EMBRYONIC DEATH INCIDENTS DUE TO HEAT STRESS AND EFFECT OF THERAPY WITH *GONADOTHROPIN RELEASING HORMONE* (GnRH) IN ACEH CATTLE

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Abstract. The aim of this study was to determine the effect of gonadotropin releasing hormone (GnRH) therapy on reducing of embryonic death in Aceh cattle due to heat stress. This study used 20 female cattle aged between 5 to 8 years with average weight of 150-250 kg, and had two times regular cycle. All cattle used in this study was provided by Aceh Livestock Breeding Center for Excellence Indrapuri, Aceh Besar which clinically healthy and has a good score that was kept in paddocks and fed with forages and concentrates. The cattle were divided into two groups; each group consisted of 10 cattle. The cattle in group one (K1) was synchronized using $PGF_2\alpha$ while those in group two (K2) was synchronized using ovsynch protocol. Artificial insemination method used for mating after examination of semen motility which obtained from frozen semen of Aceh cattle. Pregnancy rates performed by transrectal ultrasonography after 25 days post-insemination. The examination was repeated every 10 days until 55 days post-insemination. Blood samples for progesterone were collected from the jugular vein and tested by *enzyme linked immunoassay* (ELISA) at integrated research Laboratory of Veterinary Medicine Faculty, Syiah Kuala University. The result showed that 50 % of embryonic death was occurred in group K1, but none in K2 after day 25. It could be assumed that heat stress caused the death of embryos and GnRH therapeutic was the solution to decrease the deaths.

Keywords: Aceh cattle, Embryonic death, Heat stress, Progesterone

Introduction. Aceh cattle is one of the cow's germplasm in Indonesia after Bali and Madura cattle. The cattle are the result of the cross breeding between the local cows (*Bos sondaicus*) and zebu cows derivative of India (*Bos indicus*) (Martojo, 2003). The advantages of local cattle demonstrate higher productivity and maximum economic efficiency in various circumscribed conditions, therefore, its more suitable and economically developed on the traditional husbandry system (Romjali et al., 2007). Furthermore, Susilawati et al. (2002) and Romjali et al. (2007) stated that the local cattle are excellent in feed efficiency, adaptability to the Indonesia environment (heat, moisture, low feed quality, ectoparasite, and endoparasite), and carcass weight which more suitable for the needs of local markets. Gunawan (1998) added that Aceh cattle are able to live at extreme environment such as hot temperature and traditional extensive maintenance systems although these points have not been proven empirically.

However, Aceh cattle have a weakness that is low progesterone concentrations, especially at the peak luteal phase. The interesting phenomenon of progesterone concentrations in Aceh cattle is the peak concentrations only 1.54 ng/mL on day 13 of cycles (Siregar et al., 2016). This concentration is relatively low when compared with progesterone concentrations peak on the other cattle. On Punganur cattle, the peak concentration was achieved on day 15^{th} with progesterone concentrations reaching 10.66 ng/mL (Naik et al., 2013), while on Holstein cattle, the peak concentration achieved on day 14^{th} with concentration > 6 ng/mL (Henricks et al., 1970). Aceh cattle progesterone concentrations are relatively similar to the progesterone concentrations of Sahiwal cows in phase of midluteal which is reported 1.94±0.22 ng/mL (Mondal and Prakash, 2003). Wolfenson (2006) found that low concentrations of progesterone related to two major issues, the stress in the summer and the energy balance.

The progesterone hormone has an important role in the establishment and maintenance of pregnancy (Forde et al., 2009). Studies in dairy cows and cattle indicates that there are probabilities of maintaining a pregnancy with increasing concentrations of progesterone on day 7th post-insemination (Diskin and Morris, 2006; McNeill et al., 2006) and this is related with the secretion of early interferon tau (IFNT) (Mann et al., 2006). Interferon tau is a pregnancy signal generated by trophoblast tissue (bovine trophoblast tissue) on the 15th day until the 24th day of pregnancy, which prevents the release of prostaglandin F2 alpha ($PGF_2\alpha$) (Robinson et al., 1989). The low concentrations of progesterone lead to low response of uterus to IFNT making it more sensitive to release $PGF_2\alpha$ (Shaham-Albalancy et al., 2001). If a cow does not have enough progesterone concentrations, then the pregnancy will be fail maintained. The low concentration of progesterone is one of the main factors that caused the death of the embryo.

