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Research Article

Physico-Microbial Properties of Cervico-Vaginal Mucus and its Antibiotic Sensitivity Pattern in Repeat Breeding Buffaloes*

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ABSTRACT

Forty five cervico-vaginal mucus samples from repeat breeding buffaloes on examination revealed clear thin watery, clear stringy and turbid consistency of mucus in 55.55, 33.33 and 11.11 % cases, respectively; while as in 10 normal cyclic animals, 70.00 and 30.00 % samples were clear stringy and clear thin watery, respectively. Typical, atypical and nil fern pattern of oestrual mucus was observed in 33.33, 60.00 and 6.66 % samples of repeat breeders, and in 80, 20 and 0.00 % of normal cyclic buffaloes, respectively. Maximum number of normal cyclic and repeat breeding buffaloes conceived when they had clear stringy mucus (85.72 and 60.00 %), followed by those with clear thin watery mucus (33.33 and 20.00 %) and those with typical fern pattern (87.50 and 46.66 %), but none with turbid mucus and nil fern pattern. The spinnbarkeit value of mucus from repeat breeding and normal cyclic buffaloes was identical (9.76 ± 0.56 and 10.30 ± 0.93 cm), but those conceived had significantly higher ($P \leq 0.01$) spinnbarkeit value as compared to those that did not conceive (12.5 vs 8.2 cm). Of the total 37 mucus samples of repeating buffaloes cultured, 35 (94.60 %) were positive for microorganisms; while from 10 normal breeding buffaloes only 2 (20.00 %) yielded microorganisms. Out of 35 positive samples from repeating buffaloes, 31(88.57 %) had single (12 gram +ve, 19 gram –ve) and 4 (11.43 %) had mixed bacterial isolates. Single isolates were more susceptible to the entire range of drugs tested, *viz.*, ciprofloxacin (91.42 %), cephalexin (85.71 %), gentamicin (85.71 %), ceftriaxone (82.86 %), ampicillin (82.86 %), enrofloxacin (80.00 %) and oxytetracycline (77.14 %), while as mixed isolates showed highest resistance to these antibiotics. Cephalexin 4 gm and ceftriaxone 2 gm used as post-AI intrauterine infusion in 6 cases each was highly effective as 8 (66.66 %) animals conceived within 2 cycles.

Key words: Cervico-vaginal mucus, Physical properties, Microbial isolates, Drug Sensitivity, Repeat breeding, Buffaloes

INTRODUCTION

Amongst the many factors that causes repeat breeding in dairy animals the infectious agents are of major importance. Moreover, physico-microbial properties of cervico-vaginal mucus (CVM) have direct relationship with fertility status of the animals (Luktuke and Roy, 1967; Agarwal and Purbey, 1983; Panchal *et. al.*, 1994; Rangnekar *et. al.*, 2002). There are consistent and definite gradual changes in the viscosity, clarity, stretchability (Spinnbarkeit) and fern pattern of cervical mucus with the cyclic rhythm of the reproductive phenomenon and hence such parameters are considered as effective laboratory tools to predict the infertility in bovines. Moreover, a wide variety of microflora infect female genital tract and play a significant role by causing inflammation of endometrium. Various antibacterial drugs have, therefore, been tried from time to time to

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overcome the problem of conceptio failure in buffaloes with variable results, as the majority of repeat breeding cases are due to low grade bacterial infections (Krishnamurthy *et. al.*, 1974; Younis *et. al.*, 1987; Singh *et. al.*, 1996; Metwelly, 2002; Dinesh *et. al.*, 2006). The present study was therefore attempted to know the physical properties of CVM samples from repeat breeding buffaloes as well as type of bacterial isolates and their *in vitro* drug sensitivity pattern, so as to recommend suitable remedies for the same.

MATERIALS AND METHODS

This study was carried out during the period starting from the month of December 2006 to February 2007 at three villages *viz.*, Chikhodra, Bedva and Sarsa of Anand district. In all, 60 repeat breeding and 10 normal fertile buffaloes presented for artificial insemination (AI) were screened thoroughly for their genital health and reproductive status through past history and gynaeco-clinical examinations per rectum.

