

Level of sodium chloride (NaCl) and profile of cervical mucus of dairy cattle at various age synchronized by prostaglandine

Y. S. Ondho, F. A. Akbar, D. A. Lestari and D. Samsudewa*

Faculty of Animal and Agricultural Sciences, Diponegoro University,

Tembalang Campus, Semarang 50275 – Indonesia

*Corresponding E-mail: daudreproduksi@gmail.com

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ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi kadar Natrium Klorida (NaCl) dan profil lendir serviks yang meliputi *Potential of Hydrogen* (pH), kelimpahan, *spinnbarkeit* dan *ferning* lendir serviks saat estrus pada sapi perah dengan umur berbeda. Penelitian ini menggunakan 32 ekor sapi perah berumur 2 tahun (n = 2 ekor), 3 tahun (n = 18), 4 tahun (n = 8 ekor) dan 5 tahun (n = 4 ekor), yang disinkronisasi dengan hormon Prostaglandin 50 mg/ekor. Pengambilan data dilakukan pada jam ke-48, 72, 78, 84, 90, 120, 144 dan 408 setelah pemberian hormon. Data yang diperoleh dianalisis menggunakan analisis statistik non parametrik Kruskal-Wallis H-Test, apabila ada perbedaan nyata maka akan diuji lanjut menggunakan Mann-Whitney U Test. Hasil penelitian menunjukkan perbedaan nyata pada kelimpahan lendir pada jam pengamatan ke-84, sedangkan pada kadar Natrium Klorida, *spinnbarkeit*, pH dan *ferning* lendir serviks tidak terdapat perbedaan yang nyata pada sapi perah dengan umur yang berbeda. Kesimpulan dari penelitian ini adalah umur sapi perah tidak mempengaruhi kadar NaCl, *spinnbarkeit*, pH dan *ferning* lendir serviks, namun mempengaruhi kelimpahan lendir pada jam ke-84 setelah penyuntikan prostaglandin.

Kata kunci: Sapi perah, umur, sinkronisasi berahi, lendir serviks

ABSTRACT

Objectives of this study were to compare the percentage of Sodium Chloride (NaCl) and cervical mucus profile which includes potential of Hydrogen (pH), abundance of cervical mucus, *spinnbarkeit* and *ferning* of dairy cattle at different age during estrus cycle. Thirty Two head of dairy cattle were used as materials which are 2 years old (n = 2), 3 years old (n = 18), 4 years old (n = 8) and 5 years old (n=4). Dairy cattle were estrus synchronized using 50 mg/head of prostaglandin. Data was collected on 48, 72, 78, 84, 90, 120, 144 and 408 hours after prostaglandin administration. The data were analyzed by using non parametric statistic which was Kruskal-Wallis H test. The significant data was tested with Mann-Whitney U test. The result showed that the abundance of cervical mucus was significantly different ($P < 0.05$; $\chi^2 = 0.011$) on 84 hours after estrus synchronization. However, level of NaCl, abundance, *spinnbarkeit* and *ferning* of cervical mucus was not significantly different. In conclusion, the age of dairy cattle did not affected to the condition NaCl percentage, *spinnbarkeit*, pH and *ferning* of cervical mucus, but affected to the abundance of cervical mucus on 84 hours after administration of prostaglandine.

Keywords: dairy cattle, age, estrus synchronization, cervical mucus

INTRODUCTION

The high demand of animal product in Indonesia, especially demand on dairy milk has not been fulfilled by domestic production. Availability of milk in Indonesia until 2016 averaged only 2.98/kg/capita/year, while domestic milk requirement was fulfilled by importing milk which reached 11.87/kg/capita/year (Agustina, 2016). Low genetic quality of dairy cattle decreased the livestock production. The artificial insemination (AI) is one of the solutions to improve the genetic quality of livestock (Susilawati, 2011). The implementation of AI is very effective in the process of repairing and distributing the good quality of livestock, but a high service per conception (S/C) in AI is still being obstacle (Sophian and Afati, 2016).

