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# Studies on Estrual Cervical Mucus of Repeat Breeding Cows with Special Reference to Ovulatory Disturbances and Genital Infection

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**ABSTRACT**

A total of 100 crossbred Jersey cows comprising of 80 repeat breeder presented for treatment and 20 clinically healthy (control group) animals presented for artificial Insemination at their first service within 60-90 days following calving were selected. Estrual cervical mucus were examined for physical properties and white side test (WST). Effects of ovulatory disturbances and non specific bacterial genital infection on physical properties of cervical mucus were also determined. The volume, colour, consistency, stringiness, fern pattern and spinnbarkeit values of cervical mucus were not affected by ovulatory disturbances, while, colour, consistency and pH showed significant ($p < 0.01$) changes due to non-specific bacterial genital infection. The results of WST showed significant ($p < 0.01$) positive correlation (0.484) with bacterial culture. It is concluded that ovulatory disturbances have no impact on altering physical characteristics of estrual cervical mucus; however, colour, consistency and pH of cervical mucus are greatly influenced by nonspecific bacterial genital infection in repeat breeding cattle.

**Keywords:** Physical properties, estrual cervical mucus, repeat breeding cow, ovulatory disturbances, bacterial infection.

Repeat breeding is one of the important causes 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 (Bhat and Bhattacharyya, 2012). Physical characteristics of cervico-vaginal mucus/estrual discharge have direct bearing on the fertility status of the animals (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 the estrual cervical mucus with the cyclic rhythm of the reproductive process (Hafez and Hafez, 2000) and hence such parameters need to be evaluated to predict infertility in bovines. Moreover these parameters may directly be influenced by ovulatory disturbances and bacterial infection. The present study was thus undertaken to evaluate estrual cervical mucus of repeat breeding and normal cows for physical characteristics and correlation of these characteristics with ovulatory disturbances and nonspecific bacterial infection of genital tract under field conditions of temperate region.

## Materials and Methods

### *Animal selection criteria*

A total of 100 crossbred Jersey cattle comprising of 80 repeat breeders presented for treatment and 20 normal clinically healthy cows presented for artificial insemination at their first service within 60-90 days after calving were selected. Cows that failed to conceive in three or more regular services with apparently healthy genitalia were considered as repeat breeders. Detailed gynaeco-clinical examination was carried out to all the repeat breeding animals and those with gross genital pathology including ovarian cysts were excluded from the study (Kumar and Singh, 2009).

Initially repeat breeding animals were clinically examined for ovulatory disturbances. They were examined per-rectally at 12 hour interval upto 6 days from onset of behavioural estrus for persistence or rupture of follicles and development of subsequent corpus luteum (CL) at the ruptured site. Another single per-rectal examination was done at mid cycle (10-12 days) for ascertaining the presence of mature CL. Animals with persistent follicle upto 4 days from onset of estrus but not beyond that and a mature CL at mid cycle were considered as delayed ovulators; whereas, animals with persistent follicle beyond 4 days following estrus and no CL at mid cycle were considered as anovulators. The repeat breeding animals that did not show any ovulatory disturbances were put into the category of normal ovulation with infection. Thus the repeat breeding animals were divided into 3 groups i.e. anovulation, delayed ovulation and normal ovulation with infection. Repeated per rectal palpation at 12 hour intervals were also conducted to the animals of control group and any animal showing ovulatory disturbances were excluded from the study and replaced with other normal animals to keep numbers of animals constant (n=20) in that group. Then estrual cervical mucus collected from all the repeat breeding animals and animals of control group were put to bacterial culture. To compare physical properties of estrual cervical mucus (volume, colour, consistency, stringiness, fern pattern, spinnbarkeit values and pH) amongst the four groups (*i.e.* anovualtion, delayed ovulation and normal ovulation with infection and control group), animals with positive bacterial culture of estrual cervical mucus were excluded from the group of anovulation, delayed ovulation and control group to avoid the technical error. Animals included in the group of normal ovulation with infection, if showed no bacterial growth in their estrual cervical mucus in bacterial culture, were also excluded. However, for recording results of WST and bacterial culture the animals in their respective groups were kept as such.

