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Physical Properties of Cervical Mucus of Repeat Breeder Aceh Cattle

(*PERUBAHAN FISIK MUKUS SERVIKS PADA SAPI ACEH YANG MENGALAMI KAWIN BERULANG*)

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ABSTRACT

This study aimed to investigate the physical property changes of cervical mucus in repeated breeder Aceh cows. Six cows consisting three normal (K1) and three repeat breeders (RB) (K2) of adult Aceh cows were used. The characteristics of K1 group were at > 2 months post parturition, successful pregnancy following once insemination, and twice regular estrus cycle twice. The K2 were cows with normal estrus but failed to be pregnant after three times artificial inseminations. Cervical mucus samples (50 ml each) were collected by aspiration using a sterile catheter and disposable syringe. The comparisons of the physical properties of cervical mucus between fertile and repeat breeder cattle were as follows.. Cervical mucus unavailability/in small quantity was 0.00 vs. 66.67%, cloudy color was 0.00 vs. 66.67%, thick consistency was 0.00 vs. 100%, fern pattern was 0.00 vs 66.67%, spinnbarkeit was 5.16±1.60 vs. 2.83±2.02 cm and pH values was 7.33±0.57 vs 9.33±1.52. in conclusion, the physical properties of cervical mucus in repeat breeder Aceh were less in quantities, more cloudy, higher in viscosity, and higher pH as compared to normal fertile cows.

Key words: cervical mucus; Aceh cattle; repeatbreeder

ABSTRAK

Penelitian ini bertujuan mengetahui perubahan sifat fisik mukus serviks sapi aceh yang mengalami kawin berulang (*repeat breeding,* RB). Dalam penelitian ini digunakan enam ekor sapi aceh betina yang terdiri dari tiga ekor sapi normal (K1) dan tiga ekor sapi yang mengalami RB (K2). Sapi K1 merupakan sapi >2 bulan pascapartus yang mempunyai riwayat berhasil bunting dengan sekali inseminasi dan mempunyai dua kali siklus reguler, sedangkan K2 terdiri atas sapi yang didiagnosis mengalami RB, yaitu sapi yang gagal bunting setelah lebih dari tiga kali inseminasi namun memiliki siklus estrus normal. Sampel mukus serviks dikoleksi dengan metode aspirasi menggunakan kateter steril dan *disposible syringe* 50 mL. Kateter dimasukan melalui vagina yang yang diiringi dengan palpasi rektal untuk mengarahkan kateter masuk ke serviks uterus. Hasil pemeriksaan sifat fisik mukus serviks pada sapi fertil vs sapi RB menunjukkan bahwa kuantitas mukus tidak ada/sedikit (0,00 vs 66,67%), warna keruh (0,00 vs 66,67%), konsistensi kental (0,00 vs 100%), pola pakis (0,00 vs 66,67%), *spinnbarkeit* (5,16±1,60 vs 2,83±2,02 cm) dan nilai pH (7,33±0,57 vs 9,33±1,52). Dari hasil penelitian dapat disimpulkan bahwa sapi aceh yang mengalami RB memiliki kuantitas mukus lebih sedikit, warna lebih keruh, konsistensi kental, dan pH yang lebih tinggi dibanding sapi aceh yang fertil.

Kata-kata kunci: mukus serviks; sapi aceh; kawin berulang

INTRODUCTION

Reproduction productivity can be enhanced if estrus cycle and schedule were all monitored and recorded. Detection of estrus can be done by several ways, one of them by doing macroscopic and microscopic examinations on cervical mucus produced during estrus cycle, in particular around estrus peak (Silaban *et al*., 2012).

Cervical mucus is mucus secreted by cervix uterus during estrus period (Abidin *et al*., 2012). Estrogen that works on female sexual organs enhance secretion of cervical mucus that then sticks on some parts of the vulva. This hormone is produced by follicles that develop in the ovary of estrus animals and plays important roles in estrus intensity (Ismail, 2009).

The properties of cervical mucus are influenced by semen depositional capacity in female reproduction tracts and directly related to their fertility status (Rangnekar *et al*., 2002). Under the influence of gonadal hormones that fluctuate during estrus cycle, cervical mucus undergoes physical and chemical properties changes (Eltohamy *et al*., 1990). Physical properties of cervical mucus that cover color (appearance), consistency, elasticity, fern pattern, and pH may change in repeat breeder cattle (Mohanty *et al*., 1996).

