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Original article

Cervical opening during estrus in Aceh Local Cows based on camera-equipped artificial insemination endoscope

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ABSTRACT

Twelve healthy non-pregnant local cows, aged 3-6 years with parity of 1-3, were used in this study. The cows were synchronized using PGF2α (5 ml/cow, intramuscularly), twice at 11 days intervals. Estrus detection based on the scoring of van Eerdenburg was conducted 24 hours after the second prostaglandin injection. Estrus was detected three times daily for three days. The changes in cervical dilation were observed at 0, 6, and 12 hours after the onset of estrus using camera-equipped artificial insemination endoscope. The results showed that 8 out of 12 cows were in estrus after two injections of PGF2α. All cows in estrus showed slight cervical dilation with clear liquid mucus (cervical opening score 1) at 0-hour observation. Six hours later, 62.50% of cows remained at cervical opening score 1, and 37.50% changed to cervical opening score 2 (optimum cervical dilation with clear thick mucus). At 12 hours after the onset of estrus, 25% of estrus cows had cervical closure (cervical opening score 0, closed cervix with thick and cloudy mucus), and 75% changed to cervical opening score 2. In this study, 75% of the cows experienced an optimum cervical opening at 12 hours after the onset of estrus. It could be concluded that the opening of the external cervical canal of local Aceh cows is optimal at 12 hours after the onset of estrus.

Keywords : cervical opening, PGF2α, estrus, camera-equipped artificial insemination endoscope

INTRODUCTION

Artificial insemination is one of the efforts to increase cattle productivity and genetic quality (Poli *et al*., 2020). The Indonesian government implemented a massive artificial insemination program in cattle from 2014 to 2019 to increase the population. Initially, this program involved as many as three million acceptors in UPSUS SIWAB and SIKOMANDAN. UPSUS SIWAB (Special Efforts for Obligatory Breeding Cows) is a national movement that continues the previous year's activities to further encourage the growth of cattle and buffalo as outlined in the Minister of Agriculture Regulation (Permentan) Number 48/Permentan/PK.210/10/2016.

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SIKOMANDAN (Buffalo Cattle Mainstay of the Country) is a National Program of the Ministry of Agriculture, Directorate General of Livestock and Animal Health, for increasing the cattle population through improving livestock reproduction services (Ditjen PKH, 2020).

Artificial insemination is a process of depositing spermatozoa using an insemination gun by means of the rectovaginal technique into the cervical canal in estrus cows (Ax *et al*, 2000; Ismaya, 2014). Dilation of the cervix occurred during the follicular phase (proestrus and estrus) when estrogen predominates. It was closed again in the luteal phase when progesterone levels predominate (Dwarkasing et al., 2000). Cervical opening in estrus cows will determine the non return rate and pregnancy rate after artificial insemination (Susilawati, 2011). An artificial insemination endoscope with a camera has been created to facilitate the task of inseminators. This instrument is connected to a smartphone to display the location and condition of the external cervical os. The use of this kind of device for an artificial insemination aid and visualization of the external cervical os opening have never been reported. The results of this study are expected to be one of the parameters in determining the right time for artificial insemination. This study aimed to describe changes in the size of the cervical opening and the quality of the cervical discharge during estrus for an increased pregnancy rate of inseminated cows.

MATERIALS AND METHODS

This study was conducted at the Experimental Animals Technical Implementation Unit, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia. This study was located at Latitude: 5°57'01" N, Longitude: 95°36'97" E, in January - February 2021. The cows used were 12 healthy non-pregnant local cows, aged 3-6 years, 1-3 parity. The procedure of this study was approved by examination team for substantial and ethical aspect of animals use with reference No. 467/UN11.12.1/KPT/2021.

The cows were synchronized using PGF2α (Lutalyse®, Zoetis, Belgium) of 5 ml/cow intramuscularly, twice with 11 days intervals. Estrus detection was conducted 24 hours after the second prostaglandin injection at 07.00, 13.00, and 18.00 for 30 minutes for three days. The behavioral estrus detection was based on the scoring of van Eerdenburg (Van Eerdenburg *et al*., 2002). Cows with a minimum score of 50 points throughout the observation were determined to be in estrus (Mulfristia *et al*., 2022).

The artificial insemination camera endoscope consists of an Acrylic sleeve 0.5 inches in diameter and 38 cm in length, a Camera IP 67 diameter 6mm (resolution of 700 megapixelsMP), LED lighting with six lights focus at ± 4 cm, 2 meters Cable connector camera to a smartphone (CPH1909, OPPO), external USB camera with 900x1080 resolution, and CameraFi2 software.

