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Original article

Determination of estrus duration based on cervical mucus characteristics in Aceh cattle using camera-equipped artificial insemination endoscope

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ABSTRACT

This study aimed to determine the length of the estrus period in Aceh cows based on changes in the characteristics of cervical mucus observed using a camera-equipped artificial insemination (AI) endoscope. This study used twelve healthy and reproductively sound cows aged 4-6 years. All cows were synchronized using prostaglandin F2α (PGF2α) injected intramuscularly twice, 11 days apart. Estrus detection was conducted 24 hours following the second injection of PGF2α three times daily for 30 minutes, respectively. Estrous cows were then subjected to the examination of their cervical mucus at 0, 6, 12, and 18 hours after the beginning of estrus. This study showed that out of 12 samples, only eight cows exhibited estrus signs. Characteristics of cervical mucus of cows at 0 and 6 hours of estrus were transparent. At 12 hours of estrus, five cows showed transparency, and the remaining three cows showed cloudy cervical mucus. Meanwhile, at 18 hours of estrus, all sampled cows showed cloudy cervical mucus then regarded as the end of the estrus. That means the estrus length of Aceh cows in this study was 15.75 ± 3.11 hours. It can be concluded that the determination of the duration of estrus in Aceh cows based on changes in the characteristics of the cervical mucus can be carried out using an AI endoscope with a camera. For further research, it is suggested to apply this estrus detection technique in the implementation of AI and determine the pregnancy rate.

Keywords: cloudy cervical mucus, estrus synchronization, prostaglandin F2α, transparent cervical mucus

INTRODUCTION

Aceh cattle is one of Indonesia's local cattle, a wealth of genetic resources that needs to be protected, preserved, and developed its superiority for breeding (Suswono, 2011). Aceh cattle are small-type beef cattle, with a dominant color pattern of brick red in females and brown-red in males. Aceh cattle adapt well to tropical climates and are resistant to disease in tropical areas (Rasyid *et al*., 2017). Averages of body weight, height, body length, and chest circumference in male Aceh cattle were 170-200 kg, 101-103 cm, 101-110 cm, and 130-145 cm. The females' body size parameters were 158-175 kg, 100-101 cm, 99-106 cm, and 123-131 cm.

Aceh cattle are closer to zebu (Bos indicus) than to Bos taurus, with the nucleotide base homogenity of Aceh Cattle to Bos indicus being 94.36% and Bos taurus being 88.52% (Abdullah *et al*., 2007). The age of puberty is reached at 1-2 years, the average services per conception is 1.5, and the calving interval is 12.97 months (Novita *et al*., 2018).

Aceh cattle were reared traditionally by smallholder farmers in rural areas. Reproductive management at the farm level is a core element of herd management. A central aspect of this is accurate and timely estrus detection, mainly when artificial insemination (AI) is utilized (Adenuga *et al*., 2020). AI helps improve local cattle's genetic quality and population by insemination using an imported elite bull semen (Dahlen *et al*., 2014). However, low reproductive efficiency is the main problem faced by smallholder farmers in applying AI. Obstacles that often occur are inaccurate estrus observations through changes in behavior and in the external reproductive organs. Fixed time artificial insemination and estrus detection are the determining factors for pregnancy in artificially inseminated cows (Rao *et al*., 2013; Udin *et al*., 2016).

Cervical mucus ferning was reported as a complement to estrus detection (Silaban *et al*., 2012; Ferdiansyah *et al*., 2022). The physical properties of estrus mucus were related to conception rates in dairy cattle (Lim *et al*., 2014). An introduction of cervical endoscopic technology to dairy farm practice in Northern Kazakhstan increased the level of the herd reproduction system (Raketsky *et al*., 2021). However, up to now, the application of that technology on Aceh cattle has not been reported. This study aimed to determine the duration of estrus in Aceh cattle based on the characteristics of cervical mucus using an AI endoscope fitted with a camera.

MATERIALS AND METHODS

This research was conducted at the Technical Implementation Unit of Experimental Animals, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, from April to May 2021. Twelve cows aged 4-6 years weighed 210-260 (234.17±17.82) kg, and Body Condition Score (BCS) 3 (1-5 scale) was used in this study. The cows were fed about 20 kg grass, 2-3 kg concentrate daily, and ad libitum drinking water.

