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Rhythmic Changes in Serum Progesterone and Estradiol-17β in Surti Goats under Different Synchronization Protocols

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ABSTRACT

Background: Success of any estrous synchronization protocol to optimize production and reproduction in goats requires deeper insight of dynamic regulation of hormones progesterone and estradiol-17β. Surti goats reared mostly by marginal livestock owners are native to Gujarat. Present study was conducted to study rhythmic changes in serum progesterone and estradiol-17β in Surti goats under different synchronization protocols.

Methods: 18 Surti goats were divided equally (n=6) in 3 groups *viz*. G1, G2 and G3 (control). G1 and G2 groups were injected with GnRH analogue (day 0 and 11) and $PGF_2\alpha$ analogue (day 9). Progestogen sponge was kept intravaginal in G1 group (day 0) and removed (day 11) before GnRH analogue injection. G3 group (control) received placebo of 2 ml normal saline simultaneous to treatment groups.

Result: Levels were higher at estrus and lower at day 30 for Estradiol-17β and vice versa for progesterone. Coefficient of variation for estradiol-17β at estrus and for progesterone at day 30 was lower for G1 and higher in control. It was concluded that GPG synchronization protocol with intravaginal progestogen was better to synchronize estrus and establish reproductive cyclicity in Surti goats as it effectively minimized variability of progesterone during pregnancy and estradiol-17β at estrus.

Key words: GPG, Intravaginal progestogen sponge, Serum estradiol-17β, Serum progesterone, Surti goats.

INTRODUCTION

Surti breed of goat is a medium-sized dual-purpose breed that is mostly confined to small towns and cities on the western coastal belt of South Gujarat mainly in the tract stretching from Bharuch to Navsari district (Deshpande and Sabapara, 2010). Household women are significantly active for practices related to rearing of Surti goats as compared to men (Deshpande and Sabapara, 2010) that also highlights the women empowerment. Surti goats are raised under semi-intensive management system. Reproductive cyclicity is regulated through endocrine secretions, target receptors and feedback mechanism (Tanaka *et al*., 1992). Synchronization of estrous cycle helps to optimize and regulate hormonal levels for reproduction leading to congregation of most of the animals of a farm. Synchronization of estrus focuses on either reducing the luteal phase by the use of prostaglandins or its analogues or to extend the luteal phase by use of exogenous progesterone (Wildeus, 2000). Intravaginal sponges containing fluoroprogesterone acetate (40 mg) or medroxyprogesterone acetate (60 mg) and controlled internal drug release (CIDR) device (300 mg progesterone) are the most frequently useful treatments for estrus synchronization in small ruminants during non-breeding seasons and breeding (Ungerfeld and Rubianes, 2002).

Hormone levels are dynamic entities that vary transiently depending on species variation and other factors as well, there is always dearth of information concerning changes in serum estradiol-17β and progesterone levels during estrus synchronization. Therefore, the present study was undertaken to study the effect of various synchronization protocols on the rhythmic changes in the serum progesterone and estradiol-17β (E_2) profile in Surti goats.

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MATERIALS AND METHODS

Selection of animals

A total of 18 Surti goats were randomly selected in July, 2019 from Livestock Research Station's University Farm Flock (AICRP on Surti Goats), Navsari Agricultural University, Navsari, Gujarat, irrespective of their parity with recent usual kidding history and isolated for study. The goats under study were maintained in group housing and management system as per Bureau of Indian Standard (BIS) specifications.

Selected goats were kept on optimum nutritional and hygienic conditions and bucks as well as does were fed as per farm's routine.