The accuracy of estrus detection is a main factor that affected of a high S/C of AI (Susilawati, 2011). Estrus phase on dairy cattle only happen in 24 to 48 hours over 21 days estrus cycle. Incorrect estrus detection is related to prolonged calving interval and profit loss due to feed cost, decrease milk production and veterinary cost etc. Some research has already been conducted to observe the estrus detection including vulva, temperature and hormonal changing, unfortunately estrus detection is still being major problem (Roelofs *et al.*, 2010). During estrus phase, many physiological changes occur in female reproductive organ.

One of the physiological changes is increasing salt percentage in mucus cervix. The salt content (NaCl, KCl and CaCl₂) of the mucus that produced by the cervix ranged 0.8-1% and has a peak point at onset estrus (Samsudewa *et al.*, 2019). Research about NaCl on cervical mucus for determining the peak of estrus is limited. On the other hand, prostaglandin as luteolytic hormone is commonly used to synchronize estrus in animal due to its role as vasoconstrictor in blood vessels. This causes a drastic blood flow barrier to corpus luteum and lead to corpus luteum regression (Samsudewa *et al.*, 2019) Therefore, objectives of this study were to compare the percentage of NaCl and profile of cervical mucus which includes *potential of Hydrogen* (pH), abundance of cervical mucus, spinnbarkeit and ferning of dairy cattle with different age during estrus cycle that synchronized by prostaglandine.

MATERIALS AND METHODS

Research Unit

This research was conducted in Capita Farm, Semarang Regency, Central Java, Indonesia. Twelve (3 years old (n = 18), 4 years old (n = 8)) and 32 ((2 years old (n = 2), 3 years old (n = 18), 4 years old (n = 8) and 5 years old (n=4)) of dairy cattle were used as materials on NaCl percentage and other parameter of cervical mucus (abundance, ferning, spinnbarkeit and potential of hydrogen), respectively. The materials on the same of body condition score.

Estrus Synchronization

Fifty miligram prostaglandin hormon were used for synchronize the estrus of dairy cattle by intramuscular injection. Cervical mucus collection was done for collecting data of the percentage of NaCl, abundance of cervical mucus, ferning, spinnbarkeit and pH of cervical mucus.

Cervical Mucus Collection

Cervical mucus collection was performed by disclosing vulva using vaginoscope. Cervical mucus was then collected using a modified catheter that was inserted into the cervix. Smooth sucked was done to collect the cervical mucus. Cervical mucus collection was conducted on 48, 72, 78, 84, 90, 120, 144 and 408 hours after the estrus synchronization to represent each phase of estrus (proestrus, estrus, metestrus and diestrus) (Purwaningsih *et al.*, 2018).

Sodium Chloride (NaCl) Percentage Measurement

The cervical mucus was placed in a micro tube and stored in a preparat box. The samples were analyzed by using Mohr's titration method of the following formula (Samsudewa *et al.*, 2019):

$$\text{NaCl (\%)} = [(A \times N \times 58.54 \times V) / V \times 1,000] \times 100$$

Where A : Volume of AgNO₃ for titration (mL)

N : Normality of AgNO₃ (mgrek/mL)

V : Total mucus volume (mL)

58.46 is Molecular of NaCl

Abundance of Cervical Mucus Measurement

The abundance of cervical mucus is observed in the presence of mucus from the vulva, which were categorized into 3 score. Score "1":

Mucus is only around the vulva, Score “2”: Mucus is hanging at the tip on the vulva and is low quantity, Score “3”: The quantity of the mucus is seen hanging from the vulva and around the tip of the tail and the thighs (Tanjung *et al.*, 2015).

Ferning Assessment

Observation of ferning was begin with ferning preparation by taking mucus from modified catheter and then swiping it on a glass object, drying and observing with a microscope. The ferning of cervical mucus categorized into 6 levels based on Bishnoi *et al.* (1982) with modification. Score “1”: There was no crystallization, a thick-walled structure in the form of air bubbles, Score “2”: There was only primary stems crystallization, Score “3”: Ferning covered less than half field of view, Score “4”: Ferning covered than half field of view, Score “5”: Ferning covered more than 75% of field of view, Score “6”: The formation of a fern leaf with the stem of primary, secondary and tertiary (Tanjung *et al.*, 2015).