Estrus synchronization

Twelve cows were examined rectally to ensure the cows were not pregnant. Estrus synchronization was conducted using two doses of 25 mg/head of Lutalyse™ (Zoetis, Belgium) intramuscularly, at 11 days interval. Estrus detection was started within 24 hours after the second injection of prostaglandin F2α. Estrus detection was carried out three times daily, each for 30 minutes. Estrus was detected using the estrus behavior score method (Table 1). A cow with a total score of at least 50 was designated at the estrus phase.

Table 1 Scoring scale for observed symptoms of estrus (van Eerdenburg *et al*., 1996).

estrous symptoms	score
mucous valvular discharge	3
cajoling (flehmen)	3
restlessness	5
sniffing the vagina of another cow	10
mounted but not standing	10
chin resting	15
mounting (or attempting) other cows	35
the mounting head side of other cows	45
standing estrus	100

Examination of cervical mucus using an AI endoscope fitted with camera

The AI Endoscope consists of acrylic sleeve (0.5-inch diameter and 38 cm length), camera Ip 67 (resolution of 700 megapixels, diameter 6 mms, LED lighting with six lights, focus at ± 4 cm), two meters cable connecting the camera to the gadget Oppo A57, and software (external USB cam with 900 x 1080 pixels resolution). Duration of estrus was determined by examining the characteristics of cervical mucous at the beginning of estrus (0), followed by 6, 12, and 18 hours later. The characteristics of cervical mucus were classified as transparent, cloudy, and dirty or inhomogeneous mucus with yellow, gray, and red colors (Sharma *et al*., 2013).

Cows to be examined were restrained in cattle crushes. The cow's vulva was cleaned adequately with an alcoholic tissue and Bovivet

gel (Jorgen Kruuse A/S, Denmark) lubricant was smeared into the vagina. The endoscope was connected to a smartphone. Then, the sleeve was smeared with sufficient lubricating gel, inserted into vagina cavity, directed and focused to the front of the cervical orifice. Then pictures were taken and stored on a smartphone. The data were presented descriptively and confirmed by images of mucus in front of the cervical orifice.

RESULTS

Eight of 12 cows (66.67%) were found estrus (with an estrus score of 100) after synchronization with prostaglandin F2α. Meanwhile, four of them (33.33%) that only reached estrus scores 8, 15, 25, and 25 were not categorized in estrus. Observation of cervical mucus of estrus cows at 0 and 6 hours after the first sign of estrus showed transparent mucus. At 12 hours after the first sign of estrus, 62.5% (5/8) showed transparent mucus, and 37.5% (3/8) showed cloudy mucus. At 18 hours of estrus, all mucus of the estrus cows was cloudy (Table 2, Figure 1). Based on Table 2, the averages length of Aceh cows' estrus cycle was 15.75 ± 3.11 hours.

Table 2 Characteristics of cervical mucus of estrus cows at several time intervals of observation

no.	observation time after estrus (hours)				
	0	6	12		18
1	transparent	transparent	transparent		cloudy
2	transparent	transparent	transparent		cloudy
3	transparent	transparent	transparent		cloudy
4	transparent	transparent	transparent		cloudy
5	transparent	transparent	transparent		cloudy
6	transparent	transparent		cloudy	cloudy
7	transparent	transparent		cloudy	cloudy
8	transparent	transparent		cloudy	cloudy
percentage	100%	100%	62.5%	37.5%	100%

DISCUSSION

Estrus synchronization aims to obtain estrus simultaneously, more effortless observation of estrus, and finally, the insemination can be carried out simultaneously. PGF2α administration caused regression of the functional corpus luteum and was followed by estrus (Elmetwally *et al*., 2021). Estrus rate after double injection of PGF2α in this study (66.67%) was lower than those reported by Venkata Ramana *et al*. (2013) (82%) and Hafizuddin *et al*. (2012) (100%). However, this result was higher than 57% reported by Ahlawat *et al*., (2015). The success of the estrus synchronization is determined by intrinsic factors (proper diet, BCS, health) of cows and extrinsic factors such as correct heat detection techniques. The inadequacy of those factors resulted in the estrus synchronization to be not as expected (Bihon and Assefa, 2021). Estrus synchronization must be accompanied by careful estrus detection to achieve a high conception rate (Syafruddin *et al*., 2016).