The CVM samples from 45 repeating and 10 normal fertile buffaloes were aspirated aseptically just before AI by using 10 ml sterilized glass pipette connected to a syringe by recto-vaginal technique (Panangala *et. al.*, 1978). These samples were evaluated for the physical properties like colour and consistency, spinnbarkeit and fern pattern within 2-3 hours of collection. Colour and consistency was judged by direct examination of mucus after collection and categorized as clear/transparent, cloudy/turbid, sticky or watery. The spinnbarkeit was measured in centimeter as described by Panigrahi (1964). The fern pattern was studied in the oestrual mucus smears under low power (10X) and high power (40X) microscope and was classified as typical, atypical and nil type as per Luktuke and Roy (1967).

Bacterial isolation of CVM samples from 37 repeating and 10 normal fertile buffaloes was done as per Cruickshank (1965). The isolates were subjected to *in vitro* antibiotic sensitivity test as per the method recommended by Bauer *et. al.* (1966). Antibiotic discs supplied by HiMedia Laboratories Pvt. Ltd., Mumbai were used and the results were interpreted as per the chart furnished by manufacturer. The isolates were tested for their sensitivity to 7 antibiotics, *viz.* oxytetracycline, gentamicin, streptomycin, ceftriaxone, cephalexin, enrofloxacin and amplicillin. The results were analysed and interpreted statistically.

RESULTS AND DISCUSSION

Colour and Consistency of Mucus

The CVM from healthy cows and buffaloes was clear appearing like white of an egg. In the present study 55.55, 33.33 and 11.11 % of the CVM samples collected from 45 repeat breeding buffaloes were clear thin watery, clear stringy and cloudy/turbid, respectively; while from that of 10 normal cyclic animals, 70.00 and 30.00 % samples were clear stringy and clear thin watery, respectively (Table 1). Further, maximum number of normal cyclic and repeat breeding buffaloes conceived when they had clear stringy mucus (85.72 and 60.00 %), followed by those with clear thin watery mucus (33.33 and 20.00 %) and none with turbid or cloudy discharge (Table 2).

Present findings, particularly in repeat breeding buffaloes, corroborated with the report of Samad *et. al.* (2002) that oestrus mucus was transparent in 55.00 % of the 60 repeat breeding buffaloes, translucent in 38.33 % and whitish in 6.67 %. Moreover, they observed viscous/ stringy mucus in 38.33 % of the repeat breeders, thin consistency in 50.0 % and thick in 11.67 %. 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 discharge, respectively and that maximum percentage (54.54 %) of animals conceived from those having clear stringy mucus. Rangnekar *et. al.* (2002) reported that 35, 25 and 40 % cows showed thin, medium

and thick consistency of mucus, respectively. Majority of fertile oestrus (54.54 %) had thin consistency, while non-fertile oestrus (77.78 %) had thick consistency. These findings suggest that stringy mucus favours sperm survival and transport thereby improving the conception rate. Overall, 31.11 % of repeat breeders and 70 % of normal cyclic animals conceived to artificial insemination in that cycle.

Fern Pattern of CVM

The occurrence of typical, atypical and nil fern pattern of CVM was observed as 33.33, 60.00 and 6.66 % samples in repeat breeding buffaloes and 80, 20 and 0.00 % in normal cyclic buffaloes, respectively (Table 3). Like stringy mucus, maximum number of normal cyclic and repeat breeding buffaloes conceived when they had cervical mucus with typical fern pattern at the time of AI (87.50 and 46.66 %), followed by those with atypical pattern (00.00 and 25.92 %) and none with absence/nil type of fern pattern (Table 4).

