RESEARCH ARTICLE

Effect of Progestin based Estrus Synchronization Protocol on Estrus Parameters, Hormonal Profile and Vaginal Cytology in Surti Goats

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Abstract

This investigation was conducted to study the effect of progestin and prostaglandin-based estrous synchronization protocol on estrus parameters, hormonal profile and vaginal cytology in Surti goats. Ten non-pregnant Surti goats were selected and subjected to estrus synchronization with intra-vaginal progesterone sponges (60 mg Medroxyprogesterone acetate, MAP) for 11 days followed by $PGF_2\alpha$ injection (125 µg Cloprostenol). Whole blood was collected on day 0 (before treatment), day 5, 11 (after treatment), at proestrus, estrus and diestrus for analyzing progesterone as well as estrogen hormone concentration. The vaginal exfoliative cytology was studied at proestrus, estrus and diestrus. Estrus parameters such as induction rate was 100%, time of onset of estrus from PG injection was 29.70 ± 1.06 h and duration was 30.74 ± 0.19 h. Significantly higher population of parabasal cells and superficial cells were observed during proestrus, intermediate cells in diestrus and cornified cells during estrus. At estrus, level of progesterone was lowest and estrogen was highest. Combination of progestin and prostaglandin-based protocol is successful in synchronization of estrous cycle with 100% estrus induction rate in Surti goats. Further in addition to other signs, vaginal exfoliative cytology can also be used for determining different phases of estrous cycle.

Key words: Estrus synchronization, Estrus parameters, Hormones, Surti goat, Vaginal cytology.

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Introduction

The goat population of India ranks second in the world with 148.88 million that amounts to 27.79% of total livestock population (20th Livestock Census, 2019). Shorter span of reproductive cycles, higher growth rate, and tremendous adaptive capacity for harsh environment and requirement of minimum investment makes goat rearing a dependable source of income and livelihood especially for poor, small and marginal livestock owners. Surti breed of goat is native to south Gujarat that is commonly reared by livestock keepers of this region.

The seasonal nature of reproductive cycles in goats can be intervened and controlled by assisted reproductive tools like estrus synchronization protocols that also helps efficient goat rearing. Estrus synchronization facilitates timed mating/AI thereby reducing manpower and improved care and management of goats in estrus, during pregnancy as well as at parturition and kids during early life. This ultimately makes goat rearing more profitable for farmers. There are several reports about use of single or combination of hormones such as progestogens, estrogens, melatonin, $PGF_2\alpha$, PMSG, HCG and GnRH that can be utilized for synchronization of estrus (Yede *et al*., 2020; Choudhary *et al*., 2022; Koli *et al*., 2022). Different estrus synchronization strategies differ in their estrus induction efficiency, time taken for onset of estrus and duration of estrus. This is mainly determined by rhythmic variation of serum or plasma progesterone and estrogen hormones. These hormonal variations are also associated with changes in vaginal exfoliative cytology. Typical variation in number of parabasal, intermediate, superficial and cornified cells are predictive of different stages of estrous cycle and can serve as an aid for heat detection especially in goats with non-overt signs of estrus. The present study was undertaken to evaluate the effect of progestin and prostaglandin-based estrus synchronization protocol on estrus parameters, hormonal profile and vaginal cytology in Surti goats.

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Materials and Methods

The present study was conducted at Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Navsari (Kamdhenu University, Gandhinagar, Gujarat, India). The study was duly approved by Institutional Animal Ethics Committee (IAEC) (vide No. 092-VCN-VPY-2020).

Ten non-pregnant Surti goats irrespective of parity were randomly selected from Goat Farm of Livestock Research Station of the University in Navsari (India). Selected animals had previous history of normal kidding and were neither recently inseminated nor mated with any buck. They were maintained at the farm under standard practices of housing, feeding and management.

Intra-vaginal progesterone sponges impregnated with 60 mg Medroxyprogesterone acetate (MAP, procured from CIRG, Makhdoom, Farah, Dist. Mathura, India) were inserted deep into vagina of all the goats and kept for 11 days. Thereafter intramuscular injection of 125 µg Cloprostenol ($PGF_2\alpha$ analogue) was administered at the time of sponge removal on day 11. The detection of estrus was carried out by parading sexually active apronized buck twice a day (morning-evening). Behavioural signs of estrus such as vigorous wagging of tail, frequent micturition, clustering around buck, mounting other does, standing to be mounted, vulvar hyperemia and edema, mucus discharge, acceptance of male etc. were monitored. First acceptance of buck by doe was considered as onset of estrus. Duration of estrus was determined from first to last acceptance of buck. Estrus induction rate was expressed as percent of total goats that showed signs of estrus.

