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## Correlation of rheological properties of cervico: Vaginal with vaginal electrical impedance and fertility in repeat breeding crossbred cows

**Amit Kumar, Sudarshan Kumar, Madhu Shivhare, Ranjit Aich, Vinita and Shailendra**

**Abstract**

The present study was conducted on 36 crossbred cows with history of repeat insemination at different intervals, randomly categorized in infectious and non-infectious groups on basis of positive or negative reaction to Whiteside test belonging to the Dairy farm of College of Veterinary Science and Animal Husbandry, Mhow and clinical cases of progressive farmers brought for artificial insemination to Teaching Veterinary Clinical Complex and at the doorstep of farmers in nearby villages. These selected animals divided into 6 groups viz. GnRH analogue (Gp I), GnRH + HCG (Gp II), HCG alone (Gp III), HCG + GnRH (Gp IV), Progesterone (Gp V) and Control Group (Gp VI). The cervico-vaginal mucus samples were collected from the animals at oestrus and were immediately used for physical parameter analysis. The difference in spinnbarkeit values between hormonal treated repeat breeding and placebo treated repeat breeding cross bred cows were observed to be highly significant ( $P < 0.01$ ). The overall mean pH value found in the non-pregnant cows was  $8.72 \pm 0.22$  and the difference was statistically non-significant. The overall mean values of vaginal electrical impedance (ohms) of cervico-vaginal mucus in overall conceived crossbred cows were highly significant ( $P < 0.01$ ) at 0, 12<sup>th</sup> and 45<sup>th</sup> day, whilst in all non-conceived groups, it was highly significant ( $P < 0.01$ ) at day 0 and 12<sup>th</sup> day and non-significant at day 45<sup>th</sup>.

**Keywords:** Rheological, white side test, spinnbarkeit value, vaginal electrical impedance, repeat breeding

**Introduction**

The cattle population in our country is estimated to be approximately 190.9 million that include 151.2 million (79%) indigenous and 39.7 million (21%) crossbred and exotic cattle population (Annual Report, 2012). Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980) [2].

Repeat breeding is one of the major gynaecological problem and an important cause of infertility in cattle that results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers (Bhatt and Bhattacharyya, 2012). The cause of repeat breeding may originate either during early stages of follicular maturation and / or during preovulatory period. Oestrus, the most visible phase of the oestrous cycle is characterized by nervousness, bellowing and mounting, stands to be mounted by another cow, reduced feed intake and milk production. Vaginal Electrical Impedance (VEI) is used to detect oestrus and to determine the timing of ovulation in cattle, buffalo, horse, sheep, goat and pig. The principle behind VEI measurement is to measure changes in the ionic balance of vaginal mucosa (Bowers *et al.*, 2006).

The rheological properties of cervical mucus like pH, spinnbarkeit value and arborisation pattern are important properties in relation to fertility. The degree of mucus crystallization/arborisation pattern in cervical mucus is under the control of two ovarian steroid hormones (Verma *et al.*, 2014) [6].

**Materials and Methods**

The proposed work was carried out on 36 clinical cases brought to the AI (Artificial Insemination) center of Department of Veterinary Gynaecology & Obstetrics, College of

Veterinary Science and Animal Husbandry, Mhow and in nearby villages of Mhow, these animals are divided on 6 groups Gp I, Gp II, Gp III, GP IV, GP V and GP VI. The cervico – vaginal mucus samples were collected from the animals at oestrus and were immediately used for physical parameter analysis. Based on physical appearance, the estrual cervico-vaginal mucus was categorized as purulent, much-purulent and clear.

Spinnbarkeit value measured by take 2-3 drops of collected mucus sample were taken on a grease free glass slide and then another grease free glass slide was placed over it. The mucus was stretched between two slides by moving the second slide away from first one, until the mucus breaks. The distance between the two slides just before the breakage of the mucus string was measured by a scale (cm scale) mounted on the wall known as spinnbarkeit value (Sharma *et al.*, 2013) [7].

