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Efficiency of three different protocols of oestrus synchronization in malabari goats

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Abstract
Profitability of goat rearing depends on exploitation of fertility potential which can be achieved with assisted reproductive technology. Synchronization of oestrus with timed hormonal treatment is an extensively used assisted reproductive technology in small ruminants. The present study was conducted to evaluate the efficiency of three different oestrus synchronization protocols in Malabari goats. Oestrus synchronization is manipulation of either luteal or follicular phase of the oestrous cycle for making the animals to attain oestrus at the same time. Thirty three Malabari does with one or more parity and 2-5 years of age were scanned and confirmed as non-pregnant for the study. Oestrus was synchronized either by using double injections of natural $PGF_{2\alpha}$ (n=15), synthetic $PGF_{2\alpha}$ (n=10) or intravaginal progesterone sponge (n=8). Oestrus was observed within 48-72 h of second dose of $PGF_{2}\alpha$ and 48-72 h after sponge removal. Oestrus response was 100% in both natural and synthetic $PGF_{2\alpha}$ and 87.5% in sponge method. All oestrous females were allowed to mate with fertile Malabari buck. Service rate was 86.66%, 90% and 85.7% in natural $PGF_{2\alpha}$, synthetic $PGF_{2\alpha}$ and sponge method, respectively. The day of mating was considered as 0th day of pregnancy. Early pregnancy diagnosis was made by transrectal ultrasonography on 30th day of mating. Conception rate was 69.23%, 66.66% and 66.66% with natural $PGF_{2\alpha}$, synthetic $PGF_{2}\alpha$ and sponge method respectively. Kidding rate was 100% in all protocols. The present study revealed that in Malabari goats, all the three protocols were found to be equally efficient for oestrus synchronization.

Keywords: Malabari goat, oestrus synchronization, dinoprost, cloprostenol, intravaginal sponge, transrectal ultrasonography

Introduction
Goats are seasonally polyoestrous animals (Saribay *et al.*, 2012) [16], Most of the Indian goat breeds showed oestrus throughout the year (Mazumdar and Mazumdar, 1983) [10]. Krishnakumar (1992) [7] stated that Malabari crossbred does of Kerala shows oestrus behaviour throughout the year with a greater incidence from July to September and lesser incidence in January to March. Profitability of goat rearing depends on exploitation of fertility potential which can be achieved with assisted reproductive technology. Synchronization of oestrus with timed hormonal treatment is an extensively used assisted reproductive technology in small ruminants. Oestrus synchronization is manipulation of either luteal or follicular phase of the oestrus cycle for making the animals to attain oestrus at the same day. Principle behind oestrus synchronization is either shortening the luteal phase by use of prostaglandins or its analogues to induce premature luteolysis and or to lengthen the luteal phase by simulating the activity of natural progesterone produced by the corpus luteum (CL) by the use of exogenous progesterone (Wildeus, 2000) [21].

Materials and Methods
The research was carried out during the period of September to November 2018 at University Sheep and Goat Farm, Mannuthy, Thrissur (latitude-10.5332ºN, longitude-76.2656ºE) geographically located at centre of Kerala in south India. Tropical climatic conditions during the study were Temperature- Min of 20 ºC and Max of 32.3 ºC and annual rain fall of 3500 mm. Each doe was fed with daily ration of 200 gm concentrate/day and 5 h of grazing (from 9:00 AM to 2:00 PM) with regular deworming and vaccination. Animals were identified with ear tags. A total of 33 non pregnant Malabari goats which were kidded at least once were selected for the study. All the does were 2-5 years old and weighing 20 to 30 Kg. Non pregnancy of does was confirmed by trans-rectal and trans-abdominal ultrasonography (USG)

(*My lab Gamma, Esaote, Italy*) and oestrus was synchronized with three different protocols as follows.

Fifteen does were synchronized with double injections of 7.5 mg (1.5 ml) of natural $PGF_{2\alpha}$, Dinoprost Tromethamine (Lutalyse® 5mg/ml, Zoetis, Belgium) intramuscularly at 11 days interval, ten does with double injection of 250 µg (1ml) of synthetic $PGF_{2\alpha}$, Cloprostenol (Estrumate® 250µg/ml, Vet Pharma, Germany) intramuscularly at 11 days interval and Eight does with intravaginal sponge (Figure no.1) procured from Central Sheep and Wool Research Institute (CSWRI), Awikanagar, India (AVIKESIL-S®) containing 350 mg natural progesterone. Sponges were kept *in-situ* in the vagina of does for 11 days. One ml (125µg) of Cloprostenol was given intramuscularly 24 h prior to removal of sponge.

Physiological and behavioral signs of oestrus

All the goats were subjected for oestrus detection after 48 h of second inj. of PGF2α in each protocol by exposing teaser buck at 12 h interval for 30 minutes. The Flehmen's reaction expressed by the teaser buck was recorded. Other observed oestrous signs were, frequent bleating, vulval oedema and hyperemia, frequent tail wagging, mounting or standing to be mounted, tendency to seek buck and frequent micturation. Transrectal USG was performed in does during oestrus for presence of dominant follicles (>6mm). Vaginal speculum examination was done to ensure open external-os (Figure 2) and nature of discharge.

