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Short Communication: Relationship between interferon-tau level (IFN- τ) and embryo mortality incident in Aceh cattle

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Abstract. *Melia J, Siregar TN, Alfahmi L, Husnurrizal, Saumar H, Mukhtar, Meutia N, Panjaitan B, Armansyah T. 2020. Short Communication: Relationship between interferon-tau level (IFN- τ) and embryo mortality incident in Aceh cattle. Biodiversitas 21: 5758-5762.* The purpose of this research is to determine the relationship between interferon-tau level (IFN-τ) and the incidence of embryo mortality in Aceh cattle from the Province of Aceh, Indonesia. Data were obtained from four Aceh cattle, aged 3-5 years and body weight of 150-250 kg. The cattle were clinically healthy, with at least two regular reproduction cycles, and a pregnant status after estrous synchronization and artificial insemination. This process was carried out on a 10-day interval using the intramuscular administration of prostaglandin F2 alpha (PGF2α) hormone, at a dose of 25 mg, with double injection pattern. The blood samples were collected from the jugular vein on day 14 to 18 post-insemination for the assessment of IFN-τ concentration, which was measured using the enzyme linked-immunoassay (ELISA) method and the bovine interferon ELISA kit (Cusabio Technology LLC AII, USA). In addition, the transrectal ultrasonography method was used to test the pregnancy and embryo mortality of the cattle on the 25th day. The examination was repeated every 10 days until day 55. The IFN-τ concentrations of pregnant cows against those with embryo mortality on the 14th, 15th, 16th, 17th, and 18th day, were 12.066±8.222 vs 17.853±11.126, 12.983±3.491 vs 20.503±3.858, 12.193±4.535 vs 16.458±8.065, 10.143±5.370 vs 17.604±1.888, and 12.767±7.753 vs 15.096±6.955 pg/mL, respectively. Therefore, the embryo mortality in Aceh cattle is not related to IFN-τ.

Keywords: Aceh cattle, IFN-τ, embryo mortality

INTRODUCTION

Embryo mortality is defined as an interruption to the reproduction performance of cows which results in the death of the embryo before the 42nd day of pregnancy (Efendi et al. 2015). This is divided into two categories, in accordance with the time of death, namely early embryo mortality (EEM), which occurs until the 27th day, and late embryo mortality (LEM) between the 28th to the 42nd day) (Humblot 2001). Infection, environmental status, nutrition, management, and hormonal disorders generally cause this incident.

The success of pregnancy is influenced by embryo development, which is initiated from the blastocytes, followed by the hatch zone pellucida, and the trophoblast function, which produces components that prevent luteolytic and maintain pregnancy. There are four stages of embryo growth prior to implantation; these include proper development in the zona pellucida, hatching blastocytes, maternal recognition of pregnancy, and formation of the embryo outer membrane (Senger 2005). However, embryo death ensues when there is a failure in any one of these four stages.

Several studies have reported that early embryonic mortality was attributed to disturbances of signaling regulation between blastocyst and maternal endometrium or maternal recognition of pregnancy (MRP) (Raheem 2017). One of MRP in ruminant is interferon-tau (IFN-τ) (Imakawa et al. 2017), also known as bovine interferon-τ (bIFN-τ) in cattle (Basavaraja et al. 2017; Raheem 2017), which is produced during the first 14 days of pregnancy (Balhara et al. 2013), from initiation of blastocyst elongation (Imakawa et al. 2017) until 21 days of pregnancy (Hansen et al. 2017; Mishra and Sarkar, 2018). Implantation success is determined by the maternal recognition of pregnancy, due to the synthesis and secretion of interferon-tau (IFN-τ) (Spencer et al. 2013). Furthermore, the synergistic mechanism of action between progesterone, IFN-τ, and prostaglandins is needed for the maintenance of pregnancy in cows through a pre-implanted regulator in the endometrium (Dorniak and Spencer 2013).

Besides being used as a basis for early pregnancy diagnosis, the identification of IFN-τ in the blood has also been associated with premature embryo mortality. The injection of IFN-τ purification into the uterine lumen inhibits the luteolytic, thereby leading to early mortality of cow embryos (Martal et al. 1987; Matsuyama et al. 2012; Spencer and Bazer 2015). Furthermore, barriers and disturbances can cause corpus luteum regression and interferes in the development of chorioallantois placentas

which results in embryo mortality due to the low IFN-τ levels (Wiltbank et al. 2016).

