Research Article

Changes in Cervical Mucus as an Indicator of Fertility in Aceh Cattle

Tongku Nizwan Siregar, Teuku Armansyah, Budianto Panjaitan*, Gholib Gholib, Herrialfian Herrialfian, Amalia Sutriana, Zainal Abidin, Muhammad Aufa Reynaldi, Fathur-Razak, Yulli Artaliani, Yuswar Yuswar

Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia.

Abstract | This study aimed to investigate the relationship between cervical mucus viscosity and estrogen concentration, several blood biochemical profiles, and blood macro-mineral levels, as well as to determine the effect of cervical mucus viscosity and pH differences on pregnancy rates in Aceh cattle. Eight females Aceh cows aged 3-7 years old, with body weights of 150-250 kg were synchronized using a 25 mg single dose injection of PGF2α. Afterward, observation of estrus signs was performed for 30 minutes at 06:00 a.m. and at 06:00 p.m. Blood and cervical mucus samples were collected during the first cervical mucus secretion. The estrogen concentrations of each type of cervical mucus, namely thick, moderate, and thin were 29.39±6.29; 23.24±4.62; and 30.93 pg/mL, respectively. The pregnancy rate in cows with a cervical mucus pH of 6 and 9 was 0%. Meanwhile, the groups with a cervical mucus pH 7 and 8 had a pregnancy rate of 50%. An examination of cervical mucus showed different consistencies: five cows had mucus with a thick viscosity, with a 20% pregnancy rate; two had mucus with a moderate viscosity, with a 50% pregnancy rate and one had mucus with a thin viscosity, with a 100% pregnancy percentage. In conclusion, there is no significant relationship between estrogen concentration and cervical mucus viscosity levels in Aceh cattle. Levels of total protein, total cholesterol, and glucose were correlated to the viscosity of the cervical mucus. Higher levels of blood macrominerals (Na, K, Cl, Ca, P, and Mg) in female Aceh cattle resulted in greater viscosities of cervical mucus. The groups with cervical mucus pH of 7 and 8 had higher rates of pregnancy (50%) than the groups with cervical mucus pH of 6 and 9. The viscosity of cervical mucus also had an effect on the success of pregnancy in Aceh cattle.

Keywords | Cervical mucus, Aceh cattle, Estrogen, Pregnancy rate

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | September 18, 2018; **Accepted** | Dececmber 22, 2018; **Published** | February 02, 2019
***Correspondence** | Budianto Panjaitan, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia; **Email**: budi@unsyiah.ac.id
Citation | 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(4): 306-314.
DOI | http://dx.doi.org/10.17582/journal.aavs/2019/7.4.306.314
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Cervical mucus is produced by endocervical secretory cells. Its quality and quantity depend on the status of gonadal hormones during the estrous cycle (Tsiligianni et al., 2001). The main functions of cervical mucus is sperm transportation and its role as a barrier against microbial infections. Cervical mucus consists of 92-95% water, along with carbohydrates, amino acids, lipids, and a mixture of dissolved macromolecules such as proteins and polysaccharides (Schumacher, 1970). Cervical mucus also contains several enzymes such as alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and α -amylase (Tsiligianni et al., 2003).

Recently, fertility evaluation by the examination of fluids produced in the female reproductive tract has become a serious concern. The characteristics of cervical mucus play an important role in the success or failure of pregnancy. An indirect evaluation of cervical mucus can be used as an indicator of the current estrous cycle stage or hormonal status in domestic animals (Benbia et al., 2011). Cervical secretion also has roles in sperm endurance and transportation to the uterine cavity (Kumar et al., 2012). Changes in cervical mucus can also be used to determine the optimal time for artificial insemination (Tsiligianni et al., 2000).

Studies have shown that there is a strong correlation between cervical mucus and sperm motility (Gaddum-Rosse, 1981; Yousef, 1981). Some hormonal imbalances are exhibited in cervical mucus, with consequences for the ability of sperm to penetrate. Cervical mucus is also a mechanical barrier to uterine pathogens. Normally, cattle undergoing estrus will discharge cervical mucus from their vulva. High-quality cervical mucus is clean, comes from the cervix, and does not produce a smell. Clean cervical mucus secreted during artificial insemination has a close relationship with higher conception rates (Loeffler et al., 1999). Conversely, abnormal cervical mucus can suppress reproductive performance (Mahmoudzadeh et al., 2001). In humans, it is known that α-amylase and LDH found in cervical mucus play a role in spermatozoa capacitation (Vermeiden, 1989).

The properties of cervical mucus influence the possibility of depositing semen in the female reproductive tract. In addition, the physical properties of cervical mucus are directly related to the fertility status of animals (Rangnekar et al., 2002). Gonadal hormone changes during estrus influence its physical and chemical properties (Eltohamy et al., 1990). An infertile cow experiences changes in the physical properties of its mucus, including color (appearance), consistency, elasticity, fern pattern, and pH (Mohanty et al., 1996).

Several studies have examined the fertility of Aceh cattle based on hormonal status (Siregar et al., 2016; Thasmi et al., 2017), the presence of embryos (Siregar et al., 2017), and bacterial infections of the uterus (Rafika, 2017). These methods were relatively difficult, expensive, and required a long time. Until now there have been no reports about the relationship between changes in the physical properties of cervical mucus and infertility in Aceh cattle. Therefore, we need a study that aims to evaluate the relationship between the fertility of Aceh cattle and changes in cervical mucus during insemination.

MATERIALS AND METHODS

Animal Model

Eight adult female cows, aged 3-7 years old and weighing 150-250 kg, that had already given birth and had experienced at least two regular cycles, were synchronized with a single dose of 25 mg PGF2 alfa (LutalyseTM, Pharmacia & Upjohn Company, Pfizer Inc.) intramuscularly. All cows were Aceh cattle owned by the Experimental Animal Technical Unit, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. Rectal palpation was performed before the injection to evaluate the presence of corpora lutea.

