



Physico-chemical properties of cervical mucus discharge and endocrine profile during oestrus and metoestrus period in crossbred dairy cows with and without metoestral bleeding

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Abstract

The physicochemical properties of cervical mucus discharge (CMD), serum oestrogen and progesterone profiles during oestrus (day 0) and metoestrus (day 2) period in crossbred cows with (Group I, n=12) and without (Group II, n=12) history of profuse metoestral bleeding were studied. Majority of group I cows exhibited clear and transparent CMD (83.33 per cent) with thin consistency (100 per cent), typical fern pattern (83.33 per cent), pH (7.58±0.12) and spinnbarkeit value of 12.64±0.50 cm during oestrus. However, during metoestrus all the cows (100 per cent) exhibited bloody CMD with thin consistency (66.67 per cent), 'nil' fern pattern (0.0 per cent), mean pH of 7.96±0.10 and mean spinnbarkeit value of 9.35±0.5 cm. In group I cows, the mean pH and spinnbarkeit values of CMD (7.96±0.10 and 9.35±0.5 cm, respectively) was significantly ($p<0.01$) higher than that of group II cows (7.64±0.07 and 7.75±0.30 cm, respectively) during metoestrus. The mean serum progesterone concentration of group I cows (1.61±0.55 ng/mL) was significantly ($p<0.05$) higher than that of group II cows (0.36±0.07 ng/mL) on day of oestrus. The mean serum oestradiol concentration of group I cow (6.85±0.53 pg/mL) was significantly ($p<0.05$) lower when compared to group II cows (8.48±0.49 pg/mL) during metoestrus. In conclusion, the physicochemical properties of CMD, serum progesterone and oestrogen profiles significantly altered in group I cows during oestrus and metoestrus which showed lower conception rate than that of group II cows.

Key words: Cervical mucus discharge, oestradiol, supra-basal progesterone

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Metoestrus is the stage of oestrous cycle during which the hormonal shift occurs from oestrogen dominance to progesterone dominance due to sudden withdrawal of oestrogen after ovulation (Roberts, 1971). The phase is poorly defined as the waning of oestrus behaviour and changes due to gradual increase in the progesterone level may not be clearly exhibited. During oestrus the bovine cervical mucus possesses certain physicochemical properties such as colour, consistency, fern pattern, pH and stretchability (spinnbarkeit value). These characteristics are most evident at oestrus under the influence of oestrogen and alter as the stage progresses under the influence of progesterone (Layek *et al.*, 2013). The properties of cervical mucus are direct indicators of fertility status and hormonal changes in cows. Optimum characteristics of cervical mucus discharge play a vital role in sperm transport, gametes survival and fertilisation. Several authors studied the properties of cervical mucus discharge (CMD) at the time of oestrus and opined that cows with clear, transparent, thin CMD having typical fern pattern, alkaline pH and higher spinnbarkeit values were more favorable for sperm transport and fertilisation (Hanumant *et al.*, 2019). However, studies on the properties of CMD during metoestrus are scanty. Variations in these parameters during metoestrus might have significant impact on conception as because the ovulation in cows occurs 12 to 14 h after the end of oestrus. The present study was conducted to evaluate the changes in the properties of CMD and endocrine profiles in cows with and without profuse metoestral bleeding.

Materials and methods

Crossbred dairy cows aged between 3 to 10 years, calved once, with the history of profuse metoestral bleeding were selected for the study (Group I, n=12). Oestrous detection was done by visual inspection of animals at 12 h interval for primary and secondary oestrus signs. Cows showing regular oestrous cycles, without any visible metoestral bleeding and any infertility related problems were selected as control (Group II, n=12). The CMD was collected aseptically during oestrus (day 0) as well as at

metoestrus (day 2) from both the groups. A sterile AI gun fitted with plastic sheath and sleeve was introduced into the vagina and passed up to the level of mid cervix. The CMD was aspirated with the use of sterile disposable 20 mL syringe and transferred into sterile vials. The characteristics of CMD like colour, consistency, pH, stretchability by spinnbarkeit test and fern pattern at oestrus as well as at metoestrus were assessed as described by Samad (2002) within one hour of collection. Endometrial cytological smears were prepared by modified cytobrush technique to rule out subclinical endometritis in animals of both the groups as per standard procedures (Brodzki *et al.*, 2014). Blood samples (10 mL) were collected during oestrus, metoestrus and mid luteal phase (Day 10) for the estimation of serum progesterone and oestradiol concentrations by commercial RIA diagnostic kits. The assay sensitivity for oestradiol and progesterone was 10.41 pg/mL and 0.03 ng/mL respectively, with intra and inter assay co-efficient of variation being 8.15 and 8.66 per cent. The data obtained were tabulated and analysed using independent-t test (Table 1) and between days within a group was compared by using repeated measures ANOVA followed by least significant difference (LSD) test for pair wise comparison of least square means (Table 3). The cows belonging to both the groups were subjected for artificial insemination (AI) in subsequent cycles and overall conception rates were compared using independent t- test (Table 4).