Spinnbarkeit Measurement

Observation of stretching of the cervical mucus (spinnbarkeit) is done by placing the cervical mucus on the forefinger and thumb and pulling it down. The distance of the breaking point is then measured using the vernier calipers (Purwaningsih *et al.*, 2018).

Potential Hydrogen (pH) Measurement

Measuring of the potential of Hydrogen (pH) cervical mucus used a universal indicator pH paper with range 6.2 to 8.4. Swipe the cervical mucus directly from modified catheter to the universal indicator pH paper and compare to the standart (Makmun *et al.*, 2017).

Statistical Analysis

The data were analyzed by using non parametric statistic which was Kruskal-Wallis H test, and then significant different was tested by Mann-Whitney U test. All statistical analysis performed by using SPSS 16.0.

RESULTS AND DISCUSSION

Percentage of Sodium Chloride (NaCl)

Average values of NaCl percentage of cervical mucus are shown in Figure 1. Kruskal-Wallis H test showed no significant difference in

median values on NaCl percentage of cervical mucus on various age of cattle. NaCl percentage of cervical mucus has two peaks in the end of estrus with 12 hours interval on both of ages. The younger ages (3 years old) of dairy cattle has faster peak reaching compare with the older, but have the lower NaCl percentage of cervical mucus in the top of the peak. It was connected with secretion of the estrogen and mineralocorticoid. The level of NaCl in cervical mucus from each phase of estrus and age was affected by estrogen induced by Luteinizing hormone (LH) (Widiyono *et al.*, 2013; Makmun *et al.*, 2017; Samsudewa *et al.*, 2019). On other hand, increasing of estrogen secretion will stimulate increasing secretion of Adenocorticotrophic Hormone (ACTH) from hypofise. Increasing secretion of ACTH that are accompanied by circulation of high estrogen in the liver stimulates cortisol formation in the liver. Estrogen will be removed through urine by conjugating water-soluble estrogen, while cortisol will stimulate secretory mineralocorticoid hormone as regulation of electrolyte fluid in the adrenal cortex. The mineralocorticoid hormones increase retention of Na^+ , K^+ , Cl^- and H^+ in the kidneys. The minerals produced will be reabsorbed with free estrogen by entero-hepatic system. The minerals produced will be absorbed by the entero-hepatic system into each target organ, including the cervix as one of the constituent substances of cervical mucus (Widiyono *et al.*, 2013).

Abundance of Cervical Mucus

The modus values of scoring abundance of cervical mucus are shown in Table 1. Kruskal Wallis H test for abundance of cervical mucus showed significant difference only on 84 hours after synchronization. In this time, the highest abundance of cervical mucus was showed by 3 years old dairy cows. This is connected to the permeability and optimization of the goblet cell stimulate by estrogen after administration of prostaglandine. Administration of prostaglandine will stimulate regression of corpus luteum. Progesterone level will fell down in associate with regression of corpus luteum. Decreasing level of progesterone will give negative feedback mechanism to increasing estrogen. The estrogen will increase the growth of the genital tract, so the cervix was produce mucosal cells and the process of cervical mucus secretion is beginning. Lu and Morrese (2006) stated that transported estrogen

