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Research Article

Environmental Temperature Reduces Conception Rate and Increases Embryonic Mortality in Aceh Cattle

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ABSTRACT

This research was performed to investigate reproduction performance Aceh cattle in two periods of different environmental temperature. Twenty female Aceh cows between 5-8 years of age, 150-250 kg body weight, which were clinically healthy, non-pregnant, had minimal two regular cycles of 18-21 days, and had a good body condition score were included as subjects in this research. The cows were maintained in shepherding field and fed green and concentrate feed. The cows were divided into two groups, each containing 10 cows and was used in two different periods (May-June and November-December). All cows were synchronized by injecting 5 mg/ml PGF2α intramuscularly twice with 11 days interval. Artificial insemination was performed 12 hours after estrous symptoms appeared using Aceh cattle frozen semen. Pregnancy examination was performed using trans rectal ultrasonography on day 25 after artificial insemination. The examination was repeated every 10 days until day 55 after insemination. Blood collection for estradiol examination was performed immediately after artificial insemination. Estradiol concentration was examined using enzyme linked immunosorbent assay (ELISA). The average daily temperature (°C) and humidity (%) in May-June and November-December period were 29.5±1.29 and 88.0±5.78; 27.5±1.64 and 85.0±5.07, respectively. Intensity, onset (hour), and duration (hour) of Aceh cattle estrous in May-June versus November-December period were 4.4±1.07 versus 4.3±1.16, 30.8±19.78 versus 31.6±23.05, and 32.0±31.49 versus 28.0±23.01 hour, respectively (P>0.05). Conception rate of Aceh cattle in May-June and November-December periods were 40% and 70%. The average estrogen level of pregnant cows in May-June and November-December periods were 59.46±22.16 and 61.78±10.69 pg/mL (p>0.05), whereas estrogen level of non-pregnant cows in May-June and November-December periods were 49.51±15.71 and 45.28±11.98 pg/mL. The percentage of embryonic mortality was increased as much as 50% in May-June while there was no embryo death in November-December. This result showed that difference in environmental temperature did not affect the exhibition of estrous symptoms and the concentration of estradiol at the insemination. However, it reduced the conception rate and increased the incidence of embryonic mortality.

Key words: Aceh cattle, Environmental temperature, Embryonic mortality

INTRODUCTION

Temperature stress is an important cause of infertility and the main source of loss in cow breeding management (Silanikove, 2000; De Rensis and Scaramuzzi, 2003). The impact of this temperature stress will increase in the future that predicted from the increasing average earth and ocean's surface temperature in the last decade due to global warming (Lendrum and Woodruff, 2003). Global warming might directly reduce reproduction efficiency of the cattle and this effect will increase body temperature or temperature stress. Increasing body temperature may interfere with the growth of ovarian follicle and reduce steroidal concentration from its normal level. Under the influence of temperature stress, the duration and estrous intensity were reduced due to gonadotropin and steroid level reduction, which has been proven in cows (Wolfenson *et al*., 1995; Wijayagunawardane, 2009).

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The main reproductive disturbance caused by temperature stress is follicle development. Cows under temperature stress reduce feed intake, cause reduction of luteinizing hormone (LH) pulse frequency and prolong follicular phase. Prolonged follicular phase will cause smaller follicles to be dominant (Badinga *et al*., 1993; Sartori *et al*., 2002). Follicle is responsible for estrogen production and smaller follicles will produce less estrogen than larger follicles, thus, the dominancy of smaller follicles resulting in reduction of estrous activity (Jordan, 2003; West, 2004). Wolfenson *et al*. (2000) found that despite cooling effort had been performed to reduce environmental temperature stress, the fertility of dairy cow remained low.

Temperature stress can reduce the intensity and length of estrous phase. Nebel *et al*. (1997) found that dairy cow in the summer reduce the intensity and length of estrous phase compared to dairy cow in the winter. Thatcher and Collier (1986) showed similar alteration in Florida dairy cows in which estrous intensity in the summer increased when the cows were cooled. Temperature stress also reduced follicular estradiol (Wilson *et al*., 1998).

Temperature stress also causes reduction of steroid level, especially estrogen and progesterone. Reduction of steroid level causes reduction of estrous intensity. Estrous intensity in each cattle and between different breeds is different. Orihuela (2000) reported several factors that affected the intensity of estrous symptoms, such as social interaction, management, environment, nutrition, age, the presence of male, and genetic. Galina and Orihuela (2007) and Bo *et al*. (2003) stated that detection of estrous is difficult in cows with low estrous intensity. Inaccuracy of estrus detection causes failure in mating and reduced conception rate.