Present findings on fern pattern of CVM in repeat breeding buffaloes coincided well with the report of Samad *et. al.* (2002) who found very characteristic, characteristic and less characteristic fern pattern of CVM in 6.67, 48.33 and 45.00 % of the 60 repeat breeding buffaloes, but was in contradiction to the reports of Panchal *et. al.* (1994) and Rangnekar *et. al.* (2002), who reported higher frequency of typical fern pattern. Luktuke and Roy (1967) also concluded that conception rate was highest when there was typical fern pattern and it was zero when there was absence of fern pattern in oestrus mucus

Panchal *et. al.* (1994), however, reported the occurrence of typical, atypical and nil type of fern patterns in 71.11, 22.96 and 5.93 % of CVM samples of repeat breeding buffaloes. The Antibiotic sensitivity pattern inrepeat breeding buffaloes conception rate in typical fern pattern group was double than that of other 2 groups (19.79 % vs 10.26 %). Agrawal and Purbey (1983) reported that 66.60 % of animals conceived which had typical fern pattern of cervical mucus and in atypical fern pattern group 36.36 per cent of animals became pregnant.

Our findings on normal cyclic and repeat breeding buffaloes 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 percent, respectively. Present findings suggest that oestrual cervical mucus with classical arborization pattern due to increased salts and organic constituents favoured sperm survival and transport thereby improved the conception rate.

Spinnbarkeit Value of Mucus

The mean spinnbarkeit value of cervical mucus from repeat breeding and normal cyclic buffaloes was observed as 9.76 ± 0.56 and 10.30 ± 0.93 cm, respectively. The mean values of the spinnbarkeit in normal cyclic and repeat breeding buffaloes were identical. However, buffaloes that conceived, irrespective of normal or repeat breeding groups had significantly higher ($P < 001$) spinnbarkeit values as compared to those that did not conceive (Table 5).

These findings are in close conformity with the several previous reports in bovines. Thaker *et. al.* (1981) reported spinnbarkeit values as 7.40 ± 0.36 cm and 7.47 ± 0.20 cm in normal and repeat breeder buffaloes. Enkhia and Kohli (1982) reported mean elasticity value as 11.0 and 6.5 cm in normal and repeat breeding crossbred cows. Rangnekar *et. al.* (2002) reported mean spinnbarkeit value of cervical mucus of the 20 cows as 12.45 ± 0.64 cm and cows that had spinnbarkeit values of 11 cm or below did not conceive. Bennur *et. al.* (2004) reported the spinnbarkeit value of the CVM of pregnant and non-pregnant cows as 7.38 ± 5.60 and 8.05 ± 1.33 cm, respectively.

Bacterial Isolation of Cervical Mucus

Out of 37 CVM samples cultured from repeat breeding buffaloes, 35 (94.60 %) yielded one or the other type of microorganisms and 2 (5.40 %) were sterile. In contrast, of the total 10 samples cultured from normal breeding buffaloes,

Table 1: Nature of cervical mucus in repeat breeding and normal cyclic buffaloes

Type and No. of animals	Physical properties of CVM					
	Clear thin watery		Clear stringy		Cloudy/ Turbid	
	No.	Per cent	No.	Per cent	No.	Per cent
Repeat breeder (45)	25	55.55	15	33.33	5	11.11
Normal cyclic (10)	3	30.00	7	70.00	-	

Table 2: Fertility status in relation to nature of cervical mucus in normal cyclic and repeat breeding buffaloes

Type of mucus	Normal cyclic		Repeat breeders	
	Animal No.	Conception	Animal No.	Conception
Clear thin	3	1 (33.33 %)	25	5 (20.00%)
Clear stringy	7	6 (85.72 %)	15	9 (60.00%)
Cloudy/turbid	00	0 (00.00 %)	5	0 (00.00%)
Total	10	7 (70.00 %)	45	14 (31.11%)

Table 3: Fern pattern of cervical mucus in repeat breeding and normal cyclic buffaloes

Type and No. of animals	Fern pattern of CVM					
	Typical		Atypical		Nil	
	No.	%	No.	%	No.	%
Repeat breeder (45)	15	33.33	27	60.00	3	6.66
Normal cyclic (10)	8	80.00	2	20.00	-	-

Table 4: Fertility status in relation to fern pattern of cervical mucus in normal cyclic and repeat breeding buffaloes

Type of fern pattern	Normal cyclic		Repeat breeders	
	Animal No.	Conception	Animal No.	Conception
Typical 8	7	(87.50 %)	15	7 (46.66 %)
Atypical 2	0	(00.00 %)	27	7 (25.92 %)
Nil 00	0	(00.00 %)	3	0 (00.00 %)
Total 10	7	(70.00 %)	45	14 (31.11 %)

only 2 (20.00 %) were positive for presence of gram-negative microorganisms. Among the 35 positive samples from repeating buffaloes, the incidence of gram- positive, gram- negative and mixed isolates was 12 (38.70 %), 19 (61.29 %) and 4 (11.43 %), respectively (Table 6).