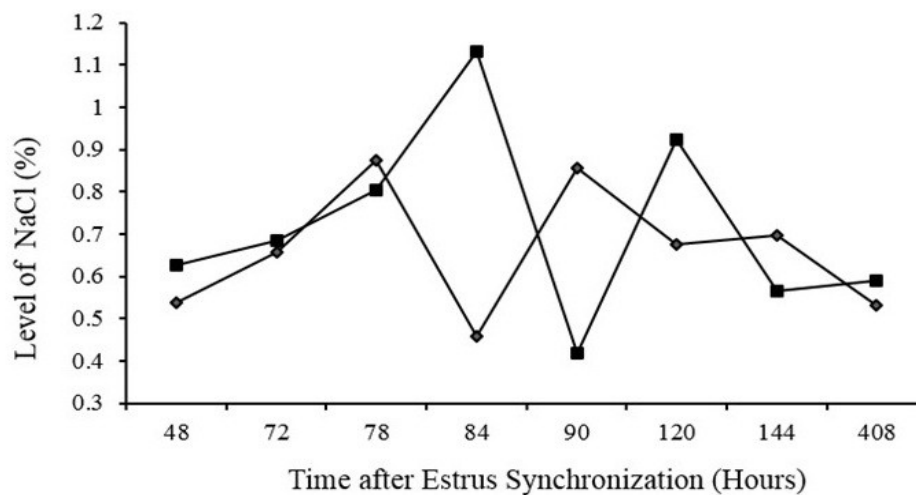


Figure 1. Natrium chloride (NaCl) percentage of cervical mucus of dairy cows on different ages after estrus synchronization with 50 mg prostaglandine. The symbols represent 3 years (◇); 4 years (□) of dairy cows

Table 1. Modus Abundance of Cervical Mucus at Various Ages and After Synchronization

Hours of Observation	Abundance of Cervical Mucus			
	Age			
	2 Years Old	3 Years Old	4 Years Old	5 Years Old
48	0	0	0	0
72	0	1	0	0
78	1	2	2	0
84	1	2	0	1
90	1	0	0	0
120	1	0	0	0
144	1	0	0	0
408	0	0	0	0

is binding to proteins in the bloodstream affects the supply of blood to the genital tract and stimulates swelling of mucus cells in the cervix, so that mucus can be produced. Estrogen in the blood affects the adrenal hormone, which increases blood pressure on the cervix and increasing permeability goblet cells inside so that mucus can come out (Tanjung et al., 2015).

Increased adrenal hormone causes the increasing of cytoplasmic fluid goblet cells. So

the goblet cells rupture due to pressure from the accumulated fluid. Makmun *et al.* (2017) reported that the increase in goblet cell size due to an increase in the amount of fluid in the cytoplasm of goblet cells causes thinning of the cell membrane, so the process of breaking goblet cells occurs and secretion of cervical mucus is beginning. Visual cervical mucus appearance is one of indicators of animal's sexual health, which related with estrus behavior. It can be used as a sign to determine the

right moment to inseminate and also associated with a higher conception rate when this mucus secretion was present at insemination (Murugavel and López-Gatius, 2009; Bernardi *et al.*, 2015).

Spinnbarkeit

Average values of Spinnbarkeit are shown in Figure 2. Kruskal-Wallis H test showed no significant difference in median values on spinnbarkeit of cervical mucus on various age of cattle. The peak of median value of spinbarkeit was shown on 84 hours for all ages of dairy cows. It was connected with the production of cervical mucus and estrogen. Spinnbarkeit is influenced by the acidity of cervical mucus and the function of cervical mucus as an antibacterial non-immune in the reproductive tract of cow. Lu and Morresey (2006) stated that estrogen secretion of cervical mucus causes uterus to resist the infection. Cervical mucus that contained with lysozyme enzyme was connected with the hydrolysis process of bacterial cell membranes. Lysozyme enzyme in cervical mucus containing N-Acetyl Glucosamine plays a role in the hydrolysis process of bacterial cells (Chimura *et al.*, 1993).

Lysozyme movement in the process of hydrolyzing bacterial cells causes a lot of the remaining membrane of bacterial cells to be damaged, leaving behind the cervical mucus. Substances contained in bacterial cell membranes are mixed in the cervical mucus. Substances that make up the structure of cell membranes are

carbohydrates, lipids and proteins. Chimura *et al.*, (1993) stated that the constituent components of cell membranes include phospholipid, glycolipid and sterol which they include in globular lipid groups. All of the constituents of the structure of cell membranes such as lipids, proteins and carbohydrates will increase the viscosity level and spinnbarkeit value of the cervical mucus during estrus.

