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Association of different biochemicals in the estrual genital discharge with pregnancy in Jersey crossbred cows

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ABSTRACT

The present study was conducted in 140 estrous periods in Jersey crossbred cows (122 in 111 normal (N) and 18 in 10 repeat breeders (RB) cows) that were inseminated. The N cows that conceived were classified to be in fertile estrus (FE); the others as non-fertile estrus (NFE) respectively. The N cows were investigated during estrus for body condition score, milk yield, extent of uterine tone, physical properties of genital discharge (color, viscosity and spinbarkait) and uterine tone. In addition, glucose (26 estrous periods), Na, K, Ca, Zn, Fe, Cu, Mg (96 estrus periods), P and total protein (122 estrus periods) were also estimated in the pre AI estrual genital discharge, in the N cows. Except for glucose, all other biochemicals were also estimated in the RB cows. Higher Zn in the FE versus NFE (1.5±0.2 versus 0.8±0.05 mg/dl) and tendency for a higher K in the FE/NFE versus RB (48.0±4.0/46.0±2.7 mg/dl versus 37.4±3.1 mg/dl) and higher Ca in the FE versus RB (5.7±0.4 versus 4.0±0.5 mg/dl) cows were the salient features of the cows getting pregnant. Biochemical analysis of the genital discharge during 20 recurrent estrus periods revealed consistently higher Mg in the 9 FE (13.2±2.3 mg/dl) than the immediately preceding 9 NFE (5.8±0.9 mg/dl) periods in the N cows, whereas, the remaining 1 cow had identical Mg (3.8 mg/dl) during the non-fertile and fertile estrus. A similarity in body condition, milk yield, genital discharge and uterine tone at the time of AI between different groups of cows adds weight to the current findings. The correlation matrix between different biochemicals revealed that in spite of established antagonism in blood; some mineral combinations, more so in the FE cows, exhibited a positive correlation.

Key words: Biochemicals, Cows, Fertility, Genital discharge

Reducing the services per conception is the ultimate motive of a sustainable cattle dairy entrepreneurship (Hoque *et al.* 2002). Availability of fertile semen and its judicious use eliminates the role of males in regulating fertility, which underscores a better understanding of lesser investigated diverse cow related factors to optimize pregnancy. Relationship between reproduction and biochemical composition of some minerals in cervical mucus has provided vital information on regulation of fertility (Sood *et al.* 2000). Deficiency or excess of certain biochemical constituents in the genital discharge can be lethal to gametes (Stegmayr and Ronquist 1982). However, a comprehensive description delineating the association of a wide variety of biochemical components in the estrual genital discharge with pregnancy in the Jersey crossbred cows is lacking, which was therefore, the intent of present study.

MATERIALS AND METHODS

The Jersey crossbred cows (exotic inheritance of 75%) in the study belonged to the University Dairy Farm. The cows were maintained on standard nutritional and management practices and had crossed the voluntary waiting period of 60 d. Estrus was detected visually (Sood and Nanda 2006). A total of 140 estrous periods [122 in 111 normal (N) cows and 18 in 10 repeat breeders (RB)] covered with AI using frozen thawed semen, were studied. The N cows conceiving to AI were classified as FE and the others as NFE, respectively.

Body condition score (BCS) (Edmondson *et al.* 1989), milk yield, extent of uterine tone (good/moderate/weak) and physical properties of the genital discharge (colour, viscosity and spinnbarkait) (Sood *et al.* 2009) expelled out of vulva during the course of transrectal examination, were ascertained in the N cows. In all, but 10 N cows, the discharge had to be

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aspirated in a sterile AI sheath with the help of a syringe; such discharge was considered non-copious. For biochemical investigations, the sample of genital discharge was collected aseptically from mid-cervix/vaginal fornix in a sterile AI sheath before AI in the N and the RB cows.

