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# Association of Ovarian Structures, Uterine Characteristics and Cervico-vaginal Mucus Attributes with Conception in Dairy Cows

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## *ABSTRACT*

This study was conducted to evaluate the association between ovarian structures, uterine characteristics and physical attributes of cervico-vaginal mucus (CVM) with conception rate in normal cyclic dairy cows (N=20) at estrus. Different physical parameters of CVM at estrus such as quantity, consistency, color, pH, Spinnbarkeit value and fern pattern were evaluated. Transrectal ultrasonography was done at estrus to carry out the morphometry of follicles and uterine horns as well as following artificial insemination, the mid-cyclic corpus luteum size and functionality were assessed on the 10th day using B-mode and color Doppler ultrasonography. Among the physical parameters, significantly higher ($p<0.05$) Spinnbarkeit values were recorded for subsequently pregnant (n=12) as compared to non-pregnant cows (n=8). The mean diameter of the preovulatory follicle at estrus and corpus luteum vascularity (%) was significantly higher ($p<0.05$) in subsequently pregnant as compared to non-pregnant cows while, no significant difference ($p>0.05$) was recorded for intraluminal uterine fluid diameter and endometrium thickness. In conclusion, higher spinnbarkeit and preovulatory follicle diameter at estrus led to a better conception rate in dairy cows.

***Key words:*** Cervicovaginal mucus, dairy cows, preovulatory follicle, spinnbarkeit, estrus.

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## INTRODUCTION

The estrous cycle constitutes a rhythmic pattern of ovarian function and causes the cows to go from non-receptivity to receptivity transition, thereby, leading to mating and later pregnancy. The cow in estrus should have appropriate structures on the ovaries and a conducive environment in the uterus and cervix for the establishment of pregnancy (Keskin *et al.*, 2016). Sonographically detectable attributes such as the diameter of the preovulatory follicle, size and

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echo texture of the luteal tissue, and shape and echo texture of the uterus can be used to evaluate the estrus phase in individual cows (Pierson and Ginther, 1988). Also, ultrasound examination of cows before artificial insemination (AI) prevents the service of proestrus and diestrus cows and decreases estrus detection errors (Luttgenau *et al*., 2015). Moreover, the evaluation of physical parameters of cervical mucus at estrus helps in depicting the fertility in cows (Rangnekar *et al*., 2002). The cervical mucus acts as a suitable medium that influences sperm viability and helps in governing the potential of cows to conceive (Beran *et al*., 2013). Furthermore, the insemination done based on the properties of cervical mucus specifically the fern pattern and spinnbarkeit value enhance the conception rate (Modi *et al*., 2011). Therefore, the study was conducted to monitor the size of ovarian structures and echotexture of the uterus along with some physical attributes of CVM at the time of estrus in dairy cows and to establish relationship with conception rate.

## MATERIALS AND METHODS

Twenty dairy cows with spontaneous estrus and no history of reproductive abnormality were enrolled in the study. The CVM was aspirated using a discharge gun at estrus and different parameters such as consistency, quantity, color, pH, spinnbarkeit and fern pattern were evaluated. The pH of CVM was measured by using pH paper. After the collection of CVM, the pH paper strip was dipped in the mucus and the change in the color of the pH paper was compared with the standard color of the pH paper strip.

The spinnbarkeit was performed by taking 2-3 drops of the discharge on the slide. Then another slide was placed on the first one, afterward both the slides were moved apart slowly until the discharge broke. The measurement was done by using the measuring scale whereas, the test was performed two times for every sample and values were expressed in cm.

The fern pattern was evaluated by taking a drop of discharge on the grease-free slide. The slide was kept at room temperature for drying and observed under the microscope at 10x. The crystallization observed was then graded into primary, secondary and tertiary fern patterns.

Trans-rectal ultrasonography of the ovaries and uterus was done on the day of estrus using B mode ultrasonography. The portable ultrasound machine equipped with a linear rectal transducer of 7.5 MHz frequency was used to evaluate the reproductive tract. The dimensions of ovarian structures and uterine horns were recorded at estrus and correlated with the conception rate. The statistical analysis was carried out using Student's t-Test with NCSS 2021, USA (Version 20.0.1).

## RESULTS AND DISCUSSION

In the present study, the conception (60%) was recorded to be more in cows that showed clear, copious, and watery discharge at the time of estrus whereas, non-pregnant cows (40%) had a scanty and viscous discharge. Similarly, the conception rate was reported to vary between 67-90% for dairy cows that had transparent vaginal mucus at estrus (Bhat *et al*., 2015).

Meanwhile, the mean pH values were recorded to be numerically ($p>0.05$) higher in conceived cows as compared to non-conceived cows (Table 1).