Zaman *et al*. (2013) reported that cervical mucus consistency of repeat breeder cattle is thicker than that of fertile cattle. The thickness of cervical mucus was affected by significant difference in glycoprotein composition that potential to influence spermatozoa penetration (Dev *et al*., 1997) so that delay fertilization. Additionally, cuprum levels in the serum and cervical mucus of fertile cattle are higher than that of repeat breeders. Cuprum is one of micro minerals that plays an important role in maintaining optimum fertility due to its function as an indicator for the secretion of follicle stimulating hormone (FSH) and luteinizing hormone as well as for estrogen activation (Modi *et al*., 2013).

Repeat breeding is a condition in the female cattle that fails to pregnant after being mated three times or more with a fertile bull without any observed abnormality (Amiridis *et al.,* 2009). Repeat breeding cases are found in almost all parts of the world with the incidence ranges from 5.5-33.3% (Gustafsson and Emanuelsson, 2002; Yusuf *et al*., 2010). The high incidence of repeat breeding is a problem in animal farming that need to be overcome due to its detrimental effects on animal farms. The change in the physical properties of cervical mucus of repeat breeder Aceh cattle has not been reported so far. Therefore, a study is required to investigate the physical properties of cervical mucus of repeat breeder Aceh cattle.

RESEARCH METHODS

Research Methodology

Six Aceh cattle raised at the Farm Animal Facility of the Faculty of Veterinary Medicine of Syiah Kuala University were used in this study. They consisted of three fertile cattle (K1) and three repeat breeders (K2). The K1 cattle >2 months postpartus cattle had a successful pregnancy by single insemination and twice regular estrus cycles whereas K2 were cattle diagnosed underwent repeat breeding i.e. cattle that have normal estrus cycle but failed to pregnant after more than three time inseminations.

Estrus Synchronization

All female cattle were synchronized using intramuscular PGF2á hormone injection of 5 mL that done twice by 10-11 days interval. Estrus detection was performed twice a day in the morning and in the afternoon for 30 minutes each. Cattle were considered estrus if showed observable estrus signals such as red and swallowing vulva, agitation, climb other cattle, and the cervical mucus secretion.

Collection of Cervical Mucus

External genitalia organs of all cattle was cleaned using light antiseptic solutions and dried using cottons. Mucus samples were collected by aspiration using a sterile catheter connected with a disposable 50 mL syringe. Catheter was inserted via vagina followed by rectal palpation to direct catheter reached the *cornua uteri*.

Mucus samples were analyzed for the quantity, color, consistency, fern pattern, spinnbarkeit, and pH. Quantity was classified into no/a little, moderate, and a lot. Mucus color was observed visually and categorized as clear, cloudy or turbid. Mucus consistency was evaluated by putting 2-3 drops of mucus onto a clean, slopped object glass and then grouped into three different categories (Deo and Roy, 1971) namely thick, medium or thin. In order to identify fern pattern, cervical mucus were put or dropped onto the surface of a glass object, air-

dried and observed using a microscope with 10x10 and 10x40 magnifications. Fern patterns were classified as typical, atypical or negative. To identify spinnbarkeit, 2-3 drops of cervical mucus were put onto the surface of a fat-free glass object. Another fat-free glass object was put on it. The two glass objects were then slowly separated. The distance between the two slides before the mucus disconnected was measured and presented in centimeter (Panigrahi, 1964). Spinnbarkeit values were grouped as 0-8; 8-16; 16-24; and >24 cm. pH values were measured using a universal indicator and classified as <7.0; 7.0-7.5; 7.5-8.0; and >8.0. The changes in physical properties of cervical mucus of normal and repeat breeder cattle were reported descriptively.

RESULTS AND DISCUSSION

The results showed that two out of three (66.7%) fertile Aceh cattle secreted a lot of cervical mucus, and one (33.3%) had moderate cervical mucus. The majority (66.7%) repeat breeder cattle had no/little cervical mucus and the rest (33.3%) secreted moderate cervical mucus. These indicated that repeat breeder cattle had lesser volume of cervical mucus than that of the fertile cattle. Increased water and electrolyte concentrations in the cervical mucus changed glycoprotein and water ratio led to the change of cervical mucus properties. Because of higher volume, cervical mucus was more viscous, translucent and thinner to be passed by spermatozoa to the fertilization site compare to those had lesser quantity mucus (Rutllant *et al*., 2005).