Artificial Insemination and Pregnancy Examination

Estrus detection was performed twice a day, at 6 a.m. and 6 p.m., for 30 minutes each time. Artificial insemination was performed when cows were considered in the estrus phase, red and swollen vulva, restlessness, mounting other cows, decreased appetite, and secretion of cervical mucus. All cows were inseminated with semen from Aceh cows that had been determined to be fertile. The pregnancy rate was determined 18-21 days after insemination using a non-return rate method.

Collection and Examination of Cervical Mucus

Cervical mucus was collected at the time of the first cervical mucus secretion. The outer part of the female genitals was cleaned using 70% alcohol and was swabbed with cotton to dry. Cervical mucus was collected using an aspiration method by inserting a 20 ml sterile pipette connected to a rubber hose into the vaginal canal, followed by palpation. Afterward, mucus was collected in a sterile tube.

The viscosity was examined by dropping 2-3 drops of the mucus onto a clean glass surface, which was then tilted at 45°. The viscosity was grouped into three different levels of consistency, namely thick (+++), moderate (++), and thin (+). Thick consistency was defined as when mucus hung without breaking and exhibited a wide diameter. Moderate consistency was defined when mucus hung without breaking but had a moderate diameter. Dilute or thin consistency was defined when mucus hanged off and exhibited a small diameter (Irfan et al., 2017).

Cervical Mucus pH Measurement

The pH of cervical mucus was directly measured in the laboratory using a universal indicator or pH meter. Mucus was categorized into four groups depending on pH: namely <7.0; 7.0-7.5; 7.5-8.0; and >8.0.

Blood Sampling

A 10 mL sample of blood was taken from the jugular vein shortly after mucus collection. The sample was put in a thermos and left for several hours in order to separate blood cells and serum into two layers. Afterward, the tube was centrifuged for 10 minutes at 3000 rpm and the serum was transferred into a micro tube. Finally, serum was stored in a -20° C freezer until hormone analysis could be carried out.

Blood Collection and Preparation

Blood plasma for the measurement of hormone levels was taken from cows' jugular veins using a 10 mL disposable syringe. Blood was collected just before insemination. The blood was put in a test tube and placed in a thermos filled with ice. The blood was then taken to the laboratory for the collection of plasma and was left for 30 minutes before centrifugation. Centrifugation was carried out at a speed of 2500 rpm for 15 minutes. The plasma was then taken from the tube with a micropipette and was placed in a microtube. The plasma was stored in a freezer with a temperature of -20 ° C until it was used for steroid analysis. Hormonal analysis was performed using an enzyme-linked immunosorbent assay (ELISA) method.

Blood Biochemistry Examination

Five μL of serum was taken and was added to 500 mg of Lab test reagent using a micropipette. The sample was then put into a spectrophotometer (Mindray BA-88A). The blood biochemical levels to be measured were total protein, total cholesterol, and glucose.

Mineral Level Examination

A micropipette was used to take 5 μl of blood serum, which was added to 500 μL of standard calibration solution reagents. Afterward, the sample was inserted into a spectrophotometer and the result was read immediately.

Measurement of Progesterone/Estradiol Concentration

For each ELISA well microplate, 25 μl of standard solution, sample, and control were added and incubated for 5 minutes at room temperature. Afterwards, the solution was mixed with 200 μl of progesterone/estradiol-HRP reagent, then incubated for 60 minutes at room temperature. The microplate was shaken quickly to remove the contents of the well. The microplate was then rinsed three times by the addition of 400 μl of washing solution to each well. Afterwards, 200 μl of the substrate solution was added to each well and the solution was incubated again for 15 minutes at room temperature. The enzymatic reaction was stopped by the addition of 100 μl of stop solution to each well. The absorbance value was read within 10 minutes, using an ELISA reader with a wavelength of 450 nm.

RESULTS AND DISCUSSION

The result showed that the percentage of cervical mucus viscosity of cattle which was categorized as thick (+++); moderate (++); and thin (+) were 62.5%; 25.0%; and 12.5%, respectively. About 75% of the mucus samples had a transparent appearance and the other 25% displayed a cloudy color. The average (±SD) estrogen concentration in cervical mucus of thick, moderate and thin viscosity was 29.39±6.29; 23.24±4.62; and 30.93 pg/mL, respectively. Data of cervical mucus viscosity, estrogen concentration, and conception rate are presented in Table 1.

Statistical analysis showed that the correlation value between estrogen concentration and cervical mucus viscosity in Aceh cattle was 0.123. The coefficient of the determinant (R2) was 1.5, indicated that as much as 1.5% of the quality of cervical mucus secretion could be explained by variations in estrogen hormone concentration, while the rest was influenced by other variables. The regression equation was as follows: y = 2.057 + 0.016x (y= viscosity of cervical mucus; x= estrogen level). A coefficient value of 0.016 meant that the addition of one pg/ml estrogen concentration would increase the cervical mucus viscosity by 0.016. This equation indicated that the greater the estrogen concentration, the more cervical mucus viscosity increased. Rangnekar et al. (2002) stated that, in addition to estrogen, progesterone can affect the physical properties of cervical mucus. Other factors such as biological variation, nutrition, number of samples (n), and time of measurement were also likely to have significant effects on viscosity. The viscosity levels of cervical mucus can also be significantly affected by changes in the composition of glycoprotein molecules, which in turn can cause difficulties in spermatozoa penetration (Dev et al., 1997). The alterations in glycoprotein composition due to the increasing of water and mineral concentration can decrease the viscosity of cervical mucus which in turn causes increased secretion and color in cervical mucus (Rutllant et al., 2005).

Table 1: Cervical mucus viscosity, estrogen concentration, and conception rate

No	Cervical mucus viscosity	Number of samples (n)	Estrogen concentration (pg/mL) (±SD)	Conception rate (%)
1	Thick (+ + +)	5	29.39±6.29	20
2	Moderate (+ +)	2	23.24±4.62	50
3	Thin (+)	1	30.93	100

Estrogen concentrations at all three cervical mucus viscosity levels in this study were relatively similar. The results of this study showed that all samples had relatively similar biological variations. Guyton (1991) stated that different hormone concentrations in animals are due to biological variations that depend on the type of animal, the animal condition, stress factors, interactions with other animals, the animal age, and the time of measurement. The estrogen concentration found in this study was relatively similar to the results of studies by Gebrehiwot et al. (2015) (25 pg/mL) and in Punganur cattle (20.24 pg/mL) (Naik et al., 2013).

