PROPERTIES OF ESTRUAL CERVICAL MUCUS IN RELATION TO PLASMA PROGESTERONE AND CONCEPTION RATES IN BUFFALOES

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ABSTRACT

The examination of estrual cervical mucus samples from 61 normal cyclic buffaloes at AI revealed clear thin watery, stringy and turbid consistency in 42.62, 37.71 and 19.67 % cases. Typical, atypical and nil fern pattern of mucus was observed in 39.34, 42.63 and 18.03 % samples, respectively. Maximum number of buffaloes (60.86 %) conceived when they had clear stringy mucus with mean plasma P_4 of 0.26 ± 0.04 ng/ml, followed by those (15.38 %) with clear thin watery mucus with P_4 of 0.54 ± 0.10 ng/ml and those with typical and atypical fern pattern (45.83 and 26.92 %). The spinnbarkeit value of mucus averaged 9.35 ± 0.66 cm. It was significantly higher ($P < 0.01$) in conceived than non-conceived buffaloes (12.94 ± 0.81 vs 7.91 ± 0.96 cm) with inverse plasma P_4 (0.21 ± 0.04 vs 0.56 ± 0.11 ng/ml). The mean spinnbarkeit value of thick stringy mucus was significantly higher ($P < 0.01$) with lower plasma P_4 than the other two grades. With regards to association, maximum animals conceived when they had both thick stringy mucus with typical fern pattern (44.44 %) followed by thick mucus with atypical fern pattern (33.33 %) and thin watery mucus with typical and atypical fern pattern (16.66 and 5.55 %). The overall first service conception rate in this study was 29.50 %. The findings clearly showed that for better conception rates in buffaloes, AI should be done when there is clear stringy mucus with higher spinnbarkeit and typical fern pattern associated with basal plasma progesterone levels.

Key words: Normal cyclic buffaloes, Cervical mucus, Physical properties, Conception rate.

INTRODUCTION

The physical properties of cervico-vaginal mucus (CVM) have direct relationship with circulatory oestrogen-progesterone levels and fertility status of the animals (Rangnekar *et al.*, 2002) as it essentially undergoes certain changes during estrus phase for the passage of spermatozoa. There are consistent and definite gradual changes in the viscosity, clarity, stretchability and fern pattern of cervical mucus along with the cyclic rhythm of the reproductive phenomenon, hence are considered as effective laboratory tools to predict the infertility in bovines. Many animals remain subfertile in the absence of any covert signs of abnormality, probably due to deviation from normal physical, and physiological changes in the cervical mucus. Agrawal and Purbey (1983) reported that 50.00, 45.85 and 4.15 % of the rural buffaloes had clear thin, stringy and turbid cervical mucus at estrus, with typical, atypical and nil fern pattern in 25.00, 45.83 and 29.17 %, with corresponding conception rates of 66.60, 36.60 and 0.00 %, respectively. The present study was therefore attempted to evaluate the physical properties of estrual cervico-vaginal mucus and its association with plasma progesterone levels and the fertility post-AI in normal cyclic buffaloes under field conditions.

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MATERIALS AND METHODS

This study was carried out during the period from December 2006 to March 2007 in 61 buffaloes selected from the three Ambulatory Clinic Centres of the College in Anand district of Gujarat. These animals were detected for estrus by behavioural signs, visual observations and rectal palpation and were inseminated using good quality frozen-thawed semen by trained lay inseminators. Before AI jugular blood samples were collected in heparinized vials and plasma samples separated out by centrifugation were stored at -20°C. The plasma progesterone (P_4) levels were estimated by using standard RIA kits of Immunotech, France (Kubasic *et al.,* 1984). The sensitivity of the assay was 0.1 ng/ml, intra-assay coefficient of variation was 5.4 %, and inter-assay variation was 9.1 %.

The estrual cervico-vaginal mucus samples were also aspirated aseptically from all these animals just before AI by using 10 ml sterilized glass pipette connected to a syringe by recto-vaginal technique. These were evaluated for the physical properties like colour and consistency, spinnbarkeit and fern pattern as early as possible (within 2-3 hours) after collection. Colour and consistency was judged by direct examination of mucus and categorized as clear/transparent, cloudy/turbid, sticky or watery. The spinnbarkeit was measured in cm as described by Panigrahi (1964). The fern pattern was studied in the estrual mucus smears under low power (10′) and high power (40′) microscope and was classified as typical, atypical and nil type (Luktuke and Roy, 1967). Conception rates were worked out by per rectal palpation of non-returned animals after 60-90 days of insemination. The data were analysed and interpreted statistically.

RESULTS AND DISCUSSION

In the present study 42.62, 37.71 and 19.67 % of the estrual mucus samples were clear thin watery, clear stringy and cloudy/turbid, respectively. Moreover, maximum number (60.86 %) of buffaloes conceived when they had clear stringy estrual mucus with mean plasma P_4 of 0.26 ± 0.04 ng/ml, followed by those (15.38 %) with clear thin watery mucus with P_4 value of 0.54 ± 0.10 ng/ml, and none with turbid or cloudy discharge with P_4 of 0.67 ± 0.12 ng/ml. The overall first service conception rate in animals studied was 29.50 %.