Synchronization protocols

The selected goats were divided uniformly into three groups (n=6) (G1, G2 and G3). Goats of G1 group were treated with i/m inj. of GnRH analogue Buserelin Acetate (0.0042 mg, Receptal® VET) with intra-vaginal progestogen sponge [60 mg Medroxyprogesterone acetate (MAP)] on day 0; inj. of natural $PGF_2\alpha$ analogue Dinoprost Tromethamine (10 mg, Lutalyse®) on the day 9th and the second inj. of GnRH analogue Buserelin Acetate on day 11th after removal of progestogen sponges. Goats in G2 group received same treatment as G1 except intra-vaginal progestogen sponge and G3 group was kept as control and intramuscular injection of 2 ml normal saline was administered on days 0, 9th and 11th as placebo. Estrus detection was performed by parading sexually active buck twice a day (morning and evening) after 11 days and visually monitoring behavioral signs of estrus. The animals were bred by natural service after being observed for estrus by selecting bucks as per usual farm practice.

Blood collection and sampling procedure

Approximately 5 ml of blood was collected from all the selected goats on day 0 (before GnRH inj.), day 5th (after GnRH inj.), day 11th (after PGF2α inj.), on the day of estrus and day 30th (post-service) aseptically by jugular vein puncture. Vacutainers containing blood samples were kept in a slanting position at room temperature for 1 to 2 hours and was centrifuged for 10 minutes at 2000 rpm to harvest sufficient quantity of serum. Serum was collected in properly labeled 5 ml sterilized plastic storage vials and stored in the deep freeze at -20°C until further analysis.

Hormone assay

Serum progesterone (P_4) and serum estradiol (E_2) concentration was measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and the procedure described by Calbiotech Inc., 1935 Cordell Court., El Cajon, CA 92020 USA. The absorbance is measured spectrophotometrically at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and the results/values were expressed as ng/ml and pg/ml, respectively.

Statistical analysis

Results obtained were compared using means by one-way ANOVA. Means were separated using Duncan's New Multiple Range test (DNMRT) at 5 and 1% level of significance. Ratio for estradiol to progesterone and coefficient of variation for both hormones in different groups were calculated.

RESULTS AND DISCUSSION

Serum progesterone (P_4) concentration

Mean serum progesterone concentration (ng/ml) in various days in different groups of does is presented in Table 1. The statistical analysis of the mean serum progesterone concentration between the groups did not differ (p>0.05) significantly at 0-day, 5th day, 11th day, on the day of estrus and at 30th day post estrus. Progesterone concentration in G1 and G2 group increased after GnRH injection and non-significantly (p>0.01) decreased after $PGF_2\alpha$ injection. The decreased levels can be attributed to lysis of the residual luteal tissue over the ovary by injection of $PGF_2\alpha$ that was given on 9th day in both groups. The reason for increased levels at day 30th can be associated with increased role of progesterone during pregnancy as all the does were found to be pregnant in each group at day 30.

Mean serum progesterone level 1.55±0.23 ng/ml and 1.44±0.17 ng/ml at 0 day before treatment in G1 and G2 groups respectively might be due to different phase of estrus cycle of the goats in each group. The mean serum progesterone concentration before treatment was observed by various research workers in their treatment protocols as 0.30 ng/ml and 6.66±3.02 ng/ml by Saribay *et al.* (2019) in Damascus goats and Singh (2016) in local goats at the time of FGA (30mg) and natural progesterone (350 mg) sponge insertion (at 0 day), respectively. Moreover, mean serum

Table 1: Serum progesterone (P_4) concentration (ng/ml) at different time intervals/days in treatment and control groups of Surti does (Mean±SEM).

Days/time intervals	Groups/treatments (n=6)			Overall	F value	P value
	(G1) Sponge with GPG	(G2) GPG	(G3) Control			
0 day (before treatment)	$1.55\pm0.23_a^X$	$1.44\pm0.17_a^X$	$1.55\pm0.07_a^X$	1.51 ± 0.09^X	0.15	0.86
5th day (during treatment)	$1.81\pm0.17_a^X$	$1.60\pm0.13_a^X$	$1.54\pm0.22_a^X$	1.65 ± 0.10^X	0.68	0.52
11th day (during treatment)	$1.65\pm0.17_a^X$	$1.48\pm0.10_a^X$	$1.84\pm0.13_a^X$	1.66 ± 0.08^X	1.67	0.22
Day of estrus	$0.75\pm0.05_a^Y$	$0.69\pm0.04_a^Y$	$0.79\pm0.03_a^Y$	0.74 ± 0.02^Y	1.40	0.28
30th day (post service)	$4.48\pm0.26_a^W$	$4.90\pm0.35_a^W$	$4.98\pm0.37_a^W$	4.79 ± 0.19^W	0.67	0.52
Overall	-	-	-	2.06±0.16	-	-
F value	55.77**	76.68**	63.60**	201.17**	-	-
P value	0.00	0.00	0.00	0.00	-	-