For fern pattern take Two to three drops of well mixed cervical mucus were spread uniformly over grease free glass slide and air dried. The air dried slide was examined under microscope using low power objective (10X) for crystallization pattern of the mucus known as fern pattern. The fern pattern of the observed mucus was grouped into three classes, viz., typical, atypical and nil as described by Luktuke and Roy (1967) [8].

- a) Typical: Fern pattern with primary, secondary and tertiary branches.
- b) Atypical: Fern pattern with primary and secondary branches.
- c) Nil: Fern pattern with no primary, secondary and tertiary branches.

The pH of cervical mucus was measured with help of pH paper. The Data analysis was done as per the standard statistical method by application of Factorial Completely Randomized Design (Snedecor and Cochran, 1994) [9].

**Result**

The spinnbarkeit value of oestrual cervico-vaginal mucus of

crossbred cows was measured in centimeter as described by Verma *et al.* (2014) [6] in the present study. Three or four consecutive readings were taken for each sample and the mean reading was considered as spinnbarkeit value of the sample.

On analysis of the cervico-vaginal mucus of normal and non-infectious repeat breeding cows, the elasticity (spinnbarkeit value) of cervico-vaginal mucus ranged from 7.19 to 20.76 cm in all groups. The mean spinnbarkeit value found in the placebo + placebo treated crossbred cows was 18.10 ± 0.66 cm while in group GnRH +GnRH, GnRH +hCG, hCG + hCG, hCG + GnRH and Inj. Progesterone treated repeat breeding crossbred cows, it was 12.28 ± 0.76, 12.23 ± 1.04, 12.48 ± 1.25, 12.34 ± 1.28 and 11.76 ± 1.55 cm, respectively (Table 1). The overall mean spinnbarkeit value found in repeat breeding cows was 13.19±0.90 cm (Table 1). The difference in spinnbarkeit values between hormonal treated repeat breeding and placebo treated repeat breeding cross bred cows were observed to be highly significant (P<0.01). The animals that subsequently became pregnant had significant marginal higher (P<0.01) mean spinnbarkeit value of 14.26 ± 0.72\*cm as against the non-pregnant where it was 11.25 ± 0.36 cm.

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**Table 1:** Spinnbarkeit values and pH values

S. No	Groups	Parameters (Mean ± SE)	
		Spinnbarkeit value (cm)	pH
1	GnRH +GnRH (n=6)	12.28 ± 0.76 <sup>b</sup>	8.58 ± 0.31
2	GnRH +hcg (n=6)	12.23 ± 1.04 <sup>b</sup>	8.48 ± 0.24
3	hCG +hCG (n=6)	12.48 ± 1.25 <sup>b</sup>	8.32 ± 0.26
4	hCG+GnRH (n=6)	12.34 ± 1.28 <sup>b</sup>	8.52 ± 0.20
5	Inj. Progesterone (P4)	11.76 ± 1.55 <sup>b</sup>	8.64 ± 0.28
6	Plasebo +Plasebo	18.10 ± 0.66 <sup>a</sup>	8.50 ± 0.24

Values bearing uncommon superscripts within the column differ significantly (P<0.01).

**Arborization pattern / Fern pattern**

Fern pattern of cervico-vaginal mucus was classified as typical, atypical and nil in the present study.

Overall fern pattern of cervico-vaginal mucus observed was typical in 77.77 per cent and atypical in 22.22 per cent cows at estrus. Nil type fern pattern was not observed. The fern pattern was correlated with conception rate. It was found that the conception rate was 57.14 per cent in typical and 50.00 per cent in atypical type of fern pattern. The results have been presented in table 2.

**Table 2:** Distribution of cows in relation to fern pattern of cervico-vaginal mucus and conception rate

Fern pattern		Conception rate
Types	Per cent	Per cent
Typical	77.77 (28)	57.14 (16)
Atypical	22.22 (8)	50.00 (4)
Nil	0.0	0.0

Figure in parenthesis indicate number of cows.