These findings corroborated with the report of Samad *et al.* (2002), who observed viscous/stringy estrual mucus in 38.33 % of the 60 buffaloes, thin consistency in 50.0 % and thick in 11.67 %. Salphale *et al.* (1993) observed that cows having cervical mucus with clear and thick consistency were mostly fertile (72.73 % CR) than those with turbid and thin mucus (42.55 % CR), which supported the present findings in buffaloes. Rangnekar *et al.* (2002) reported 35, 25 and 40 % occurrence of thin, medium and thick consistency of mucus, respectively, in repeat breeding cows. Majority of fertile estrus had thin (54.54 %) consistency, whereas majority of non-fertile estrus had thick (77.78 %) consistency.

Present study revealed the incidence of typical, atypical and nil type of fern pattern in 39.34, 42.63 and 18.03 % of the mucus samples, respectively, in buffaloes at the time of insemination, with the corresponding plasma P_4 values of 0.28 ± 0.03, 0.52 ± 0.11 and 0.73 ± 0.09 ng/ml. Further, like stringy mucus, maximum number of buffaloes conceived when they had clear cervical mucus with typical fern pattern and basal plasma P_4 at the time of AI (45.83 %), followed by those with atypical pattern (26.92 %) and none with absence/nil type of fern pattern.

Present findings on fern pattern of CVM coincided well with the report of Agrawal and Purbey (1983), who observed typical, atypical and nil fern pattern of estrual mucus in 25.00, 45.83 and 29.17 % of rural buffaloes, with the corresponding conception rates of 66.60, 36.60 and 0.00 %. Luktuke and Roy (1967) also observed better pregnancy rates in cows and buffaloes showing typical fern pattern of their estrual mucus and almost zero pregnancy rate, when there was absence of fern pattern in mucus. Rangnekar *et al.* (2002) observed typical, atypical and nil fern pattern in CVM of 60, 35 and 5 % of cows, respectively. Majority of the fertile estrus (81.82 %) had typical fern pattern of cervical mucus, whereas majority of non-fertile estrus (55.56

%) had atypical fern pattern. Our findings also compared well with the report of Kumaresan *et al.* (2001) who reported that out of 69 buffaloes, 40, 16 and 13 showed typical, atypical and nil fern pattern with conception rates of 57.50, 18.57 and 0.00 %, respectively. Inseminations at low P_4 resulted in higher conception rate. Present findings suggested that estrual cervical mucus with clear stringy consistency and classical arborization pattern due to increased salts and organic constituents favoured sperm survival and transport thereby improved the conception rate.

Of the 61 buffaloes studied for their mucus property at breeding, maximum animals conceived when they had thick stringy mucus with typical fern pattern (44.44 %) followed by thick mucus with atypical fern pattern (33.33 %) and thin watery mucus with typical and atypical fern pattern (16.66 and 5.55 %). All animals with thick stringy mucus that conceived had less than 0.15 ng/ml P_4 at AI. An inverse trend was observed for those, which failed to conceive after breeding for their mucus quality attributes, as majority of buffaloes having thin or cloudy mucus with atypical or no fern pattern returned to estrus after insemination. These findings also coincided well with the observations of most of the above workers in cows as well as buffaloes.

The pooled mean (± SE) spinnbarkeit value (elasticity) of estrual cervical mucus from 61 normal cyclic buffaloes was observed to be 9.35 ± 0.66 cm, and it ranged from 2 to 26 cm. The spinnbarkeit value of clear thin watery, clear stringy and cloudy/turbid mucus samples averaged 6.12 ± 0.53, 14.39 ± 0.95 and 6.96 ± 0.78 cm, respectively. The corresponding P_4 values were 0.54 ± 0.10, 0.26 ± 0.04 and 0.67 ± 0.12 ng/ml. The spinnbarkeit value for thick stringy mucus was significantly higher ($P < 0.01$) with lower plasma P_4 than the other two grades. Moreover, 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 with inverse plasma P_4 profile (0.21 ± 0.02 vs 0.56 ± 0.11 ng/ml).

These findings are in close conformity with the previous reports in bovines (Rangnekar *et al.*, 2002). Patil (1987) observed significantly higher spinnbarkeit value of mucus for fertile than non-fertile estruses (14.70 ± 0.57 vs 11.25 ± 0.37 cm), but Jadhav (1996) and Bennur *et al.* (2004) did not find such variation in fertile and infertile estruses (12.28 ± 0.76 vs 11.28 ± 0.78 cm) and in conceiving and non-conceiving cows (7.38 ± 5.60 and 8.05 ± 1.33 cm), respectively.

Present findings clearly indicated that physical properties of estrual mucus is directly related with plasma progesterone (and oestrogen) profile and that the favourable rheological properties of estrual cervical mucus support survival and transport of sperms in the cervical / uterine lumen to help achieve better conception rates in dairy animals.

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