Mild endometritis resulting in repeat breeding may occur as a result of non-specific microbial infection. It was interesting to note in the present study that the cervical mucus of normal cyclic buffaloes yielded only gram-negative organisms. Krishnamurthy *et. al.* (1974) recovered 190 isolates belonging to different species of bacteria from 86 % of 106 repeat breeding bovines. Ashturkar *et. al.* (1995) observed presence of gram-negative rod, i.e. *E. coli* and mixed infection in vaginal and uterine swabs from repeat breeder cases of large ruminants. Singh *et. al.* (1998)

Table 5: Mean and range of spinnbarkeit values (cm) of cervical mucus of normal cyclic and repeat breeding buffaloes

Class	Group	No. of animals	Spinnbarkeit value (cm)	
			Mean ± SE	Range
Repeat breeders	Conceived	14	12.50 ± 0.73[a]	8.00 – 18.00
	Non-conceived	31	8.18 ± 0.46[b]	4.00 – 15.00
	Pooled	45	9.76 ± 0.56	4.00 – 18.00
Normal cyclic	Conceived	7	12.57 ± 1.11[a]	9.00 – 18.00
	Non-conceived	3	7.33 ± 1.20[b]	5.00 – 9.00
	Pooled	10	10.30 ± 0.93	5.00 – 18.00

Means bearing common superscripts do not differ significantly by 't' test.

Table 6: Genital microflora isolated from normal cyclic and repeat breeding buffaloes

Nature of buffaloes	No. of animals/ samples	Samples found culturally		Nature of isolates		Single bacterial isolates		
		+ve	-ve (sterile)	MixedType	SingleType	Total No.	Gram positive	Gram negative
Repeating	37	35 (94.60%)	2 (5.40%)	4 (11.43%)	31 (88.57%)	35	12 (38.70%)	19 (61.29%)
Normal cyclic	10	2 (20.0%)	8 (80.0%)	-	2 (100%)	2	-	2 (100%)

Table 7: Sensitivity pattern of different type of organisms isolated from CVM samples of repeat breeding buffaloes

Sr. No.	Type of organism	No. of isolates	No. of isolates sensitive to						
			CL	CT	GM	OT	EF	AC	CF
1.	Gram +Ve	12	10	10	9	9	11	11	12
2.	Gram –Ve	19	18	18	19	17	17	17	18
3.	Mixed type	4	2	1	2	1	-	1	2
4.	Total	35	30	29	30	27	28	29	32

CL = Cephalexin, CT = Ceftriaxone, GM =Gentamicin, OT = Oxytetracycline, EF = Enrofloxacin, AC = Ampicillin, CF = Ciprofloxacin

studied cervical mucus from 50 repeat breeder cows, 26 of which were subsequently found to have clinical signs of disease. Bacteria were isolated in pure or mixed culture from 20 (76.9 %) of the 26 cows, and from 11 (45.8 %) of the 24 with no clinical signs. Adhau *et. al.* (1999) reported that out of 64 buffaloes, only 47 (73.44 %) CVM samples were positive, which yielded 56 bacterial isolates. Present findings are in agreement with most of these reports.