As evident from table 3 typical fern pattern was observed in 66.66, 50.00, 50.00, 66.66, 50.00, and 66.66 per cent cows belonging to group GnRH +GnRH, GnRH +hCG, hCG+hCG, hCG+ GnRH, Inj. Progesterone (P<sub>4</sub>) and Placebo +Placebo, respectively, with conception rate of 50.00, 66.66, 66.66, 50.00, 66.66, and 50.00 per cent, respectively. Atypical fern pattern (Table 3) was observed in 33.33, 50.00, 50.00, 33.33, 50.00 and 33.33 per cent crossbred cows belonging to group GnRH + GnRH, GnRH + hCG, hCG + hCG, hCG + GnRH, Inj. Progesterone (P<sub>4</sub>) and Placebo +Placebo,

respectively, with conception rate of 50.00, 33.33, 0.0, 50.00, 33.33 and 50.00 per cent, respectively. Nil type fern pattern was not observed in crossbred cows of all groups. These findings indicated that on the day of oestrus each sample of cervical secretions exhibited the fern pattern.

Fern pattern of the cervico-vaginal mucus was judged as typical in 72.77 per cent, atypical in 27.22 per cent and nil type in 0.0 per cent cows with 57.14 per cent, 50.00 per cent and 0.0 per cent conception rate, respectively. Nil type fern pattern was not observed.

**Table 3:** Cervico-vaginal mucus fern pattern and conception rate (%)

Groups	No. of cows	Typical	Conception rate	Atypical	Conception rate	Nil	Conception rate
GnRH +GnRH	6	66.66 (4)	50.00 (2)	33.33 (2)	50.00 (1)	0.0	0.0
GnRH +hCG	6	50.00M (3)	66.66 (2)	50.00 (3)	33.33 (1)	0.0	0.0
hCG +hCG	6	50.00 (3)	66.66 (2)	50.00 (3)	0.0 (0)	0.0	0.0
hCG+GnRH	6	66.66 (4)	50.00 (2)	33.33 (2)	50.00 (1)	0.0	0.0
Inj. Progesterone (P <sub>4</sub> )	6	50.00 (3)	66.66 (2)	50.00 (3)	33.33 (1)	0.0	0.0
Placebo+placebo	6	66.66 (4)	50.00 (2)	33.33 (2)	50.00 (1)	0.0	0.0

Figure in parenthesis indicate number of cows.

**Table 4:** Spinnbarkeit and pH value of estrual cervico-vaginal mucus of conceived and non-conceived crossbred cows

Groups	Parameters (Mean ± SE)	
	Spinnbarkeit value (cm)	pH
Conceived cows	14.26 ± 0.72*	8.42 ± 0.09
Non conceived cows	11.25 ± 0.36	8.72 ± 0.22

\* Significant ( $P < 0.05$ )

### pH

On analysis of the cervico-vaginal mucus of normal and non-infectious repeat breeding crossbred cows, the pH of cervico-vaginal mucus ranged from 8.00 to 9.00. The mean pH value found in Placebo +Placebo treated crossbred cows was  $8.50 \pm 0.24$  while in group GnRH +GnRH, GnRH +hCG, hCG + hCG, hCG +GnRH, Inj. Progesterone (P<sub>4</sub>) repeat breeding crossbred cows, it was  $8.58 \pm 0.31$ ,  $8.48 \pm 0.24$ ,  $8.32 \pm 0.26$ ,  $8.52 \pm 0.20$  and  $8.64 \pm 0.28$ , respectively. The overall mean pH value found in repeat breeding crossbred cows was  $8.50 \pm 0.04$ . The difference in pH values between hormonal treated and Placebo +placebo treated repeat breeding crossbred cows were observed to be non-significant ( $P > 0.05$ ). The overall mean pH value found in the pregnant cows was  $8.42 \pm 0.09$  while in non-pregnant cows, it was  $8.72 \pm 0.22$ . The difference was statistically non-significant (Table 4).

