetic progesterone is an effective technique for estrus synchronization of Black Bengal goat that not only increases the estrus response and conception rate but also preserves the overall fertility and increases the litter size. For effective estrus synchronization in Black Bengal goat 12.5 mg or 15.0 mg progesterone treatment might be used in the controlled breeding program followed by artificial insemination.

Author's contribution

A Khatun and SAM Hoque conducted the study, collected and analyzed data and wrote the manuscript. MY Ali interpreted the data and revised the manuscript. MAMY Khandoker supervised all aspects of the study design, wrote and revised the manuscript.

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