Original article

**pH and ferning score of cervical mucus related to gestation and calf gender of Holstein Friesian crossbreed cows**

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**ABSTRACT**

This study aimed to compare the ferning score based on the pH of the cervical mucus and its relation with gestation and the calf gender of cows. Ten Holstein Friesian crossbreed cows which were detected in estrus and about to be artificially inseminated, were used for this study. Sample of cervical mucus was collected using Metricheck for pH and ferning observations. Pregnancy diagnosis was conducted two months after insemination, and the gender of the calves was recorded at parturition. This study indicated that cervical mucus pH did not affect the ferning characteristics, ferning score, and pregnancy of cows. However, higher ferning scores tended to result in the pregnancy of cows. Also, alkaline pH tended to result in male calves and vice versa. Further study is needed with a more significant number of samples related to the application of ferning display as an indicator of estrus to obtain a higher pregnancy rate.

**Keywords**: alkaline pH, calf gender, cervical mucus, estrus, gestation

**INTRODUCTION**

In Indonesia, dairy cattle reared are generally Holstein Friesian (HF) cattle (Sutarno and Setyawan, 2016). Most dairy farmers in Indonesia are smallholder farmers; and the national milk demand have not been met. The high demand for milk in Indonesia still cannot be met by domestic milk production, both in quantity and quality (Purwantini et al., 2021). The low productivity of dairy cattle occurred due to a lower knowledge and lower skill of managing and reproducing dairy cattle (Ayele and Leta, 2021).

Artificial Insemination (AI) technology is a reproductive technology that has successfully produced good quality offspring in large numbers by utilizing superior males (Parkinson and Morrell, 2019). The success of AI is determined, among others, by heat detection accuracy. The signs of a cow in heat are mucus from the cervix, which flows through the vagina and vulva, restlessness, the base of the tail is slightly raised, the vulva becoming reddish, and appetite and drinking decrease. The vulva will swell, redden, and be filled with transparent mucus secretions that hang from the vulva or are visible at the base of the tail. The estrus intensity score shows the cumulative value of the appearance of the vulva and behavior. The reproductive efficiency of cattle can be improved if the estrus cycle is observed and appropriately recorded (LeRoy et al., 2018). One effort that can be made is to detect some
signs of estrus. Cervical mucus can be used to detect estrus, especially at the peak of estrus. Ferning is a microscopic view like branches in the form of ferns formed from the crystallization process of NaCl contained in cervical mucus that has been dried (Cortés et al., 2014). The pH of cervical mucus is one of the crucial factors determining pregnancy success in livestock because cervical mucus is a transport medium for sperm (Siregar et al., 2019). This study aimed to compare the ferning pattern of HF crossbreed cattle based on the pH of cervical mucus.

MATERIALS AND METHODS

This study was conducted from February 2020 to January 2021 in Village Unit Cooperative Dadi Jaya, Purwodadi, Pasuruan District, East Java. Purwodadi is located on 6°59'21.98"S latitude and 112°33'23.56"E longitude at an altitude of 500-1000 meters above sea level. The temperature ranges 17-23°C, humidity 70-95%. The annual rainfall ranges from 1,000-1,700 mm per year, with the number of rainy days ranging from 70–120 days per year.

Ten HF crossbreed cows which were detected in estrus and would be artificially inseminated soon, were used for this study. Sample of cervical mucus was collected using Metrichcheck for pH, consistency and ferning observations. pH was measured using digital pH meter (Sartorius PB-10). Cervical mucus consistency was assessed using categories of thick, moderate, and thin. Mucus sample was homogenized and dropped on a pre-cleaned object glass, spread and air dried. Slides were then observed under a light microscope (Nikon E 100) at 100 and 400x magnification. Scoring of cervical mucus was based on World Health Organization (WHO) (2010) reference for cervical mucus ferning score, which were based on the complexity of fern-like appearance formed and ferning coverage density (Figure 1). Scoring criteria covered no crystallization, atypical ferning, primary and secondary stem ferning, tertiary and quaternary stem ferning (score 0 - 3) (Atrio et al., 2015; Najmabadi et al., 2021).

Inseminator inseminated the cows whose cervical mucus samples were taken. Pregnancy diagnosis was conducted two months after insemination, and the gender of the calves was recorded at parturition. The data of sampling time, physical characteristics, scores of ferning pattern, and the results of pregnancy diagnosis and gender of calves were presented descriptively based on the pH of cervical mucus.

RESULTS

Cervical mucus was collected in 3-5 hours after the appearance of signs of estrus. pH measurement showed a range of 6.8-8.2 for the ten samples. All cervical mucus samples were clear and transparent with 80% of them had moderate consistency while the rest was thin (sample no. 1, pH 6.8 and no. 10, pH 8.2). Three samples with ferning score 3 were confirmed pregnant (30%) with male and female calves, while cows with ferning score 2 and 1 were 50% and 20% respectively and they were confirmed non-pregnant (Table 1).
Table 1 Results of examination of cervical mucus samples of HF crossbreed cows