The presence of estrogen greatly affects animal physiology during the estrus phase and causes the animal to be able to copulate. Tsiligianni et al. (2011) reported that super ovulated cows in the follicular phase had a minimum estrogen level of 30.95 pg/mL and a maximum level of 54.77 pg/mL. The increase in estrogen concentration in blood circulation reaches the anterior pituitary and stimulates LH release. Moreover, estrogen also affects the nervous system, causing animals to be restless and to mount other cows. Estrogen also causes the uterus to contract gently, which allows spermatozoa to be transported to the female reproductive genitals after insemination and natural copulation. Other effects of high estrogen concentration include dilatation of blood vessels in genital organs and mucus secretion by cervical and vaginal glands (Ramli et al., 2016).

Cervical mucus is a substance produced by the cervix during estrus and consists of mucin and plasma. Cervical mucus sterilizes microbes in the uterus and can be used as an indicator of the estrous cycle in animals. Characteristics of the mucus depend on the hormones produced in the estrus phase (Benbia et al., 2011). Estrogen-influenced cervical mucus secretion results in thick and clear mucus (Lim et al., 2014).

The pH levels of cervical mucus in Aceh cows ranged from 6.0 to 9.0, with an average pH of 7.6, as presented in Table 2. The pH range of Aceh cows in this study was relatively greater than those reported for different types of livestock and breeds. Bernardi et al. (2016) reported that the pH level of cervical mucus in Holstein cattle ranged from 7.0-7.6 with no significant differences between natural and induced estrus. Dogra et al. (2017) stated that the pH of cervical mucus during the estrus phase in goats ranged from 6.8-7.0 and was relatively similar in both induced and natural estrus. Mahmoud (2013) also stated a consistent result that the cervical mucus pH in estrus-induced sheep was 6.74, while the cervical mucus pH in natural estrus was 6.80.

Table 2: pH level of cervical mucus and pregnancy rate in Aceh cattle

No	pH of mucus	Sample (n)	Pregnancy rate (%)
1	6.0	1	0
2	7.0	2	50
3	8.0	4	50
4	9.0	1	0

The pH difference in this study was likely to be related to the measurement method used and differences in estrogen levels in each cow. Suharto (2003) stated that environmental conditions at the time of measurement could also affect the pH of cervical mucus. When cervical mucus pH was measured outside the body (*in vitro*) in an environment with high humidity, the mucus pH tended to be more alkaline. Cervical mucus pH can also be influenced if the cervical mucus is exposed to atmospheric air for 5-10 minutes (Correa et al., 2001). Furthermore, Salisbury and Vandenmark (1985) stated that the pH level of cervical mucus which was measured *ex situ* tended to be alkaline, ranging from 9.0-9.2. In this study, pH level was measured *ex situ*, which could be the reason that five out of eight cows had alkaline pH.

Besides differences in measurement methods, differences in pH levels of cervical mucus could also be affected by hormones such as estradiol levels during the estrus cycle (Prasdini et al., 2015; Suharto, 2003). The presence of OH elements in the structure of estradiol could cause mucus pH towards neutrality or alkalinity. The present study supported that finding in which the estradiol concentration of cervical mucus of Aceh cows with pH below 7 was higher (22.7 ng/mL) compared to estradiol concentration of cervical mucus with a pH above 7 (25.32 pg/mL).

At this study, the most commonly found pH level of cervical mucus was 8. All groups in this study showed a NRR value of 0% at pH 6 and pH 9 group and NRR value of 50% at pH 7 and pH 8 group. Hamana et al. (1971) found similar results, showing that the highest pH level of cervical mucus was 6.0-7.2. Zaman et al. (2013) stated that the pH value of cervical mucus in normal cows was 7.35, which was coupled with a conception rate of 90%. Furthermore, Berger (1951) stated that highest pregnancy rates had cervical mucus that was slightly alkaline.

Hafez and Hafez (2000), stated that excessive acidity or alkalinity in cervical mucus could reduce sperm motility and cause fertilization failure. According to Modi et al. (2011), the pH levels of cervical mucus of fertile cows ranged from 7.3-9.1, while pH levels of 4.7-7.9 tended to breed repeatedly. In this study, cows with relatively acidic cervical mucus (pH 6) and extremely alkaline cervical mucus (pH 9) had NRR values of 0%.

Cervical mucus with an optimum pH could help the survival of spermatozoa (Fordney-Settlage, 1981), but when the cervical mucus pH beyond the optimum level, it will reduce the fertility of spermatozoa. Branigan and Larry (2008), stated that a pH range of 7.0-8.5 was the optimal range to support sperm viability and motility. Bernardi et al. (2016) observed that the spermatozoa penetration ability in FH cows with cervical mucus pH of 7.4±0.1 (induced estrus) and 7.0±0.0 (natural estrus) were not significantly different, and the value recorded were 78.58 dan 88.45%, respectively. Zavos and Melvin (1980) stated that a mucus pH of ≥6.1 increased sperm viability, while pH values of ≤6.0 tended to reduce the survival rates of spermatozoa. Acidic mucus usually had pH values between 3 and 4. Fur thermore, at pH levels below 6 could lead to decreases in sperm motility (Agarwal et al., 2008; Peek and Colin (1986).

The cervical mucus pH probably correlated with the estrogen concentration, thus it might be related to the estrus cy

Table 3: Profile of several blood biochemical levels in Aceh cattle with different viscosities of cervical mucus

No.	Mucus criteria	Number of cows	Total protein (g/dL)	Total cholesterol (mg/dL)	Glucose (mg/dL)
1.	Thin	1	8.2	123	39
2.	Moderate	2	9.75±0.9	146±2.8	32.5±10.60
3.	Thick	5	9.48±1.1	162.2±39.90	38.8±18.24

cle of cows. Dodamani et al. (2010) stated that pH levels of cervical mucus in Deoni cows during the estrus phase were around 8.83-8.91. According to Suharto (2008), pH levels during the estrus phase ranged from 7.5-9.8. Tsiligianni et al. (2011) conclude that the high estrogen concentration will induce the pH reduction while the low estrogen concentration will increase the cervical mucus pH. This condition has attributed to the peroxide activity changes which induced by the different estrogen concentration (Farley et al., 1992).

