

## RESEARCH ARTICLE

# Electrolytes Profile of Estrual Mucus of Gir Cows with Reference to Body Condition Score and Fertility

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### ABSTRACT

Fifty Gir cows of different reproductive status (10 = normal cyclic, 20 = repeat breeders and 20 = induced estrus) were selected to determine the association of electrolytes mainly the macro-minerals profile of their estrual cervico-vaginal mucus (CVM) to their body condition score (BCS) and fertility post-AI. The overall pooled mean concentrations of calcium, inorganic phosphorus, magnesium, sodium and potassium obtained in CVM of Gir cows were  $14.27 \pm 0.33$  mg/dL,  $1.87 \pm 0.13$  mg/dL,  $4.24 \pm 0.10$  mEq/L,  $159.85 \pm 5.52$  mEq/L and  $29.50 \pm 0.78$  mEq/L, respectively. The repeat breeding cows had significantly ( $p < 0.01$ ) higher calcium and lower inorganic phosphorus, sodium and potassium in their CVM than the normal cyclic and/or induced estrus cows. Similarly, the conceived cows, overall and of repeater group, had significantly ( $p < 0.05$ ) lower calcium and higher phosphorus, sodium and potassium than the non-conceived cows. However, no such variation was found in the magnesium content of CVM of these groups. Further, the levels of calcium in CVM showed an increasing trend, while other elements showed a decreasing trend with an increase in BCS from 2.5 to 3.5 (on 0-5 point scale) in all three categories of animals, but the differences were not significant for BCS within the group/category. It was thus inferred that significantly increased or widened Ca:P ratio and absolute lower levels of sodium and potassium in CVM could be responsible for conception failure and repeat breeding in dairy cows.

**Keywords:** BCS, Estrual cervical mucus, Fertility, Gir cows, Macro-minerals profile.

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### INTRODUCTION

The significance of various biochemical and mineral constituents of cervico-vaginal and uterine fluids has been well recognized, as their deficiency or excess adversely affect the viability and fertilizing ability of sperms (Vadodaria, 1987; Gohel *et al.*, 2012a). The higher or lower level of proteins and minerals in the cervical mucus was one of the responsible factors of the repeat breeding (Hawk, 1979; Adnane *et al.*, 2018). The rheological properties of CVM appears to be determined primarily by the nature of its macro-molecules, and their magnitude is regulated by the concentration of salts, of which NaCl forms the major salt which greatly influences the ionic strength of mucus. Together with K<sup>+</sup>, it is responsible for the crystallization phenomenon in cervical mucus (Verma *et al.*, 2014; Bernardi *et al.*, 2016). The crystallization phenomenon of CVM also varies significantly between pregnant and empty animals, and for natural and induced estrus (Tsingiani *et al.*, 2002; Verma *et al.*, 2014; Bernardi *et al.*, 2018). The proteins in CVM improve sperm transport and regulate its osmolarity, consistency, threadability and buffering capacity (Goel *et al.*, 1974; Gohel *et al.*, 2012b). Inorganic phosphorus is essential for energy transformation at the cellular level and is associated with maintenance of sperm glycolysis and respiration (Panchal *et al.*, 1994). This study was therefore aimed to evaluate and correlate the macro-minerals profile of estrual cervical mucus in Gir cows of different body condition score (BCS) and reproductive status.

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### MATERIALS AND METHODS

The study was conducted from November 2009 to April 2010 on Gir cows maintained at Livestock Research Station, AAU, Anand, and Heifers Development Project of the Baroda District Co-operative Milk Producers' Union Ltd., Itola, Vadodara, Gujarat. The estrual cervico-vaginal mucus (CVM) samples from 50 Gir cows of 3 different reproductive status (10 = normal cyclic, 20 = repeat breeders and 20 = CIDR induced estrus) were collected aseptically just before insemination by "Pipette and Syringe Method" (Gohel *et al.*, 2012a). The CVM samples were diluted with triple glass

distilled water @ 1:3 and stored at -20°C until analyzed for macro-minerals profile (Vadodaria, 1987). The BCS of each cow was assessed on 0 to 5 point scale (Edmonson *et al.*, 1989). The cows in mid estrus were inseminated with good quality frozen-thawed semen soon after aspiration of mucus. Pregnancy was confirmed per rectum in non-return cases 60 days after AI.