Embryonic death is not only attributed to the low of progesterone concentrations, but also due to the environmental heat stress. Embryonic death in cattle increases when exposed to heat and more susceptible in the middle period after the onset of oestrus and early period after insemination. Putney et al. (1989) states that the embryo development will be disrupted on heifers

which given a heat stress ten hours after the onset of oestrus, a period after the luteinizing hormone (LH) surge and before ovulation. Ealy et al. (1993) found that heat stress one day after matting also reduce the development of the embryo.

In Aceh cattle, the low conception rates and the high embryonic death rates is assumed due to low concentrations of progesterone in peak luteal phase. The provision of gonadotropin releasing hormone (GnRH) is expected to reduce the risk of embryonic death in cattle Aceh due to heat stress. It relates to the ability of GnRH which increases the concentration of progesterone through the formation of corpus luteal accessories (Pursley et al., 2012). The increasing concentration of progesterone is associated with the increasing of survival embryo. Cows with a low concentration of progesterone on post-ovulation will cause the death of the embryo, which is one reason of repeated mating disruption. Progesterone concentration which low in the early luteal phase will be repeated from one cycle to the next cycle (Parr et al., 2010).

Based on the above assumptions, it is needed a study to determine the effect of heat stress on the environment to the Aceh cattle embryo mortality and the effect of GnRH therapy on the incidence of embryonic death on Aceh cattle due to environmental heat stress. The result is expected to be a source of information and recommendations in an effort to increase the productivity of Aceh cattle.

The aim of this study was to determine the effect of GnRH therapy on reducing of embryonic death in Aceh cattle due to heat stress

Materials and Methods

Animals and flocks management

In this study 20 Aceh cows were used with the criteria as follows; adult female, aged between 5 to 8 years, 150-250 kg of body weight, and had at least two times regular cycle. All the cows were provided by Aceh Livestock Breeding Center for Excellence Indrapuri (BPTU), Aceh Besar. The cows were clinically healthy with a good body condition, kept in paddocks and fed with forages and concentrates. The cows were grouped into two treatment groups, ten cows in each group were used on two different treatments (May-June, 2015) to examine the effects of environmental heat on reproductive performance of cows Aceh. Temperature measurement which located at BPTU Indrapuri was done 3 times a day, in the morning (09.00 am), noon (14:00 am) and afternoon (18:00 pm). The time selection was based on the information obtained from the Meteorology, Climatology and Geophysics (BMKG) of Indrapuri, that estimate the temperature in May and June was the extreme temperatures in the province of Aceh.

Oestrus synchronization

All cattle were divided into two groups, each group consisted of ten cows. In group 1 (K1), the cattle were synchronized oestrus using 25 mg $PGF_2\alpha$ (LutalyseTM, Pharmacia & Upjohn Company, Pfizer Inc.) intramuscularly with double injection pattern at intervals of 11 days, whereas in group 2 (K2), oestrus was synchronized using protocols ovsynch. Ovsynch protocol start on day 0 by injection of 100 mg GnRH (Fertagyl^{TM}, Merck Animal Health) which contain 50 pg/mL gonadoreline diacetate tertahydrate. On day 7th, the cows were injected with 25 mg $PGF_2\alpha$ (Lutalyse^{TM}, Pharmacia & Upjohn Company, Pfizer Inc.) and on the day 9th, re-injected with 100 pg GnRH, intramuscularly.

Artificial insemination

The artificial insemination was performed using frozen semen from Aceh cow. Before the insemination, frozen semen motility was examined and the motility > 70% was used in this study. The Insemination was carried out by a certified inseminator and within 12 hours after oestrus symptoms appeared.

Pregnancy examinations and diagnosis of early embryonic death

Pregnancy examination was performed by transrectal ultrasonography on day 25th post- insemination. The examination was repeated every 10 days until day 55th post-insemination in accordance with the method of Chaudhary and Purohit (2012). Transrectal ultrasonography was performed using an ultrasound scanner equipped with a 5 MHz transducer (Ultrascan 90 Alliance, Quebec C). The cows pregnancy were detected on the 25th day post-insemination based on fluid anechoic with visualization of the embryo and the heartbeat on one cornua uteri while the death of the embryo were diagnosed on day 35th based on non-embryo visibility, absence of pregnancy positive signs, or embryo degeneration signs.