MATERIALS AND METHODS

The samples used in this study was 20 adult female Aceh cattle, 5-8 years old, weighed 150-250 kg, non-pregnant, and had minimal two regular cycles of 18-21 day. The cows were divided into two groups, 10 cows in each group, which were studied in two different periods to examine the effect of environmental temperature toward reproduction performance of Aceh cattle. The research time was selected based on information from Department of Meteorology, Climatology, and Geophysics (BMKG) of Indrapuri, which estimated May-June as an extreme temperature period and November-December as a normal temperature period in Aceh Province.

Measurement of cowshed temperature

Cowshed temperature was measured using digital thermometer. The measurement was performed three times a day at 10 am, 2 pm, and 6 pm. Humidity data was obtained from Department of Meteorology, Climatology, and Geophysics of Aceh Besar.

Estrous synchronization

The estrous of all cows were synchronized by injecting 5 mg/ml PGF2α (Lutalyse^{TM}, Pharmacia & Upjohn Company, Pfizer Inc.) intramuscularly twice with an interval of 12 days. The non-pregnant cows, which had corpus luteum were selected. The cows were fed with green fodder twice daily and concentrate once daily, while water was given ad libitum. The day when cow showed estrous symptoms was considered as day 0.

Measurement of estrous performance

Estrus detection as response to synchronization was performed 24 hours after last synchronization injection, which was performed for ±1 hour. Cows showing signs of primary and secondary estrous, such as standing heat, mounting other cows, anxious, red and swollen vulva, secretion of cervical mucus, and decrease appetite, were rated with score from 0 to 5. Sonmez *et al*. (2005) established the criteria for scoring of 5 to 0 as follows, 5 = excellent (standing, mounting other cows, anxious, red and swollen vulva, secretion of cervical mucus, and decreased appetite), 4 = good (standing, mount other cow, anxious, red and swollen vulva, and secretion of cervical mucus), 3 = normal (red and swollen vulva, secretion of cervical mucus, and decreased appetite), 2 = fair (red and swollen vulva and decreased appetite), 1 = poor (decreased appetite), and 0 = no estrous.

Artificial insemination

The cows that showed estrous symptoms in the morning (standing heat) during observation were inseminated in the evening. On the other hand, cows that showed estrous symptoms in the evening were inseminated in the next morning. Mating was performed using artificial insemination using Aceh cow frozen semen. Prior to use, the motility of frozen semen was examined and the semen with a minimal >70% of motility was used.

Pregnancy examination

The 25 days after artificial insemination, pregnancy examination was performed using ultrasonography (USG) with ultrasound scanner and 5 MHz transducer (Ultrascan 90 Alliance, Quebec Canada). The cows were considered pregnant at 25-day post artificial insemination when anechoic fluid was detected together with embryo and heart beat visualization in one of the uterine horn. Embryo death examination was performed using USG on day 35, 45, and 55 days after insemination.

Blood collection and estradiol concentration examination

Blood sample for estradiol examination was collected from jugular vein shortly after artificial insemination. The drawn blood was inserted into tube containing anticoagulant (EDTA) and placed in cool box. Afterwards, the blood was carried to the laboratory for centrifugation (to separate and withdraw the plasma). Centrifugation was performed at 2500-rpm speed for 15 minutes and the plasma was withdrawn from the tube. The plasma was stored in the freezer at -20°C until hormonal analysis was performed.

Steroid concentration examination was performed using enzyme linked immunosorbent assay (ELISA) for estradiol (Estradiol Kit, (DRG Instrument Gmbh, Germany). The procedure was started by inserting 25 µl of standard solution, sample, and control in each well, and then incubated for 5 minutes at room temperature.

Subsequently, 200 µl of progesterone-HRP or estrogen-HRP conjugate reagent was added and the mixture was incubated for 60 minutes at room temperature. The mixture was shaked rapidly to release the content of wells and the wells were rinsed three times using 400 µl cleansing solution for each well. In each well, 200 µl of substrate solution was added and the mixture was incubated for 15 minutes at room temperature. Enzymatic reaction was stopped using 100 µl stop solution for each well. Absorbance level was read on ELISA reader within 10 minutes at wavelength 450±10 nm.

