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Physical properties of estrual mucus in repeat breeder buffaloes

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Abstract
Forty four repeat breeder buffaloes were classified into infectious (n=20) and non-infectious (n=14) groups based on the result of white side test. . Ten buffaloes were kept as control (Group V). The infectious buffaloes Group I received Exapar-N® 100 ml, Janova® and Mintrus® and Group II Exapar-N® 50 ml, Janova® and Mintrus®. The non-infectious buffaloes Group III received GnRH 10µg post AI and Group IV GnRH 10µg post AI + GnRH 10µg post insemination. The color of estrual mucus in repeat breeder buffaloes was 23.27% transparent, 38.63% translucent and 34.09% whitish. 36.36%, 43.18% and 20.45% of repeat breeder buffaloes were having thick, viscous and thin consistency of estrual mucus. The mean pH value was 8.45±0.10 in infectious and 7.37±0.04 in non-infectious repeat breeder buffaloes. The arborization pattern in repeat breeder buffaloes was 32.00% typical, 54.00% atypical and 14.00% nil type. Out of 44 repeat breeder buffaloes, 45.45% showed positive and 54.54% negative result for sub-clinical metritis through white side test. Conception rate in buffaloes is more when cervico-vaginal mucus on the day of estrus is transparent, viscous, with typical fern pattern, spinnbarkeit value more than 10 cm and pH ranges 7-8.

Keywords: Repeat breeder buffaloes, estrual mucus, arborization pattern, spinnbarkeit, white side test

Introduction
In buffalo, estrus behaviour has a lower intensity than in cows and is much more difficult to detect. Several factors such as climate, temperature, photoperiod and nutrition, have been shown to affect the length of estrous cycle and the degree of heat expression. Acceptance of the male is considered as the most reliable oestrus indicator. Frequent urination, bellowing, vulvar swelling and mucus discharge are referred to be salient signs of oestrus. The cervical mucus is a complex secretion with major production from the secretory cells under the influence of estrogen (Albrecht *et al.*, 1985) [1]. Thus, characterization of cervical mucus can be used as a tool for indirect assessment of hormonal status in females (Phatak *et al.*, 1980) [2]. Several studies indicated the importance of arborization, spinnbarkeit and pH of cervico-vaginal mucus as potential diagnostic tool in ascertaining the estrus and predicting the fertility of dairy animals (Pandey *et al.*, 1983 and Vadodaria and Prabhu, 1989) [3, 4]. White side test is performed on the cervico-vaginal mucus (CVM) for the detection of uterine infection in animals. White side test can be employed to differentiate subclinical cases of metritis from normal animals. Hence, the white side test is a simple, quick, reliable and accurate test which is highly helpful for veterinarians in the field conditions to differentiate the normal healthy animal from subclinical and clinical cases of metritis (Mohankumar *et al.*, 2006) [5].

Materials and Methods
The study was carried out in 44 repeat breeder buffaloes belonging to members of Bidar-Gulbarga Milk Producers Union, buffaloes presented to VGO-OPD, Veterinary College, Bidar and APMC Veterinary Hospital Bidar. The buffaloes, which failed to conceive to 2 to 3 or more insemination using good quality frozen semen / served by healthy fertile buffalo bull at regular cycling estrus without any reproductive tract abnormalities were selected for the study. These buffaloes were diagnosed for repeat breeding on the basis of history obtained by the animal owner (age, parity, number of AI / natural service done, history of dystocia / abortion / RFM, regularity of estrous cycle), per rectal examination, white side test and records.

Physical characteristics of estrual mucus

Following confirmation of estrus, the cervico-vaginal mucus from buffaloes was collected aseptically in a sterile vial for studying various physical parameters / tests viz. color, consistency, pH, spinnbarkeit, arborization and white side test.

1. **Color:** Color of cervico-vaginal mucus was observed and graded as transparent, translucent and whitish.
2. **Consistency:** Consistency of cervico-vaginal mucus was observed and graded as viscous, thin and thick.
3. **pH:** The pH of cervico-vaginal mucus was recorded using universal indicator pH paper.
4. **Spinnbarkeit value:** A drop of cervico-vaginal mucus was spread on a clean glass slide and was lifted with the help of another glass slide along the side of a scale fixed vertically. The point of breaking up of mucus while lifting is the spinnbarkeit value recorded in centimeters.
5. **Arborization pattern:** A drop of cervico-vaginal mucus was spread on a clean glass slide and air dried then observed under low power objective under microscope for appearance and presentation of crystallization pattern. The type of arborization was classified as per Vadodaria and Prabhu, (1989) [4].
6. **White side test:** One ml of cervico-vaginal mucus was mixed with equal volume of 5% Sodium hydroxide (NAOH) solution and heated upto the boiling point in a water bath for two minutes as described by Pateria and Rawal (1990) [6].