The results of this study showed variation in the average profile of several biochemical levels in blood, as well as the viscosity of cervical mucus of Aceh cattle, as presented in Table 3. Out of a total eight cows, five cows has mucus with thick viscosity, two cows had mucus with moderate viscosity, and one cow had mucus with thin viscosity.

The average (±SD) total blood protein levels of cows with cervical mucus with thin viscosity, moderate viscosity, and thick viscosity were 8.2; 9.75±0.9; and 9.48±1.1 g/dL, respectively. According to Mitruka and Rawnsley (1981), the total normal protein concentration of cow blood is 7.56 ±0.50 g/dL. The total blood protein level in this study that was closest to normal levels was found in cows with thin-viscosity mucus. Siregar et al. (*unpublish*) reported that the levels of total blood protein in Aceh cows that experienced repeat breeding and in fertile cows were 6.9±1.97 and 6.6±1.56 g/dL (P>0.05), respectively.

The results of this study showed that the higher the total blood protein, the greater the viscosity of the cervical mucus. Khan et al. (2010) stated that low protein levels could cause disturbances in gonadotropin synthesis. Therefore, the high protein levels found in this study were expected to increase gonadotropin synthesis. Manas et al. (2012) stated that amino acids and proteins were needed for the biosynthesis of GnRH and LH, which started ovulation. The increase of gonadotropin synthesis could increase steroid concentration in blood, and steroid concentration has a relationship with the physical properties of cervical mucus (Rangnekar et al., 2002).

The average (±SD) total blood cholesterol in cervical mucus with thin viscosity, moderate viscosity, and thick viscosity was 123.0; 146.0±2.8; and 162.2±39.90 mg/dL, respectively. Cholesterol is a fatty substance that is produced by the liver and circulates in the blood (Murray et al., 2003). Normal cows have a total blood cholesterol in the range of 80-170 mg/dL (Mitruka et al., 1977). Total cholesterol levels in this study were found in the normal range. According to Murray et al. (2003), cholesterol is an important element in plasma membranes and a chief element in all other steroids synthesized in the body, such as adrenal cortex hormones and sex hormones, as well as vitamin D and bile acids.

The results of this study showed that the higher the total cholesterol level, the greater the viscosity of the cervical mucus. Pereek and Dien (1985) and Arosh et al. (1998) found a close relationship between low cholesterol levels and decreased levels of steroidogenesis. Highshoe et al. (1991) reported that cholesterol acted as a precursor to the biosynthesis of androstenedione, progesterone, and estrogen by granulose cells under the influence of LH. Increased steroidogenesis increases the concentration of steroids, which in turn affects the viscosity of cervical mucus (Lim et al., 2014).

Average (±SD) blood glucose levels in cervical mucus with thin viscosity, moderate viscosity, and thick viscosity were 39.0; 32.5±10.60; and 38.8±18.24 mg/dL, respectively. The results showed that there was no clear relationship between blood glucose levels and viscosity of cervical mucus. Therefore, it was suspected that the viscosity of cervical mucus in this study was more influenced by the levels of total protein and cholesterol.

Mitruka and Rawnsley (1981) reported that a normal cow blood glucose level was 89.0±22.0 mg/dL. Blood glucose levels from cows with three different cervical mucus viscosities in this study were low. Low blood glucose levels could cause a decrease in hypothalamic GnRH secretion, due to the lack of ATP, which plays the role of activating cAMP as an intracellular messenger (Murray et al., 2003). The decrease in GnRH secretion was followed by a decrease in the synthesis of FSH and LH hormones, which resulted in the absence of ovarian follicle growth or ovarian hypofunction, reduced insulin and Insulin Growth Factor 1 (IGF-1) concentrations, and reduced estradiol production by ovarian follicles (Mulligan et al., 2006).

The average blood macromineral levels at different cervical mucus viscosities are presented in Table 4.

Table 4: Macromineral levels of Aceh cattle blood serum at different cervical mucus viscosities

No	Mucus criteria	Number of cows	Na (mmol/L)	K (mmol/L)	Cl (mmol/L))	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)
1.	Thin	1	112	3.1	128	8.2	7.2	2.1
2.	Moderate	2	222±1.41	6.5±0.07	258±1.41	16.9±0.21	15.6±0.42	4.1±0.07
3.	Thick	5	582±5.59	17.5±0.3	620±2.91	43.6±0.22	35.8±2	30.3±8.91

From a total sample of eight cows, five had cervical mucus with a thick viscosity, all of which was transparent in color. Two cows showed a moderate mucus viscosity, in which the mucus color was transparent and cloudy. There was one cow with mucus with thin viscosity and a turbid color. The average macromineral levels of Na, K, Ca, P, Cl, and Mg in Aceh cows with thick vs moderate vs thin viscosity were as follows: Na (582±5.59 vs 222±1.41 vs 112 mmol/L); K (17.5±0.3 vs 6.5±0.07 vs 3.1 mmol/L); Ca (43.6±0.22 vs 16.9±0.21 vs 8.2 mg/dL); P (35.8±2,0 vs 15.6±0.42 vs 7.2 mg/dL); Cl (620± 2.91 vs. 258±1.41 vs. 128 mmol/L); and Mg (30.3±8.91 vs. 4.1± 0.07 vs. 2.1 mg/dL), respectively.

The macromineral concentrations in the blood serum of female Aceh cattle with thin mucus viscosities were relatively normal, whereas for moderate and thick viscosities, the macromineral concentrations were high. Therefore, higher macromineral concentrations in blood serum could cause higher cervical mucus viscosity. This shows the specific influence of macromineral concentration on cervical mucus viscosity. There are numerous factors which make macromineral concentration affect cervical mucus viscosity, although Piliang (2002) stated that body mineral requirements were small, only about 4%. The body macromineral that plays roles in reproduction, particularly in the estrus phase, is NaCl. In the estrus phase, NaCl levels are high and can cause an increase in estrogen levels. Estrogen plays a role in the secretion of cervical mucus.