About 3 mL of whole blood from jugular vein was collected without anticoagulant from all the animals on day 0 (before treatment), day 5, 11 (after treatment), at proestrus, estrus and diestrus (6 days post-estrus). Serum was harvested and stored at -20°C for analysis of progesterone and estrogen hormones by standard Caprine specific ELISA kits (BT LAB, Jiaxing Korain Biotech Co., Ltd, Zhejiang, China).

Stages of estrus were characterized by vaginal exfoliative cytology. Vaginal swabs were collected at different stages of estrous cycle and smears were made. Smears were air-dried, fixed with methanol and stained with Giemsa (Brar *et al*., 2000). The smears were observed under 100 x and cells were identified based on their morphological and stained characteristics. They were categorized into parabasal, intermediate, superficial and cornified anucleated superficial cell.

Descriptive statistics as well as ANOVA was used for analyzing the data obtained. Means were tested by Duncan's New Multiple Range Test (DNMRT) at 5% significance level.

Results and Discussion

Estrus Parameters

In the present study using progestin plus PG protocol, the estrus induction rate, estrus onset and estrus duration were 100%, 29.70±1.06 h and 30.74±0.19 h, respectively. Like present findings, 100% estrus induction rate was also recorded earlier in local goats with similar estrus synchronization protocol (Pawshe, 2012). However, unlike our observations, the higher mean estrus onset interval of 34.25±1.06 h in local goats (Bind, 2016) and 35.82±0.41 h in Black Bengal goats (Dash *et al*., 2017) has been reported, with comparable estrus synchronization protocols; while Pawshe (2012) recorded lower mean estrus onset (20.91±0.27 h) in local goats treated with same protocol for 10 days duration. The mean duration of estrus observed in the present study was 30.74±0.19 h. Similar duration was also observed by Romano (1996) in Nubian does. In contrast, a lower (23.80±0.70 h) and a higher (36.20±0.63 h) duration of estrus has been recorded in Beetal x Dwarf goats (Kausar *et al*., 2018) and Black Bengal goats (Dash *et al*., 2017), respectively.

Exfoliative Vaginal Cytology

Comparison between different stages of estrous cycle (Table 1) revealed that parabasal cells were significantly higher ($p \leq 0.05$) in proestrus as compared to estrus and diestrus. Intermediate cells were significantly higher ($p \leq 0.05$) in diestrus as compared to proestrus followed by estrus. Superficial cells were significantly higher ($p \leq 0.05$) in proestrus and significantly lower in diestrus. Estrus phase showed significantly highest numbers of cornified cells followed by diestrus and proestrus. Results of exfoliated vaginal cells indicated successful differentiation of estrous cycle stages.

Vaginal cytology findings of dominating superficial cells during proestrus in present study are in coherence with studies that have shown dominance of superficial cells and fewer cornified cells in local non-descript goats (Sahu, 2007) and predominance of neutrophils, superficial cells, and few cornified cells in Jamunapari goats (Patil, 2009). Lakhera *et al.* (2014) also reported appearance of neutrophils, intermediate cells, and few cornified cells in vaginal smears of Jamunapari goats. Significantly increased superficial cells during proestrus, followed by intermediate cells and few cornified cells in the present study indicated that cyclicity and estrus was about to commence.

Table 1: Mean (± SE) vaginal exfoliated cells during proestrus, estrus and diestrus in Surti goats after estrus synchronization

Exfoliative vaginal cells	Proestrus	Estrus	Diestrus
Parabasal cells	11.90^{a}±0.38	7.50^{b}±0.37	6.50^{b}±0.43
Intermediate cells	34.00^{b}±0.45	25.00^{c}±0.92	42.10^{a}±0.84
Superficial cells	44.20^{a}±0.70	15.70^{b}±0.75	11.60^{c}±0.34
Cornified cells	9.90^{c}±0.31	51.80^{a}±0.74	39.80^{b}±0.88

Mean bearing different superscripts across row differ significantly at $p \leq 0.05$

Superficial cells of vagina get transformed to keratinized cells (nuclear) and cornified cells (anucleated) under the influence of estrogen as the estrus approaches. Dominance of cornified cells during estrus has also been reported by other workers such as by Kumar (2017[a]), who reported significantly higher (61.73±12.45%) cornified cells and few (23.33±5.05%) intermediate cells during estrus in Sirohi goats. Sahu (2007) also found majority of cornified cells at estrus in local non-descript goats. In present study intermediate cells were significantly higher (p≤0.05) in diestrus.