Fig 1: Intravaginal progesterone sponges removed after 11 days

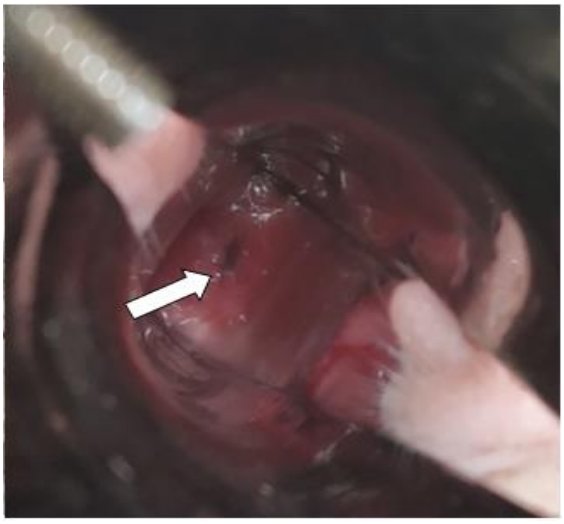

Fig 2: External os of cervix (open)

Fig 3: Natural mating by fertile Malabari buck

Number of does that showed oestrous signs out of the total number of does synchronized for oestrus is expressed in percentage and referred as oestrous response rate. Number of does that successfully completed the natural service out of total number of does showed oestrous signs is expressed in percentage and referred as service rate. Number of does that became pregnant out of the total number of does mated expressed in percentage and referred as conception rate. The percentage of does kidded normally out of the total number of does conceived is referred as kidding rate (Madhusudhan, 2017) [8].

All oestrous females were allowed to mate with proven fertile Malabari buck during behavioural oestrus or after 48 -72 h of second PGF2α injection or sponge removal (Figure 3). The day of natural mating was considered as 0th day of pregnancy. Pregnancy was confirmed trans-rectally using conventional B mode USG (7.5 MHz frequency) on 30th day of mating. Amniotic vesicle and conceptus observed is depicted in figure no. 4 and 5 respectively.

Results and Discussion

There are several protocols for synchronization of oestrus in goats including non-hormonal and single or combined use of hormones such as GnRH, prostaglandins, progestagens (by means of oral, injections, or intravaginal releasing devices), gonadotropins, oestrogens, melatonin (Cinar *et al.*, 2017) [4]. The present study compared the efficiency of three oestrus synchronization protocols in Malabari goats. Oestrus response, service rate, conception rate and kidding rate observed in three protocols are presented in table 1. Results were analyzed using chi square test with help of SPSS software version 24.0, chi square value obtained was 0.087.

Table 1: Comparison of oestrus synchronization protocols in Malabari goats

Oestrus synchronization protocol	Oestrus response rate (%)	Service rate (%)	Conception rate (%)	Kidding rate (%)	Chi square value
Double inj. Dinoprost at 11 days interval (n=15)	100 (15)	86.66 (13)	69.23 (9)	100 (9)	0.087
Double inj. Cloprostenol at 11 days interval (n=10)	100 (10)	90 (9)	66.66 (6)	100 (6)	
Intravaginal progesterone sponge with Cloprostenol 24 h prior to removal (n=8)	87.5 (7)	85.7 (6)	66.66 (4)	100 (4)	
Overall total (n=33)	96.96 (32)	87.5 (28)	67.85 (19)	100 (19)	
Percentage means do not differ significantly in each column					

