Ovozoa 11 No. 1, April 2022 p-ISSN 2302-6464 e-ISSN 2722-967X
Available at https://e-journal.unair.ac.id/OVZ/index

Original article

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

Muhammad Billy Ferdiansyah[1*], Iwan Sahrial Hamid[2], Herry Agus Hermadi[3], Abdul Samik[3], Tatik Hernawati[3]

[1]Faculty of Veterinary Medicine, [2] Division of Veterinary Basic Medical Science, [3] Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga
[*] Corresponding author, e-mail: bilferdiansyah@gmail.com

Open access under CC BY – SA license, DOI: 10.20473/ovz.v11i1.2022.22-26
Received March 2 2022, Revised March 7 2022, Accepted March 13 2022
Published online April 2022

How to cite this article: Ferdiansyah MB, Hamid IS, Hermadi HA, Samik A, Hernawati T. 2022. pH and ferning score of cervical mucus related to gestation and calf gender of Holstein Friesian crossbreed cows. Ovozoa: Journal of Animal Reproduction 11: 22-26.

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 Metricheck 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

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

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

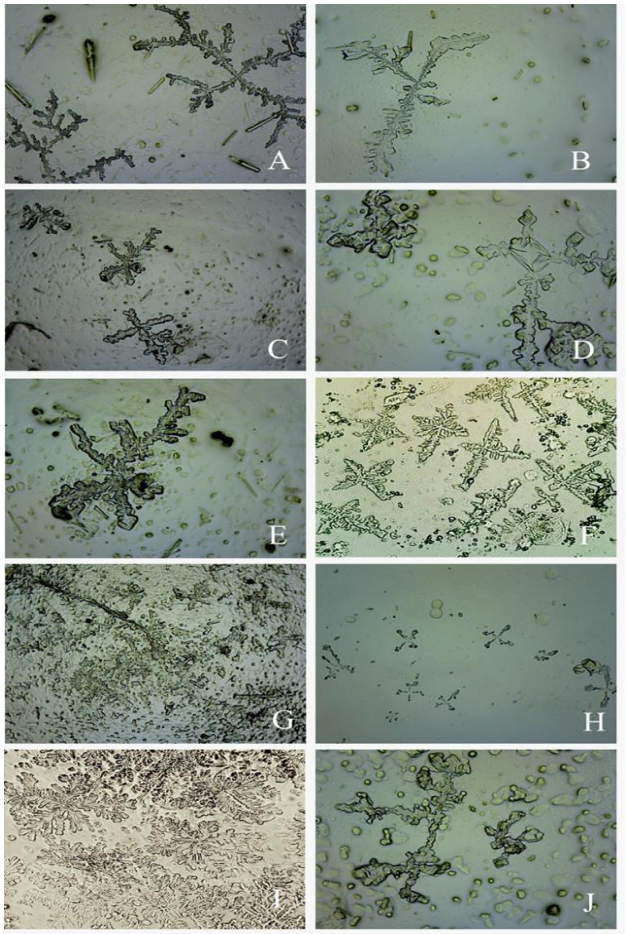

Figure 1 Ferning pattern of crystallized cervical mucus of HF crossbreed cows; A: pH 6.8, ferning score 3; B: pH 6.9, ferning score 2; C: pH 7.2, ferning score 2; D: pH 7.3, ferning score 1; E: pH 7.5, ferning score 2; F: pH 7.7, ferning score 3; G: pH 7.8, ferning score 1; H: pH 7.9, ferning score 2; 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 becameclearer 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 $CaCl_2$. 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 (Yıldız, 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.

REFERENCES

Adnane M, Meade KG, O'Farrelly C. 2018. Cervico-vaginal mucus (CVM) - an accessible source of immunologically informative biomolecules. Vet Res Commun. 42: 255-63.

Abebe E, Anumba DOC. 2018. The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. Front Med (Lausanne) 5: 181.

Atrio J, Stek A, Vora H, Sanchez-Keeland L, Zannat F, Natavio MT. 2015. The effect of protease inhibitors on the cervical mucus of HIV-positive women taking norethindrone contraception. Eur J Contracept Reprod Health Care 20: 149-53.