### *Collection of estrual cervical mucus*

Estrual cervical mucus from all the estrous animals was collected at 8-12 hours after the onset of behavioural estrus. For the purpose, animals were properly restrained in the service crate. Full sleeved left hand lubricated with sterile glycerin was inserted per rectum and back racking was done for evacuation of rectal faeces. Perineal region including vulva was thoroughly washed with soap and running tape water, dried with soft absorbable cotton and disinfected with 70 % alcohol by cotton swab. After a gap of 15 to 20 minutes, disinfection of area was repeated with 70% alcohol while both the vulvar lips were held apart by an assistant. A sterilized 10 ml glass pipette with pointed end directed outwards was inserted in the vagina. The external pointed end of the pipette was connected to a 20 ml disposable syringe with a rubber adopter for aspiration. The pipette was guided through the vaginal canal to the os or mid cervix by the left hand already introduced per rectum. Cervical mucus was aspirated from the os or mid cervix and then transferred to a sterilized test tube.

### Evaluation of estrual cervical mucus for physical characteristics

Estrual cervical mucus was evaluated for volume, colour, consistency, stringiness, pH, spinnbarkeit value, and fern pattern within 2 to 3 hours of collection.

Volume was recorded as profuse, scanty and absent. Colour of the estrual cervical mucus was graded by direct visual examination into transparent, opaque, milky and yellowish. Consistency was visually assessed and categorized as thick and thin. Stringiness was measured on the basis of stretchability or elasticity of the mucus and assessed by noticing break or continuity of mucus from vulva of the cow to the floor and was graded as more stringy (when mucus appeared like a cord/ rope touching the ground) and less stringy (when the rope not touching the ground). pH was determined using pH paper strips suitable for assessing pH (6.5 – 9.0). Spinnbarkeit value was assessed by the technique described earlier (Dhillon *et al.,* 2006). Fern pattern in the dried smear prepared from estrual mucus was examined under low power (10 × 10X) microscope and classified as typical, atypical and nil type fern pattern.

### *White side test*

For conducting white side test (WST), 1 ml of estrual cervical mucus was heated with equal volume of 5 % sodium hydroxide upto boiling point and after cooling the intensity of colour changes were studied and graded as:

| | |
|---|---|
| 1. Normal | Turbid or No colour |
| 2. Mild infection | Light yellow colour |
| 3. Moderate infection | Yellow colour |
| 4. Severe infection | Dark yellow colour |

**Bacterial culture**: The collected mucus were evaluated for isolation and identification of the non-specific organisms on the basis of cultural, morphological, colony characteristics, motility and biochemical reactions (Cowen and Steel, 1970).

The data obtained regarding the physical characteristics of estrual cervical mucus (volume, colour, consistency, stringiness and fern pattern) were subjected to statistical analysis by Z test. Data regarding WST and bacterial culture were expressed in percentage. Correlation between bacterial culture and physical characteristics of estrual discharge (volume, colour, consistency, stringiness and fern pattern) and results of WST was calculated by Spearman's rank correlation method. Spinnbarkeit values and pH were subjected to one-way ANOVA. To test the significance of difference between means post-hoc analysis by LSD was done.

## Results

On the basis of repeated per rectal examination and bacterial culture, out of 80 repeat breeding animals 6 suffered from anovulation, 2 anovulation with infection, 14 delayed ovulation and 4 delayed ovulation with infection. All the remaining 54 animals showed bacterial growth in their estrual mucus. Contrarily, none of the animals of control group was affected with ovulatory disturbances, but 3 animals showed bacterial growth. In order to compare physical properties of estrual cervical mucus these 9 animals (2 anovulation with infection, 4 delayed ovulation with infection and 3 infected animals of control group) were excluded from their respective groups. Thus anovualtion, delayed ovulation, normal ovulation with infection and control group (normal ovulation without infection) comprised of 6, 14, 54 and 17 animals in each group, respectively. For recording WST and bacterial examination animals in their respective group were kept as such i.e. anovulation, delayed ovulation, normal ovulation with infection and control group consisted of 8, 18, 54 and 20 animals respectively.