The estimation of calcium, magnesium, and inorganic phosphorus content in mucus samples was done using standard procedures and assay kits of Crest Biosystems, Goa, India, on an auto-analyzer. The sodium and potassium contents were estimated by flame photometry (Flame photometer 128). Data were analyzed statistically using a completely randomized design and critical difference test as well as unpaired 't' test (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

The relationship of BCS and conceived and non-conceived status of normal cyclic, repeat breeder and induced estrus cows with the macro-minerals profile of their CVM has been shown in Table 1 and 2.

### Calcium-Phosphorus Concentration

The mean calcium concentration in the CVM of repeat breeding cows was significantly ( $p < 0.01$ ) higher ( $15.44 \pm 0.62$  mg/dL) and inorganic phosphorus concentration was lower ( $1.26 \pm 0.14$  mg/dL) than the normal cyclic and/or induced estrus cows (Table 1). This resulted into a wider (almost double) Ca:P ratio in the CVM of repeat breeders (approx. 11:1) than the other two groups of cows (approx. 5.6:1). These findings of higher calcium and lower phosphorus compared well with the earlier reports in cattle and buffaloes (Wani *et al.*, 1979; Enkhia *et al.*, 1983; Sharma and Tripathi, 1987; Shukla and Sharma, 2006;

Modi *et al.*, 2011). Some researchers (Panchal *et al.*, 1994; Sood *et al.*, 2000; Bernardi *et al.*, 2018), however, noted identical calcium and/or phosphorus levels in CVM of fertile and infertile cows/heifers, and even between CVM of induced and spontaneous estrus.

Further, the conceived cows in comparison to non-conceived ones had significantly ( $p < 0.01$ ) lower calcium ( $13.27 \pm 0.32$  vs.  $15.67 \pm 0.53$  mg/dL) and higher phosphorus ( $2.15 \pm 0.14$  vs.  $1.48 \pm 0.25$  mg/dl) concentration in CVM, overall, and in repeat breeding group also (Table 1), with altered Ca:P ratio (approx. 10:1 vs. 6:1) in conceived and non-conceived groups. Thus, the reproductive failure in repeat breeding cows could be due to altered calcium-phosphorus levels resulting into wider Ca:P ratio in their CVM, which is not congenial for the survival of gametes. Others have also recorded similar higher phosphorus content in CVM of fertile cows as compared to infertile cows/buffaloes (Reddy and Abdullakhan, 1977; Vadodaria, 1987; Panchal *et al.*, 1994). Like our findings, Tsiligianni *et al.* (2002) also found significantly higher calcium in CVM of PG induced estrus than spontaneous estrus cows, while Bernardi *et al.*, (2018) documented almost identical values in CVM of induced and natural estrus in HF heifers. Yanagimachi and Usui (1974) opined that calcium plays role in the induction of acrosomal reaction in mammalian spermatozoa, and it is also involved in sperm motility. Inorganic phosphorus is essential for energy transformation at the cellular level and is associated with the maintenance of sperm glycolysis and respiration (Panchal *et al.*, 1994).

The calcium-phosphorus levels were not only related to reproductive/cyclical status, but also with the BCS of cows. There was a clear trend of increasing levels of calcium and decreasing levels of phosphorus resulting into wider Ca:P ratio with increase in BCS from 2.5 to 3.5 in all three categories

**Table 1:** Mean ( $\pm$  SE) macro-minerals profile in cervico-vaginal mucus of normal cyclic, repeat breeder and induced estrus cows in relation to their fertility