Cervical mucus of repeat breeder cattle had higher (66.7%) turbidity levels than that of fertile cattle that had clear (100%) color. This finding was in agreement with those reported by Zaman *et al*. (2013), that maximum conception was found among cattle had clear cervical mucus. Clear cervical mucus was more conducive for spermatozoa penetration and motility. Steroid hormones produced by ovaries induced physicochemical changes of mucus characteristic such as color to facilitate spermatozoa motility. According to Enkhia and Kohli (1982) the color of cervical mucus was a common factor reported influenced spermatozoa penetration and conception rate. Turbidity of cervical mucus might retard spermatozoa motility to reach fertilization site. To obtain optimum fertility, the colour of cervical mucus must be clear and transparent (Lopez-Gatius *et al*., 1993).

Prevalence of thick, moderate and thin consistencies of cervical mucus of fertile cattle was 0.00, 33.3, and 66.7%, respectively. All (100%) repeat breeder cattle had thick mucus. This result was in agreement with those reported by Sukh and Roy (1971), that high (100%) conception rate was observed in fertile cattle had thin consistency of cervical mucus. Cervical mucus had thin consistency increased transportation continuity and survival of spermatozoa. According to Mufti *et al*. (2010) conception rate of 47.5% occurred in cattle had cervical mucus consistency ranged from thin to moderate, that in turn increased migration of spermatozoa inside female reproductive organs. Combination between *mucoprotein* and weak spermatozoa penetration might suppress progressive movement of spermatozoa and then caused lower conception rate in cattle had thick cervical mucus.

Two out of three (66.7%) fertile cattle showed atypical fern pattern and one (33.3%) showed no fern pattern. Repeat breeder cattle, on the other hand, showed either typical (66.7%) or atypical (33.3%) fern pattern. These results were different from those stated by Modi *et al*. (2011) that maximum conception rate was showed by fertile cattle had typical fern pattern compared to repeat breeder cattle had atypical or no fern pattern.

Fern pattern was formed by crystallization produced by molecules aggregation led to the formation of crystal nuclei (nucleation). Cervical mucus could be crystallized due to it contained *mucoprotein* (such as mucins) or other organic compounds and electrolytes in particular salts such as NaCl, KCl, and CaCl. Sodium chloride is even the main salt occurs in cervical mucus (Cortes *et al*., 2014).

Fern typology of cervical mucus may show different pattern according to estrus cycle. Closer to ovulation, the pattern becomes much clearer due to increased concentration of estrogen. Crystallization pattern of cervical mucus is related to the activity of ovaries. Crystallization pattern will continuously form before, during and after estrus. Noonan *et al.* (1975) stated that fern pattern of dried vaginal and cervical mucus occurred in much higher levels during estrus day compare to during other phases of estrus cycle. During estrus cycle, concentration of vaginal and cervical mucus reaches minimum values in estrus day and maximum in mid-

estrus. Collection of cervical mucus outside mid-estrus might potentially result in the formation of atypical fern pattern in fertile cattle.

Average spinnbarkeit values of cervical mucus of fertile and repeat breeder cattle were 5.16±1.60 and 2.83±2.02 cm, respectively. Spinnbarkeit values of fertile cattle ranged from 0-8 cm (67.8%) and 8-16 cm (33.3%) whereas those of repeat breeder cattle were only ranged from 0-8 cm (100%). Modi *et al.* (2011) suggested that conception rates were higher among cattle had larger spinnbarkeit values.

In fertile cattle, pH of cervical mucus during estrus period ranged from 7.0-8.5 with an average of 7.33±0.57. This finding was in agreement with those obtained in observation done by Methai *et al.* (2005). The pH of cervical mucus in repeat breeder cattle ranged from 8.0-11.0 with an average of 9.33±1.52. This was also in agreement with the report of Rane *et al.* (1992) that the pH of cervical mucus of repeat breeder cattle was higher than that of fertile cattle. The pH of cervical mucus showed the status of uterus environment during fertilization. Hafez and Hafez (2000) said that over acidity or alkalinity of cervical mucus reduces spermatozoa motility that in turn results in fertility failure. Acid or bases pH was commonly caused by biophysical and biochemical conditions of cervical mucus controlled by hormonal changes during estrus cycle. This mechanism causes each estrus cycle has characteristic pH value (Noakes *et al.*, 2003).

CONCLUSION

It can be concluded that cervical mucus of repeat breeder Aceh cattle has less amount of mucus with cloudy color, more viscous consistency, and higher pH than that of fertile Aceh cattle.

SUGGESTION

Further study is suggested to evaluate the physical properties of cervical mucus in female Aceh cattle with RB through increasing the sample number and repeated examination of estrus period.

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