The pH of cervico-vaginal mucus ranged from 8.00 to 9.00. The mean pH value found in Placebo +Placebo treated crossbred cows was  $8.50 \pm 0.24$  while in group GnRH + GnRH, GnRH +hCG, hCG+hCG, hCG +GnRH, Inj. Progesterone (P<sub>4</sub>) repeat breeding crossbred cows, it was  $8.58 \pm 0.31$ ,  $8.48 \pm 0.24$ ,  $8.32 \pm 0.26$ ,  $8.52 \pm 0.20$  and  $8.64 \pm 0.28$ , respectively. The overall mean pH value found in repeat breeding crossbred cows was  $8.50 \pm 0.04$ .

## Discussion

### Spinnbarkeit

The findings are in close accordance with Siddique (1980), Dodamani *et al.* (2010) [11] and Modi *et al.* (2011) [12]. The elasticity (spinnbarkeit value) of cervico-vaginal mucus ranged from 7.19 to 20.76 cm in all groups. The observations are in conformity with the earlier reports of Gavit (2010) and Zaman *et al.* (2013) [14] where the higher spinnbarkeit values were correlated with better conception rate in cows; however Bennur *et al.* (2004) [4] failed to notice any significant

difference in the spinnbarkeit value of estrual mucus in fertile and infertile cows.

### Fern Pattern

The findings indicated that on the day of oestrus each sample of cervical secretions exhibited the fern pattern. The finding clearly indicates that animals subsequently became pregnant exhibited considerably higher percentage of typical fern pattern. These findings are in line with the observations made by Dodamani and Honnappagol (2004) [15] in Deoni cows. On contrary Bennur *et al.* (2004) [4] did not notice any difference in fern pattern of estrual cervico-vaginal mucus in the pregnant and non-pregnant animals.

Typical fern pattern is said to be indicative of ovulatory heat, whereas, in silent or weak estrus, atypical pattern is observed (Galhotra *et al.*, 1971) [16]. Majority of fertile oestrus had typical fern pattern of cervical mucus and majority of non-fertile oestrus had atypical fern pattern of cervical mucus (Ragenekar *et al.*, 2002).

pH The observation was recorded by Dodamani *et al.* (2004). Values observed by Modi *et al.* (2011) [12] were slightly higher than the value recorded in the present study. The overall mean pH value found in the pregnant cows was  $8.42 \pm 0.09$  while in non-pregnant cows, it was  $8.72 \pm 0.22$ . The difference was statistically non-significant.

pH value is comparable to the findings of Bennur *et al.* (2004) [4]. Gavit (2010) found significantly higher mean pH value in conceived cows as compared to non-conceived cows. Zaman *et al.* (2013) [14] found lower value than the value recorded in the present study. The pH values did not depict any bearing on fertility thus confirming earlier report of Dodamani *et al.* (2010) [11]. Hafeez and Hafeez (2000) [18] stated that acidity or excessive alkalinity of cervical mucus reduces the sperm motility, thereby causing failure of fertilization. The 7.0 - 9.0 pH range of cervico-vaginal mucus was found to be satisfactory to maintain the progressive forward movement and the viability of spermatozoa (Pattabiraman *et al.*, 1967) [19].

### Conclusions

Based on the results it is concluded that the measurement of vaginal electrical impedance (VEI) could serve as an indicator of the follicular and luteal status in crossbred cows and daily

impedance measurements are necessary to confirm stage of the oestrous cycle, The overall mean spinnbarkeit value found in the crossbred cows ( $14.26 \pm 0.72$  cm) may be indicative of good conception rate, The overall fern pattern of cervico-vaginal mucus observed was typical in 77.77 (28) per cent and atypical in 22.22 (8) per cent in crossbred cows, to achieve good conception rate. Fern pattern was correlated with conception rate. It was found that the conception rate was 57.14 (16) per cent in typical and 50.00 (4) per cent in atypical types of fern pattern, respectively.

