



Characteristics of Cervical Mucus for Estrus Detection in Murrah Buffaloes (*Bubalus bubalis*)

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ABSTRACT

The objective of the experiment was to study the changes in different characteristics of cervical mucus of Murrah buffaloes in relation to occurrence of estrus. The Murrah buffaloes maintained at Livestock Research Centre (LRC), ICAR-NDRI, Karnal were classified into three groups viz. heifers, primipara and pluripara. Cervical mucus was collected on the day of estrus prior to the artificial insemination by aspiration from the mid cervix using blue sterile sheath and Universal artificial insemination gun through recto-vaginal method. Immediately after collection, mucus sample was taken to the laboratory various physical and chemical parameters were assessed. From the present study it can be concluded that the changes in cervical mucus characteristics can be used for prediction of estrus time and subsequently time of artificial insemination. Murrah buffaloes exhibited higher proportion (70%) of copious discharge, 86 % buffaloes had clean appearance and 68% of buffaloes showed moderate consistency of cervical mucus. The spinnbarkeit value of cervical mucus of 46% of estruses were in 8-16 cm and 58% showed the typical fern pattern predominantly with primary, secondary and tertiary venation. pH of cervical mucus were within 7.5-8.0 and conductivity in a range of 13.50-15.00 mS/cm.

Keywords: Cervical mucus, estrus, spinnbarkeit value

Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by inherent late maturity, poor estrus expression in summer, distinct seasonal reproductive patterns, long postpartum ovarian activity and prolonged intercalving intervals (Madan and Raina, 1984; Suthar and Dhami, 2010). They have been traditionally regarded as a poor breeder having poor fertility (Kanai and Shimizu, 1983; Barile, 2005; Suthar and Dhami, 2010) as they have lower number of primordial follicles in their ovary varying from 10,000 to 19,000 (Samad *et al.*, 1988) compared with 150,000 in cattle. So, an important factor to consider in improving the productive and reproductive life in dairy buffaloes is heat detection (Sastry, 1983) and it is a major problem that decreases the high reproductive efficiency in national dairy herd (Senger, 1994). The success of estrus detection not only increases conception rates, but also raises the milk production of the herd (Diskin and Srenan, 2000;

Tsai *et al.*, 2014). Most of these problems crop up from the lack of or poor estrus detection and use of the “out of breeding season” mating technique, carried out to meet the market demand (Suthar and Dhami, 2010). Onset of estrus, different stages of estrus and ovulation time can be predicted by observing different properties of cervical mucus of cattle and buffaloes (Alena *et al.*, 2008). The present study was undertaken to study the changes in properties of cervical mucus in relation to occurrence of estrus in Murrah buffaloes.

MATERIALS AND METHODS

The present study was conducted on Murrah buffaloes maintained at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal, Haryana from June, 2015 to February, 2016. The experimental animals were maintained in loose housing system under group

management practice. Buffaloes were classified into heifers, primipara and pluripara depending on parity. The feeding mangers and resting area were covered with asbestos sheets at moderate height with low slope inclination. At one corner of paddock, there was provision of drinking water trough with running fresh tap water. There were trees shades within the paddock for shelter of the animals as per their preferences. The flooring of the paddock was “brick on edges” and the manger area is concrete with grooves. Buffaloes were fed as per requirements the available green fodders (maize, jowar, berseem, lucerne and oats) with concentrates based on their body weight and milk yield. Milking buffaloes were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield and maintenance ration. The concentrate to the milking animals was fed in divided allowances during times of milking.

Cervical mucus was collected on the day of estrus prior to the artificial insemination by aspiration from the mid cervix using blue sterile sheath and Universal artificial insemination gun through recto-vaginal method. Immediately after collection, mucus sample was taken to the laboratory and following parameters were assessed:

Quantity of discharge

Quantity was assessed on the basis of amount of mucus discharge observed at the day of estrus before AI and classified into three groups such as copious, moderate and absent/ scanty.