**Table 1. Comparison of physical characteristics of estrual cervical mucus between repeat breeding and normal cyclic (control group) animals.**

| **Parameters** | **Anovulation** (n=6) | | **Delayd ovulation (n=14)** | | **Normal ovulation** with infection (n=54) | | **Control group** (n=17) | |
|---|---|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| Volume-Perofuse | 3 | 50.00 | 11 | 78.57 | 43 | 79.63 | 15 | 88.24 |
| - Scanty | 3 | 50.00 | 3 | 21.43 | 11 | 20.37 | 02 | 11.76 |
| - Absent | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| Colour - Transparent | 5 | 83.33 | 12 | 85.71 | 20 | 37.04 | 16 | 94.12 |
| - Opaque | 1 | 16.67 | 2 | 14.29 | 25 | 46.30 | 1 | 5.88 |
| - Milky | 0 | 0.00 | 0 | 0.00 | 7 | 12.96 | 0 | 0.00 |
| - Yellowish | 0 | 0.00 | 0 | 0.00 | 2 | 3.70 | 0 | 0.00 |
| Consistency-Thin | 3 | 50.00 | 12 | 85.71 | 18 | 33.33 | 15 | 88.24 |
| - Thick | 3 | 50.00 | 2 | 14.29 | 36 | 66.67 | 2 | 11.76 |
| Stringiness –More | 2 | 33.33 | 4 | 28.57 | 22 | 40.774 | 14 | 82.35 |
| - Less | 4 | 66.67 | 10 | 71.43 | 32 | 59.26 | 3 | 17.65 |
| Fern pattern-Typical | 3 | 50.00 | 9 | 64.29 | 43 | 79.63 | 15 | 88.24 |
| - Atypical | 2 | 33.33 | 4 | 28.57 | 9 | 16.67 | 1 | 5.88 |
| - Nil | 1 | 16.67 | 1 | 7.14 | 2 | 3.70 | 1 | 5.88 |

The volume, colour, consistency, stringiness, fern pattern and spinnbarkeit values of cervical mucus were not affected by ovulatory disturbances, while, colour, consistency and pH showed significant ($p < 0.01$) changes due to non-specific bacterial genital infection (Table 2 and 3).

**Table 2. Correlation of bacterial culture with physical characteristics of estrual cervical mucus and results of WST.**

| | Volume | Colour | Consistency | Stringiness | Fern pattern | WST |
|---|---|---|---|---|---|---|
| Bacterial Culture | 0.015 | 0.487** | 0.40** | -0.077 | 0.105 | 0.484** |

** Correlation is significant (p< 0.01).

**Table 3. Comparison of spinnbarkeit value and pH of estrual cervical mucus between repeat breeding and normal cyclic (control group) animals.**

| | Repeat Breeder (74) | | | |
|---|---|---|---|---|
| **Parameter Mean ±SE** | **Anovulation (n = 6)** | **Delayed ovulation (n= 14)** | **Normal ovulation with infection (n= 54)** | **Control group (n = 17)** |
| Average Spinnbarkeit value (cm) | 15.83±0.95 | 15.57±0.94 | 15.63±0.45 | 16.88±0.81 |
| Spinnbarkeit value in cm (range) | (13 - 19) | (12 - 23) | (11 - 24) | (10 - 25) |
| pH | $7.75{\pm}0.11^{a}$ | $7.89{\pm}0.06^{a}$ | $8.19{\pm}0.06^{b}$ | $7.79{\pm}0.07^{a}$ |
| pH (range) | (7.50 – 8.00) | (7.50 - 8.00) | (7.50 - 9.00) | (7.00 - 8.00) |

(Figures within parenthesis indicate range)
Means with different superscripts within trait in a row differ significantly ($p < 0.05$)

The results of WST revealed that only 15% animals of control group showed infection and remaining 85% animals were free of infection; however majority of repeat breeding animals showed infection (72.25%) and only 28.75 % animals were free of infection (Table 4).