In blood, the normal concentration of Na is 137-148 mmol/L and the normal concentration of K is 3.8-5.2 mmol/L (Anderson and Rings, 2009). In this study, normal values were only found in cows with thin mucus viscosity, whereas in cows with moderate and thick mucus viscosities, the values were higher. A deficiency of Na in the body will cause hypofunction in ovaries, which results in repeated breeding (Robert, 1971). Meanwhile, excessive administration of K can result in delayed puberty and ovulation, impaired development of corpora lutea, and increased incidence of anestrus in cows (Velladurai et al., 2016).

Normal Cl concentration in cow blood is 90-110 mmol/L (Clark, 2001) and normal Ca concentration is 8-12 mg/dL. If calcium levels drop to 4-5 mg/dL, hypocalcemia is indicated (Goff et al., 2005). The highest peak of Ca concentration is on day 0 of the estrous cycle (the estrus phase). Ca levels will decrease significantly on day 7, and will rise slowly on day 25 of the estrous cycle (Kekan and Shirbhate, 2015).

The normal concentration of P in blood plasma ranges from 4.24 to 7.58 mg/dL (Hazzimusic and Krinic, 2014). In this study, a normal value was only found in a cow with a thin mucus viscosity, whereas in cows with moderate and thick viscosities, the values were high. P plays a role in the metabolic process and in the reproductive system. Low P levels can reduce fertility (productivity) and cause irregular estrus, which can result in repeated breeding (McDonald et al., 2002).

The normal concentration of Mg in blood plasma is 1.8-2.4 mg/dL (Kincaid, 2008). In this study, a normal value was only found in a cow with thin mucus viscosity, whereas in cows with moderate and thick viscosities, the values were high. Mg deficiency results in decreased appetite, leading to overall decreases in nutritional intake. The decrease in total nutritional intake can result in indirect reproductive disorders. Magnesium usually does not have a direct effect on livestock reproductive disorders, but Mg has an antagonistic relationship with Ca. Therefore, any changes in Ca-P-Mg homeostasis will affect the reproductive status of livestock (Kumar, 2003).

NaCl and Mg percentages in cervical mucus are 0-0.003% and 0.05%, respectively. In the estrus phase, levels of NaCl increase because there is an increase in estrogen. Estrogen affects mineralocorticoids, adrenaline, and oxytocin. Mineralocorticoids are steroid hormones that are responsible for the maintenance of sodium levels, as well as the balance of sodium, potassium, and hydrogen that circulating in the body, including circulation to Goblet cells through nerve cells (Makmum et al., 2017).

The pregnancy rates in cows with thick, moderate, and thin cervical mucus viscosities were 20%; 50%; and 100%, respectively (Table 1). This result was consistent with a study by Deo and Roy (1971), which observed a higher conception rate in fertile cows with thin cervical mucus (100%). Thin cervical mucus facilitates spermatozoa transport and increases its lifespan. According to Mufti et al. (2010), a 47.54% conception rate occurred in cows with thin to moderate cervical mucus consistencies, which caused an increase in migration of spermatozoa in the female reproductive tract. Bernardi et al. (2016) reported that the success rates of artificial insemination in Holstein cows categorized by cervical mucus viscosity were as follows: 9% for cows with thick cervical mucus, 30% for cows with moderate cervical mucus, and 61% for cows with thin cervical mucus. During the pre-ovulation phase, cervical mucus has a low viscosity and the amount of cervical mucus and the changes that occur are influenced by body hormone levels (Tsiligiani et al., 2011). Mellado et al. (2015) found that the conception rate of cows with transparent mucus is higher than that of cows with turbid mucus (30.6% vs 22%). The combination of mucoprotein and weak sperm penetration will prevent progressive movement of spermatozoa, which causes a lower conception rate in cows with thick cervical mucus.

The amount and consistency of mucus will change depending on the estrous cycle phase and variations in hormone levels (Verma et al., 2014). During the estrous cycle, changes in the physical characteristics of cervical mucus can be used to determine the optimal time for artificial insemination (Tsiligiani et al., 2011). The natural fertilization process generally occurs when animals are in estrus, in which one of the signs in female animals is the accumulation of mucus in the vagina. This mucus seems to be a moderate that helps spermatozoa reach the fertilization site, as well as becoming a selector of spermatozoa (Cox et al., 1997; Hafez and Hafez, 2000).

From a sample of eight cows, one cow showed a thin cervical mucus viscosity. A thin mucus consistency facilitates the migration of sperm cells in the female reproductive tract. Bernardi et al. (2016) reported that fertile cows with thin and clear mucus had a pregnancy rate of 65%, while cows with thick mucus had a pregnancy rate of 40%.

Thinner cervical mucus causes faster penetration of sperm cells to the ovum cell, resulting in a higher pregnancy rate. This is in accordance with the results of an in vitro study by Murase et al. (1990), which found that spermatozoa moved linearly with the direction of estrus mucus streaks. The movement of spermatozoa may be facilitated by the presence of channels in the mucus that are easily penetrated and guide the spermatozoa forward. This situation occurs due to the influence of estrogen at the time of estrus, which acts to regulate glycoprotein macromolecules so that the distance between mucus molecules becomes 2-5 μm. In this situation, a channel forms and can be traversed by spermatozoa (Hafez and Hafez, 2000).

ACKNOWLEDGEMENTS

This work was supported by the rector of syiah kuala university, banda aceh, indonesia under the project "hibah guru besar, 2017."

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

The manuscript was written by Tongku Nizwan Siregar, Muhammad Aufa Reynaldi, Fathur Razak, Yulli Artaliani, Yuswar Yuswar, Zainal Abidin and edited by Teuku Armansyah, Budianto Panjaitan, Gholib Gholib, Amalia Sutriana and Herrialfian Herrialfian.