Appearance of mucus discharge

The mucus obtained was observed visually to check the appearance, which was classified into two classes (Deo and Roy, 1971) as clear / transparent and cloudy / translucent.

Consistency of mucus discharge

Consistency of the mucus was detected by placing few drops of collected sample into a grease free glass slide and the slide was inclined to 45°. The movement of the mucus was observed and accordingly the sample was grouped into three different consistencies (Deo and Roy, 1971) such as thin, moderate and thick.

Spinnbarkeit value of mucus: Few drops of cervical mucus were taken on a grease free glass slide; then another grease free glass slide was placed over it; then the upper

glass slide was slowly moved away from the first slide; the mucus was stretched between two slides; the slide was moved until the mucus breaks and the distance between the two slides was measured just before the breakage of the mucus string through a scale (cm scale) mounted on the wall (Panigrahi, 1964). The obtained spinnbarkeit values were grouped into three groups like 0-8 cm, 8.1-16 cm and 16.1-24 cm.

Arborisation pattern of mucus discharge

Few drops of well mixed cervical mucus sample were placed on a grease free glass slide, spread uniformly over the slide and air dried. The air dried slide was examined under microscope using low power objective (10X) for crystallization pattern of the mucus, known as fern pattern. The fern pattern of the observed mucus were grouped into three groups such as typical, atypical and nil.

Conductivity of mucus

Cervical mucus was vortexed in a vortex mixture (Spinix Corporation, CA, USA) until the sample got lysed and became a free flowing liquid. Then the conductivity was measured in the lysed cervical mucus using a pH-Conductivity Benchtop (Orion 4 star, Thermo Electron Corporation, USA). The conductivity was recorded in mili-Siemens/cm unit (mS/cm). The obtained conductivities of the samples were grouped into three groups such as 9-13.5 mS/cm, 13.6-15 mS/cm and >15 m.

pH of Mucus

pH of cervical mucus was measured using pH-Conductivity Benchtop (Orion 4 star, Thermo Electron Corporation, USA). The observed pH were categorised into three categories like 7.0-7.5, 7.6-8.0 and above 8.0.

Estrus stage was confirmed by serum progesterone concentration, i.e. if the serum progesterone concentration was < 0.1 ng/ml, then the animal was said to be in heat (Batra *et al.*, 1979).

RESULTS AND DISCUSSION

Characteristics of cervical mucus

Physical properties of cervical mucus

The frequency distribution of some physical properties of cervical mucus viz. quantity (amount), appearance

(colour), consistency, spinnbarkeit value, and arborisation pattern (fern pattern) are given in the Table 1.

Quantity

In present study majority of the estrus periods (64%) were associated with copious mucus discharge during AI. The higher proportion (70%) of copious discharge was found in pluripara as compared to primipara (57.14%) and heifers (50%) as shown in Table 1. For the characteristic changes in the cervical mucus during the estrus threshold estrogen level is required may be low in case of pluripara than primipara which may be the reason for more copious discharge in pluripara. Moreover, calving history and the postpartum changes have the direct effect on the endometrial, cervical and vaginal glands contributing to the quantity of cervical mucus.

In the present study the values observed are higher than the report of Verma, 2012, who reported that 56.38% estruses were associated with copious mucus discharge in Murrah buffaloes. However, the findings are lower than

the findings of Layek (2010) who reported that 70.17% estruses were associated with copious mucus discharge. Moreover he also reported that pluriparus cows were found with higher proportion of copious discharge (75.61%) as compared to primiparous (56.25%) in Sahiwal cow.