RESULTS

The average of daily temperature (°C) and humidity (%) in May-June versus November-December period were 29.5±1.29 and 88.0±5.78 versus 27.5±1.64 and 85.0±5.07, respectively. Base on the temperature humidity index (THI) the temperatures during May-June were included as moderate stress, while during November-December was categorized as mild stress.

Performance of estrous (intensity, onset, and duration) in Aceh cows in May-June and November-December is presented at Table 1. Table 1 showed that the intensity of Aceh cows estrous in May-June and November-December period were 4.4±1.07 and 4.3±1.16 (P>0.05). Estrous intensity in this research is relatively similar to the report of (Thasmi *et al*., 2017) in fertile cows which found that estrous intensity and repeated breeding of Aceh cattle were 4.0±1.0 and 2.9±1.1, respectively. Onset and duration of estrous of Aceh cattle in May-June and November-December period were 30.8±19.78 versus 31.6±23.05 hours and 32.0±31.49 versus 28.0±23.01 hours, respectively (P>0.05).

The average of pregnant cows estrogen levels were 59.46±22.16 in May-June and 61.78±10.69 pg/mL in November-December (P>0.05), while in non-pregnant cows were 49.51±15.71 and 45.28±11.98 pg/mL in May-June and November-December, respectively. Although there was no significant difference between the two periods of treatment, estradiol levels of pregnant cows (59.46±22.16 pg/mL in May-June versus 49.51±15.71 pg/mL in November-December) were higher than non-pregnant cows (61.78±10.69 pg/mL in May-June versus 45.28±11.98 pg/mL November-December).

DISCUSSION

In this study, estrous intensity in Aceh cattle was not affected by environmental temperature as seen in Table 1. This is different from previous finding by Lucy *et at*. (1992), which observed that temperature stress could reduce duration and intensity of estrous. Sankar and Archunan (2012) stated that temperature >80ºF would decrease estrous expression resulting in decreased efficiency of estrous detection. The different estrous intensity in this research might be due to the different of cow's breeds. In this regard, Paula-Lopes *et al*. (2013) reported that difference breed affects cows response on the temperature stress, i.e. *Bos indicus* breed is more tolerant toward temperature stress compared to *Bos sondaicus*. Cow breeds that are more tolerant toward temperature stress have wider thermoregulatory and thermo resistance ability, thus base on the estrous intensity result, Aceh cows possibly had good tolerance toward temperature stress.

Onset and duration of estrous of Aceh cattle in May-June and November-December period were relatively similar as shown in Table 1. Thasmi *et al*. (2017) reported that the onset of estrous in Aceh cattle were longer (46.4±29.1 hour) than this research. According to Sonjaya *et al*. (1993), variation of estrous onset was affected by ovarian activity, particularly the presence of active corpus luteal and normal reproductive cycle. Estrous duration of Aceh cattle in November-December was shorter than May-June period, although it was statistically insignificant (P>0.05). In line with this study, Sankar and Archunan (2012) reported that duration of estrous was shorter in winter and spring than in summer (18.73±0.18; 18.04±0.19; and 21.02±0.35 hours), respectively. However, Takahashi (2012) reported a different result, whereby temperature stress reduced intensity and duration of estrous through ovarian function disorder. Singh *et al*. (2008) reported that 63.5% cows from repeated breeding, had prolonged estrous. This condition was caused by high concentration of progesterone, causing inhibition of LH surge.

Payne and Wilson (1999) stated that the most important element of climate that played a role in reproductive system was temperature and humidity. The cows, which were kept in air temperature of 25-35º C, shorten the duration of estrous up to 11 hours, while in 17-18º C the average estrous duration was 20 hours. Yousef (1985) also stated that temperature stress would prolong the estrous cycle and shorten estrous period. However, in Aceh cattle, temperature stress did not affect estrous duration.

Conception rate of Aceh cattle in May-June and November-December periods were 40% and 70%, respectively. Schuler *et al*. (2014) reported that conception rate was reduced by 5% in cows exposed to THI index>73 for 1 hour. Mellado *et al*. (2013) also reported a similar finding in which increasing the THI index from ≤70 to ≥95 unit reduced conception rate from 47% to 26%. The increase of THI index resulted in the increase of heat stress which in turn lowered estrus expression and decreased the estradiol secretion, thus reduced the cow fertility (De Rensis and Scaramuzzi, 2003).