The results of bacterial culture showed similar trend with that of WST (Table 5). Out of 80 repeat breeding animals 60 (75.00 %) yielded bacterial isolates while as 20 (25.00 %) samples were free of bacteria. In the control group only 3 (15.00%) showed bacterial growth on the culture, whereas 17 (85.00 %) samples did not show any bacterial growth (Table1).

In our study, results of WST showed a significant ($p < 0.01$) correlation of 0.484 with bacterial culture (Table 2).

**Table 4. Results of WST showing grades of infection based on colour intensity between repeat breeding and normal cyclic (control group) animals.**

| Colour intensity | Grade of infection | Repeat Breeder ( n= 80) | | | | | Control group (n=20) | |
|---|---|---|---|---|---|---|---|---|
| | | Anovulation (n= 8) | Delayed ovulation (n= 18) | Normal ovulation (n= 54) | Overall (n=80) | | | |
| | | | | | n | % | n | % |
| No colour | 0 (Normal) | 4 (50.00 %) | 10 (55.56 %) | 9 (16.67%) | 23 | 28.75 | 17 | 85 |
| Light yellow | + (Mild infection) | 4 (50.00 %) | 6 (33.33 %) | 36 (66.67%) | 46 | 57.50 | 3 | 15 |
| Yellow | ++ (Moderate infection) | 0 (0.00 %) | 1 (5.56 %) | 5 (9.26%) | 6 | 7.50 | 0 | 0 |
| Dark yellow | +++ (Severe infection) | 0 (0.00 %) | 1 (5.56 %) | 4 (7.41%) | 5 | 6.25 | 0 | 0 |
| Overall | | 8 (100 %) | 18 (100%) | 54 (100%) | 80(100%) | | 20 (100%) | |

**Table 5. Results of bacterial culture between repeat breeding and normal cyclic (control group) animals.**

| Parameters | Repeat Breeder (80) | | | | | | | | Control group (n = 20) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Anovulation (n = 8) | | Delayed ovulation (n= 18) | | Normal ovulation with infection (n= 54) | | Overall (n = 80) | | | |
| | n | % | n | % | n | % | n | % | n | % |
| No. of samples positive for bacterial isolates | 2 | 25.00 | 4 | 22.22 | 54 | 100 | 60 | 75.00 | 3 | 15.00 |
| No. of samples free from bacterial isolates | 6 | 75.00 | 14 | 77.78 | 0 | 0.00 | 20 | 25.00 | 17 | 85.00 |
| No. of samples having single bacterial isolate | 2 | 3.33 | 3 | 5.00 | 41 | 68.33 | 46 | 76.67 | 3 | 100.0 |
| No. of samples having 2 bacterial isolates | 0 | 0.00 | 1 | 1.67 | 11 | 18.33 | 12 | 20.00 | 0 | 0.00 |
| No. of samples having 3 bacterial isolates | 0 | 0.00 | 0 | 0.00 | 2 | 3.33 | 2 | 3.33 | 0 | 0.00 |
| Overall no. of samples having mixed infection | 0 | 0.00 | 1 | 1.67 | 14 | 21.67 | 14 | 23.33 | 0 | 0.00 |

## Discussion

### *1. Volume*

Volume did not change between the animals of impaired ovulation and normal ovulation. The volume of estrual mucus showed non significant correlation (0.015) with bacterial culture indicating that infection can't be judged on the basis of the estrual discharge volume.