Interferon-τ is produced between the 12-19th days of pregnancy, with the highest levels being seen on the 15 to 16th days. This is followed by a gradual decrease until the 19th day or between the 14 and 21st days, with the highest level seen on the 16th day (Farin et al. 1980; Sheikh et al. 2018). IFN-τ is a pregnancy marker produced to influence the inhibition mechanism of luteolytic by the endometrium. It maintains the corpus luteum in an attempt to continuously produce progesterone, thereby having a significant effect on embryonic growth (Bazer et al. 2010). Approximately 10-15% of pregnancy failure, and consequently embryo mortality, in cows, is caused by the inadequate production of IFN-τ required to maintain the corpus luteum in the luteolytic phase (Spencer et al. 1995). This reinforces the need to acquire more adequate knowledge in the field of mortality detection specifically for the development of a diagnostic technique that pertains to the increase of cow productivity.

Lucy and Pooks (2012) reported that IFN-τ cannot be assayed directly in blood. On the contrary, Antoniazzi et al. (2012) stated that IFN-τ can be detected in the uterine veins of sheep on the 15th day of pregnancy. Since the IFN-τ action occurs through the paracrine and endocrine mechanisms, it was assumed that IFN-τ could be detected in peripheral blood. In our preliminary study also proved that IFN-τ could be detected in blood serum of Aceh cows on 14th day of pregnancy. Therefore, this study was conducted to determine the level of IFN-τ in the blood samples of Aceh cows with the intention of maintaining pregnancy and preventing early embryo mortality.

MATERIALS AND METHODS

Materials

A total of four cows between the ages of 3-5 years and body weight of 150-250 kg, were artificially impregnated via insemination. Furthermore, they were characterized to be clinically healthy with normal reproductive organs and experienced at least two regular cycles. The cows were examined in accordance with the criteria of the Ministry of Agriculture Decree Number 2907/Kpts/OT.140/6/2011.

Research procedure

Estrous synchronization and artificial insemination

The double injection pattern with a 10-day interval was used on all female cows, with the prostaglandin (Lutalyse^{TM}, Pharmacia & Upjohn Company, Pfizer Inc.) injected intramuscularly at a dose of 25 mg. Also, estrus detection was performed in the morning (8:00) and afternoon (16:00) for 30 minutes, and determined by the presence of a red and swollen vulva, as well as transparent cervical mucus discharge, which indicated their readiness to mate (Sudarmaji et al. 2012). The artificial insemination was carried out 12 hours after the cows showed estrus behavior.

Blood sampling

Approximately 10 mL of blood samples were taken from the jugular vein from 14th day to 18th day, daily after insemination. The samples were further placed in a blood tube and left for several hours to allow for the separation of serum. The samples were then centrifugated at a speed of 3000 rpm for 10 minutes. This preparation was transferred into the microtube and stored in the freezer at a temperature of -20°C.

Measurement IFN-τ Concentration

The serum was examined by the enzyme linked-immunoassay (ELISA) method, using Bovine Interferon ELISA Kit (Cusabio Technology LLC AII, USA). Furthermore, all *reagent*s, standard solutions, and samples were prepared and 100 µL placed into each microplate well, which was subsequently incubated for 2 hours at 37°C. In addition, the solution was removed from the well microplate with 100 µl of Biotin-antibody (1x) added and incubated for 1 hour at 37°C. These were all aspirated, with the process repeated twice and washed 3 times using a 200 µL wash buffer. Consecutively, 100 µl of HRP-avidin (1x) was added to each well microplate, and incubated for 1 hour at 37ºC. It was further aspirated and washed 5 times, followed by the addition of 90 µL TMB substrate, which was incubated for 15-30 minutes at a temperature of 37°C in the absence of light. Finally, a 50 µL *stop solution* was added to the well microplate, and read at 450 nm after 5 minutes.

Pregnancy checking and diagnosis of embryo mortality

Pregnancy examination was conducted using transrectal ultrasonography on the 25th day after artificial insemination, and repeated every 10 days to the 55th day, in accordance with Caudhary and Purohit (2012) instructions. In addition, transrectal ultrasonography was carried out using Mindray DP 10 Vet linear transrectal probe 50L60EAV (Shenzhen Mindray Bio-Medical Electronics Co., Ltd*.)*. Cows are considered pregnant on the 25th day, based on the presence of anechoic fluid, with visualization of the embryo and heart rate in the cornual located in the uterus. However, embryo mortality was diagnosed on the 35th day which was characterized by the absence of embryonic visibility, positive signs of pregnancy, and the presence of degeneration. The data were analyzed descriptively.

RESULTS AND DISCUSSION

A total of four pregnant Aceh cows were used to carry out this experiment, which was examined for embryo mortality. The results showed that two (50%) pregnancies survived until the 55th day, while the remaining cows experienced embryo mortality due to late embryonic mortality/LEM, on the 35th and 45th day, respectively. The total concentration of IFN-τ in LEM cows from days 14 to 18 were higher than pregnant cows as shown in Figure 1**.** The ultrasonography result showed the survival rate up to the 55th day, as seen in Figure 2 and Figure 3 for LEM Aceh cattle.