Planning and execution of this work were under the supervision of Tongku Nizwan Siregar. Muhammad Aufa Reynaldi, Fathur Razak, Yulli Artaliani, Yuswar Yuswar, Zainal Abidin collected the samples and conducted an experiment under the supervision of Tongku Nizwan Siregar, Teuku Armansyah, Budianto Panjaitan, and Gholib Gholib. All authors read and approved the final manuscript.

REFERENCES

- Agarwal A, Bragais FM, Sabanegh E (2008). Assesing sperm function. Urol. Clin. North. Am. 35(2): 157-171. https://doi.org/10.1016/j.ucl.2008.01.012
- Anderson DE, Rings M (2009). Current Veterinary Therapy. Food Animal Practice St. Louis, Saunders Elsevier, USA.
- Arosh AJ, Kathiresan D, Devanathan TG, Rajasundaram RC, Rajasekaran J (1998). Blood biochemical profile in normal cyclical and anoestrous cows. Indian J. Anim. Sci. 68(1): 1154-1156.
- Benbia S, Kalla A, Yahia M, Belhadi K, Zidani A (2011). Enzymes activity in bovine cervical mucus related to the time of ovulation and insemination. J. Biol. Agricult. Food Biotechnol. Engineering. 5(11): 664-666.
- Berger PC (1951). The pH of bull semen and the vagina of cows as related to fertility. Tesis. Master of Science. Oregon State College, USA.
- Bernardi S, Rinaudo A, Marini P (2016). Cervical mucus characteristics and hormonal status at insemination of holstein cows. Int. J. Vet. Res. 17(1): 45-49.
- Branigan RE, Larry IL (2008). Sperm transport and capacitation. London. Global Library of Women's Medicine. United of Kingdom.
- Clark JH (2001). Nutrient Requirement of Dairy Cattle. 7th ed. Washington DC. National Academy Press.
- Correa CHM, Mattos ALG, Ferrari AN (2001). In situ variation of cervical mucus pH during exposure to atmospheric air. Braz. J. Med. Biol. Res. 6(34): 767-770. https://doi.org/10.1590/S0100-879X2001000600011
- Cox JF, Martinez C, Lagos S, Saravia F, Sasmay R (1997). Sperm migration in cervical mucus in goats. II Relationship with colonization of the oviduct and fertilization efficiency. Theriogenology. 47(1): 254. https://doi.org/10.1016/S0093-691X(97)82381-3
- Deo S, Roy DJ (1971). Investigations on repeat breeding cows

and buffaloes studies on physical properties of cervical mucus. J. Indian Vet. 48: 479-484.