Blood collection and examination progesterone concentrations

The blood sample for progesterone examination was collected from the jugular vein on the 7th day post-insemination. The blood was collected in a test tube which was placed in a flask containing ice. The blood then was brought to the laboratory for collection of plasma and allowed to stand for 30 minutes prior to centrifugation. The centrifugation was done at 2,500 rpm within 15 minutes. The plasma was then separated, placed in a micro tube and stored in -20º C freezer until used for hormonal analysis.

The examination of steroid concentrations was conducted by using absorbant enzyme linked immunoassay (ELISA). The kit used was DRG Progesterone ELISA (DRG Instruments GmbH, Germany). In each well ELISA plate was inserted 25 mL standard liquid, sample, and control then incubated for five minutes at room temperature. Thereafter, the solution was mixed with 200 mL of reagent-HRP conjugate progesterone or oestrogen HRP, incubated for 60 min at room temperature, shaken quickly to unload the well and rinse well three times by adding 400 mL of washing solution on each well. About 200 mL solution of the substrate solution were put in each well and incubated for 15 min at room temperature. The enzymatic reaction was stopped by adding 100 mL stop solution to each well. The absorbance value was read with ELISA reader within 10 minutes with a wavelength of 450 nm.

Data analysis

Estrus performance data and concentration of progesterone in pregnant cows were analysed using T-test. The relationship between progesterone concentrations on day 5th post-insemination was analysed descriptively.

Results

The average temperature and humidity in May-June were 29.5±1.29 and 88±5.78, respectively, and its classified as stress category when converted at the temperature humidity index (THI). Aceh cattle reproductive performance in these conditions was treated with two different oestrus synchronization protocol which is presented in Table 1.

From 10 cows in group K1 and K2, only 4 individuals were successfully pregnant in each group. Two cows in group K1 have not succeeded to maintain a pregnancy because of the embryonic loss on ultrasound examination on day 35th and 45th. The concentration progesterone on day 5th of pregnancy was higher (P<0.05) in K2 than K1 (4.20±1.40 vs 2.4±0.42, respectively) and progesterone concentration in cattle with embryonic loss on day 5th of pregnancy in group K1 was lower than cattle which able to maintain pregnancy (2.4±0.42 vs 1.93±0.30 ng/mL).

Table 1. **Aceh cattle reproductive performance at environmental heat stress conditions which treated with estrus synchronization protocol with PGF2α and ovsynch**

Reproductive parameters	Treatment	
	K1, May-June, prostaglandin	K2, May-June, ovsynch
Conception Rate (%)	40	40
Late embryonic mortality, LEM (%)	50	0
Average level of progesterone (ng/mL) in pregnant cow (day 5th)	2.4±0.42[a]	4.20±1.40[b]
Average level of progesterone (ng/mL) in LEM cow (day 5th)	1.93±0.30	-
[a,b] Different superscripts in the same column raws the real difference (P<0,05)		

Discussion

The conception rate in this study was relatively similar to the previous study carried out by Forro et al. (2012) which observed 44.4% conception rate in cattle crossbreed Friesian Holstein (FH), after received similar synchronization protocol with ovsynch. However, the present result shows higher conception rate as compared to the previous studies that used similar synchronization protocol (Aali et al., 2008; Ghuman et al., 2014) which reported that the conception rate was 31% in Holstein cows and 30% in buffalo, respectively.

The data of Aceh cattle conception rate with synchronization protocol with $PGF_2\alpha$ and progesterone has been reported previously by Siregar et al. (2015) that showed the conception rate was higher than the present result. Siregar et al. (2015) obtained the conception rate of cattle that was synchronized using CIDR-B and $PGF_2\alpha$ were 80.0 and 80.0%, respectively. The difference was likely due to the results of research time difference of treatment. This study was conducted in May-June, when the temperature and humidity in Aceh Province reach the highest peak that is likely to cause environmental heat stress and affect the conception rates.