The estimation of biochemicals included glucose (n= 26 estrous periods in 26 N cows), Na, K, Ca, Zn, Fe, Cu, Mg (96 estrous periods in 85 N cows), P and total protein (TP) (in 122 estrous periods in 111 N cows). Except for glucose, all the aforementioned biochemicals were also estimated in the RB cows. Glucose estimation was carried out within 1h of sample collection, whereas the remaining biochemicals were estimated after thawing of the samples stored at –20°C.

Glucose, P and TP were estimated using standard kits in spectrophotometer, Na and K were estimated in a flame photometer, whereas Mg, Cu, Fe, Zn and Ca were analyzed in atomic absorption spectrophotometer. All the inseminated cows were examined for pregnancy after 60 d of AI by transrectal examination of genitalia and analysed retrospectively with the biochemical composition.

Statistical analysis: The observations in average and percentage were compared for significance using Student's *t*–test and chi-square analysis, respectively. The Pearson's correlation coefficient between the biochemical constituents was also determined in FE and NFE cows. For statistical significance between 2 values, a difference of $P<0.05$ (at least) was considered significant, whereas $P<0.10$ was considered as a tendency for difference. The entire statistical analysis was performed using software package for social sciences (SPSS version 16.0).

RESULTS AND DISCUSSION

It is intriguing to ascertain the cause of repeat breeding in cows without any palpable genital lesions, purulent discharge and abnormalities of estrous cycle. Diverse cow related factors are required to be understood to harness the single ovum. In present study, different biochemicals in the estrual genital discharge in face of pregnancy outcome were investigated in normal and repeat breeder cows.

The overall PR in the N cows in the present study was 43%. The average BCS and milk yield did not differ in the FE versus NFE cows. The BCS (Starbuck *et al.* 2004) and high milk yield (Tenhagen *et al.* 2000) reduce pregnancy in cows. Also, the genital discharge was clear and stringy in the entire N cows, whereas it was copious in all, but 10 estrus periods. Clear, copious and stringy genital discharge at estrus is essential for viability, transport and survival of spermatozoa initially and embryos later (Sreenan and Behran 1974). The uterine tone during estrus was moderate and good, respectively, in 41 and 81 estrus periods, with corresponding pregnancy rates of 42.0% and 44.0%, respectively. This corroborates to study of Singh *et al.* (2008).

Biochemical constituents in genital discharge: The average composition of different biochemicals in the genital discharge in the FE versus NFE versus RB cows is presented in Table 1. Although glucose (Wani *et al.* 1979), Na (Roberts 1982), Fe (Sidhu and Guraya 1985), Cu (Barnea *et al.* 1985), Mg (Salisbury *et al.* 1978), P (Pugh *et al.* 1985) and total protein (Wiebold 1988) in genital discharge affect reproduction in dairy cows, but their similar concentrations among different groups exclude their influence on pregnancy in the current study.

Table 1. Comparison of average (mean±SEM) concentration of certain biochemical constituents of the estrual genital discharge between fertile (FE), non-fertile (NFE) and repeat breeder (RB) cows

Biochemical (mg/dl)	Status of the cow at estrus/insemination		
	Normal		Repeat breeder
	FE	NFE	RB
Glucose	15.5±1.2 (11)	16.6±2.0 (15)	-
Na	229.5±11.7 (41)	242.0±11.0 (55)	228.2±15 (18)
K	48.0±4.0 ^{a} (41)	46.0±2.7 ^{a} (55)	37.4±3.1 ^{b} (18)
Ca	5.7±0.4 ^{a} (41)	5.3±0.5 (55)	4.0±0.5 ^{b} (18)
Zn	1.5±0.2 ^{c} (41)	0.8±0.05 ^{d} (55)	1.1±0.2 (18)
Fe	2.4±0.4 (41)	2.1±0.3 (55)	2.7±0.6 (18)
Cu	0.05±0.01 (41)	0.05±0.01 (55)	0.05±0.01 (18)
Mg	1.6±0.4 (41)	1.2±0.1 (55)	0.9±0.2 (18)
P	2.2±0.1 (52)	2.2±0.1 (70)	2.0±0.6 (18)
TP	1500.0±30.0 (52)	1600.0±30.0 (70)	1400.0±20.0 (18)

The values in parenthesis indicate the number of estrus periods. $^{a,b;\ c,d}$ values with different superscripts within same row vary at $P<0.10$ and $P<0.01$, respectively.