**Table 1:** Relationship between physical parameters of cervicovaginal discharge and pregnancy status in dairy cows (Mean ±S.E.)

| | Cervico-vaginal discharge properties (CVD) | |
|---|---|---|
| **Cows (N=20)** | **pH** | **Spinnbarkeit (cm)** |
| **Pregnant (n=12)** | 8.00 ± 0.16 | $9.63\pm0.80^{x}$ |
| **Non pregnant (n=8)** | 7.68±0.09 | $6.50\pm0.91^{y}$ |

$^{x,y}$ Values with different superscripts within the same column differ significantly ($p<0.05$)

However, Hanumant *et al.* (2019) reported the mean pH values in the cervical mucus of subsequently conceived cows to be significantly higher ($p<0.01$) than non-conceived cows. However, no significant ($p>0.05$) variation was reported in the mean pH values of cervicovaginal mucus between conceived and non-conceived cows (Yildiz, 2021).

The mean spinnbarkeit (cm) values in conceived cows were recorded to be significantly ($p<0.05$) higher as compared to non-conceived cows (Table 1). In concurrence with the present findings, significantly ($p<0.01$) higher mean spinnbarkeit values in conceived cows were reported by Hanumant *et al.* (2019) and Yildiz (2021).

Meanwhile, the tertiary fern pattern was mostly recorded in subsequently pregnant as compared to non-pregnant cows and the findings were in concurrence with other researchers as typical tertiary arborization patterns in dairy cows led to a higher conception rate (Ningwal *et al*., 2018).

The results of the present study illustrated that the mean diameter of the preovulatory follicle at estrus was significantly higher ($p<0.05$) in pregnant cows as compared to non-pregnant cows (Table 2).

**Table 2:** Relationship between ovarian follicular, luteal, and uterine characteristics at estrus phase in subsequently pregnant and non-pregnant dairy cows (Mean ±S.E.)

| S. No. | Status of cows (N=20) | | Mean diameter (mm) |
|---|---|---|---|
| 1 | **Preovulatory follicle** | Pregnant (n=12) | 12.60±0.49$^{x}$ (10.0-15.3) |
| | | Non-pregnant (n=8) | 10.84±1.25$^{y}$ (8.10-14.55) |
| 2 | **Intra luminal uterine fluid diameter** | Pregnant (n=12) | 7.26±0.99 (3.70-23.00) |
| | | Non-pregnant (n=8) | 6.11±0.46 (4.25-8.00) |
| 3 | **Endometrial thickness** | Pregnant (n=12) | 7.68±0.38 (5.35-8.15) |
| | | Non-pregnant (n=8) | 7.94±1.21 (4.75-12.15) |
| 4 | **Mid cyclic CL** | Pregnant (n=12) | 17.29±0.67 (15.9-19.65) |
| | | Non-pregnant (n=8) | 16.01±1.34 (13.35-17.6) |
| 5 | **Vascular perfusion index (%)** | Pregnant (n=12) | 14.43±0.93$^{x}$ (12.80-16.01) |
| | | Non-pregnant (n=8) | 8.23±1.26$^{y}$ (6.05-10.41) |

$^{x,y}$ Values with different superscripts within the same column for the same parameter differ significantly ($p<0.05$)

Similarly, the large-size preovulatory follicles resulted in higher conception rates than in cows with small-size preovulatory follicles (Keskin *et al*., 2016; Kapse *et al*., 2017). However, higher conception rates were reported in cows with small and young ovulatory follicles which was contrary to our current findings (Lynch *et al*., 2010) whereas, Brusveen *et al*. (2009) reported no effect of follicle size on the conception rates in dairy cows. Moreover, no significant ($p>0.05$) difference was recorded in other uterine parameters such as intraluminal uterine fluid and endometrial thickness (Table 2). Although, Ahmadi *et al*. (2019) reported better pregnancy rates in cows with a higher uterine diameter which was not present in the present study.

The mean diameter of the mid-cyclic corpus luteum (CL) was recorded to be non-significantly ($p>0.05$) higher in pregnant cows as compared to non-pregnant ones (Table 2). The current study also monitored the vascularity of mid-cyclic CL (vascular perfusion index) and was recorded to be significantly ($p<0.05$) higher in pregnant when compared to non-pregnant cows (Table 2). According to Acosta and Miyamoto (2004), the growth and function of the CL depend on blood supply, and therefore, a positive correlation between plasma progesterone concentration and CL blood flow was reported during the mid-luteal phase of the estrous cycle (Herzog and Bollwein, 2007).

# CONCLUSIONS

In conclusion, the higher spinnbarkeit values and tertiary fern pattern were mostly recorded in subsequently pregnant as compared to non-pregnant cows. Similarly, the preovulatory follicle size and corpus luteum vascularity was recorded to have a significantly positive effect on the conception rate while, no variation was found for other uterine and ovarian parameters i.e., intraluminal horn fluid, endometrial thickness and corpus luteum size.

# CONFLICT OF INTEREST

The authors declare no conflict of interest.

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