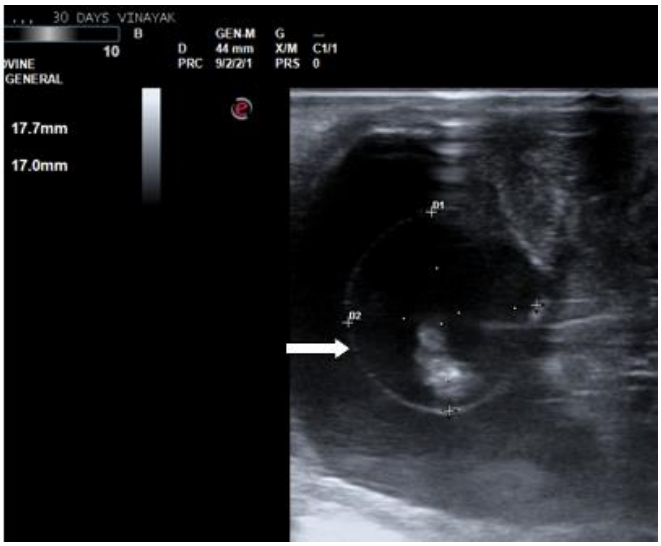

Fig 4: Amniotic vesicle on 30th day of mating

Fig 5: Embryonic mass on day 30 of gestation

Oestrous response rate in present study following double inj. of Dinoprost was 100% (15/15) which finds agreement with the oestrus response recorded by Madhusudhan (2017) [8] with 10 mg Dinoprost at 12 days apart in Malabari goats. However, lower oestrous response of 84% was reported by Ogunbiyi *et al.* (1980) [12] in Red Sokoto goats with 7.5 mg Dinoprost at 11 days apart and 95.33% by Afsal *et al.* (2003) [2] in Malabari crossbred does. 90% by Omentese *et al.* (2014) [13] in Red Sokoto does. Service rate in present study by double inj. of Dinoprost was 86.66% (13/15). Whereas, Madhusudhan (2017) [8] found 90% (18/20) service rate with double inj. of Dinoprost at 12 d interval in Malabari goats. Conception rate was 69.23% (9/13) in double inj. of Dinoprost. This result was in agreement with Tibary (2009) [19] who obtained similar conception rate in Boer goats (65 to 75 %) by using double inj. of $PGF_{2\alpha}$ at 14 days apart during breeding season. Further, Madhusudhan (2017) [8] obtained 66.67% (12/18) conception rate in Malabari does with double Inj. of Dinoprost at 12 days apart. On the other hand, Afsal *et al.* (2003) [2] obtained much lower conception rate than the present study (22.92%) in Malabari crossbred does. However, some authors obtained higher conception rate than the present study, Ogunbuyi *et al.* (1980) [12] 90% in Red Sokoto goats; Ahmed *et al.* (1998) [3] 77.8% in Sudanese Nubian goats; Riaz *et al.* (2012) [14] 78% in Beetal and Dwarf goats.

In the present study 100% (10/10) oestrous response was found with double inj. of Cloprostenol. This was in agreement with Medan *et al.* (2004) [11] in Sheeba goats (n=18) by single dose of Cloprostenol after confirmation of active CL on either of ovary by USG, and also agreement with Romano (1998) [15] in Nubian goats by double inj. of Cloprostenol at 12 days apart, irrespective of the breeding season. Whereas, authors obtained lower oestrous response were, Voh Jr. *et al.* (2003) [20] 92.86% in Red Sokoto goats; Siqueira *et al.* (2009) [18] 88.7% in Toggenberg goats with double inj. of Cloprostenol at 10 d interval and Maia *et al.* (2017) [9] from 88.2% to 96.9% in dairy goats. Service rate in present study by double inj. of Cloprostenol was 90% (9/10) and conception rate was 66.66% (6/9). However, a lower conception rate of 49.1% was reported by Siqueira *et al.* (2009) [18] in Toggenberg goats with double inj. of Cloprostenol at 10 days apart. Whereas, Maia *et al.* (2017) [9] reported Conception rate as 26.7% and 93.6% in trail-1 and 2 respectively by using 37.5µg Cloprostenol at 11.5 days interval in dairy goats.

By using this sponge with Cloprostenol 24 h prior to sponge removal, oestrous response rate obtained was 87.5%. Compared to other two protocols, oestrous response rate was decreased in this protocol. It was not agreement with the De K *et al* (2015) [6] and De K *et al* (2016) [5] who used same sponge with inj. eCG on 12th day, followed by fixed time artificial insemination (FTAI) at 48 h and 56 h of sponge removal and observed lower oestrous response as 79.4% (374/471) and 83.85% (514/613) respectively in sheep. Serin *et al.* (2010) [17] used synthetic progesterone sponge and observed 100 % oestrous response in 15 Saanen goats by 20 mg FGA (Flurogesterone acetate) for 11 days with 500 IU of eCG and 0.5 ml Cloprostenol i/m inj. 48 h prior to sponge removal. Whereas, Abdel-Ghani *et al.* (2016) [1] obtained lower oestrous response rate 82.5% (33/40) with 40 mg FGA sponge for 5days in 40 Egyptian local goats, along with i/m Inj. of Dinoprost (2.5mg) and 10.5 µg Busereline acetate at time of sponge removal. Service rate in the present study using this sponge along with Cloprostenol was 85.7% (6/7) and Conception rate was 66.66 % (4/6). Whereas, Serin *et al.* (2010) [17] found 60 % conception rate by using 20 mg FGA sponge with inj. Cloprostenol in 15 Saanen goats. The present result was not in agreement with Abdel-Ghani *et al.* (2016) [1] who got higher (82.5%) conception rate.

Comparison of these three protocols for oestrus synchronization hasn't been done widely in goats, particularly in Malabari breed. In the present study there was no significant difference between three protocols in oestrus response rate, service rate, conception rate and kidding rate ($P \leq 0.05$). All the three protocols found equally effective in synchronization of oestrous cycle in Malabari goats. Close monitoring of the oestrous cycle using a buck as well as USG evaluation of ovaries for dominant follicles might be the reason for better results in all the three protocols studied. Further studies with more number of animals have to be performed in future for further confirmation.

Conclusion

The present study revealed that, in Malabari goats, double inj. of natural and synthetic PGF2α and progesterone sponge with Cloprostenol was found to be equally efficient for oestrus synchronization.

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