Potassium: The K concentration in the FE and NFE tended to be higher than the RB cows. This finding corroborates to marginally higher K concentration of 27.6±2.2 mg/dl in normal than 23.2±1.6 mg/dl in repeat breeder cows, respectively (Sharma and Tripathi 1989). However, similar K concentrations of 46.8±1.1 mg/dl in normal and 48.4±1.5 mg/dl in repeaters, is also on record (Wani *et al.* 1979). Potassium is the major intracellular cation and plays a very important role in the crystallization pattern of genital discharge (Roland 1962).

Calcium: The Ca content in the N cows corroborates to a previous finding of 5.0±0.3 mg/dl during estrus (Sood *et al.* 1999). Few other studies have reported Ca concentration in the estrual discharge to range from 7.6±2.1 mg/dl to 8.7±2.8 mg/dl during estrus (Sharma and Tripathi 1989, Bhosrekar *et al.* 1995). Finding higher Ca in the N than the RB cows in present study is in line with significantly higher Ca in the conceived *versus* the non-conceived cows (Sood *et al.* 2000). Extremely high Ca of 16.8±1.4 mg/dl in repeat breeders compared to 9.4±1.6 mg/dl in the normal cows, was considered a cause of repeat breeding (Wani *et al.* 1979). Hence, marginally higher Ca favour conception and extremely high concentrations are detrimental *per se* (El-Naggar and Baksai-Horvath 1971). Optimal Ca in genital discharge maintains the selective permeability of cell membrane (Banerjee 2008), stimulates glycolysis thereby sustaining the viability, motility and metabolism of sperms (Sidhu and Guraya 1985).

Zinc: The average Zn concentration was significantly higher in the FE than the NFE cows. Importantly, Zn concentration in the genital discharge of cows has not been reported previously. However, higher Zn concentration was reported in day 7 uterine flushing of cows with abnormal than normal embryos (Wiebold 1988); this was interpreted as a consequence to unutilized Zn by the abnormal embryos.

Zn affects the membrane stability, mechanical properties of accessory fibers, tail morphology and sperm motility (Hidiroglou and Knipfel 1984). In cows, Zn regulates the pituitary gonadotropins and Zn dependent "N-terminal zinc-fingers" in the follicular and luteal tissues that control ovarian steroidogenesis. The zinc-fingers mediate specific binding to target DNA sequences, termed ligand-responsive elements. In the absence of ligand, steroid hormone receptors are thought to be weakly associated with nuclear components (Apagar 1985). The peripheral Zn concentration was higher in cows with normal ovulation than those with anovulation (Das *et al.* 2009). The estimation of Zn in plasma could have discriminated the systemic versus localized effect of Zn vis-a-vis pregnancy rate in present study. However, the timing of low Zn in the estrual genital discharge of NFE cows during estrus remains a novel finding of present study.

Magnesium: For 20 recurrent estrus periods, it was only Mg which exhibited consistently higher concentrations for 9 FE periods than the immediately preceding 9 NFE periods. The remaining 1 cow, however, had similar Mg concentration during the FE and NFE period. The higher Mg could have benefitted pregnancy due to Mg stimulated spermatozoal motility (Wani *et al.* 1979) by acting as a catalyst in sperm glycolysis (Salisbury *et al.* 1978).

Correlation between different biochemicals in the FE and NFE cows: The correlation between different biochemicals is presented in Table 2. All the biochemicals in the FE and majority in the NFE, were positively correlated. However, P with Fe and Zn with Na, K, Ca, Fe, P and TP shared a negative correlation in the NFE cows.