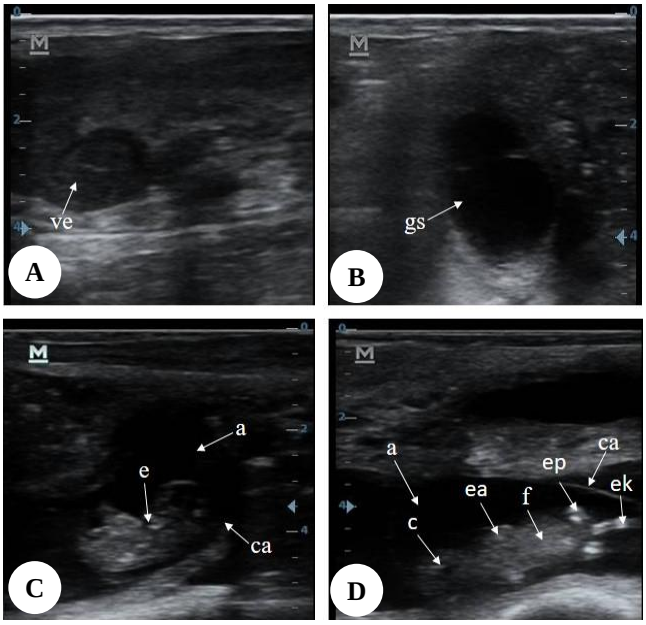

Figure 2. Image of uterus sonography in pregnant Aceh cattle. Note: A. Uterus pregnant day 25, B. Uterus pregnant day 35, C. Uterus pregnant daily, D. Uterus pregnant day 55 (ve: embryonic vesicle, gs: Gestational sac, ea: anterior extremities, ep: posterior extremities, ek: tail; a: amnion, c: cranium e: embrio, f: fetus; bar = 0.5 cm)

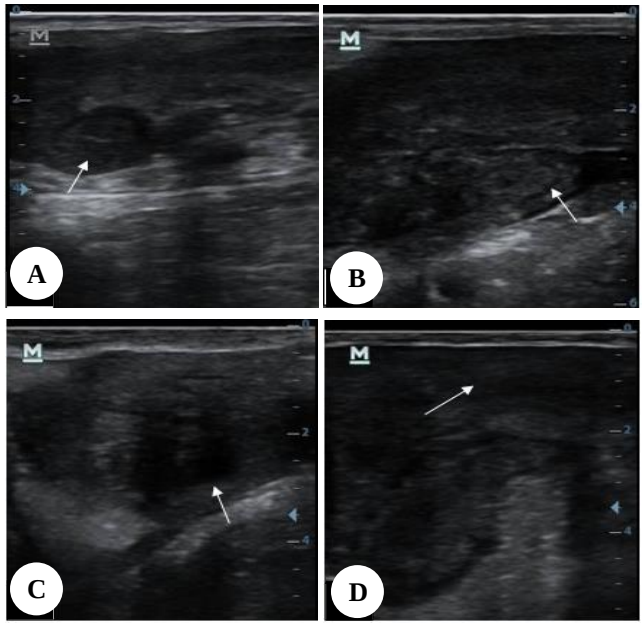

Figure 3. Image of uterus sonographic in Aceh cattle experiencing late embryonic mortality (LEM). Note: A. Pregnant uterus day 25, B. LEM 3day 35, C. LEM day 45, D. LEMday 55

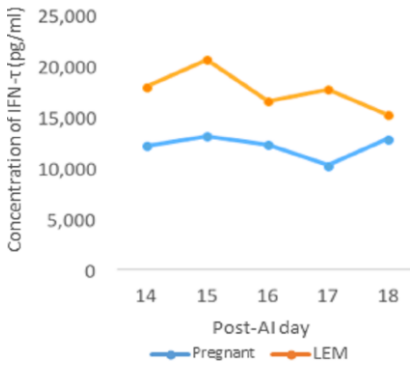

Figure 1. Interferon-τ concentration in pregnant cows and late embryonic mortality/LEM

The final IFN-τ secretions in pregnant and LEM cows achieved on the 15th day were 12.983±3.491 pg/mL and 20.503±3.858 pg/mL, respectively. The results differed from the report of Bazer et al. (2009), which concluded that the IFN-τ secretion climax occurred on the 16th and 14th day by 136.09 pg/mL, and 82.70 pg/mL in LEM cow. Farin et al. (1980) reported on the occurrence of climax, which took place between 15-16th day of pregnancy, with differences in time and concentration ensuing due to the variation in breeds and the number of cows. In this research, 16 breeds Karan fries were used to determine the differences in management (Bazer et al. 2009).