The artificial insemination endoscope was connected to a smartphone using the CameraFi2 software. The acrylic sleeve was covered with plastic and lubricated sufficiently to get a clear image. The cows to be observed for estrus were placed in a restraining cage. The cow's vulva was cleaned using a tissue, and the vaginal cavity was smeared with lubricant (Bovivet gel™ lubricant gel, Kruuse, Denmark).

The artificial insemination endoscope was inserted 45° through the vulva, then directed until it reached the external cervical os. The plastic wrap was opened for good image focus quality, and then the light intensity was adjusted at a distance of ± 4 cm from the external cervical os. Images taken were stored on the smartphone. The examination was carried out three times at 0, 6, and 12 hours after the onset of estrus. Observations were made by using a speculum to open the vagina and taking photos of the external cervical os observed with the camera.

Table 1 Cervical opening scoring points based on the degree of cervical dilation (Kumbhar and Markandeya, 2017)

score	cervical opening
0	closed
1	slightly open
2	optimally open

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The parameters of this study were changes in cervical dilation at 0, 6, and 12 hours after the onset of estrus. The cervical opening was classified into three categories (Table 1). Data on the cervical opening during the estrus in local cattle were analyzed descriptively.

RESULTS

Eight out of 12 cows came into estrus after twice PGF2α injection. Data on the cervical opening score of estrus cows at 0, 6, and 12 hours of estrus can be seen in Table 2.

Table 2 Percentages and score of cervical opening in estrus cows observed at 0, 6, and 12 hours after the onset of estrus

score	observation time		
	0	6	12
0	-	-	25% (2/8)
1	100% (8/8)	62.5% (5/8)	-
2	-	37.5% (3/8)	75% (6/8)

score 0: closed cervix with thick cloudy mucus; score 1: slight cervical dilation with clear watery/liquid mucus; score 2: optimum cervical dilation with clear thick mucus

Based on observations using an artificial insemination endoscope with a camera, an overview of changes in the local cow's cervical opening during the estrus phase at 0, 6, and 12 hours can be seen in Figure 1. Cows not in estrus have a closed external cervical os, while it constantly opened during the estrus phase. A slightly open external cervical os is an early sign of estrus (Figure 1a). The cervix appears slightly dilated and begins to secrete mucus that looks liquid and clear. Five cows showed a slightly open external cervical os up to 6 hours of estrus observation (Figure 1b). Furthermore, an optimally open external cervical os (Figure 1c1), it can be seen that the cervix is optimally dilated. At this stage, the mucus secreted became thick and clear. Towards the end of estrus, the external cervical os will be closed again. A total of 2 cows experienced cervical closure at 12 hours after the onset of estrus (Figure 1c2).

In this study, 75% of the cows experienced an optimum cervical opening at 12 hours after the onset of estrus. This situation is considered the right time for artificial insemination. This followed the guidelines for artificial insemination: the best time for artificial insemination is 12 hours after the cow is in estrus (Stout, 2018). The time required for the external cervical os from being closed in the luteal phase to optimally dilated (approximately 1 cm) in estrus is seven hours (Kumbhar and Markandeya, 2017).

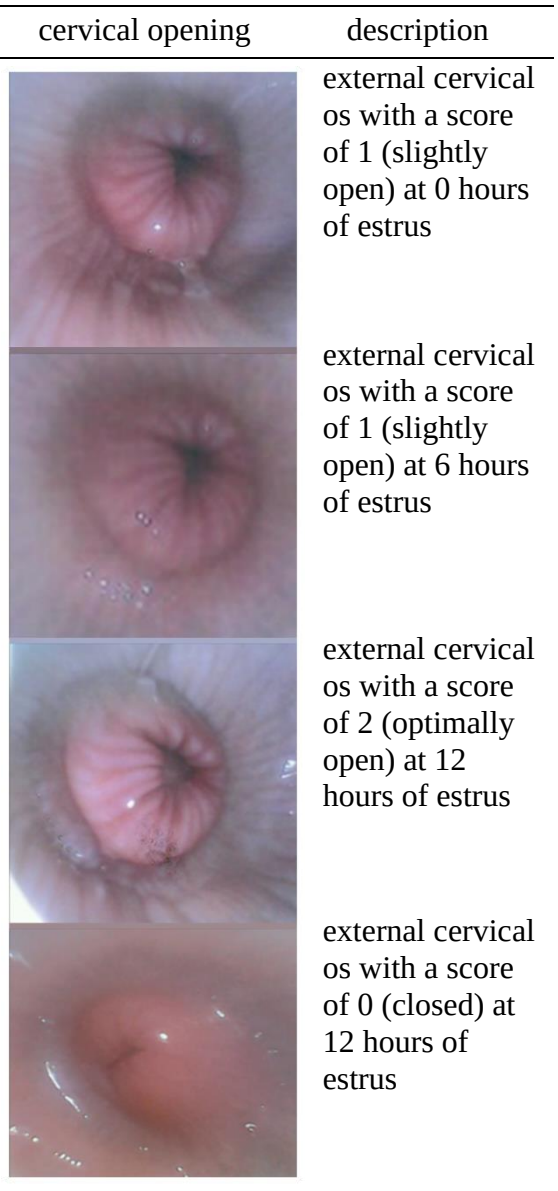

Figure 1 Changes in cervical opening score at 0, 6, and 12 hours after the onset of estrus