- Dev S, Pangawkar GR, Sharma RK, Verma HK (1997). Sperm penetration in relation to physical characteristics of buffalo oestrual mucus. Inter. J. Anim. Sci. 12: 89-91.
- Dodamani MS, Mohteshamuddin K, Awati SD, Tandle MK, Honnappagol SS (2010). Evaluation of pre and post artificial insemination effect of GnRH hormone on conception of repeat breeder Deoni cows. Vet. World. 3(5): 209-211.
- Dogra P, Dhaliwal GS, Kaswan S (2017). Estrous behaviour, physio chemical properties and vaginal cytology of cervical mucus in beetal goats during induced estrus. Indian J. Anim. Reprod. 38(1): 41-44.
- Eltohamy MM, Zakaria AD, Taha NA (1990). Changes in the contents of buffalo cervical mucus during different phases of the estrous cycle. Anim. Reprod. Sci. 22: 203-211. https://doi.org/10.1016/0378-4320(90)90060-S
- Farley DB, Ford SP, Rosazza JPN (1992). Increase in uterine peroxidase activity in the rat uterus during oestrogen hyperaemia. J. Reprod. Fert. 95: 551-558. https://doi.org/10.1530/jrf.0.0950551
- Fordney-Settlage D (1981). A review of cervical mucus and sperm interactions in humans. Int. J. Fertil. 26(3): 161-169.
- Gaddum-Rosse P (1981). Some observations on sperm transport through the uterotubal junction of the rat. American J. Anat. 160: 333-341. https://doi.org/10.1002/aja.1001600309
- Gebrehiwot G, Gebrekidan B, Weldegebriall B (2015). The effect of one shot prostaglandin on estrus synchronization of local and Holstein friesian cattle in and around Wukro Kilte Awulaelo District, Northern Ethiopia. J. Biol Agricult. Healthcare. 5(7): 99-106.
- Goff DC, Sullivan LM, McEvoy JP (2005). A comparison of ten-year cardiac risk estimates in schizophrenia patients from the CATIE study and matched controls. Schizophrenia Res. 80: 45-53. https://doi.org/10.1016/j.schres.2005.08.010
- Guyton AC (1991). Buku Teks Fisiologi Kedokteran (Diterjemahkan oleh Adji Dharmadan P. Lukmanto). EGC, Jakarta.
- Hafez B, Hafez ESE (2000). Reproduction in Farm Animals. 7th Ed. Lippincott William and Wilkins. Philadelphia. https://doi.org/10.1002/9781119265306
- Hamana K, Elbama AA, Hafez ESE (1971). Sialic acid and some physico-chemical characteristic of bovine cervical mucus. Cornell Vet. 61: 104-113.
- Hazzimusic N, Krnic J (2012). Values of calcium, phosphorus and magnesium concentrations in blood plasma of cows in dependence on the reproductive cycle and season. J. Fac. Vet. Med. Istanbul Univ. 38(1): 1-8.
- Highshoe AP, Cochran RC, Corah LR, Kiracofe GH, Harmon DL, Perry RC (1991). Effects of calcium soaps of fatty acids on postpartum reproductive functions in beef cows. J. Anim. Sci. 69: 4097-4103. https://doi.org/10.2527/1991.69104097x
- Irfan, Wahjuningsih S, Suliswati T (2017). Pengaruh karakteristik lendir serviks sebelum inseminasi buatan (IB) terhadap keberhasilan kebuntingan sapi komposit. J. Ternak Tropikal. 18(1): 24-28. https://doi.org/10.21776/ub.jtapro.2017.018.01.2
- Kekan PM, Shirbhate RN (2015). Biochemical status during oestrus cycle in regular and repeat breeding cows. Theriogenology Insight. 5(3): 213-217. https://doi.org/10.5958/2277-3371.2015.00023.6
- Khan S, Thangavel A, Selvasubramaniyan S (2010). Blood biochemical profile in repeat breeding cows. Tamilnadu J. Vet. Anim. Sci. 4(2): 90-102.
- Kincaid R (2008). Changes in the concentrations of minerals in blood of peripartum cows. Mid-South Ruminant Nutrition Conference: 1-8.
- Kumar A, Mehrotra S, Dangi SS, Singh G, Singh M, Mahla AS (2012). Amylase activity in cervical mucus and serum during estrus in normal and repeat breeder cattle. Vet. World. 5(8): 486-488. https://doi.org/10.5455/vetworld.2012.486-488
- Kumar S (2003). Management of infertility due to mineral deficiency in dairy animals. In: Proceedings of ICAR summer school on "Advance diagnostic techiques and therapeutic approaches to metabolic and deficiency diseases in dairy animals". Held at IVRI, Izatnagar, UP (15th July to 4th Aug):128-137.
- Lim H, Son J, Yoon H, Baek K, Kim T, Jung Y, Kwon E (2014). Physical properties of estrus mucus in relation to conception rates in dairy cattle. J. Emb. Trans. 2(29): 157-161. https://doi.org/10.12750/JET.2014.29.2.157
- Loeffler SH, de Vries MJ, Schukken YH, de Zeeuw AC, Dijkhuizen AA, de Graaf FM, Brand A (1999) Use of technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. Theriogenology 52: 1267-1284. https://doi.org/10.1016/S0093-691X(99)00071-0
- Mahmoud GB (2013). Physical and chemical properties of ewes cervical mucus during normal estrus and estrus induced by intravaginal sponges. Egyptian J. Anim. Prod. 50(1): 7-12.
- Mahmoudzadeh AR, M Tarahomi, H Fotoohi (2001). Effect of abnormal vaginal discharge at oestrus on conception rate after artificial insemination in cows. Anim. Sci. 72: 535- 538 https://doi.org/10.1017/S1357729800052061.
- Makmum AD, Samsudewa, Ondho YS (2017). Kadar NaCl dan pH lendir serviks rusa timor (Rusa timorensis) betina yang mendapatkan suplementasi mineral selama siklus estrus. J. Sain Peternakan Indonesia. 12(3): 209-307.
- Manas KR, Mondal MK, Samik Kr., SK Paul (2012). Haematobiochemical profile in repeat breeding crossbred cows. Explor. Anim. Med. Res. 2(1): 60-65.
- Mardiati SM (2003). Kadar Garam Na Lendir Serviks serta Kadar Garam Na dan K Lendir Mulut pada Berbagai Struktur Daun Pakis (*Tes ferning*). Thesis. Universitas Diponegoro, Semarang.
- McDonald P, Edwards RA, Greenhalgh JFD (2002). Animal Nutrition. 6th ed. Longman, London and New York.
- Mellado M, Lara LM, Veliz FG, Santiago MAD, Reyes LA, Herrera CM, Garcia JE (2015). Conception rate of artificially inseminated Holstein cows affected by cloudy vaginal mucus under intense heat conditions. Pesq. Bras. Brasilia. 50(6): 492-498. https://doi.org/10.1590/S0100-204X2015000600008
- Mitruka BM, Rawnsley HM, Vadehra BV (1977). Clinical biochemical and hematological reference values in normal experimental animals. Masson Publishing, Inc. New York.
- Mitruka BM, Rawnsley HM (1981). Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals and Normal Humans. 2nd ed. Year Book Medical Publishers Inc. Chicago.
- Modi LC, Suthar BN, Nakhashi HC, Sharma VK, Panchasara HH (2011). Physical characteristic of estrual cervical mucus and conception rate in repeat breeder kankrej cattle. IJAVMS. 5(4): 416-423. https://doi.org/10.5455/ijavms.20110801094641