It is established that certain minerals are antagonistic and therefore logically share a negative correlation. In the perspective of present study, P with Fe, Na with K and Zn with Cu, P, Ca and Fe, are recognized antagonists (Banerjee 2008) and therefore should possess a negative correlation. Instead, all the aforementioned mineral combinations shared a positive correlation in the FE cows. Hence, more number of positive correlations amongst minerals in the FE cows, considered to be more favourable for the pregnancy, as recorded hitherto, indicates that the biochemical constituents in the genital discharge are not only the product of simple diffusion from blood, but also a result of localized secretion from the genital tissues. In confirmation, out of 96 estrus

Table 2. Pearson correlation coefficients between different biochemical constituents in the FE and the NFE cows

Biochemical	Na		K		Ca		Zn		Fe		Cu		Mg		P		TP	
	FE	NFE	FE	NFE	FE	NFE	FE	NFE	FE	NFE	FE	NFE	FE	NFE	FE	NFE	FE	NFE
Na	1.00	1.00	0.96***	0.97***	0.88***	0.32*	0.89***	–0.07	0.90***	0.08	0.37*	0.34**	0.50**	0.54***	0.27	0.26	0.97***	0.99***
K			1.00	1.00	0.91***	0.34**	0.93***	–0.10	0.97***	0.12	0.31*	0.35**	0.53**	0.55***	0.27	0.25	0.90***	0.98***
Ca					1.00	1.00	0.95***	–0.10	0.88***	0.04	0.37*	0.10	0.34*	0.12	0.21	0.15	0.83***	0.36**
Zn							1.00	1.00	0.96***	–0.04	0.33*	0.01	0.52**	0.01	0.15	–0.13	0.81***	–0.08
Fe									1.00	1.00	0.26	0.20	0.53*	0.17	0.17	–0.05	0.82***	0.11
Cu											1.00	1.00	0.18	0.70***	0.12	0.27*	0.38*	0.35**
Mg													1.00	1.00	0.25	0.37**	0.47**	0.54***
P															1.00	1.00	0.29	0.27*
TP																	1.00	1.00

*, **, *** indicate a significant association between 2 variables across the corresponding row and column, respectively, at P<0.05, P<0.01 and P<0.001.

periods in the N cows, concentration of K, Na, Mg and Ca in the genital discharge exceeded the upper concentration limits in blood (Kaneko 1989) in 92, 10, 8 and 3 estrous periods, respectively.

In conclusion, recording of relatively higher Zn, K, Ca and Mg in the estrual genital discharge of the cows getting pregnant suggested modulation of dietary regimens so that optimal concentrations of all the aforementioned minerals in the genital discharge circumvent reproductive wastage in cows.

REFERENCES

Apagar J. 1985. Zinc and reproduction. *Annual Review of Nutrition* **5**: 43–49.

Banerjee G C. 2008. Animal nutrition. *A Textbook of Animal Husbandry*. 8th edn, pp. 491. Oxford and IBH Publishing Company Private Limited, New Delhi.

Barnea A, Cho G and Colombani-Vidal M. 1985. A role for extracellular copper in modulating prostaglandin E_2 (PGE_2) action: facilitation of PGE_2 stimulation of the release of gonadotropin-releasing hormone (GnRH) from median eminence explants. *Endocrinology* **117**: 415–21.

Bhosrekar M R, Phadnis Y P, Mokashi S P and Mangurkar B R. 1995. Biochemical composition of cervical mucus and arborisation patterns of oestrus synchronised cattle and buffaloes. *Indian Journal of Animal Sciences* **65**: 887–88.

Das J M, Dutta P, Biswas R K, Sarmah B C and Dhali A. 2009. Comparative study on serum macro and micro mineral profiles during oestrus in repeat breeding crossbred cattle with impaired and normal ovulation. http: //www.lrrd.org/lrrd21/5/das21072.htm.

Edmondson A J, Lean I J, Weaver L D, Ferver T and Webster G. 1989. A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* **72**: 68–78.

