ESTROUS BEHAVIOUR, PHYSIO-CHEMICAL PROPERTIES AND VAGINAL CYTOLOGY OF CERVICAL MUCUS IN BEETAL GOATS DURING INDUCED ESTRUS

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ABSTRACT

Sixty post-partum anestrus beetal goats were randomly divided into three groups of 20 each to compare the efficacy of norgestomet with or without prostaglandin ($PGF_{2\alpha}$) or eCG or both for the induction of estrus. Each goat subcutaneously received half norgestomet implant (1.5 mg) for 11 days either with eCG 500 IU on day 9 (Gp Norg+eCG), with $PGF_{2\alpha}$ 50 μg on day 10 (Gp Norg+PG) or with both eCG and PG on day 9 and 10 of protocol, respectively (Gp Norg+eCG+PG). Subsequent to implant removal, estrus was detected twice a day using aproned buck and animals in estrus were mated twice at 12 h interval. The animals in estrus showed bleating, wagging of tail and standing heat. The cervical mucus discharge during induced estrus was more copious in goats that became pregnant, while it was scanty in their counterparts failing to conceive ($p<0.05$). The cervical mucus during induced estrus was clear with high degree of arborization and spinnbarkeit in majority of the goats. The pH of cervical mucus during induced estrus was 6.8 - 7.0 with dominance of superficial cells (72%). In brief, the exhibition of estrous behaviour during induced estrus was similar to natural estrus.

Keywords: Beetal goat, Estrus behaviour, Induced estrus, Norgestomet, Vaginal cytology

INTRODUCTION

A major constraint in Indian goat breeding is longer post-partum interval to first estrus (Neeru *et al*., 2003). Thus, to minimize the kidding interval, estrous synchronization protocols were put into use to induce estrus (Whitley and Jackson, 2004). However, it is not well known whether the properties of induced estrus mimic the spontaneous estrus or not in goats (Endo *et al*., 2016). Moreover, the information regarding vaginal cytology and physio-chemical properties of cervical mucus during induced estrus is scanty in goats. Hence, there is a need to study the exhibition of estrus symptoms and alterations in vaginal cytology and physical characteristics of cervical mucus during induced estrus.

MATERIALS AND METHODS

Sixty post-partum anestrus goats were divided into three groups of 20 each. Each goat was subjected to half norgestomet (Crestar, 1.5mg, MSD Animal Health) implant subcutaneously on the upper side of an ear and left *in situ* for 11 days. In addition, group Norg+eCG goats received 500 IU eCG (Folligon, MSD Animal Health) on day 9, group Norg+PG received 50 μg $PGF_{2\alpha}$ (Cloprostenol, Virbac India) on day 10 and, group Norg+eCG+PG received 500 IU eCG on day 9 and 50 μg $PGF_{2\alpha}$ on day 10 of the protocol.

After removing the implant, estrus was detected twice a day by visualizing the estrus behaviour signs and observing the reactions of females to an aproned buck. The goats were recorded in estrus if they stood to be mounted. The estrus signs like bleating, frequent micturition, mucus discharge, wagging of tail and stand to be mounted were noted in goats induced to estrus. Vaginal smears were prepared during induced estrus by using vaginal swab method and Leishmen stain. Every dried smear was observed for different cells *viz*. superficial, intermediate and parabasal. A total of

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100 cells were counted from 5 fields of each slide and percentage of each cell type was recorded.

During induced estrus, cervical mucus was collected by using a glass pipette, and was used to evaluate the physical characteristics like pH, colour, fern pattern and spinnbarkeit. Colour of the mucus was detected visually and was categorized as clear or cloudy. The pH of the mucus was estimated by using pH paper. For spinnbarkeit, drop of mucus was taken in between the forefinger and the thumb of a hand and, stringiness of the mucus was determined and graded by separating the forefinger and thumb as high, medium and low spinnbarkeit. For arborization (fern like pattern), a dried drop of cervical mucus was observed under a microscope (40X), and the pattern was graded using classification of cattle that ranged from 0 to 4 on a scale (Bishnoi *et al.,* 1982).

Data regarding vaginal cytology and pH were analyzed by one-way ANOVA. Other physio-chemical properties of vaginal mucus and estrus behaviour were analyzed using chi square test, and differences were significant when $p<0.05$.

