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Effect of season on sperm oestrual-mucus penetration in buffaloes

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ABSTRACT

The study was conducted to understand the effect of season on sperm oestrualmucus interaction in buffaloes through *in vitro* sperm penetration test in summer (n=35) and winter (n=55). The oestrual mucus was collected at the time of AI by recto-vaginal method. Sperm penetration tests (SPT) and biochemical analysis of oestrual mucus were performed to establish its relation with sperm-penetration distance (SPD). The *in vitro* SPD differed significantly between the 2 seasons (21.22±1.08 mm/20 min in summer vs 32.67±1.28 mm/1.28 mm/20 min in winter). Biochemical studies of oestrual mucus revealed significantly higher contents of Na, Cl, Cu and Mn during winter than that in summer whereas pH was lower in winter. The K, Ca, Mg and Zn contents of mucus varied nonsignificantly between the 2 seasons. The SPD values were significantly correlated with oestrual mucus levels of Na, Cl, Ca and Mg during winter. A similar correlation was observed between SPD value and levels of Na and Cl in summer. Significantly low conceptions occurred during summer than that in winter. The buffaloes that conceived during winter and summer had significantly different concentration of Na, Cl (higher) and Ca (lower) in their oestrual mucus than those failed to conceive. The K concentration was lower and Mg, Cu, Zn and Mn were higher in oestrual mucus of conceived buffaloes. From the present study, it was concluded that season influenced certain biochemical constituents of oestrual mucus, thereby, altering sperm mucus penetration and resulting in poor conception during summer.

Key words: Buffalo, Biochemical profile of mucus, Oestrual mucus, Season, Sperm mucus penetration.

Cervical mucus is a conglomerate of uterine, tubal and follicular fluids, as well as blood transudate and cervical epithelial secretions. Chemical and physical changes in cervical mucus affect *in vivo* transport of spermatozoa from the site of ejaculation to the site of fertilization (Zavos and Cohen 1980). Poor interaction between sperm and cervical mucus is associated with infertility (Eggert-Kruse *et al.* 1993). Raut and Kadu (1989), Zaaijer *et al.* (1993) and Dev (1995) reported positive correlation between *in vitro* sperm mucus penetration and fertility in different species. Season might influence buffalo reproduction by changing levels of biochemical constituents of oestrual mucus and thereby altering sperm migration through these secretions. However, the information regarding climatic variations in relation to sperm migration through oestrual mucus is lacking in buffaloes. Therefore, the present study was carried out to find effect of season on sperm oestrual-mucus interaction in buffaloes through *in vitro* sperm penetration test.

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MATERIALS AND METHODS

Normal cyclic healthy buffaloes (90) maintained at the University farm during winter (November-January, n=55) and summer (April-June, n=35) were studied. They were kept in loose housing system, comprising half-walled *pucca* open sheds. They were fed with green fodder, wheat straw, concentrates including mineral mixtures throughout the year according to the standard schedule followed at Dairy Farm. Drinking water was provided *ad lib*. Oestrus detection was carried out by parading a vasectomized bull twice a day and later on confirmed by rectal palpation.

Oestrual mucus samples were collected asceptically prior to insemination by rectovaginal method from tubular genital tract with a sterilized glass pipette and syringe. The samples were stored at –20°C in screw capped plastic vials till analyzed.

All the animals detected in oestrus were inseminated artificially with frozen-thawed semen having more than 40% post-thaw motility. Pregnancy was confirmed per rectum following 60 days of AI.

Biochemical analysis of oestrual mucus

Sodium and potassium in oestrual mucus were estimated by flame photometry (Oser 1965). Chloride was estimated by the method of Schales and Schales (1941). The macro (calcium and magnesium) and micro (copper, zinc and manganese) minerals in oestrual mucus were estimated on wet oxidized samples using spectrophotometer (Ludmilla 1976).