Results and discussion

Colour

In the present study, 83.33 per cent of cows in group I exhibited clear and transparent CMD while, 16.67 per cent cows exhibited turbid and opaque CMD on the day of oestrus. During metoestrus (day 2), all the cows (100 per cent) in group I voided CMD containing blood at varying proportions. The first service conception and overall conception rates obtained in cows with metoestral bleeding were 8.33 and 33.33 per cent, respectively (Table 1). The CMD of normally cycling cows appeared clear and transparent during early phase of oestrus, which became turbid at mid-oestrus and finally

changed to translucent at the end of oestrus. Reports suggested that the clarity of CMD is a conducive factor for sperm penetration and conception, while turbidity could affect the sperm motility, fertilization and conception (Layek *et al.*, 2013; Hanumant *et al.*, 2019). In the present study, the bloody CMD during metoestrus might have contributed to reduced conception rate and it was in agreement with the observations of Mahmoudzadeh *et al.* (2001) and Kutty (2002) who opined that the presence of blood might have created unfavourable environment for gametes.

Consistency and Spinnbarkeit test value (cm)

Most of the animals in both the groups voided thin/watery consistency CMD during oestrus while higher proportion of thick/viscous CMD was seen during metoestrus period in group II cows (83.33 %) (Table 1). However, most of the group I cows (66.67 %) had thin/watery consistency CMD during metoestrus period also. The mean spinnbarkeit value of group I cow (9.35 ± 0.5 cm) during metoestrus was significantly ($p < 0.01$) higher than that of group II cows (7.75 ± 0.3 cm) (Table 2).

The elasticity of CMD may vary with the stage of oestrous cycle and it will be watery with minimal viscosity at the time of ovulation in cows. Due to the oestrogen dominance during follicular phase, the secretion of cervical mucus increases in volume and becomes watery in nature whereas, it becomes opaque,

thick, viscous and scant during luteal phase of cycle under the influence of progesterone. Kutty (2002) stated that the consistency of discharge during metoestral bleeding was thin mucinous to viscid and often hanged from the vulva however, in our study majority of the cows showed thin consistency discharge. In contrary, Chacko (2003) reported that 60 per cent cows had thick blood-tinged mucus discharge during metoestrus. In the present study, even during the metoestrus, majority of cows had thin consistency bloody CMD which might be due to prolonged oestrogen activity from persistent follicle which was observed in group I cows. According to Bhat *et al.* (2015) majority of cows exhibited thin consistency of oestral mucus in anovulation and delayed ovulation conditions whereas, thick consistency was recorded in cows had normal ovulation after oestrus period.

Fern pattern

In the present study, majority of cows (83.33 %) showed typical fern pattern on the day of oestrus in both the groups whereas, during the period of metoestrus all the group I cows showed 'nil' type pattern of CMD with RBCs while in group II cows, atypical and 'nil' type pattern was observed in 83.33 and 16.67 per cent cows, respectively.

Bovine cervical mucus is characterised by unique 'arborisation' or 'ferning phenomenon' that occurred due to the change in sex steroid hormone levels (Cortes *et*

Table 1. Colour and consistency of cervical mucus discharge during oestrus and metoestrus in cows with and without metoestral bleeding.

Characteristics of CMD		(Oestrus) Day 0		(Metoestrus) Day 2	
		Group I (n=12)	Group II (n=12)	Group I (n=12)	Group II (n=12)
		No (%)	No (%)	No (%)	No (%)
Colour	Clear and transparent	10 (83.33)	9 (75.0)	0 (0.0)	8 (66.67)
	Turbid and opaque	2 (16.67)	3 (25.0)	0 (0.0)	4 (33.33)
	Bloody and opaque	0 (0.0)	0 (0.0)	12 (100.0)	0 (0.0)
Consistency	Thick /viscous	0 (0.0)	1 (8.33)	4 (33.33)	10 (83.33)
	Thin/ watery	12 (100.0)	11 (91.67)	8 (66.67)	2 (16.67)
Fern pattern	Nil	0 (0.0)	0 (0.0)	12 (100.0)	2 (16.67)
	Atypical	2 (16.67)	2 (16.67)	0 (0.0)	10 (83.33)
	Typical	10 (83.33)	10 (83.33)	0 (0.0)	0 (0.0)