Appearance

In 86 % estruses cervical mucus was clean in appearance and in rest (14%) mucus was cloudy in appearance. Higher proportion of pluripara (90%) as compare to heifers (66.67%) and primipara (85.71%) showed clear mucus during estrus (Table 1) No animal in the present study was observed with dirty discharge. Gill *et al.* (1974) reported 82.42% clear mucus discharge in Murrah buffaloes which is similar to the present study. Agarwal and Purbey (1983) reported a little higher value (95.83%) whereas Deo and Roy (1971) and Gunasekaran *et al.* (2007) reported a lower proportion (67.9% and 62.86% respectively) of clear discharge in Indian buffaloes. As per the reports of Verma

Table 1: Physical properties of cervical mucus in Murrah buffaloes at the time of AI (n=50) (In the parenthesis the no. of animals are given)

	Heifer (n= 6)	Primipara (n= 14)	Pluripara (n= 30)	Total (50)
Quantity				
Copious	50(3)	57.14(8)	70(21)	64(32)
Moderate	33.33(2)	28.57(4)	23.33(7)	26(13)
Scanty	16.67(1)	14.29(2)	6.67(2)	10(5)
Appearance				
Clear	66.67(4)	85.71(12)	90(27)	86(43)
Cloudy	33.33(2)	14.29(2)	10(3)	14(7)
Consistency				
Thin	33.33(2)	14.29(2)	16.67(5)	18(9)
Moderate	66.67(4)	78.57(11)	63.33(19)	68(34)
Thick	0.00(0)	7.14(1)	20.00(6)	14(7)
Spinnbarkeit value				
0-8cm	33.33(2)	7.14(1)	16.67(5)	16(8)
8-16cm	50.00(3)	57.14(8)	40.00(12)	46(23)
16-24 cm	16.67(1)	35.71(5)	38.00(13)	38(19)
Arborisation Pattern				
Typical	50.00(3)	57.14(8)	60.00(18)	58(29)
Atypical	33.33(2)	28.57(4)	36.67(11)	34(17)
Nil	16.67(1)	14.29(2)	3.33(1)	8(4)

(2012) 85.10% estruses cervical mucus were clean in appearance and in rest mucus was cloudy in appearance which is close to the findings of the present study. In the present study cervical mucus was collected by blue sheath method.

In the present study relatively higher proportion of cloudy cervical mucus was found in heifers (33.33%) as compare to primiparous (14.29%). The higher proportion of cloudy discharge in heifers may be due to late estrus or infection. But the higher proportion of cloudy discharge is a normal phenomenon in heifers.

Consistency

Higher proportion (68%) of buffaloes showed cervical mucus with moderate consistency. In primipara (78.57%) buffaloes cervical mucus was more of moderate consistency as compared to heifers (66.67%) and pluriparus (63.33%) buffaloes (Table 1).

Findings are almost similar to that of Gunasekaran *et al.* (2007) in Murrah buffaloes (68.57%). Deo and Roy (1971) and Agarwal and Purbey (1983) reported a higher proportion of thin mucus (61.8% and 54.17%, respectively) as compare to our findings (18%) in buffaloes. As per the reports of Verma (2012) 64.68% estruses cervical mucus were moderate in consistency which is close to the present finding. The consistency of mucus basically depends on the stage of estrus when the sample is collected and that may be the reason of difference between the studies because the animals brought for insemination may not be at the same stage of estrus in all studies. The consistency is subjective property and the definition of different level of consistency depends on the observer/experimenter which may be probable cause in difference between the studies. In the present study, most of the primiparous buffaloes are associated with moderate consistency of cervical mucus which replies that majority of primipara were in mid estrus during the time of insemination.

Spinnbarkeit Value

Cervical mucus of 46% of estruses were in 8-16 cm. range where as 38 % of estruses were in 16.1-24 cm. range (Table 1). The higher proportion of primiparous (57.14%) and pluriparous buffaloes (40.00%) buffaloes were found in 8-16 cm. The findings of the present study are close to the

reports of Verma, 2012, who reported that cervical mucus of 41.49% of estruses were in 8-16 cm. range where as 39.36% of estruses were in 16-24 cm in Murrah buffaloes.