The difference in conception rate between the two periods in this study might be caused by exposure toward different environmental temperature for a relatively long period of two months. Al-Katanani *et al*. (1999) stated that response toward temperature stress was observed when cows were exposed for 12 days before estrous, particularly 2 days before mating. Nabenishi *et al*. (2011) reported that maximal environmental temperature on the day after insemination had a negative relationship with the conception rate. Effect of temperature stress before mating was correlated with low dominant follicle diameter and alteration of follicular fluid biochemistry Shehab-El-Deen *et al*. (2010), which produced poor quality of oocyte and granulocyte (Ferreira *et al*., 2011; Payton *et al*., 2011). Another effect of temperature stress toward reduced conception rate were delayed of ovulation, oocyte aging, and embryo death (Sartori *et al*., 2002).

Table 1: Comparison of aceh cattle reproductive performance in different temperature and humidity period

Reproductive Parameter	Treatment Period	
	May-June	November-December
Estrous Performance		
- Estrous intensity	4.4±1.07[a]	4.3±1.16[a]
- Estrous onset (hour)	31.6±23.05[a]	31.60±23.05[a]
- Estrous duration (hour)	32.0±31.49[a]	28.0±23.01[a]
Conception rate (%)	40.0	70.0
Late embryonic mortality, LEM (%)	50.0	0.0
Average estrogen level (pg/ml) during insemination in pregnant cows	59.46±22.16[a]	61.78±10.69[a]
Average estrogen level (pg/ml) during insemination in non-pregnant cows	49.51±15.71[a]	45.28±11.98[a]

[a] The same superscript in the same row showed not significant difference (P>0.05).

Low conception rate in May-June period might be related to fertilization failure and early embryo death. In this research, pregnancy test was performed from day 25 to 55 after insemination. Humbolt (2001) divided embryo mortality into 2 categories: early embryo mortality (occurred until day 27 after insemination) and late embryo mortality (occurred from day 28 to 42 after insemination). Late embryo mortality in this research was diagnosed properly; they were 40.0% and 0% for May-June period and November-December period, respectively. However, fertilization failure and early embryo mortality could not be diagnosed due to technological limitation. This supports the findings of Sartori *et al*. (2002), who found that fertilization rate decreased significantly in the summer. Furthermore, Putney *et al*. (1989) also found that temperature stress affected early embryo development. Quality and development of embryo flushed from the reproductive tract on the seventh day showed a reduction under temperature stress. In contrast, Ryan *et al.* (1993) reported that temperature stress did not affect fertilization rate, but it affected embryo mortality after 7 days of pregnancy.

Aside from poor embryo quality, Thatcher *et al*. (2010) reported that temperature stress also inhibited embryo ability to secrete interferon-tau (signal that prevent corpus luteal regression) and stimulated PGF2α secretion, which is a pregnancy antagonist. In Aceh cattle, temperature stress may cause embryo mortality because Aceh cattle tend to have a lower progesterone level in its peak luteal phase (Siregar *et al*., 2016). The percentage of late embryo mortality in this study was 50.0% in May-June period and 0.0% in November-December period. This result was similar to the report of Garcia-Ispierto *et al*. (2006), which stated that temperature increase will increase late embryo mortality. The percentage of pregnancy loss on THI index of 55, 55-59, 60-64, 65-69, and > 69 were 0, 1, 2, 8, and 12%; respectively.

The average of pregnant cows and non-pregnant cows estrogen levels were not different between May-June and November-December as performed in Table 1. This finding supported Bridges *et al*. (2005), who stated that reduction of dairy cow fertilization might be related to premature differentiation of follicular cell, which were responsible to produce estradiol. Concentration obtained in this study were relatively lower than estradiol concentration in Aceh cattle during the estrous peak, which was reported by Thasmi *et al*. (2017) and Siregar *et al*. (2016), at level of 110.4±20.1 and 223.13±9.50 pg/mL, respectively. Different level of estradiol level among the same cow breed could not be explained in this study. However, the result of this study was similar to finding of Domenech *et al*. (2011), which found estradiol level of 26.75±8.63 pg/mL from fresh blood samples and 52.91±12.99 pg/mL from frozen blood samples. It can be concluded that an increase in temperature did not affect the estrous performance and estradiol level of Aceh cows. However, it decreased conception rate and increased early embryo mortality.

Conclusions

This result concluded that difference in environmental temperature did not affect the exhibition of estrous symptoms and the concentration of estradiol at the insemination. However, it reduced the conception rate and increased the incidence of embryonic mortality.

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