Group	Reproductive status	Calcium (mg/dL)	Inorganic phosphorus (mg/dL)	Magnesium (mEq/L)	Sodium (mEq/L)	Potassium (mEq/L)
Normal Cyclic (10)	Conceived (8)	$12.27 \pm 0.63$	$2.29 \pm 0.19$	$4.41 \pm 0.35$	$172.54 \pm 09.48$	$32.77 \pm 0.81$
	NC (2)	$13.29 \pm 0.40$	$2.22 \pm 0.63$	$4.06 \pm 0.35$	$158.29 \pm 02.94$	$28.50 \pm 2.85$
	Pooled (10)	$12.47 \pm 0.52^x$	$2.43 \pm 0.25^y$	$4.34 \pm 0.28$	$169.69 \pm 07.73^y$	$31.91 \pm 0.91^y$
Repeat Breeder (20)	Conceived (7)	$13.47 \pm 0.64^a$	$1.71 \pm 0.22^a$	$4.09 \pm 0.28$	$186.40 \pm 06.58^a$	$27.29 \pm 0.90^a$
	NC (13)	$16.50 \pm 0.75^b$	$1.01 \pm 0.15^b$	$4.33 \pm 0.23$	$116.16 \pm 10.28^b$	$22.64 \pm 0.53^b$
	Pooled (20)	$15.44 \pm 0.62^y$	$1.26 \pm 0.14^x$	$4.25 \pm 0.17$	$140.75 \pm 10.35^x$	$24.27 \pm 0.68^x$
Induced Estrus Group (20)	Conceived (14)	$13.59 \pm 0.37$	$2.37 \pm 0.22$	$4.30 \pm 0.17$	$183.16 \pm 07.92^a$	$34.78 \pm 1.05^a$
	NC (6)	$14.65 \pm 0.32$	$2.25 \pm 0.70$	$3.90 \pm 0.10$	$152.71 \pm 05.81^b$	$30.56 \pm 1.07^b$
	Pooled (20)	$13.91 \pm 0.29^{xy}$	$2.29 \pm 0.25^y$	$4.18 \pm 0.13$	$174.03 \pm 06.55^y$	$33.52 \pm 0.90^y$
Overall (50)	Conceived (29)	$13.27 \pm 0.32^a$	$2.15 \pm 0.14^a$	$4.28 \pm 0.14$	$181.01 \pm 04.85^a$	$32.42 \pm 0.81^a$
	NC (21)	$15.67 \pm 0.53^b$	$1.48 \pm 0.25^b$	$4.18 \pm 0.15$	$130.61 \pm 07.66^b$	$25.17 \pm 0.94^b$
	Pooled (50)	$14.27 \pm 0.33$	$1.87 \pm 0.13$	$4.24 \pm 0.10$	$159.85 \pm 5.52$	$29.50 \pm 0.78$

The figures in parentheses indicate number of animals, NC = Non-conceived.

Means bearing uncommon superscripts differ significantly ( $p < 0.05$ ) between groups (x, y) and sub-groups (a, b) within the column.



**Table 2:** Macro-minerals profile (Mean  $\pm$  SE) of cervico-vaginal mucus of normal cyclic, repeat breeder and induced estrus cows in relation to their body condition score

Group	BCS	No. of animals	Calcium (mg/dl)	Inorganic phosphorous (mg/dl)	Magnesium (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)
Normal Cyclic (10)	2.5	3	11.73 $\pm$ 1.57	2.78 $\pm$ 0.16	5.17 $\pm$ 0.78	171.57 $\pm$ 13.47	32.85 $\pm$ 0.81
	3.0	6	12.77 $\pm$ 0.49	2.00 $\pm$ 0.22	3.91 $\pm$ 0.12	170.15 $\pm$ 11.40	32.49 $\pm$ 1.08
	3.5	1	12.89 $\pm$ 0.00	2.40 $\pm$ 0.00	4.42 $\pm$ 0.00	161.23 $\pm$ 00.00	25.65 $\pm$ 0.00
Repeat Breeder (20)	2.5	2	14.14 $\pm$ 1.79	2.24 $\pm$ 0.25	4.01 $\pm$ 0.07	144.08 $\pm$ 12.73	25.21 $\pm$ 0.64
	3.0	5	13.89 $\pm$ 1.08	1.79 $\pm$ 0.15	4.13 $\pm$ 0.30	137.01 $\pm$ 20.90	23.81 $\pm$ 1.45
	3.5	13	16.00 $\pm$ 0.80	1.29 $\pm$ 0.16	4.33 $\pm$ 0.25	135.51 $\pm$ 13.28	24.30 $\pm$ 0.91
Induced Estrus Cows (20)	2.5	6	13.61 $\pm$ 0.63	2.34 $\pm$ 0.52	4.31 $\pm$ 0.35	183.01 $\pm$ 13.99	34.14 $\pm$ 2.10
	3.0	11	13.91 $\pm$ 0.42	2.31 $\pm$ 0.36	4.10 $\pm$ 0.14	170.51 $\pm$ 07.02	33.40 $\pm$ 0.92
	3.5	3	14.50 $\pm$ 0.20	2.09 $\pm$ 0.37	4.18 $\pm$ 0.15	168.97 $\pm$ 26.87	32.69 $\pm$ 3.69

The figures in parenthesis indicate number of animals.

of animals, though the differences within the group were non-significant, perhaps due to varying and limited number of animals under each score (Table 2).