Table 8: Per cent effectiveness of the antibiotics on different bacterial isolates

Sr. No.	Name of the antibiotics	Sensitive		Resistance	
		No. of isolates	Percentage	No. of isolates	Percentage
1	Cephalexin	30	85.71	5	14.29
2	Ceftriaxone	29	82.86	6	17.14
3	Gentamicin	30	85.71	5	14.29
4	Oxytertracycline	27	77.14	8	22.86
5	Enrofloxacin	28	80.00	7	20.00
6	Ampicillin	29	82.86	6	17.14
7	Ciprofloxacin	32	91.42	3	8.57

Drug Sensitivity Test

A total of 35 isolates that included 12 gram-positive, 19 gram-negative and 4 mixed types were examined for *in vitro* antibiotic sensitivity (Table 7). The isolates showed wide variation in their sensitivity to seven antibacterial drugs ranging from 77.14 to 91.42 percent (Table 8). Comparatively gram-negative organisms and gram-positive organisms were found more susceptible to the entire range of drugs tested (Table 7). Among the mixed type, highest samples showed resistance to different antibiotics, particularly ampicillin, enrofloxacin, oxytetracycline, and even ceftriaxone. The result of drug-wise per cent sensitivity / resistance pattern (Table 8) revealed that ciprofloxacin was the most effective antibiotic (91.42 %) followed by cephalexin (85.71 %), gentamicin (85.71 %), ceftriaxone (82.86 %), ampicillin (82.86 %), enrofloxacin (80.00 %) and oxytetracycline (77.14 %).

Out of the six animals each sampled for cultural isolation before post-AI intrauterine infusion of cephalexin 4 gm and ceftriaxone 2 gm, 3 each yielded gram negative bacteria, 2 each gram -positive and 1 each of mixed type. Moreover, all the gram -positive and negative isolates were sensitive *in vitro* to both the antibiotics used in the treatment, while mixed isolates showed resistance to cephalexin. Further, five animals conceived in the same treatment cycle and three more in first cycle post-treatment, suggesting beneficial role of these antibiotics in combating uterine infection by way of post-insemination infusion.

Benjamin *et. al.* (1983) reported that majority of the organisms isolated from the cases of repeat breeder and metritis cases were sensitive to oxytetracycline and tetracycline. Kharde and Kulkarni (1983) found that the CVM isolates from 40 repeat breeding cows and 10 healthy cows were sensitive to gentamicin and kanamycin. Dhabale *et. al.* (1997) observed that 100, 42.9, 28.6, 57.1, 50.0 and 85.7 % of bacterial isolates of cervical mucus were resistant to ampicillin, chloramphenicol, gentamicin, tetracycline, streptomycin and nitrofurazone, respectively, while Adhau *et. al.* (1999) reported that 91.07, 85.71, 69.94, 64.28, 32.64, 8.92 and 5.53 % isolates of CVM samples of repeat breeding buffaloes were sensitive to ciprofloxacin, gentamicin, norfloxacin, chloramphenicol, nitrofurazone, furazolidone, ampicillin and oxytetracycline, respectively.

Ramaswamy *et. al.* (1998) isolated gram-positive and gram-negative bacteria in nearly equal numbers from repeat breeding cows (110) and buffaloes (6). These were resistant to polymixin-B, ampicillin, tetracycline, cephalexin, chloramphenicol and nitrofurantoin, and sensitive to only gentamicin and streptomycin. 73.50 % of the bacteria mostly gram-negative exhibited multiple drug resistance to two or more drugs. Metwelly (2002) observed that enrofloxacin, oxytetracycline and gentamicin were active against 85.7, 75.0 and 69.6 % bacterial isolates, respectively from CVM of buffaloes. In the present study, all the seven antibiotics tested *in-vitro*

appeared effective against genital microflora of repeat breeding buffaloes, and there was not much variation among them for sensitivity pattern.

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ISSAR NEWS

ISSAR executive and Editorial board of IJAR along with ISSAR West Bengal Chapter cordially felicitates Dr. Jaharlal Chakraborty Ph.D (LM No. 1132) for having taken over as Director of Animal Husbandry and Veterinary Services in the Directorate of Animal Resources and Animal Health, Govt. of West Bengal on 2nd April, 2008. Dr. Chakraborty is an active life member of the ISSAR and intensily involved in various animal welfare organizations. We all look forward for overall development of veterinary science and animal husbandry under his dynamic leadership in West Bengal. We wish him all success in his endeavour.