Table 2. Comparison of pH and Spinnbarkeit value of cervical mucus discharge during oestrus and metoestrus period in cows with and without metoestral bleeding

Variables	Group I (n=12)		Group II (n=12)	
	Days of oestrous cycle		Days of oestrous cycle	
	Day 0	Day 2	Day 0	Day 2
pH	7.58 ± 0.12 ^a	7.96 ± 0.10 ^{b**}	7.47 ± 0.09 ^a	7.64 ± 0.07 ^{c*}
Spinnbarkeit value(cm)	12.64 ± 0.50 ^a	9.35±0.5 ^{b**}	13.13 ± 0.33 ^a	7.75±0.3 ^{c**}

** Significant (P< 0.01); * significant (P<0.05)

Means bearing different alphabets as superscripts differ significantly *pH*

Table 3. Comparison of serum oestradiol (pg/mL) and progesterone (ng/mL) concentrations during different periods of oestrous cycle in cows with and without metoestral bleeding

Day of oestrous cycle	Serum oestradiol concentration (pg/mL)		Serum progesterone concentration (ng/mL)	
	Group I	Group II	Group I	Group II
Day 0	10.37 ± 0.78 ^{a**}	12.06 ± 0.82 ^{a**}	1.61 ± 0.55 ^b	0.36 ± 0.07 ^{c*}
Day 2	6.85 ± 0.53 ^c	8.48 ± 0.49 ^{b*}	1.17 ± 0.31 ^b	0.99 ± 0.34 ^b
Day 10	4.63 ± 0.44 ^c	5.47 ± 0.45 ^c	24.13 ± 4.24 ^{a**}	13.97 ± 3.77 ^{a**}

** Significant (p< 0.01); * significant (p<0.05)

Means bearing different alphabets as superscripts differ significantly

Table 4. Comparison of conception rate during three consecutive oestrous cycles and overall conception rate in cows with and without metoestral bleeding

Groups	Number of animals conceived			Overall CR (%)
	In 1 st AI	In 2 nd AI	In 3 rd AI	
	No (%)	No (%)	No (%)	
Group I	1 (8.33)	1 (9.09)	2 (20.00)	33.33
Group II	5 (41.66)	4 (57.14)	1 (33.33)	83.33

al., 2014). Oestrogen promotes the fern pattern of cervical mucus whereas progesterone alters it. The crystallisation of several organic components such as mucins, colloids, electrolytes and chlorides causes the ferning of CMD. This observation was in agreement with Chacko (2003) who observed that thin blood tinged mucus discharge displayed 'nil' pattern with plenty of RBCs in 40 per cent of cows with metoestral bleeding. The cervical mucus with typical fern pattern promotes good sperm survivability, sperm transport on the day of oestrus which results in higher conception rates while no conception was observed in cows with 'nil' patterns due to higher serum progesterone concentration (Layek *et al.*, 2013).

On day of oestrus, the pH of CMD in both the groups did not vary significantly whereas, on day 2 the cows with metoestral bleeding had significantly (p<0.01) higher pH (7.96±0.10) than that of the cows without

metoestral bleeding (7.64±0.07). The pH during metoestrus period was also significantly (p<0.01) higher than that of oestrus (Table 2).

Optimum pH of cervical mucus is essential for the survival of spermatozoa and plays a vital role in maintaining the uterine environment for fertilisation. According to Rangnekar *et al.* (2002), the average pH of oestral mucus in crossbred cattle was 7.71±0.17, the pH of oestral mucus in fertile cows ranged from 7.5 to 8.5 and that of cows with infertility issues ranged from 6.5 to 9.0. Present findings on lower conception rate in group I cows were in agreement with Zaman *et al.* (2013) as the pH value of cervical mucus in normal cows without infertility problems (7.35±0.167) was significantly (p<0.01) lower than that of repeat breeding cows (7.95±0.096). In the present study, increased pH of CMD might be because of altered uterine environment due to presence of blood in the uterus which could

be responsible for lower conception rate (Table 4). During oestrus, the likelihood of bacterial contamination increases due to ascending infection through the cervix; and the presence of blood in the uterus may also provide a favourable medium for bacterial proliferation. Bhat *et al.* (2015) opined that increase in pH of CMD favours the growth of organisms in the reproductive tract which might have resulted in endometritis or repeat breeding condition. The increased pH (7.5-8.0) of vaginal discharge could be due to bacterial contamination of the uterus that contribute to a hostile environment for the survival of spermatozoa and embryo.