The failure of pregnancy in this study was assumed due to the failure of fertilization or embryo mortality before day 25 (early embryonic mortality) with a percentage of 60%. This result is consistent with the findings of Diskin et al. (2011) that embryonic death was particularly occurred until 16 days after mating. On cattle, it was reported that the artificial insemination fertilization rate was about 90-100% (Sreenan et al., 2001). Therefore, it can be assumed that the failure of pregnancy in this study was mostly associated with early embryonic death related to environmental heat stress at the time of treatment. This study also prove that ovsynch protocol did not increase the conception rates compared to $PGF_2\alpha$ treatment (40 vs 40%). This result supports the finding of Vassilev et al. (2005), that the pregnancy rate and early embryonic death is not affected by ovulation synchronization using ovsynch methods.

Death percentage of embryos in this study reached 50% in K1 which was higher than previously reported. Vassilev et al. (2005) reported that the incidence of early embryonic death was 5.45 to 13.2% which was observed until 35 days. Furthermore, Fricke et al. (1999), Mee et al. (1994), and Vasconcelos et al. (1997) reported that the incidence of embryo mortality was 10-16% in cattle that have shown positive results of pregnancy on the 28th day.

The reason of the high incidence of embryo mortality in this study may be related to environmental heat stress during the research carried out. The average temperature and humidity at the time of each study (May and June) was 29.5±1.29º C and 88±5.78, respectively. According THI index, these temperatures included into the category of stress. Ghanem and Nishibori (2015) stated that heat stress can inhibit endocrine function characterized by a decreasing in estrus intensity of preovulatory LH which ultimately reduce fertility. Wolfenson (2006) state that the stress in the summer causing damage follicular function, followed by sub-optimal CL function. Sub-optimal luteal function after artificial insemination was associated with lower progesterone secretion or early luteal regression. This situation resulted in the death of the embryo.

In this study, the K2 group did not experience the embryonic death after 25 days, indicating that ovsynch protocols could prevent the disorder in which the pregnancy rate after 25 days was higher than K1 group. The increase in pregnancy rate is related to the ability of GnRH which increases the concentration of progesterone through the formation of corpus luteal accessories

(Pursley et al., 2012). Similarly, Yildiz et al. (2009) reported that administration of GnRH on day 9th post-insemination could improve pregnancy rate about 77.7 and 50.0%, respectively, compared with control.

This study also showed that progesterone concentration in Aceh cattle which was able to maintain pregnancy in the group that injected with double $PGF_2\alpha$ and ovsynch were 2.4±0.42 and 4.20±1.40 ng/mL, respectively, while in the group that experienced the embryonic death was only of 1.93±0.30 ng/mL. Mee et al. (1993) reported that cows injected with GnRH during estrus have greater progesterone concentration after ovulation, and during the 40 days after GnRH administration, the cattle are able to maintain pregnancy due to the increased ratio of theca cells in the corpus luteal.

On Aceh cattle, lower pregnancy rate is caused by factors progesterone concentration which were relatively low at luteal peak phase. Mann and Lamming (1992) suggested the low concentration of progesterone results in low conception rates. Willard et al. (2003) added that one of the main causes of the embryonic death which responsible for the lower pregnancy rate is inadequate luteal function as indicated by low concentrations of progesterone. The result of this study indicates that progesterone concentration in Aceh cattle which experienced embryonic death was of 1.93±0.30 ng/mL, while in those cattle that were able to sustain pregnancy after treated with double injection $PGF_2\alpha$, increased to 2.4±0.42 ng/mL. Mann et al. (2006) reported that in dairy cows experiencing embryonic death have relatively lower concentrations of progesterone during the early luteal phase and the elevation of progesterone concentration during the early luteal phase reduces the risk of the embryonic death.

Conclusion

Based on the result, it can be concluded that the estrus performance of Aceh cattle in environmental heat stress condition is not affected by method of oestrus synchronizing with $PGF_2\alpha$ or ovsynch. Ovsynch protocol was not able to improve the conception rates in Aceh cattle compared with $PGF_2\alpha$ treatment but might reduce the occurrence of embryo mortality due to environmental heat stress.

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