### Magnesium Concentration

The magnesium concentration in CVM of normal cyclic cows was slight higher than that in repeat breeders and induced estrus cows. It was also non-significantly higher in CVM of conceived than non-conceived cows (4.28  $\pm$  0.14 vs 4.18  $\pm$  0.15 mEq/dL). Further, as with fertility status, magnesium levels of CVM did not reveal any specific trend with BCS of cows in any of the categories studied (Table 1 and 2). In contrast to our findings, Wani *et al.* (1979) reported a significantly higher level of magnesium in infertile/repeat breeders than in normal cyclic cows. Similarly, Tsiligianni *et al.* (2002) noted significantly higher magnesium in CVM of cows with induced estrus than spontaneous estrus, the difference in the present study was, however, non-significant, while Bernardi *et al.* (2018) recorded significantly higher magnesium in CVM of spontaneous estrus as compared to hormonally induced estrus HF heifers. Vadodaria (1987) and Bernardi *et al.* (2018) further recorded significantly higher magnesium in non-conceived than conceived buffaloes and HF heifers, respectively, while Panchal *et al.* (1994) found a reverse trend in conceiving and non-conceiving repeat breeding buffaloes.

### Sodium-Potassium Concentration

Both the sodium and potassium concentrations were significantly ( $p < 0.01$ ) lower in the estrual CVM of repeat breeding cows (140.75  $\pm$  10.35 and 24.27  $\pm$  0.68 mEq/L, respectively) as compared to normal cyclic and induced estrus cows. Further, the levels of both the elements were higher in conceived than non-conceived cows with significant differences in overall means (181.01  $\pm$  4.85 vs 130.61  $\pm$  7.66 mEq/L and 32.42  $\pm$  0.81 vs 25.17  $\pm$  0.94 mEq/L) and also in repeat breeders as well as induced estrus groups (Table 1). Thus, the ratio of Na:K remained more or less constant in CVM of all the categories of cows, irrespective of their reproductive

status. Hence, it can be inferred that absolute lower levels of sodium and potassium could be the cause of reproductive failure in repeat breeders rather than their ratio. Previous researchers (Wani *et al.* 1979; Enkhia *et al.* 1983; Sharma and Tripathi, 1987) also reported higher levels of sodium and potassium in the cervical mucus of normal cows as compared to repeat breeder cows. However, Siddiquee (2006) did not observe any difference in potassium concentrations of mucus samples obtained from normal and repeat breeder crossbred cows. Similarly, Vadodaria (1987), Panchal *et al.* (1994), and Bennur *et al.* (2004) reported slightly higher concentrations of sodium and potassium in the estrual CVM of conceived cows as compared to non-conceived cows and buffaloes. However, in a recent study, sodium in CVM was reported to be significantly higher and potassium lower in pregnant than empty HF heifers, both at spontaneous and induced estrus (Bernardi *et al.* 2018). Furthermore, Tsiligianni *et al.* (2002) and Bernardi *et al.* (2018) observed significantly higher levels of both sodium and potassium in CVM of cows with induced estrus than spontaneous estrus, which, however, did not differ in our study.

Moreover, like inorganic phosphorus, the levels of both sodium and potassium in CVM showed a trend of gradual and insignificant decline with increase in the BCS from 2.5 to 3.5 in all three categories of animals (Table 2). The rheological properties of cervical mucus appears to be determined primarily by the nature of its macro-molecules, and their magnitude is regulated by the concentration of salts, of which NaCl forms the major salt which greatly influences the ionic strength of mucus. Together with K<sup>+</sup>, it is responsible for the crystallization phenomenon in cervical mucus (Panchal *et al.* 1994; Verma *et al.* 2014; Bernardi *et al.* 2016). Thus in general, it can be inferred that significantly altered Ca:P ratio and absolute lower levels of sodium and potassium in CVM could be responsible for conception failure and repeat breeding in dairy cows.

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