Means bearing different superscripts within a column (between time intervals/days) differ significantly (**p<0.01) and means bearing common subscripts within a row (between the group) did not differ significantly (p>0.05).

progesterone concentration was also observed as 1.47±0.11 ng/ml by Takle (2018) in Osmanabadi goats and 3.09±4.38 ng/ml by Panjaitan *et al.* (2020) in Kacang goatsat 0 day before GnRH injection in their respective GPG protocols.

In G1 and G2 groups, the mean serum progesterone level were 1.81±17 ng/ml and 1.60±0.13 ng/ml at 5th day after GnRH injection and decreased non-significantly (p>0.01) to 1.65±0.17 ng/ml and 1.48±0.10 ng/ml at 11th days after $PGF_2\alpha$ injection given at 9th day, respectively. Similar decrease in mean serum progesterone level was reported as from 5.64±2.08 ng/ml to 1.90±0.26 ng/ml by Gupta *et al.* (2019) in Salem black goats and 15.6 ng/ml to 1.9 ng/ml reported by Holtz *et al.* (2008) in Boer goats by using 250 mcg Cloprostenol and 3.75 mg Luprostiol, respectively in their GPG protocols.

The mean serum progesterone level on the day of estrus in G1 and G2 groups were 0.75±0.05 ng/ml and 0.69±0.04 ng/ml, respectively. Mean serum progesterone concentration on the day of estrus was observed by various research workers in their treatment protocols as 0.23 ng/ml by Teleb and Ashmawy (2007) in Damascus Baladi goats; 0.42±0.03 ng/ml by Bonia *et al.* (2015) in Assam local goats; 0.9 ng/ml by Holtz *et al.* (2008) in Boar goats and 1.28 ng/ml by Anonymous (2012) in Surti goats at RBRU (Anand). Additionallythe mean serum progesterone level at 30th day post estrus in G1 and G2 groupswere 4.48±0.26 ng/ml and 4.90±0.35 ng/ml, respectively and these findings corroborated with mean serum progesterone level reported as 3.75±0.17 ng/ml by Al-Sobaiyl (2010) at 30th day post mating in Aradi goats; 4.3±11.0 ng/ml by Khanum *et al.* (2008) during gestation in Dwarf goat; 4.6±2.8 ng/ml by Gaafar *et al.* (2005) at week 2 of gestation in Damascus goats. Goats are the species in which corpus luteumis required during pregnancy and placenta acts as extra source of progesterone which can explain the levels for control group.

Serum estradiol-17β (E_2) concentration

The mean serum estradiol-17β concentration (pg/ml) on days in different groups of animals is presented in Table 2. Mean serum estradiol-17β concentration in the treatment and control groups did not differ (p>0.05) significantly between each other at 0-day, 5th day, 11th day, day of estrus and at 30th day post estrus. In G1 and G2 treatment groups, the mean serum estradiol-17β level was variable before GnRH injection. The levels increased non-significantly (p>0.01) at 5th day after GnRH injection and subsequently increased significantly (p<0.01) at 11th day after $PGF_2\alpha$ injection (given at 9th day). Further the levels increased significantly (p<0.01) on the day of estrus to higher level

Table 2: Serum estradiol-17β (E_2) level (pg/ml) at different time intervals/days in treatment and control groups of Surti does (Mean±SEM).