RESULTS AND DISCUSSION

In the present study, estrus signs observed during induced estrus included frequent urination, mucus discharge, bleating, wagging of tail and standing heat. However, bleating, wagging of tail and standing heat were the prominent estrus signs and were observed in all the goats (Table 1). The percentage of goats voiding frequent urination and mucus discharge was similar between groups ($p>0.05$). In general, the overall impression of behavioural signs was that the quantity of mucus secretion was very less during induced estrus and signs like bleating, frequent urination and wagging of tail were more evident in the presence of buck. Others also found decreased mucus secretion in ewes during induced estrus (Rexroad and Barb, 1977). Moreover, earlier studies in goats also showed that the estrus behaviour during spontaneous estrus became more pronounced in the presence of buck (Haulenbeek and Katz, 2011 and Endo *et al*., 2016). Since the induced estrus signs were similar to spontaneous estrus, therefore, the induced estrus signs are more or less physiological. Nevertheless, the comparison of pregnant and non-pregnant animals within the three groups, retrospectively, revealed that the frequency of goats showing frequent urination was similar ($p>0.05$, Table 1). However, the frequency of mucus discharge was higher in pregnant goats of all the groups, with the differences reaching significance ($p<0.05$) only in groups Norg+eCG and Norg+PG (Table 1). Therefore, it could be suggested that the mucus discharge was more in goats that became pregnant while it was scanty in those which fail to conceive.

In the cervical mucus of induced estrus, the pH varied between 6.8 - 7.0 ($p>0.05$, Table 2). Contrary to present study, higher pH (7.9) was reported in ewes

Table 1: The comparison of behavioural estrus exhibited by Beetal goats conceiving or failing to conceive following mating at norgestomet-based estrus induction protocols. Norg, Norgestoment; eCG, equine chorionic gonadotropin; PG, Prostaglandin $F_{2\alpha}$

Group	Pregnancy status	**Frequent urination**, n (%)	**Mucus discharge**, n (%)
Norg + eCG	**Pregnant,** n=10	5 (50)	7 (70)[a]
	Non-pregnant, n=6	3 (50)	2 (33.3)[b]
Norg + PG	**Pregnant**, n=14	7 (50)	10 (71.3)[a]
	Non-pregnant, n=3	2 (66.7)	1 (33.3)[b]
Norg + eCG + PG	**Pregnant**, n=10	8 (80)	6 (60)
	Non-pregnant, n=9	5 (55.6)	4 (44.4)

Values bearing different superscripts differ significantly ($p<0.05$) within a group

Table 2: The physio-chemical properties of cervical mucus and vaginal cytology of Beetal goats during norgestomet-based estrus induction. Norg, Norgestoment; eCG, equine chorionic gonadotropin; PG, Prostaglandin $F_{2\alpha}$

Parameters		**Norg + eCG**, n=16	**Norg + PG**, n=17	**Norg + eCG + PG**, n=19
pH, Mean±SE		6.95±0.05	6.9±0.04	6.77±0.03
Colour, n (%)	Clear	13 (81.3)	13 (76.5)	16 (84.2)
	Cloudy	3 (18.7)	4 (23.5)	3 (15.8)
Spinnbarkeit, n (%)	High	4 (25)	3(17.6)	2 (10.5)
	Medium	4 (25)	8 (47.1)	7 (36.8)
	Low	8 (50)	6 (35.3)	10 (52.7)
Arborization pattern, n (%)	Score 1	-	-	1 (5.3)
	Score 2	2 (12.5)	2 (11.8)	2 (10.5)
	Score 3	8 (50)	11 (64.7)	12 (63.2)
	Score 4	6 (37.5)	4 (23.5)	4 (21)
Vaginal Cytology, Mean±SE	Superficial	72.3±0.9	71.8±0.8	71±0.8
	Intermediate	16.2±0.7	17±0.7	18±0.6
	Parabasal	11.5±0.5	11.2±0.5	11±0.5

induced to estrus using vaginal sponges (Manes *et al*., 2016). These differences in pH could be due to the use of different progestagen sources. During induced estrous, the colour of cervical mucus of majority (75-85%) goats was clear (Table 2). The present results regarding clear mucus were in accordance with the results (80%) in bovines (Sharma *et al*., 2011). A high to medium spinnbarkeit was found in cervical mucus of 75-89% goats (Table 2). Contrary to our study, lower spinnbarkeit was recorded in the cervical mucus of ewes induced to estrus (Rexroad and Barb, 1977). The arborization pattern of score 3 and/or 4 of cervical mucus was observed in >80% goats (Table 2). Similar to our results, higher arborization scores in maximum animals was reported in the cervical mucus of MGA-induced estrus cows (Boyd *et al*., 1972). Nevertheless, the color of mucus discharge, spinnbarkeit and arborization pattern was similar between the induced estrus groups of the present study (p>0.05, Table 2).

The vaginal cytology revealed the superficial cells as the dominated cells in the vaginal smears of all the goats in all the groups (Table 2), but the differences were not significant (p>0.05). Earlier studies also reported the dominance of superficial cells during spontaneous (Ola *et al*., 2006) and induced estrus (Leigh *et al*., 2010) in goats. Therefore, the vaginal cytology could be used as effective tool for breeding management in goats, especially if estrus signs are not overt enough to be detected by the observer or the buck, or the mucus discharge is scanty.

It could be concluded that exhibition of estrus behaviour during induced estrus was similar to behaviour exhibited during natural estrus. The mucus discharge was copious in goats that became pregnant, while it was scanty in those which fail to conceive. Moreover, the vaginal cytology could be used to detect estrus in goats exhibiting scanty estrous especially during induced estrus.

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