The concentration of IFN-τ was lower in pregnant cows compared to LEM, as shown in Figure 1. However, Bazer et al. (2009) stated a reverse report. This variation is due to differences in the number of samples, with a possibility that the occurrence of LEM is not related to the level of IFN-τ. The cause of embryo mortality was due to infection (endometritis), the environment, nutrition, management, and hormonal disorders (Prihatno et al. 2013). Also, long-lasting endometritis leads to repeated breeding in cows and embryo mortality. This possibility is supported by research carried out by Rafika (2017) which stated that repeat breeding is identified by the presence of *Escherichia coli*, *Pseudomonas sp.*, *Klebsiella sp.*, and *Enterobacter sp.* in the uterus which has the potential to cause embryo mortality. A poor uterine environment after parturition facilitates the entry of microbes into the uterine lumen, which is characterized by pollution of the uterine environment, and subsequent mortality (Noakes et al. 2001).

According to previous studies, embryo mortality is caused by the low production of IFN-τ by the embryonic trophectoderm (Matsuyama et al. 2012). In addition, the concentration of 12.983 pg/mL on the 15th day after artificial insemination, was able to maintain pregnancy till the 55th day, therefore making it an MRP. This helps to maintain the productivity of progesterone by the corpus luteum which is known to affect embryo growth (Bazer et al. 2010). Despite the occurrence of MRP in LEM cows, embryo mortality occurred as a result of the low secretion of IFN-τ (Matsuyama et al. 2012). Its role was examined in embryo formation within the uterus, characterized by the regulation of complement genes present in the parent endometrium (Arosh et al. 2016). In addition, during early

pregnancy, the embryo extends leading to the production of more progesterone (Bazer et al. 2009). These IFN-τ are produced by the paracrine embryo trophoblast tissue, which impedes the action of oxytocin receptors (OTRs) in the endometrial epithelium, thereby inhibiting the release of endometrial pulses in the form of prostaglandins (PGF2α) (Spencer et al. 2013). Consequently, there is also a marked increase in the production of endometrial lutein-protective mediators of prostaglandin (PGE2) and the interferon-tau show to play an important role in embryo extension and the continuity of pregnancy (Spencer et al. 2013; Arosh et al. 2016).

The findings in the present study differed from the previous study. Serrano-Pérez et al. (2019) reported that the expression of interferon-tau *stimulated gene* (ISG15) was higher in pregnant cows compared to the non-pregnant cows. Yaginuma et al. (2019) stated that the concentration of bIFN-τ expression of mRNA from ISG15) in pregnant cows (which have been determined to be repeat breeders) was significantly higher than the non-pregnant cows with the same repeat breeding patterns. Guo et al. (2020) also detected an increase of bIFN-τ concentration at days 14 post AI. Furthermore, Zhu et al. (2017) observed an increase of bIFN-τ concentration in pregnant cows compared to non-pregnant cows.

Figure 2 shows a description of uterine ultrasonography in Aceh cattle which indicates pregnancy, due to the presence of embryonic vesicles (Amrozi and Setiawan 2011). Furthermore, it shows an isoechoic to hyperechoic appearance, which surrounds the hypoechoic fluid, in contrast with the study conducted by Chaudary and Purohit (2012) and Sayuti et al. (2016). Cows are considered pregnant on the 25th day after insemination based on the presence of anechoic fluid and adequate visualization of the embryo and heart rate in the cornual of the uterus. This research showed that pregnant cows showed embryonic vesicles due to the presence of various breeds. Also, the ultrasonography conducted on the 35th day showed the presence of a gestational sac (a pregnancy bag filled with anechoic and amniotic fluid, with a chorioallantois hypoechoic as well as hyperechoic membrane), and an embryo on the 45th day. Furthermore, the gestational sacs and fetuses were visible with hyperechoic colored heads, as well as extremities and tails on the 55th post insemination day.

Figure 3a shows the embryonic vesicles of pregnant cows on the 35th day, while Figure 3b demonstrates the degenerated uterus and embryonic vesicles decay, indicated by hypoechoic to hyperechoic colors. Figure 3c shows the absorption of the dead embryo by the mother as the sonographic picture disappeared, despite the presence of the amniotic fluid with anechoic to hypoechoic coloration on the 45th day. Figure 3d shows the absorption of amniotic fluid by the body on the 55th day, which is characterized by the uterine lumen shrinkage and endometrial thickening. In conclusion, embryo mortality in Aceh cattle was not shown to be related to IFN-τ.

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