- Mohanty BN, Dash RN, Mohanty DN, Girl SC (1996). Physico-biochemical properties of cervical mucus in normal and repeat breeding cows. XIII National Convention of ISSAR and National Symposium on Animal Biotechnology, December 4-6, 1996. G.B. Pant University of Agriculture and Technology, Pantnagar, U.P. (India).
- Mufti MMR, Alam MK, Sarker S, Bostami AB, Das N (2010.) Study on factors affecting the conception rate in red Chittagong cows. Bangladesh. J. Anim. Sci. 39 (1&2): 52-57.
- Mulligan FJ, O'Grady L, Rice DA, Doherty ML (2006). Nutrition and fertility in dairy cows. Irish Vet. J. 60: 15-20.
- Murase T, Okuda K, Sato K (1990). Assessment of bull fertility using mucus penetration test and a human chorionic gonadotrophin stimulation test. Theriogenology 34(5): 801-812. https://doi.org/10.1016/0093-691X(90)90552-5
- Murray RK, Granner DK, Mayes PA, Rodwell VW (2003). Biokimia. 25th ed. Penerbit Buku Kedokteran EGC. Jakarta.
- Naik R, Kumar AVNS, Bramhaiah KV, Ravi A, Chakravarthi VP (2013). Estrogen and progesterone hormone levels in Punganur cattle. IOSR-JAVS. 2(5): 50-53. https://doi.org/10.9790/2380-0255053
- Peek JC, Colin M (1986). The pH of cervical mucus, quality of semen, and outcome of the postcoital test. Clin. Reprod. Fertil. 4(3): 217-225.
- Pereek PK, Dean AC (1985). Certain blood constituents level in anestrus cows. Indian J. Anim. Reprod. 6: 33-35.
- Piliang WG (2002). Nutrisi Vitamin. Volume 1. 5th ed. Institut Pertanian Bogor Press. Bogor.
- Prasdini WA, Sri R, Mochammad SD (2015). Level of estrogen and cervical mucus pH as indicator of estrus after calving towards the provision of selenium vitamin ETM on dairy cow Frisien holstein (FH). Int. J. Chem. Tech. Res. 1(7): 190-195.
- Rafika I (2017). Isolasi dan identifikasi bakteri gram negatif pada uterus sapi aceh yang mengalami repeat breeding. Tesis. Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia.
- Ramli M, Siregar TN, Thasmi CN, Dasrul, Wahyuni S, Sayuti A (2016). Hubungan antara intensitas estrus dengan konsentrasi estradiol pada sapi aceh pada saat inseminasi. J. Med. Vet. 1(10): 27-30. https://doi.org/10.21157/j.med.vet..v10i1.4032
- Rangnekar MN, Dhobel RL, Gacche MG, Ingawale MV, Sawale AG, Jadhov JM (2002). Physical properties of estrual cervical mucus in repeat breeding crossbred (HF) cows with reference to fertility. Indian. J. Anim. Sci. 72: 1122-1124.
- Roberts SJ (1971). Veterinary Obstetrics and Genital Disease. 2nd ed. CBS Publishers and Distributors (India), New Delhi.
- Rutllant J, Lopez-Bejar M, Lopez-Gatius F (2005). Ultrastructural and rheological properties of bovine vaginal fluid and its relation to sperm motility and fertilisation: a review. Reprod. Domest. Anim. 40: 79-96. https://doi.org/10.1111/j.1439-0531.2004.00510.x
- Salisbury GW, Vandenmark NL (1985). Fisiologi Reproduksi dan Inseminasi Buatan Pada Sapi. Gajah Mada University Press, Yogyakarta.
- Schumacher GFB (1970). Biochemistry of cervical mucus. Fert. Steril. 21: 697-700. https://doi.org/10.1016/S0015-0282(16)37800-1
- Siregar TN, Agustina I, Masyitah D, Al-Azhar, Dasrul, Thasmi CN, Sulaiman R, Daud R (2017). Physical properties of cervical mucus of repeat breeder Aceh cattle. J. Vet. 3(18): 378-382. https://doi.org/10.1155/2016/3976125
- Siregar TN, Melia J, Rohaya R, Thasmi CN, Masyitha D, Wahyuni S, Rosa J, Nurhafni N, Panjaitan B, Herrialfian H (2016). Determining proportion of exfoliative vaginal cell during various stages of estrus cycle using vaginal cytology techniques in aceh cattle. Vet Med Int, http://dx.doi.org/10.1155/2016/3976125.
- Siregar TN, Wajdi F, Akmal M, Fahrimal Y, Adam M, Panjaitan B, Sutriana A, Daud R, Armansyah T, Meutia N (2017). Embryonic death incidents due to heat stress and effect of therapy with gonadothropin releasing hormone (GnRH) in aceh cattle. Vet. Med. Zoot. 75(97): 70-74.
- Suharto K (2003). Penampilan potensi reproduksi sapi perah friesian holstain akibat pemberian kualitas ransum berbeda dan infusi larutan iodium povidon 1% intra uterin. Fakultas peternakan UNDIP. Semarang.
- Suharto K (2008). Pengaruh waktu inseminasi buatan (IB) dalam hubungannya dengan waktu ovulasi terhadap angka kebuntingan dan rasio seks pada kambing peranakan etawah (PE). Dissertation. Universitas Gadjah Mada. Yogyakarta.
- Thasmi CN, Siregar TN, Wahyuni S, Aliza D, Hamdan H, Panjaitan B, Asmilia N, Husnurrijal H (2017). Estrus performance and steroid level of repeat breeding aceh cattle synchronized with Pgf2 alfa. Veterinaria. 66(1): 36-41.
- Tsiligianni A, Karagiannidis P Saratsis, Brika S (2003). Enzyme activity in bovine cervical mucus during spontaneous and induced estrus. Canadian J. Vet. Res. 67(3): 189-193. Tsiligianni TH, Amiridis GS, Dovolou E, Menegatos I, Chadio S, Rizos D, Adan AG (2011). Association between physical properties of cervical mucus and ovulation rate in superovulated cows. Canadian J. Vet. Res. 75: 248-253.
- Tsiligianni TH, Karagiannidis IA, Brikas P, Saratsis PH (2000). Enzyme activity in bovine cervical mucus during spontaneous and induced estrus by progesterone and/or PGF2α. Canadian J. Vet. Res. 67:189-193.
- Tsiligianni TH, Karagiannidis IA, Brikas P, Saratsis PH (2011). Physical properties of bovine cervical mucus during normal and induced (progesterone and/or PGF2ά) estrus. Theriogenology. 55(2): 629-640. https://doi.org/10.1016/S0093-691X(01)00431-9
- Velladurai C, Selvaraju M, Napoleon RE (2016). Effects of macro and micro minerals on reproduction in dairy cattle a review. Int. J. Sci. Res. Sci. Technol. 2(1): 68-70.
- Verma KK, Prasad S, Kumaresan A, Mohanty TL, Layek SS, Patbandha TK, Chand S (2014). Characterization of physico-chemical properties of cervical mucus in relation to parity and conception rate in Murah buffaloes. Vet. World. 7(7): 467-471. https://doi.org/10.14202/vetworld.2014.467-471
- Vermeiden JPW, Bernardus RE, Brug CS, Statema-Lohmeijer C, Willemsen-Brugma AM (1989). Pregnancy rate is significantly higher in in vitro fertilization procedure with spermatozoa isolated from nonliquefying semen in which liquefaction is induced by α-amylase. 51(1): 149-152.
- Yousef A (1981). Note on the biochemical aspects of bovine cervical mucus. Indian J. Anim. Sci. 51: 1082-1085.
- Zaman MI, Sharma U, Kumar S, Kumar S (2013). Studies on physical properties of cervical mucus of repeat breeding crossbred cows. Indian J. Anim. Reprod. 34(2): 6-8.
- Zavos PM, Melvin RC (1980). The pH of cervical mucus and the postcoital test. Fertil. Steril. 34(3): 234-238. https://doi.org/10.1016/S0015-0282(16)44953-8