The characteristics of the cervical mucus in dairy cows are transparent mucus at the beginning of estrus, which gradually turns semi-transparent in the middle of estrus and cloudy at the end of estrus. The cloudy cervical mucus indicates that the cow's estrus period has ended (Bernardi *et al*., 2016). Estrus generally appeared in 10-18 hours (Gilbert, 2018). The estrus length of Aceh cows in this study was 12-18 hours. At 12 hours from the onset of estrus, 62.5% of cows showed transparent mucus, while the remaining 37.5% showed cloudy mucus, and at 18 hours from the onset of estrus, the cervical mucus of all cows was cloudy. This was different from the cervical mucus in buffalo that was still transparent at 12 hours from the onset of estrus (Sharma *et al*., 2013).

hours		cervical mucus
0		transparent
6		transparent
12		transparent
12		cloudy
18		cloudy

Figure 1 Characteristics of cervical mucus at 0, 6, 12, and 18 hours from the onset of estrus of Aceh cows.

The cervix connects the vagina and the uterine lumen. The cervical wall is composed of mucosa, muscular, and serosa. The cervical mucosa is arranged in folds with high columnar epithelium (Chase and Kaushik, 2019). The composition of cervical mucus will vary throughout the estrus cycle. This increase in cervical mucus production wss caused by increased paracellular permeability of the ectocervical cells. Estrogen produced from follicles in the ovaries increased the volume, thinness, and clarity of cervical mucus (Widiyono *et al*., 2011). During estrus, the cervix dilated due to high estrogen levels, and the cervical goblet cells produced watery and transparent cervical mucus that comes out of the vulva. While in the luteal phase, high progesterone levels caused the cervical rings to close, and the cervical goblet cells produce thick mucus to prevent microorganisms from entering the uterine lumen (Bernardi *et al*., 2016).

The amount of cervical mucus also depended on the level of estrogen in the circulation (Ma'ruf *et al*., 2017). The higher the estrogen was followed by an increase in the volume of cervical mucus (Widiyono *et al*., 2011). Estrogen induced the secretion of adrenaline and oxytocin hormones. Adrenaline affected the occurrence of estrus behavior in cows, while oxytocin caused the endothelial cells of blood vessels to become permeable, which increased the activity of goblet cells. The water accumulation in the goblet cell was followed by the release of mucus to the cervical canal (Pluta *et al*., 2012).

The physical properties of cervical mucus differ depending on estrogen and progesterone level along the estrous cycle (Han *et al*., 2020). The concentration of estrogen decreased 24 hours after estrus (Tsiligianni *et al*., 2011). Thin cervical mucus was related to the higher estrogen concentrations, and thick cervical mucus was related to the lower estrogen concentrations (Siregar *et al*., 2019). The viscoelasticity of cervical mucus was influenced by a gel-forming content of mucin or Mucin type 5 (MUC 5B) (Portal *et al*., 2017). This mucin was more commonly found in cervical mucus collected in the follicular phase than in the luteal phase (Soleilhavoup *et al*., 2014). Several aspects were assessed on cervical mucus, such as ferning (fern), viscosity, elasticity, thixotropy, and stickiness (Cortés *et al*., 2014). The estrus phase showed fewer microbes in the cervical mucus (Nur *et al*., 2020). The more cervical mucus produced, the higher the level of the lysozyme enzyme; thereby, the cervical mucus looks liquid and transparent due to the decreased number of bacteria (Abd-ElHafeez *et al*., 2020).

Cervical mucus produced by the endocervix played an essential role in Aceh cows' fertility (Siregar *et al*., 2019). Estrogen caused a lowering of the viscosity and maximized the

ferning of cervical mucus during estrus. Cervical mucus showed a form that resembled a fine thread hanging from the vulva (Nakano *et al*., 2015). Cervical mucus ferning was also related to cow's gestation and calf gender (Ferdiansyah *et al*., 2022). Progesterone caused cervical mucus appeared thick and intact, the cervical canal closed tightly, and the myometrium relaxed (Nakano *et al*., 2015). Estrus duration has influenced the hormones used to synchronize estrus (Handayani *et al*., 2014). The estrus length of Aceh cows in this study was 15.75 ± 3.11 hours, which means that the a.m./p.m. guideline could be applied to Aceh cattle. On the cow's estrus observed in the a.m. hours, AI should be conducted at the p.m. hours, and vice-versa (Stout, 2018).

CONCLUSION

The determination of the duration of estrus in Aceh cows based on changes in the characteristics of the cervical mucus could be carried out using an AI endoscope with a camera fitted, with an average length of estrus of 15.75 ± 3.11 hours. Further research can be carried out on the effect of the cow's age on the duration of estrus based on the characteristics of cervical mucus.

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