Days/time intervals	Groups/treatment (n=6)			Overall	F value	P value
	G1 Sponge with GPG	G2 GPG	G3 Control			
0 day (before treatment)	$06.74±0.72_a^Y$	$08.20±0.88_a^Y$	$08.06±1.10_a^Z$	$07.66±0.52^Y$	0.78	0.47
5th day (during treatment)	$07.95±1.46_a^Y$	$10.13±1.01_a^Y$	$07.15±1.36_a^Z$	$08.41±0.76^Y$	1.43	0.26
11th day (during treatment)	$15.68±0.66_a^X$	$15.44±1.11_a^X$	$17.63±1.34_a^X$	$16.24±0.63^X$	1.23	0.31
Day of estrus	$34.11±1.04_a^W$	$32.92±1.31_a^W$	$35.69±2.10_a^W$	$34.24±0.88^W$	0.81	0.46
30th day (post service)	$15.77±2.03_a^X$	$18.84±2.25_a^X$	$12.88±1.41_a^Y$	$15.83±1.20^X$	2.39	0.12
Overall	-	-	-	16.46±1.07	-	-
F value	72.42**	49.04**	60.13**	165.16**	-	-
P value	0.00	0.00	0.00	0.00	-	-

Means bearing different superscripts within a column (between time intervals/days) differ significantly (**p<0.01) and means bearing common subscripts within a row (between the group) did not differ significantly (p>0.05).

Table 3: Serum estradiol-17β (E_2) to progesterone (P_4) level ratio at different time intervals/days in treatment and control groups of Surti does (Mean±SEM).

Serum estradiol-17β (E_2) to progesterone (P_4) ratio						
Days/time intervals	Groups/treatments (n=6)			Overall	F value	P value
	Sponge with GPG (G1)	GPG (G2)	Control (G3)			
0 day (before treatment)	$4.78±0.71_a^Y$	$5.95±0.7_a^{XY}$	$5.37±0.98_a^{XY}$	$5.37±0.45^Y$	0.52	0.60
5th day (during treatment)	$4.91±1.22_a^Y$	$6.43±0.63_a^{XY}$	$4.97±0.92_a^{XY}$	$5.44±0.55^Y$	0.81	0.46
11th day (during treatment)	$10.15±1.34_a^X$	$10.89±1.45_a^X$	$9.63±0.47_a^X$	$10.22±0.65^X$	0.29	0.75
Day of estrus	$46.21±2.64_a^W$	$48.61±3.48_a^W$	$46.03±3.79_a^W$	$46.95±1.83^W$	0.18	0.83
30th day (post service)	$3.51±0.37_{ab}^Y$	$3.86±0.4_a^Y$	$2.65±0.33_b^Y$	$3.34±0.23^Y$	2.89	0.08
F value	152.4	116.7	100.6	392.0	-	-
P value	0.00	0.00	0.00	0.00	-	-

Means bearing different superscripts within a column (between time intervals/days) differ significantly (**p<0.01) and means bearing different subscripts within a row (between the group) differ significantly (p>0.05).

and later on decreased significantly ($p<0.01$) at 30th day post estrus (Table 2). The estradiol-17β concentration was found to be increased after GnRH injection has been reported by Gupta *et al.* (2019) in Salem Black goats. Significant ($p<0.01$) increase in mean serum estradiol-17β level after $PGF_2\alpha$ injection could be due to lysis of residual luteal tissue by $PGF_2\alpha$ injection on 9th day thereby further minimizing the levels as well as effects of progesterone and thus enhancing the follicular activity. The levels decreased significantly at 30th day post estrus since most of the does were found to be pregnant in each group.