Potential Hydrogen (pH)

Average median values of pH on cervical mucus of various ages of cattle are shown on Figure 3. Kruskal-Wallis H test showed no significant difference in median values on pH of cervical mucus on various age of cattle. The pH of cervical mucus is affected by lysozyme enzyme. Lysozyme is an enzyme that fights bacterial cells in the cervical mucus. Lu and Morresey (2006) stated that lysozyme is a protection against gram-positive bacteria by hydrolysis, lysozyme naturally contained in tears, mucus, milk and saliva. Lysozyme in the cervical mucus acts as a local defense mechanism against bacteria that get in through the cervix from female external genitalia. Chimura *et al.*, (1993) stated that lysozyme is an antibacterial agent that acts as a non-immune defense factor that fights incoming infection from the external genitalia in the cervical mucus.

Lysozyme enzymes fight bacteria by damaging bacterial cell membranes causing

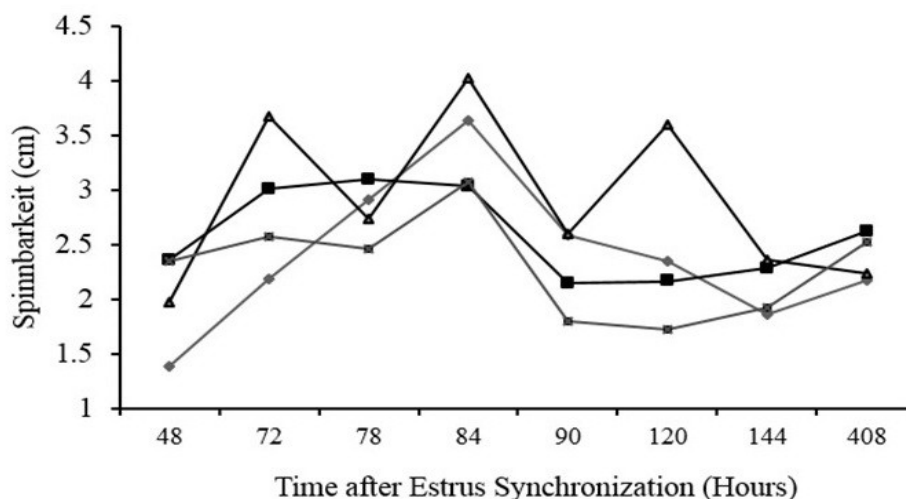


Figure 2. Spinnbarkeit of cervical mucus of dairy cows on different ages after estrus synchronization with 50 mg prostaglandine. The symbols represent 2 years (◇); 3 years (□); 4 years (Δ) and 5 years (x) of dairy cows

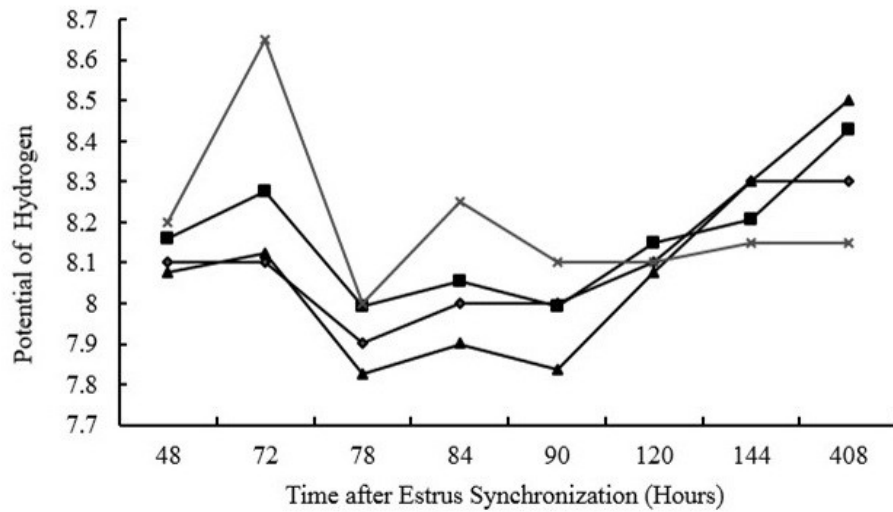


Figure 3. Potential hydrogen (pH) of cervical mucus of dairy cows on different ages after estrus synchronization with 50 mg prostaglandine. The symbols represent 2 years (◇); 3 years (□); 4 years (Δ) and 5 years (x) of dairy cows