Serum oestradiol concentration

The mean serum oestradiol concentration of group I cow (10.37 ± 0.78 pg/mL) was lower than that of group II cows (12.06 ± 0.82 pg/mL) on the day of oestrus which was non-significant ($p > 0.05$). However, the mean serum oestradiol concentration of group I cow (6.85 ± 0.53 pg/mL) was significantly ($p < 0.05$) lower than that of group II cows (8.48 ± 0.49 pg/mL) during metoestrus period.

The concentration of oestradiol was found to be increased due to progressive development of the dominant follicle and the serum oestradiol- 17β concentrations in cyclic cows were found associated with waves of ovarian follicular development. In the present study, the serum oestradiol concentration in both the groups during oestrus did not vary significantly. However, a significant ($p < 0.01$) reduction in oestradiol concentration was observed during the period of metoestrus in group I cows than that of group II cows. The lower concentrations of oestradiol- 17β on day 2 might be due to sudden withdrawal of oestrogen during metoestrus period; however, the ovulation was delayed in this group. The results are in accordance with Bloch *et al.* (2006) as they reported a reduced oestradiol concentrations prior to oestrus likely due to a delayed LH surge which might be responsible for extended time interval from oestrus to ovulation. Similarly, Mogheiseh *et al.* (2019) remarked that low levels of oestradiol- 17β at breeding are found associated with reduced fertility and repeat breeding in dairy cows.

Serum progesterone concentration

The mean serum progesterone concentration of group I cows (1.61 ± 0.55 ng/mL) was significantly ($p < 0.05$) higher on day of oestrus (day 0) than that of group II cows (0.36 ± 0.07 ng/mL). During metoestrus the mean serum progesterone concentration was non-significantly higher in group I cows (1.17 ± 0.31 ng/mL) than that of group II cows (0.99 ± 0.34 ng/mL). A significantly ($p < 0.01$) higher progesterone concentration was observed on day 10 in both the groups. On day 10, progesterone level in group II cows was found lower than that of group I cows however, the values varied non-significantly ($p > 0.05$).

Progesterone levels were found elevated or declined in accordance with the development and regression of the CL, as it was the main source of progesterone. Kishore (2006) recorded the mean serum progesterone concentrations of 0.64 ± 0.06 ng/mL and 0.92 ± 0.05 ng/mL in crossbred dairy cows on days 0 and day 3 of oestrous cycle, respectively. The serum progesterone concentration during follicular phase will be < 1 ng/mL and during luteal phase the concentration will be > 1 ng/mL in dairy cows. In the present study an elevated serum progesterone concentration at oestrus could be regarded as suprabasal progesterone which might be due to inadequate luteolysis of the previous CL. Few authors (Shakir, 2018; Arun *et al.*, 2020) reported higher progesterone level (> 0.5 ng/mL) on the day of oestrus in repeat breeding cattle with prolonged oestrus than normally cycling animals. Bage *et al.* (2000) reported that the presence of suprabasal progesterone at oestrus could be due to continuous adrenal stimulation influenced by physical, managerial or environmental stress. This will inhibit the positive feedback mechanism of oestradiol and results in delayed LH surge and ovulation. However, Honparkhe *et al.* (2010) opined that an elevated progesterone concentration in crossbred dairy cows could be due to inadequate luteolysis of previous CL or from extra-gonadal source. The suprabasal progesterone at oestrus might be responsible for prolonged exhibition oestrus signs, delayed LH surge and ovulation which lead to aging of gametes, fertilisation failure and reduced conception rate.

Conclusion

Metoestrus period is one of the critical stage for fertilisation of gametes and implantation. Altered physicochemical properties of CMD during oestrus and metoestrus might create unfavorable uterine environment for the gametes survival and fertilisation. Altered endocrine milieu such as suprabasal progesterone and lower oestradiol concentration at oestrus and metoestrus, could affect timely ovulation and fertilisation of gametes. Crossbred cows with profuse metoestral bleeding showed lower conception rate than that of cows without metoestral bleeding.

Conflict of interest

The authors declare that they have no conflict of interest.

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