## References

1. Annual Report, Basic Animal Husbandry Statistics. Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India, New Delhi, India, 2012, 52-53.
2. Zemjanis R, Repeat breeding or conception failure in cattle. In: DA. Morrow (ed.) Current Therapy in Theriogenology, WB. Saunders, Publishing Co., Philadelphia, 1980, 205-213.
3. Bhatt FA, Bhattacharya HK, Oestrus duration and status of reproductive organs in repeat breeding cows. Iranian Journal of Applied Animal Science. 2012; 2(3):295-299.
4. Bennur PC, Honnappagol SS, Tandle MK, Effect of physico-chemical properties of cervico-vaginal mucus on fertility in cow, The Indian Veterinary Journal. 2004; 81(9):1069.
5. Bowers S, Gandy S, Graves K, Moore A, Willard S. Relationships between measurements of vaginal electrical impedance, uterine involution and hormonal profiles in postpartum dairy cows, Journal of Animal and Veterinary Advances. 2004; 5(7):552-558.
6. Verma KK, Prasad S, Kumaresan A, Mohanty TK, Layek SS, Patbandha TK *et al.* Characterization of physico-chemical properties of cervical mucus in relation to parity and conception rate in Murrah buffaloes, Veterinary World. 2014; 7(7):467-471.
7. Sharma V, Prasad S, Gupta HP, Studies on physical and rheological properties of cervico-vaginal mucus during early pregnancy in buffaloes (*Bubalus bubalis*). Veterinary world. 2013; 6(8):508-511.
8. Luktuke SN, Roy DJ, Studies on cervical mucus pattern in relation to fertility in bovine. Indian Journal of Veterinary Sciences and Animal Husbandry. 1967; 37:26-31.
9. Snedecor GW, Cochran WG, Statistical Methods, 7th Edn, Oxford and IBH Publishing Co, New Delhi. 1994, 312-317.
10. Siddique GM, Studies on cervico-vaginal mucus and exfoliate vaginal cytology of normal and repeat breeding crossbred cows. MV Sc. thesis (Gynaecology and Obstetrics), Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, 1980.
11. Dodamani MS, Mohteshamuddin K, Awati SD, Tandle, MK, Honnappagol SS. Evaluation of pre and post artificial insemination effect of GnRH hormone on conception of repeat breeder Deoni cows, Veterinary World. 2010; 3(5):209-211.
12. Modi LC, Suthar BN, Nakhasi HC, Sharma VK, Panchasara HH. Physical characteristics of oestrial cervical mucus and conception rate in repeat breeder Kankrej cattle. International Journal for Agro Veterinary and Medical Sciences. 2011; 5(4):416-423.
13. Gavitt SK. Studies on physico-biochemical characteristics of oestrial cervico-veginal mucus with reference to body condition score and fertility in rural crossbred cows. MV Sc. thesis (Gynaecology and Obstetrics), Anand Agricultural University, Anand, 2010.
14. Zaman MI, Sharma U, Kumar S, Kumar S. Studies on physical properties of cervical mucus of repeat breeding crossbred cows. Indian Journal of Animal Reproduction. 2013; 34(2):6-12.
15. Dodamani MS, Honnappagol SS. Therapeutic management of repeat breeder HF X JR cows employing busarelin acetate, Karnataka Journal of Agriculture Science. 2004; 17(2):306-308.
16. Galhotra AP, Tyagi RPR, Banerjee AK. Diagnostic Significance of arborization of cervical mucus in buffaloes and heifers. Haryana Agriculture University Journal of Research. 1971; 1: 97-104.
17. Rangnekar MN, Dhoble RL, Gacche MG, Ingawale MV, Sawale AG, Jadhav JM *et al.* Physical properties of oestrial mucus in repeat breeding crossbred (Holstein-Friesian) cows with reference to fertility. Indian Journal of Animal Sciences. 2002; 72(12):1122-1124.
18. Hafeez B, Hafeez ESE, Reproduction in Farm Animals. 7th Edition, Gopson papers Ltd., Noida. 2000, 26-27.
19. Pattabiraman SR, Venkataswamy V, Krishnamoorthy R. Physio-chemical properties of oestrial mucus of cows, Indian Veterinary Journal. 1967, 44:413.