Results and Discussion

Color

Out of 44 repeat breeder buffaloes, 27.27% exhibited whitish, 38.63% translucent and 34.09% transparent color of estrual mucus. In contrast, Samad *et al.* (2002) [7] reported that, estrus mucus was transparent in 55.0%, translucent in 38.33% and whitish in 6.67% repeat breeder buffaloes. Enkhia and Kohli (1982) [8] found 50% transparent, 30% translucent and 20% yellowish in repeat breeder cows. Infectious repeat breeder buffaloes exhibited 60% whitish, 40% translucent and 0% transparent color. However, non-infectious repeat breeder buffaloes exhibited 0% whitish, 37.5% translucent and 62.5% transparent color. Mehta (1986) [9] reported that, 54.17% of repeat breeder animals had clean and transparent cervical mucus. Out of 22 repeat breeder buffaloes, 54.54% conceived when the estrual mucus color was transparent followed by 40.90% in translucent and 4.54% in whitish. Out of 22 repeat breeder buffaloes having 50.00% whitish, 36.36% translucent and 13.63% transparent did not conceive. In contrast, Vadodaria and Prabhu (1990) [10] reported that, conceived Mehsani buffaloes had 26.56% transparent, 29.69% translucent and 43.75% whitish color of mucus. However, non-conceived Mehsani buffaloes had 21.05% transparent, 36.84% translucent and 42.11% whitish color of mucus. These findings corroborated with Enkhia and Kohli (1982) [8]. Gill *et al.* (1973) [11] recorded 78% conception rate associated with translucent color of cervical mucus in buffaloes.

Consistency

In present study, the consistency of mucus was 36.36% thick, 43.18% viscous and 20.45% thin in repeat breeder buffaloes. These results are in agreement with Sharma *et al.* (2011) [11]. In contrast, Samad *et al.* (2002) [7] found that, consistency of estrus mucus was viscous in 38.3%, thin 50% and thick in 11.67% repeat breeder buffaloes. The consistency of cervical mucus of repeat breeder buffaloes was found to be thin in 55.55% and thick in 44.44% (Kumar *et al.,* 2011) [13]. Thin estrual mucus observed in most of repeat breeder cows (Enkhia and Kohli, 1982: Pandey *et al.,* 1983) [8, 3]. Das *et al.* (2010) [14] reported that, thin consistency of vaginal mucus was found to be more number in animals with delayed ovulation and anovulation, while most of the animals with normal ovulation had thick consistency in repeat breeding cattle.

The estrual mucus consistency was thick in 60%, viscous in 40% infectious buffaloes whereas it was thick in 16.66%, viscous in 45.83% and thin in 37.5% non-infectious buffaloes. Kumar *et al.* (2012) [15] recorded turbid, thick and mucopurulent vaginal discharge in infectious repeat breeder buffaloes, which could be due to uterine and cervical infection (Saini *et al.,* 1995 and Singla *et al.,* 2004) [16, 17]. Higher pregnancy rate of 59.09% was recorded in buffaloes having viscous type of estrual mucus discharge followed by thick (27.72%) then followed by 18.18% in thin mucus discharge. These results are in agreement with Vadodaria and Prabhu (1990) [10]. In contrast, Sharma *et al.* (2011) [11] recorded 44.44% in viscous, 33.33% in thick and 22.33% conception rate in thin vaginal mucus. Deo and Roy (1971) [18] reported 59.10% and 32.90% of conception rates in buffaloes with thin and thick cervical mucus respectively. Gebhard and Schumacher (1970) [19] reported that, profuse, watery and clear cervical mucus was favourable for sperm penetration and thick, scanty and opaque cervical mucus was unfavourable for sperm penetration.

pH

The mean pH values of estrual mucus in groups I, II, III, IV and V were 8.29±0.21, 8.42±0.17, 7.35±0.07, 7.35±0.08 and 8.20±0.0, respectively. The pH values are towards alkaline side. This is in agreement with Vadodaria and Prabhu (1990) [10], Salphale *et al.* (1993) [20], Samad *et al.* (2002) [7], Kumar *et al.* (2011) [13] and Ramsingh *et al.* (2013) [21] but Tsiligianni *et al.* (2001) [22] reported slightly acidic i.e. 6.5-6.7 pH. The mean value of pH was 8.45±0.10 in infectious and was 7.375±0.04 in non-infectious repeat breeder buffaloes. Pateria and Rawal (1990) [6] reported the pH of the uterine discharge was also found to be higher (8.8±0.06) in clinical and subclinical metritis cases than in normal buffaloes. Salphale *et al.* (1993) [20] reported that, the cervical mucus of repeater animals had higher mean pH (8.027±0.11) than that of normal animals. Reason for this might be infectious organism present in genital tract of repeat breeder animals which cause inflammation and denudation of uterine mucosa. In addition, metabolites of bacteria and inflammatory exudates might have altered the pH of uterine and cervical fluid to the alkaline side resulting in failure of conception due to death of spermatozoa. Out of all repeat breeder buffaloes 72.72% conceived when the estrual mucus pH ranged from 7 to 8 followed by 13.63% conception rate in pH of both 7 and 8-9. Higher conception rate was noticed in repeat breeder buffaloes showing pH of 7-8 in estrual mucus. Vadodaria and Prabhu (1990) [10] reported pH increased in cervical mucus of conceived group and decreased in cervical mucus of non-conceived group.