The mean serum estradiol-17β levels of 06.74±0.72 pg/ml and 08.20±0.88 pg/ml at 0 day before treatment in G1 and G2 groups, respectively was due to their existence indifferent phase of estrus cycle. Mean serum estradiol-17β level of07.95±1.46 pg/ml and 10.13±1.01 pg/ml at 5th day before $PGF_2\alpha$ injection increased significantly ($p<0.01$) to 15.68±0.66 pg/ml and 15.44±1.11 pg/ml at 11th days after $PGF_2\alpha$ injection (given at 9th day), respectively. Similarly, the mean serum estradiol-17β level before GnRH treatment was observed as 4.56±0.82 pg/ml and increased from 9.18±2.29 pg/ml to 11.97±3.93 pg/ml when comparison was made before and after $PGF_2\alpha$ injection reported by Gupta *et al.* (2019) using Ovsynch protocol in Salem Black goats.

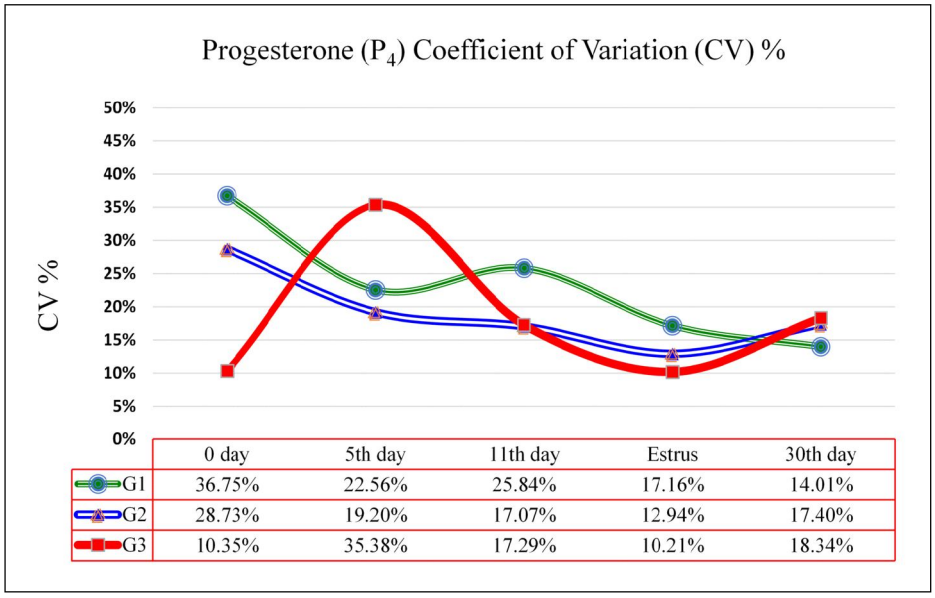
The mean serum estradiol-17β level on the day of estrus in G1 and G2 groups were 34.11±1.04 pg/ml and 32.92±1.31 pg/ml, respectively. Different levels of the mean serum estradiol-17β on the day of estrus was reported as 46.10±5.9 pg/ml by Gaafar *et al.* (2005) in Damascus goat; 27.90±1.30 pg/ml by Leigh and Muoma (2016) in West African dwarf goatsand 40.20±2.30 pg/ml by Moeini *et al.* (2015) in Markhoz goats. At 30th day post estrus the mean serum estradiol-17β level in G1 and G2 groups were 15.77±2.03 pg/ml and 18.84±2.25 pg/ml, respectively. Moreover, the mean serum estradiol-17β level was 10.0±0.0 pg/ml also reported by Pandya (2009) at 30th day of pregnancy in the Surti goats.

Estrogen: progesterone ratio

The results for estrogen: progesterone ratio for all groups is mentioned in Table 3. The estrogen progesterone ratio did not differ significantly ($p>0.05$) between all groups at 0, 5th, 11th and on the day of estrus and differ significantly ($p<0.05$) at 30th day post estrus. While, the estrogen: progesterone ratio differs significantly ($p<0.05$) within groups at different time intervals. However, it was higher *i.e.* 46.21±2.64, 48.61±3.48 and 46.03±3.79 for G1, G2 and G3, respectively on the day of estrus indicating estradiol-17β dominance and lowest *i.e.* 3.51±0.37, 3.86±0.4 and 2.65±0.33 for G1, G2 and G3 on day 30th of the study that showed progesterone dominance.