<table>
<thead>
<tr>
<th>sample no.</th>
<th>pH</th>
<th>time of sampling</th>
<th>ferning characteristics</th>
<th>ferning score</th>
<th>pregnancy (sex of calf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8</td>
<td>5</td>
<td>ferning with primary, secondary, and tertiary stems</td>
<td>3</td>
<td>pregnant (female)</td>
</tr>
<tr>
<td>2</td>
<td>6.9</td>
<td>5</td>
<td>only one ferning with primary, secondary, and tertiary stems</td>
<td>2</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>3</td>
<td>7.2</td>
<td>4</td>
<td>ferning with primary and secondary stem</td>
<td>2</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>4</td>
<td>7.3</td>
<td>4</td>
<td>small ferning and uneven</td>
<td>1</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>3</td>
<td>only one ferning with primary, secondary, and tertiary stem</td>
<td>2</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>6</td>
<td>7.7</td>
<td>3</td>
<td>ferning with primary, secondary, and tertiary stems</td>
<td>3</td>
<td>pregnant (male)</td>
</tr>
<tr>
<td>7</td>
<td>7.8</td>
<td>4</td>
<td>ferning was small and scattered, with air bubbles</td>
<td>1</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>8</td>
<td>7.9</td>
<td>4</td>
<td>ferning was small and uneven</td>
<td>2</td>
<td>non-pregnant</td>
</tr>
<tr>
<td>9</td>
<td>8.0</td>
<td>3</td>
<td>ferning was evenly distributed, with primary, secondary, and tertiary stems</td>
<td>3</td>
<td>pregnant (male)</td>
</tr>
<tr>
<td>10</td>
<td>8.2</td>
<td>4</td>
<td>ferning was small and uneven; only stems</td>
<td>2</td>
<td>non-pregnant</td>
</tr>
</tbody>
</table>

Time of sampling: interval (hours) between the appearance of signs of estrus and sampling

Figure 1 Ferning pattern of crystallized cervical mucus of HF crossbreed cows; A: pH 6.8, ferning score 3; B: pH 6.9, ferning score 2; C: pH 7.2, ferning score 2; D: pH 7.3, ferning score 1; E: pH 7.5, ferning score 2; F: pH 7.7, ferning score 1; G: pH 7.8, ferning score 1; H: pH 7.9, ferning score 2; I: pH 8.0, ferning score 3; and J: pH 8.2, ferning score 2 (100x magnification).

DISCUSSION

Cervical mucus pH and time of sampling did not affect the ferning characteristics, ferning score, and pregnancy of cows. Higher ferning scores had a tendency to be followed by pregnancy of cows, while alkaline pH of mucus sample was followed by the birth of male calf, and vice versa. The clinical symptoms of estrus included cervical mucus characteristics which depended on the high or low blood estrogen produced by mature follicles (Crowe, 2016). The most important physical characteristics of cervical mucus were pH, viscosity, elasticity, and crystallization (Verma et al., 2014). Acidic
or alkaline pH was often caused by mucus biophysical and biochemical conditions controlled by hormonal changes during the estrus cycle (Prasdini et al., 2015). In the middle of the cycle, the usual range of endocervical pH was 7-8, resulting in a higher pregnancy rate than those of with pH 6 and 9 (Siregar et al., 2019). Under the control of estrogen, amino acid secretion increased but its concentration in mucus decreased. These properties helped spermatozoa live longer in cervical mucus (Adnane et al., 2018). The factor that caused the pH of the mucus to be alkaline was that goblet cells affected the lysozyme enzyme to reduce bacteria in the vagina, metabolizing glycogen and forming lactic acid, which was responsible for the low pH of the vagina, so when lactic acid decreased it became alkaline (Nakano et al., 2015). High estrogen states promoted the preservation of a homeostatic vaginal microenvironment by stimulating the maturation and proliferation of vaginal epithelial cells and glycogen accumulation. A glycogen-rich vaginal milieu is a haven for the proliferation of Lactobacilli facilitated by lactic acid production and decreased pH (Amabebe and Anumba, 2018).

This study revealed a higher ferning score followed by the pregnancy of cows. The appearance of the ferning pattern indicated the cow’s estrus level and is related to the ovarian activity (Cortés et al., 2014). As ovulation approaches, the pattern became more distinct due to increased estrogen concentration. Crystallization in bovine cervical mucus in ferning leaves was found during the follicular phase and disappeared during the luteal phase (Bernardi et al., 2016; Siregar et al., 2017). Ferning is the formation of a fern leaf structure in cervical mucus due to calcium, zinc, magnesium, potassium, and sodium. The higher the salt concentration, the more apparent the ferning pattern (Savia et al., 2021). Crystallization of NaCl was highly dependent on the concentration of estrogen. The fern pattern was formed by the crystallization produced by the aggregation of molecules, leading to a crystal nucleus (nucleation). Cervical mucus could crystallize because it contained mucoproteins or other organic compounds and electrolytes in certain salts such as NaCl, KCl, and CaCl2. Sodium chloride was the main salt in cervical mucus (Cortés et al., 2014).

Cervical mucus is a medium for sperm survival and determines the occurrence of a cow's pregnancy. Fixed time insemination based on cervical mucus characteristics may increase the pregnancy rate. The cervical mucus score for non-conceived cows reduced fern pattern. The highest conception rate was reached in score three for all parameters of the cervical mucus. The pH of the cervical mucus at the time of AI did not influence the cows' pregnancy rates (Yildiz, 2021). This study indicated that the alkaline pH of cervical mucus tends to result in male calf offspring. It was similar to an earlier report that an alkaline pH is more favorable for the survival of Y-bearing sperm cells (Oyeyipo et al., 2017).

CONCLUSION

This study revealed that cervical mucus pH did not affect ferning characteristics, ferning score, and pregnancy of cows. Higher ferning scores tended to result in the pregnancy of cows; alkaline pH tended to result in male calves and vice versa. Further study is needed with a more significant number of samples related to the application of ferning display as an indicator of estrus to obtain a higher pregnancy rate.

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