El-Naggar M A and Baksai-Horvath E. 1971. Biochemical changes in the cervico-vaginal mucus of infertile cows. *Acta Veterinaria Academiae Scientiarum, Hungaricae* **22**: 31–37.

Hidiroglou M and Knipfel J E. 1984. Zinc in mammalian sperm: a review. *Journal of Dairy Science* **67**: 1147–59.

Hoque M A, Baik D H, Hussen M S and Rahman M S. 2002. Effect of breeding groups and environment on conception in Pabna and its Crossbred cows in Bangladesh. *Pakistan Journal of Biological Science* **5**: 612–15.

Kaneko J J. 1989. *Clinical Biochemistry of Domestic Animals*. 4th edn. Academic Press, Incorporation, San Diego, USA.

Pugh D G, Elmore R G and Hembree T R. 1985. A review of the relationship between mineral nutrition and reproduction in cattle. *Bovine Practice* **20**: 10–18.

Roberts S J. 1982. Physiology of female reproduction. *Veterinary Obstetrics and Genital Diseases*. 2nd edn, pp. 366. CBS Publishers and Distributors, New Delhi.

Roland M. 1962. The office application of the fern test. *Clinical Obstetrics and Gynaecology* **5**: 218–34.

Salisbury G W, Denmark N L V and Lodge J R. 1978. Metabolism of bull spermatozoa. *Physiology of Reproduction and Artificial Insemination of Cattle*. 2nd edn, pp.351. CBS Publishers and Distributors, Delhi.

Sharma V K and Tripathi S S. 1989. Biochemical studies on oestral mucus of cows. *Indian Journal of Animal Sciences* **59**: 1532–33.

Sidhu K S and Guraya S S. 1985. *Buffalo Bull Semen*. 1st edn, pp. 131. USG Publishers and Distributors, Ludhiana.

Singh N, Nanda A S and Singh J. 2008. Assessment of factors affecting conception rate in crossbred cows following artificial insemination under field conditions. *Indian Journal of Animal Reproduction* **29**: 211–15.

Sood P and Nanda A S. 2006. Effect of lameness on estrous behavior in crossbred cows. *Theriogenology* **66**: 1375–80.

Sood P, Singh M M, Vasishta N K and Nigam J M. 1999. Studies on some biochemical attributes of cervical mucus and blood serum in cows of Himachal. *Indian Journal of Animal Reproduction* **20**: 28–30.

Sood P, Vasishta N K, Singh M and Pathania N. 2009. Prevalence and certain characteristics of mid-cycle estrus in crossbred cows. *Veterinarski Arhiv* **79**: 143–49.

Sood P, Vasishta N K, Singh M M and Nigam J M. 2000. Relationship of certain biochemical attributes in cervical mucus with conception rate in cows. *Indian Journal of Animal Reproduction* **21**: 57–58.

Sreenan J and Behran D. 1974. Egg transfer in the cow: pregnancy rate and egg survival. *Journal of Reproduction and Fertility* **41**: 497–99.

Starbuck M J, Dailey R A and Inskeep E K. 2004. Factors affecting retention of early pregnancy in dairy cattle. *Animal Reproduction Science* **84**: 27–39.

Stegmayr B and Ronquist G. 1982. Stimulation of sperm motility by organelles in human seminal plasma. *Scandinavian Journal of Urology and Nephrology* **16**: 85–89.

Tenhagen B A, Drillich M and Heuwieser W. 2000. Analysis of cow factors influencing conception rates after two timed breeding protocols. *Theriogenology* **56**: 831–38.

Wani G M, Tripathi S S and Saxena V B. 1979. Studies on biochemical attributes of cervical mucus in normal and repeat-breeding crossbred cows. *Indian Journal of Animal Sciences* **49**: 1034–38.

Wiebold J L. 1988. Embryonic mortality and the uterine environment in first-service lactating dairy cows. *Journal of Reproduction and Fertility* **84**: 393–99.