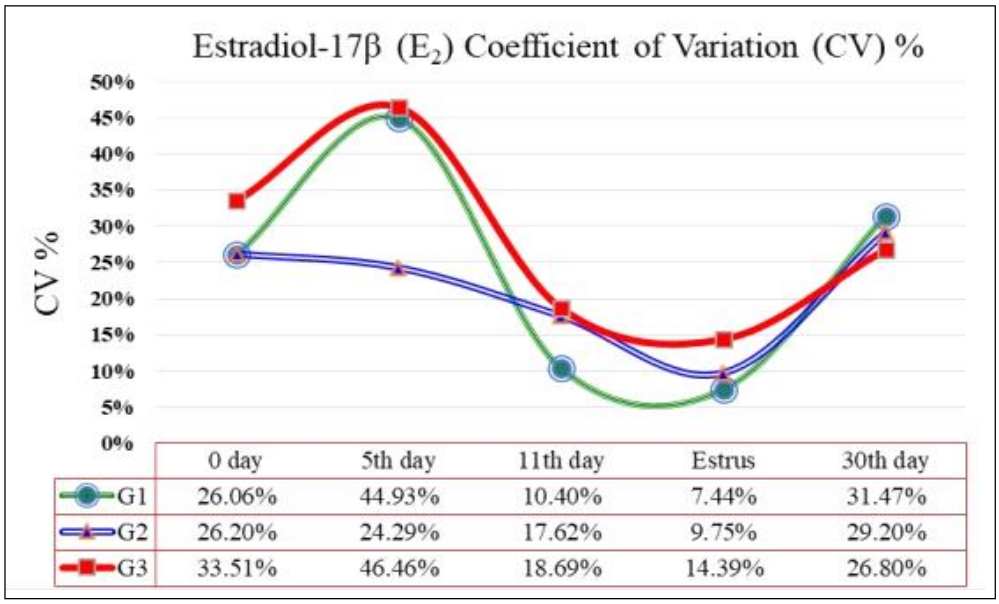
Variability levels of progesterone and estradiol-17β

The results for coefficient of variation in percent (CV%) for progesterone (P_4) and estradiol-17β are represented in Fig 1, 2. The coefficient of variation for progesterone (P_4) was lesser for G1 (14.01%) and G2 (17.40%) as compared to G3 group (18.34%) at day 30 of study. The coefficient of variation for estradiol-17β on the day of estrus was lesser for G1 (7.44%) and by G2 (9.75%) as compared to G3 (14.39%) group. Estrus synchronization efficacy is gazed by minimum variability obtained by both types of treatment protocol in the present study. Coefficient of variation for estradiol-17β at estrus and for progesterone at day 30 was lowest for G1 as compared to G2. Coefficient of variation was highest in control.



	0 day	5th day	11th day	Estrus	30th day
G1	36.75%	22.56%	25.84%	17.16%	14.01%
G2	28.73%	19.20%	17.07%	12.94%	17.40%
G3	10.35%	35.38%	17.29%	10.21%	18.34%

Fig 1: Serum progesterone (P_4) level coefficient of variation percentage (CV%) at different time intervals/days in treatment and control groups of Surti does.



	0 day	5th day	11th day	Estrus	30th day
G1	26.06%	44.93%	10.40%	7.44%	31.47%
G2	26.20%	24.29%	17.62%	9.75%	29.20%
G3	33.51%	46.46%	18.69%	14.39%	26.80%

Fig 2: Serum estradiol-17β (E_2) level coefficient of variation percentage (CV%) at different time intervals/days in treatment and control groups of Surti does.

CONCLUSION

GPG synchronization protocol (at 0, 9 and 11th day) effectively minimizes the variability of estradiol-17β at estrus and progesterone during pregnancy to synchronize estrus and establish reproductive cyclicity in Surti goats. The efficacy to synchronize hormones was better for GPG protocol with intravaginal progestagen/medroxyprogesterone acetate as compared to GPG protocol alone.

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