Ayele B, Leta S. 2021. Knowledge and preventive practices of livestock farmers on infectious causes of reproductive disorders in dairy cows in the Kembata Tambaro zone southern Ethiopia. Anim Dis. 1: 1-8.

Bernardi S, Rinaudo A, Marini P. 2016. Cervical mucus characteristics and hormonal status at insemination of Holstein cows. Iran J Vet Res. 17: 45-9.

Cortés ME, González F, Vigil P. 2014. Crystallization of Bovine Cervical Mucus at Oestrus: An Update. Rev Med Vet. 28: 103-8.

Crowe MA. 2016. Reproduction, Events and Management: Estrous Cycles: Characteristics, Reference Module in Food Science, Elsevier. 428-33.

LeRoy CNS, Walton JS, LeBlanc SJ. 2018. Estrous detection intensity and accuracy and optimal timing of insemination with automated activity monitors for dairy cows. J Dairy Sci. 101: 1638-47.

Najmabadi S, Schliep KC, Simonsen SE, Porucznik CA, Egger MJ, Stanford JB. 2021. Cervical mucus patterns and the fertile window in women without known subfertility: a pooled analysis of three cohorts. Hum Reprod. 36: 1784-95.

Nakano FY, Leão RBF, Esteves SC. 2015. Insights into the role of cervical mucus and vaginal pH in unexplained infertility. Med Express (São Paulo, online) 2: M150207.

Oyeyipo IP, van der Linde M, du Plessis SS. 2017. Environmental exposure of sperm sex-chromosomes: A gender selection technique. Toxicol Res. 33: 315-23.

Parkinson TJ, Morrell JM. 2019. Artificial insemination. In: Noakes DE, Parkinson TJ, England GCW (Eds.) Veterinary Reproduction and Obstetrics. 10th Ed. Elsevier Publishing, USA. 746-59.

Prasdini WA, Rahayu S, Djati MS. 2015. Level of estrogen and cervical mucus pH as indicator of estrus after calving towards the provision of selenium vitamin ETM on dairy cow Frisian Holstein. Int J Chem Tech Res. 7: 190-5.

Purwantini TB, Saliem HP, Ariningsih E, Erwidodo E, Anugrah IS, Suryani E,Irawan AR, Hetherington JB. 2021. The performance of smallholder dairy farms in West Java. IOP Conf. Ser.: Earth Environ. Sci. 892: 012098.

Savia CL, Osorio JS, Rodríguez JV, Guibert EE, Rinaudo A. 2021. A simple and reliable refractometric method to determine the total solids concentration of the cervico-vaginal bovine mucus samples. Heliyon 7: e07010.

Siregar TN, Agustina I, Masyitah D, Azhar A, Dasrul D, Thasmi CN, Sulaiman R, Daud R. 2017. Physical Properties of Cervical Mucus of Repeat Breeder Aceh Cattle. J Vet.18: 378-82.

Siregar TN, Armansyah T, Panjaitan B, Gholib G, Herrialfian H, Sutriana A, Abidin Z, Reynaldi MA, Razak F, Artaliani Y, Yuswar Y. 2019. Changes in cervical mucus as an indicator of fertility in Aceh cattle. Adv Anim Vet Sci. 7: 306-14.

Sutarno S, Setyawan AD. 2016. The diversity of local cattle in Indonesia and the efforts to develop superior indigenous cattle breeds. Biodiversitas 17: 275-95.

Verma KK, Prasad S, Kumaresan A, Mohanty TK, Layek SS, Patbandha TK, Chand S. 2014. Characterization of Physico-chemical properties of cervical mucus in relation to parity and conception rate in Murrah buffaloes. Vet World 7: 467-71.

World Health Organization. 2010. WHO laboratory manual for the examination and processing of human semen. 5th Ed. WHO Press.

Yıldız A. 2021. The validity of the scores of cervical mucus during artificial insemination for estimating the probability of conceiving in clinically healthy cows. Vet Sci Res Rev. 7: 58-65.