### *2. Colour*

Earlier workers reported occurrence of more clear discharge in normal and less clear or turbid or cloudy in repeat breeding cows (Selvaraj *et al*., 2002; Satheshkumar and Punniamurthy, 2007) and buffaloes (Sharma *et al*., 2008). Purulent discharge in estrual mucus of repeat breeding cow (Methai *et al*., 2005) and buffaloes (Samad *et al*., 2002) was also observed earlier. Clear estrual mucus is conducive for sperm penetration and thereby providing better conception (Hamana *et al*., 1971), whereas turbidity arrests sperm motility in estrual mucus (Dev *et al*., 1997) and thereby lowering conception. This study clearly indicates that any alterations in the colour of estrual mucus will indicate genital infections. However, colour is not affected by ovulatory disturbances.

### *3. Consistency*

The thin consistency of estrual cervical mucus was recorded in majority of the animals of anovulation, delayed ovulation and control group; however thick consistency was recorded in the animals of normal ovulation with infection. Available literature revealed thicker consistency of cervical mucus in repeat breeding cows and buffaloes (Agarwal and Datta, 1979; Satheshkumar and Punniamurthy, 2007). This study indicated that consistency of estrual cervical mucus is not affected by ovulatory disturbances but significantly affected by genital infections.

### *4. Stringiness*

In our study stringiness did not show significant variation amongst the animals of anovualtion, delayed ovulation, normal ovulation with infection and control group. Significant correlation of stringiness with bacterial culture was also not noticed as reported earlier (Satheshkumar and Punniamurthy, 2007). It is thus concluded that genital infection had no impact on altering stringiness of the estrual mucus.

### *5. Fern pattern*

No marked variation in the type of fern pattern was observed between the animals of repeat breeding and control group. This finding simulates the earlier finding in crossbred cow (Selvaraj *et al*., 2002). It is concluded that ovulatory disturbances and genital infections do not alter fern pattern of estrual discharge.

### *6. Spinnbarkeit value*

The mean spinnbarkeit values (cm) amongst the groups of anovulation, delayed ovulation, normal ovulation with infection and control group varied non significantly. Although one study revealed significantly higher spinnbarkeit value in fertile cows (Shelar *et al*., 2002); but most studies reported non-significant variation of spinnbarkeit value in repeat breeding cows and buffaloes (Mehta *et al*., 1986; Sharma *et al*., 2008). Our study suggests that repeat breeding due to impaired ovulation and genital infection in cows does not change spinnbarkeit value of estrual cervical mucus.

### *7. pH*

The pH in normal ovulation with infection group was significantly ($p< 0.05$) higher when compared to other groups (Table 3). This indicates that pH of estrual mucus increases as the growth and multiplication of organisms occurs in the genitalia resulting in endometritis or repeat breeding condition. Due to infection and inflammation the pH would increase (Boiter *et al*., 1980). Several earlier workers recorded more alkaline pH in repeat breeding cow (Pateria and Rawal, 1990, Methai *et al*., 2005) and buffalo (Kumar *et al*., 2011). High alkalinity of cervical mucus could be due to bacterial contamination and is not suitable for survival/ penetration or mobilization of spermatozoa (Hafez and Hafez, 2000; Sheldon *et al*., 2006). No variation in pH of cervical mucus between repeat breeding (7.42±0.09) and normal crossbred cows (7.48±0.09) was also reported (Siddiquee, 2006). This study suggested that pH of the estrual cervical mucus was significantly ($p < 0.05$) affected by bacterial infection but not by ovulatory disturbances.

### *8. Results of white side test (WST) and Bacterial culture:*

The normal uterine discharge has too low number of leucocytes to cause any colour change; where as discharges in repeat breeders due to clinical or sub-clinical metritis contain increased number of leucocytes causing a moderate colour change (Bhattacharyya *et al*., 2011). In the present study results of WST showed a significant correlation of 0.484 with bacterial culture and thus indicating the importance of WST under field conditions where (particularly developing countries) optimum facility for microbial isolation is not available.

It is concluded that colour, consistency and pH of estrual cervical mucus were significantly affected by bacterial infection but not by ovulatory disturbances in repeat breeding cattle. The WST can be used effectively as a preliminary test to judge the bacterial infection of genital organ under field conditions and thereby restricting the indiscriminate use of antibiotics.

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