Hormonal Parameters

Serum progesterone and estrogen levels obtained at different stages are depicted in Table 2, The periodic progesterone level varied significantly (p≤0.05) with lowest value observed at estrus, followed by proestrus and diestrus. Highest value for progesterone was observed at day 11 of estrus synchronization.

In comparison to progesterone value noted at day 0, the lower values were recorded in Osmanabadi goats (Gangaram, 2013; Takle, 2018), local does (Kumar, 2017 [b]) and Black Bengal goats (Dehury *et al*., 2017); and higher values were reported in Black Bengal does (Dash *et al*., 2017) and Surti goats (Chaudhary, 2017).

As compared to current findings lower mean blood progesterone levels at estrus in Boar goats (Greyling and Van Niekerk, 1991), Osmanabadi goats (Gangaram, 2013), and Black Bengal goats (Dash *et al*., 2017; Dehury *et al.,* 2017) and higher values in local goats (Kumar, 2017 [b]; Singh *et al*., 2018) were recorded

The significant (p≤0.05) increase in progesterone levels on day 5 and 11 after estrus synchronization may be attributed to slow release of progesterone from inserted intra-vaginal progesterone sponge (IVPS). Such increase in progesterone due to IVPS has been reported by Kusina *et al.* (2000). After removal of IVPS, progesterone levels declined during proestrus to reach lowest level at estrus followed by increasing levels with commencement of diestrus phase.

Table 2: Serum hormones (Mean±SE) in Surti goats after estrous synchronization

Parameter	Progesterone (ng/mL)	Estrogen (pg/mL)
0 day	1.44^{c}±0.03	5.61^{e}±0.20
5th day	1.81^{b}±0.01	7.44^{d}±0.25
11th day	1.87^{a}±0.01	14.39^{c}±0.18
Proestrus	1.11^{d}±0.01	16.40^{b}±0.12
Estrus	0.63^{e}±0.01	32.40^{a}±0.36
Diestrus	1.13^{d}±0.01	13.79^{c}±0.10

Mean bearing different superscripts across column differ significantly at p≤0.05

Circulating progesterone and its analogues cause blocking of luteinizing hormone (LH) release from the anterior pituitary by negatively impacting preovulatory follicles as well as ovulation rate. Sudden removal of this hormone leads to pronounced estrous cycle (Dogan *et al.,* 2004). Simultaneously administration of prostaglandins or related substances accelerates luteal regression followed by ovulation.

Serum levels of estrogen were significantly (p≤0.05) highest at estrus and lowest at day 0 of estrus synchronization in the present study. Conversely, higher oestradiol-17β level before treatment has been observed as 13.93±0.67 and 14.08±0.38 pg/ml by Dehury *et al.* (2017) and Dash *et al.* (2017) in Black Bengal goats and 34.78±3.88 pg/ml by Chaudhary (2017) in Surti goats. However lower values at estrus have also been reported in Markhoz goats (Talebi *et al*., 2012) and Beetle x Dwarf goats (Kausar *et al*., 2009; Kausar *et al*., 2018).

The peak of estrogen levels in the present study might be due to presence of mature follicles. This results in preovulatory surge of LH followed by ovulation and estrus. Spike in estrogen during proestrus can be attributed to an increase in pituitary drive caused by $PGF_2\alpha$ luteolytic impact. Positive feedback process of LH release leading to ovulation is favored by an increase in estrogen concentration during estrus (Dehury *et al.,* 2017).

Conclusions

Combination of progestin and prostaglandin-based protocol is successful in synchronization of estrous cycle with 100% estrus induction rate in Surti goats. Further in addition to other signs, vaginal exfoliative cytology can also be used for determining different phases of estrous cycle.

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