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Changes in the cervical opening at 0, 6, and 12 hours of estrus (Figure 1) were followed by changes in mucus discharge. Mucus secretions that were initially liquid and clear turned into thick and cloudy. This difference in the duration of estrus and the degree of cervical dilation could be influenced by the levels of estrogen and progesterone in circulation (Arimbawa *et al*., 2012). High estrogen levels and low progesterone levels were followed by an opening of the cervical canal (Dwarkasing *et al*., 2000).

The development of antral follicles, i.e., the growth phase of the antrum, is characterized by rapid follicle growth which is highly gonadotropin dependent. FSH-influenced theca interna cells continued to differentiate into theca interstitial cells, which produced more androstenedione; therefore, the estrogen produced also increased (Sakaguchi *et al*., 2019). Graafian follicles were formed in this phase of determining or selecting one dominant follicle that is about to ovulate. Decreased FSH levels caused the smaller antral follicles to undergo atresia while the dominant follicle grew by accumulating numbers of granulosa cells and their FSH receptors. Graafian follicles enlarged and matured, and the ovum underwent maturation (Adams *et al*., 2008). In this phase, estrogen levels increase, stimulates the central nervous system to trigger estrus behavior and increasing vascularization to the reproductive organ; signs of estrus appeared, such as a reddened and swollen inner vagina, warm and swollen vulva (Sumiyoshi *et al*., 2014). During estrus, cervical mucus looks clear, like egg white (Sharma *et al*., 2013). The cow's cervix is more relaxed during the follicular phase than the luteal phase. In the follicular phase, there are structural changes in the collagen connective tissue in the cervix. Collagen is the main component of connective tissue, where the pattern of distribution, content, and degree of degradation of collagen can affect the texture of the cervix (Kershaw *et al*., 2007). The percentage of collagen in the superficial layer is higher than in the deep layer. Even though both collagen layers have the same high content, the percentage of collagen denaturation plays an essential role in determining the texture of collagen tissue (Dwarkasing *et al*., 2003). High estrogen levels affected the nervous system, causing signs of estrus, such as an anxious cow that wanted to be ridden by another cow. Apart from that, it also caused an increase in blood flow to the genital organs and mucus secretion by the cervical glands, which made it easier for spermatozoa to enter the female reproductive organs after insemination or natural mating (Tiro *et al*., 2020). Estrogen also played a role in positive feedback to the luteinizing hormone (LH), which triggerred ovulation (Suartini *et al*., 2013).

The increased estrogen levels in the follicles provided positive feedback to the pituitary to produce the LH surge. The LH surge caused the formation of progesterone in the granulosa cells. FSH, LH, and Progesterone stimulated proteolytic enzymes to degrade collagen in the follicular wall so that it easily ruptured (Li *et al*., 2007). In cows showing an open external cervical canal, estrogen concentrations remained high during estrus (Tsigilianni *et al*., 2011). Increased estrogen also caused FSH activity in the follicle to be amplified, providing negative feedback to the pituitary to inhibit FSH secretion as well as facilitating the influence of FSH in the form of LH receptors on granulosa cells. Ovulation occurred at the end of the estrous cycle under the influence of LH and adenohypophysis (Orlowski and Sarao, 2022). The granulosa cells of the follicles, which have ruptured in the ovulation, were immediately luteinized. The luteinizing hormone stimulates the luteinization of the theca and granulosa cells into luteal cells and begins to produce progesterone. After ovulation, estrogen levels in the circulation system would decrease drastically, and the remaining ovulation tissue would gradually undergo luteinization to form a corpus luteum which produced progesterone (Holesh *et al*., 2022). Under the effect of progesterone, the cervical canal was closed. The closing stage of the external cervical os was characterized by the gradual reduction of cervical dilation and the change in cervical mucus to become thick and cloudy around the vaginal fornix (Bernardi *et al*., 2016).

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Progesterone played a role in closing and keeping the cervix tightly closed (Wiltbank *et al*., 2014).

CONCLUSION

Based on the observations using an artificial insemination endoscope equipped with a camera, it could be concluded that the external cervical canal opening of local Aceh cows reached optimum at 12 hours after the onset of estrus.

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