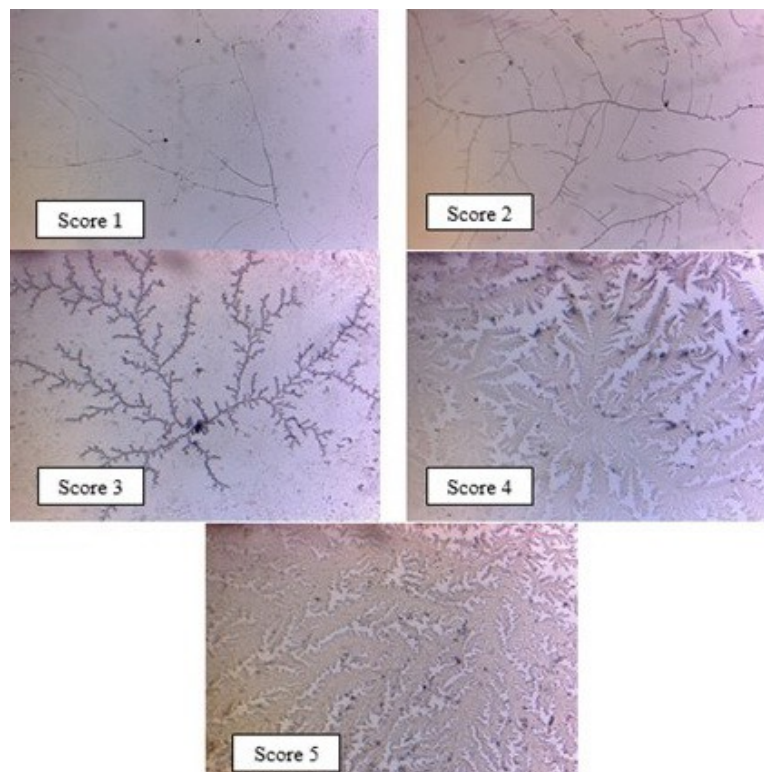


Figure 4. Ferning Crystallization of Dairy Cows

Table 2. Modus Ferning of Cervical Mucus at Various Ages and After Synchronization

Hours of Observation	Ferning of Cervical Mucus			
	Age			
	2 Years Old	3 Years Old	4 Years Old	5 Years Old
48	1	1	1	2
72	1	2	2	2
78	2	2	2	2
84	2	2	2	2
90	2	2	2	2
120	2	2	2	2
144	2	2	2	2
408	1	1	1	1

metabolic changes in bacteria and form lactic acid bacteria. Lysis bacteria condition changes glycogen metabolism in these bacterial cells, so that the bacteria transform into lactic acid bacteria. The formation of lactic acid bacteria continues to grow along with the number of bacteria causing the condition of the acidity / pH of the mucus to decrease

Ferning

Modus values of ferning on cervical mucus of various ages of cattle are shown on Figure 4. Kruskal-Wallis H test showed no significant difference in modus values on ferning of cervical mucus on various age of cattle. Ferning is a visual representation of the content of NaCl in the cervical mucus. NaCl in the mucus will crystallize and form an arrangement like fern leaves. Yahari *et al.*, (2009) stated that NaCl is crystallizing and dissolving with water. The crystal is the result of the crystallization of NaCl which is mainly influenced by the estrogen hormone. Tanjung *et al.*, (2015) stated that ferning is the crystallization of NaCl in cervical mucus which is affected by the estrogen hormone in estrous conditions.

Ferning will be different in each phase of estrus. It is related to the secretion of estrogen as a stimulation of NaCl in the cervical mucus (Table 2). Yahari, *et al.*, (2009) stated that when approaching ovulation, ferning will be apparent because of the increased concentration of the hormone estrogen secreted.

CONCLUSION

In conclusion, the age of dairy cattle only affected on the abundance of cervical mucus during 84 hours after administration of prostaglandine. The age of dairy cattle showed no significance different for other parameters.

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