Arborisation pattern

Cervical mucus in higher proportion of estruses (58%) had shown the typical fern pattern (Table 1) predominantly with primary, secondary and tertiary venation. Among the remaining estruses, 34% represented the atypical fern pattern and there was no arborisation in 8 % estrus periods. The present finding is slightly lower than the findings observed by Kumar (1989) in buffaloes (60.29%) and Rangnekar *et al.* (2002) in HF cows (60%) whereas the typical fern pattern value of present finding is higher than the values reported by Deo and Roy (1971) and Agarwal and Purbey (1983) in buffaloes (42.1 and 25%, respectively). Higher amount of pluriparous (60.00%) and primiparous buffaloes (57.14%) had shown typical fern pattern, as compared to heifers (50.00%). The findings are close to the reports of Verma, (2012), where he stated that higher proportion of estruses (54.25%) show typical fern pattern and more number of pluriparous (57.63%) and primiparous buffaloes (54.54%) had shown typical fern pattern, as compared to heifers (38.47%).

Chemical properties of cervical mucus

In the present study pH and electrical conductivity (EC) was measured and values are presented in Table 2.

pH

Cervical mucus of higher proportion of estruses (58.00%) were within pH range 7.5-8.0 while 34.00 % and 8.00% cervical mucus were in pH range >8 and 7.0-7.5 respectively (Table 2). Cervical mucus of higher number of buffalo heifers (50.00%), primiparous (78.57%) and pluriparous (50.00%) were in pH range 7.5-8.0. The findings are close to the reports of Verma, 2012, who stated that higher number of buffalo heifers (53.85%), primiparous (72.73%) and pluriparous (69.49%) were in pH range 7.5-8.0.

Electrical conductivity

The little variation is found in different studies than

Table 2: Chemical properties of cervical mucus in Murrah buffaloes at the time of AI (n=50) (In the parenthesis the no. of animals are given)

Parameters	Heifer (n= 6)	Primipara (n= 14)	Pluripara (n= 30)	Total (50)
pH				
7-7.5	16.67(1)	7.14(1)	6.67(2)	8.00(4)
7.6-8	50.00(3)	78.57(11)	50.00(15)	58.00(29)
> 8	33.33(2)	14.29(2)	43.33(13)	34.00(17)
Conductivity				
9-13.5	16.67(1)	28.57(4)	30.00(9)	28.00(14)
13.6-15	66.67(4)	57.14(8)	60.00(18)	60.00(30)
> 15	16.67(1)	14.29(2)	10.00(3)	12.00(6)

our study may be due to lysis of cervical mucus before the measurement of conductivity and method of measurement. Cervical mucus in higher number of estruses (60.00%) was in the conductivity ranged between 13.50-15.00 mS/cm (Table 2). While rest of estrus periods (28.00% and 12.00%) were associated with either in the range 9-13.5 mS/cm or > 15.00 mS/cm. Cervical mucus of higher proportion of heifers (66.67%), primiparous (57.14%) and pluriparous (60.00%) buffaloes were in the conductivity range 13.50-15.00 mS/cm. The higher value of conductivity was reported by Bishnoi *et al.* (1983) in crossbred cattle (14.758 mS/cm in early estrus and 12.485 mS/cm in mid estrus). The findings of the present study are close to the values (13.9 mS/cm.) reported by Sopelink (1971).

CONCLUSION

From the present study it can be concluded that the changes in cervical mucus characteristics can be used for prediction of estrus time and subsequently A.I. time. The physical properties like copious discharge, clean in appearance, moderate consistency was found in higher proportion buffaloes. The spinnbarkeit value fell in the range of 8-16 cm. Cervical mucus in higher proportion of estruses showed the typical fern pattern predominantly with primary, secondary and tertiary venation. The chemical properties of cervical mucus showed a pH range 7.5-8.0 and conductivity in a range of 13.50-15.00 mS/cm in highest proportion of the Murrah buffaloes.

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