Spinnbarkeit value

The mean spinnbarkeit values of groups I, II, III, IV and V were 7.29±1.72, 6.71±1.17, 10.20±1.30, 9.10±1.45 and 8.10±1.50 cm respectively. The difference between the groups was non-significant (p>0.05). The mean value of spinnbarkeit

in infectious was 6.75±0.81 and 9.87±0.88 cm in non-infectious repeat breeder buffaloes. The difference between the infectious and non-infectious group of animal was significant ($p<0.05$). Sharma *et al.* (2011) [11] reported average spinnbarkeit value of mucus was 9.35±0.66 cm which is in close conformity with Rangnekar *et al.* (2002) [24]. Dodamani (2000) [25] reported higher spinnbarkeit value (24.67±1.32 cm) in repeat breeding cows this value was in conformity of with Mohanty *et al.* (1996) [26] and Chouduri *et al.* (1997) [27]. Among all experimental repeat breeder buffaloes, 54.54% conceived when the spinnbarkeit value was more than 10 cm followed by 27.27% conception rate in spinnbarkeit value ranged from 6-10 cm and 18.18% conception rate in spinnbarkeit value from 1-5 cm respectively. Patil (1987) [28] and Sharma *et al.* (2011) [11] reported that, buffaloes that conceived had significantly higher ($p<0.01$) mean spinnbarkeit value (12.94±0.81 vs 7.91±0.96 cm) as compared to those that did not conceive. But Jadhav (1996) [29] and Bennur (2004) [30] did not find such variation in fertile and infertile estruses and conceiving and non-conceiving cows (7.38±5.60 and 8.05±1.33 cm)

Arborization pattern

The arborization patterns in repeat breeder buffaloes was 32.00% typical, 54.00% atypical and 14.00% nil type. Out of all experimental buffaloes, 45.83% typical arborization was seen in non-infectious whereas, 15% in infectious cases. Atypical arborization was seen in 55% and 54.16% estrual mucus of infectious and non-infectious buffaloes respectively. Also 30% infectious buffaloes showed nil type of arborization pattern. Luktuke and Roy (1967) [31] reported the cervical mucus fern pattern as typical (72.7%), atypical (18.2%) and nil type (9.1%). reported 25.1% more pregnancies in typical pattern. Pandya *et al.* (1987) [32] reported 35.71% typical, 44.24% atypical and 19.05% nil type of fern pattern in buffaloes. Kumaresan *et al.* (2001) [33] reported that, out of 69 buffaloes, 40, 16 and 13 showed typical, atypical and nil fern pattern. Conception rate of 57.5% and 18.57% were obtained in buffaloes with typical and atypical fern pattern. Crystallization or arborization or the fern phenomenon is not specific for cervical mucus. Tsiligianni *et al.* (2001) [22] revealed that, cervical mucus crystallization is distinctly dependent on the action of estrogen to increase its occurrence and progesterone to reduce its occurrence. Samad *et al.* (2002) [7] indicated that, fern pattern was typical in 55% atypical in 45.0% and 0% nil in repeat breeder buffaloes. Sharma *et al.* (2011) [11] reported typical, atypical and nil fern pattern of mucus was observed in 39.34%, 42.63% and 18.03% buffaloes.

Among all experimental repeat breeder buffaloes, typical arborization was seen in 54.54%, atypical 40.90% and nil 4.54% pregnant repeat breeder buffaloes vs 9.09% typical, 68.18% atypical and 22.72% nil type arborization in non-pregnant repeat breeder buffaloes respectively. Luktuke and Roy (1967) [31] reported 25.1% more pregnancies in typical pattern. Pandya *et al.* (1987) [32] reported 73.33% of buffaloes conceived when the fern pattern was typical followed by 26.67% in atypical and zero % in nil type. Kumaresan *et al.* (2001) [33] reported conception rate of 57.5% and 18.57% were obtained in buffaloes with typical and atypical fern pattern.

White side test

Out of 44 repeat breeder buffaloes, 20 buffaloes (45.45%) showed positive result and 24 (54.54%) showed negative result for sub-clinical metritis through white side test. Similar findings were noticed by Mohankumar *et al.* (2006) [5] who recorded the incidence of 45.72% sub-clinical metritis in buffaloes and Gupta *et al.* (2011) [34] performed white side test on the uterine discharge of 96 animals revealed that, 45.83% of positive results and Bhattacharya *et al.* (2011) [35] reported 39.29% of sub-clinical metritis in repeat breeding cows diagnosed based on the results of white side test. Thus, white side test can be of some diagnostic value in preliminary detection of sub-clinical endometritis as it is a simple and quick test to be carried at field level at least to differentiate between normal healthy animals from sub-clinical cases of metritis (Mohankumar *et al*., 2006) [5].

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