Sperm mucus penetration test (SMPT)

The test was carried out as per Kremer (1965) with little modifications (Dev 1995). Oestrual mucus was thawed for about one- and a half-hour at 37°C before use. Thawed mucus was aspirated into 2 plain haematocrit round capillary tubes using a mucus sucking device. The mucus was cut at free end of the tube to detach it from the mucus pool. One end of the capillary tube was then sealed with modelling clay and kept at 37°C in the incubator. The frozen-thawed semen (0.5 ml) was placed in a semen reservoir cup attached to glass slide. The open end of capillary tubes filled with mucus were then immersed in the pool of semen taken in the cup. The whole assembly was incubated at 37°C for 20 min. Immediately after incubation, the capillaries were removed and cooled quickly in crushed ice to give cold shock to spermatozoa. The capillary tube was then placed on microslide and examined under phase contrast micrscope (×300). The distance travelled by the foremost sperm was measured with the scale fixed on microscopic stage. Sperm penetration distance (SPD) in oestrual mucus was designated in millimetre per 20 min. All the SMPTs were conducted within 1 month of oestrual mucus collection.

The data were analyzed with Student's t-test and correlation coefficients (r) of SPD with other biochemical traits were worked out as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The overall mean values of sperm penetration distance (mm/20 min) and biochemical parameters of oestrual mucus are presented in Table 1. The significantly (P<0.05) higher conception rate and SPD values were recorded in winter (63.63% and 32.67±1.28 mm/20 min, respectively) as compared to those in summer (31.42% and 21.22±1.08 mm/20 min, respectively). The SPD values recorded in winter are in accordance with the results of others (Akhtar *et al.* 1980, Dev 1995). The significant difference observed in SPD values between the 2 seasons might be because of variable biochemical attributes of the oestrual mucus (Khajuria *et al.* 2004). During winter, the mean values of pH were significantly (P<0.05) lower while, the mean quantities of Na, Cl, Cu and Mn were significantly (P<0.05) higher than those in summer. The lower pH values of oestrual mucus favoured spermatozoal penetration in bovines (Shehata *et al.* 1978, Wani *et al.* 1979). The significantly higher values of certain biochemicals (Na, Cl, Cu, Mn) in the oestrual mucus during winter might be correlated with better expression of oestrus in buffaloes during winter (Takkar *et al.* 1979, Reddy *et al.* 1987). The K ion concentration was nonsignificantly lower in oestrual mucus during winter than that in summer. Lower K concentration in oestrual mucus might favour the sperm capacitation, as high concentration of it in epididymal fluids lowers its functional activity (Mann and Mann 1981).

The correlations of SPD values with the biochemical profiles of oestrual mucus during winter and summer in Table 1. During winter, significant (P<0.05) positive correlations were observed between SPD values and Na, Cl, Ca and Mg contents. Whereas, during summer similar correlations were observed for Na and Cl only. The K values were negatively correlated with the SPD values during both the seasons. Nair and Kharche (1988) and Dev (1995) recorded similar observations in bovines.

The biochemical profiles of oestrual mucus of conceived and non-conceived groups of buffaloes during both the

Table 1. Effect of biochemical profile of oestrual mucus of buffaloes on SPD during winter and summer and their interactions

Season and No. of animals	Conception rate (%)	SPD (mm /20 min)	pH	Na (mg%)	K (mg%)	Cl (mg%)	Ca (mg%)	Mg (mg%)	Cu (mg%)	Zn (mg%)	Mn (mg%)
Winter	63.63[a]	32.67	7.25	372.1	31.9	504.3	6.51	3.61	0.28	2.50	0.92
(n=55)		±1.28[a]	±0.03[a]	±6.43[a]	±1.12	±23.08[a]	±0.54	±0.24	±0.01[a]	±0.31	±0.06[a]
Correlation coefficient (r) with SPD	–	–	–	0.88*	–0.32*	0.87*	0.39*	0.33*	0.24	0.14	0.14
Summer	31.42[b]	21.22	7.84	312.6	34.9	368.5	6.90	3.50	0.14	1.90	0.38
(n=35)		±1.08[b]	±0.08[b]	±15.11[b]	±1.49	±20.64[b]	±0.73	±0.32	±0.01[b]	±0.49	±0.01[b]
Correlation. coefficient (r) with SPD	–	–	–	0.79*	–0.43*	0.86*	0.31	0.21	0.07	0.08	0.03

Means bearing different superscripts in a column differ significantly (P<0.05); (*)–significantly (P<0.05) correlated with SPD values.

Table 2. Mean (±SE) SPD values and biochemical profiles of oestrual mucus of conceived (C) and non-conceived (NC) groups of buffaloes during winter and summer

Parameters	Winter		Summer		Overall	
	C (n=35)	NC (n=20)	C (n=11)	NC (n=24)	C (n=46)	NC (n=44)
SPD (mm/20 min)	37.43±1.16[a]	26.96±1.89[b]	26.93±1.24[a]	16.95±0.76[b]	33.93±1.14[a]	22.51±1.21[b]
pH	7.35±0.44	7.18±0.04	7.70±0.12	7.95±0.09	7.46±0.05	7.52±0.07
Na (mg%)	384.49±8.50[a]	358.59±9.00[b]	380.00±17.45[a]	262.06±15.17[b]	383.00±8.13[a]	315.69±11.02[b]
K (mg%)	31.70±1.81	32.10±1.53	28.41±1.26[a]	39.72±1.79[b]	30.60±1.30	33.50±1.43
Cl (mg%)	524.96±35.13[a]	479.52±27.40[b]	456.65±27.63[a]	301.80±19.02[b]	502.19±25.62[a]	400.53±21.85[b]
Ca (mg%)	6.10±0.14[a]	7.66±0.35[b]	6.00±0.13[a]	7.06±0.23[b]	6.04±0.15[a]	7.46±0.28[b]
Mg (mg%)	4.13±0.42	3.48±0.42	4.00±0.57	3.15±0.36	4.08±0.34	3.33±0.28
Cu (mg%)	0.29±0.02	0.25±0.03	0.17±0.04	0.13±0.03	0.26±0.02	0.22±0.02
Zn (mg%)	2.57±0.41	2.43±0.47	1.91±0.91	1.90±0.52	2.25±0.67	2.17±0.49
Mn (mg%)	0.96±0.25	0.85±0.08	0.38±0.01	0.37±0.02	0.82±0.13	0.59±0.05

Means bearing different superscripts in a row differ significantly (P<0.05) between groups

seasons are given in Table 2. The conceived buffaloes (winter, n=35; summer, n=11) revealed significantly (P<0.05) higher SPD values than the non-conceived ones during both the seasons (overall, 33.93±1.14 vs 22.51±1.21 mm/20 min). The Na and Cl were also significantly (P<0.05) higher in conceived than that in non-conceived groups during both the seasons. The higher values of Na and Cl in oestrual mucus might be responsible for better crystallization and sperm penetrability (Aboul-Ela *et al.* 1983) and hence the conception rate. The K content in oestrual mucus of conceived group was nonsignificantly lower in winter whereas, it was significantly (P<0.05) lower during summer. Lower K concentration in oestrual mucus might favour the sperm capacitation (Mann and Mann 1981) and the conception. Significantly (P<0.05) lower Ca content was observed in oestrual mucus of animals which got conceived than non-conceived ones in both the seasons. High Ca level is detrimental for sperm survival and interferes with the process of fertilization leading to low conception rate (Nair and Kharche 1988). The micro-minerals (Cu, Zn, Mn and Mg) in oestrual mucus were nonsignificantly higher in conceived than non-conceived buffaloes during both the seasons. Though no clear cut relationship of these micro-elements with conception and fertility has been established yet their significance in normal physiological functions can not be undermined as their deficiency hampers the normal reproduction in affected animals (Hidiroglou 1979). The overall values of SPD, Na and Cl were significantly (P<0.05) higher and that of Ca lower in oestrual mucus of conceived buffaloes than the non-conceived ones. The Mg, Mn and Zn were also higher though nonsignificantly in conceived than non-conceived buffaloes (Table 2). Hence, it could be concluded that season influenced certain biochemical constituents of oestrual mucus, thereby, altering sperm mucus penetration and resulting in poor conception during summer in buffaloes.

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