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Accuracy of early pregnancy diagnosis using interferon-tau (IFN-τ) in Aceh cows

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ABSTRACT: Interferon-tau (IFN-τ) is a pregnancy signal produced by embryonic trophoblast cells at the time of implantation in a mammal's endometrial wall, which is useful for conveying a message that the mammal can accept the presence of the foreign object (embryo). This study aims to determine the accuracy of early pregnancy diagnosis in Aceh cows by measuring the concentration of IFN-τ. This study involved eight cows aged 3-5 years, weighing 150-250 kg, clinically healthy, and having normal reproduction (have had calves and at least two regular cycles). All cows were estrous-synchronized twice using PGF2α at a dose of 5 ml with 11 days intervals before artificial insemination (AI). Serum collection was performed on days 14, 15, 16, 17, 18 after AI. Based on ultrasound examination, out of eight cows, four (50%) cows were diagnosed as pregnant, and four (50%) cows were diagnosed as not pregnant. Mean (± SD) IFN-τ of pregnant vs. non-pregnant cows on day 14, 15, 16, 17, and 18 were 14.96±8.65 pg/ml vs. 6.14±5.54 pg/mL; 16.74±5.28 pg/mL vs. 4.44±3.51 pg/mL; 14.33±5.9 pg/mL vs. 5.78±5.20 pg/mL; 13.87±5.42 pg/mL vs. 4.38±3.76 pg/mL; and 13.93±6, 16 pg/mL vs. 3.24±2.63 pg/mL, respectively. The lowest IFN-τ concentration in cows that were successfully pregnant was 7.88±1.84 pg/mL. The best timing of IFN-τ for pregnancy diagnosis was on day 15 after AI, with an accuracy of 87.5%, specificity of 100%, and sensitivity of 80%.

Keywords: IFN-τ; Aceh cows; Diagnosis of pregnancy

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INTRODUCTION

It is essential to diagnose pregnancy in livestock after the cattle have been mated, either by natural mating or artificial insemination (AI). Pregnancy diagnosis is carried out as early as possible to identify pregnant or non-pregnant animals immediately after mating so that infertile livestock can be handled appropriately to increase the economic value of management. Nova, Riady, and Melia (2014) explained that early pregnancy diagnosis is needed for several things: immediately identifying whether the cattle are pregnant or not after mating or AI; as one of the considerations if livestock must be sold or culled; reducing the cost of mating, especially those using estrus synchronization techniques; economic ration management; and identifying pregnant cattle after mating in order to be given special treatment to reduce the risk of abortion.

One of the common pregnancy detections is by observing estrus. The estrus observation method was carried out by detecting female cattle that experienced estrus after mating. Cattle are considered pregnant if they do not return to the oestrus cycle. This method is called not return to estrus (Siregar and Hamdan, 2008). This method is considered less efficient because it can only be done after pregnancy reaches a certain age (Wardani, Suyadi, and Nuryadi, 2018).

The other popular diagnostic method in cattle is rectal palpation, effective from day 35 of gestation (Balhara *et al*., 2013). The application of this method is challenging to implement because it requires sufficient operator expertise and experience and the risks posed by poor handling. In the field, the amount of human resources for the application of this method is very limited. Another method of pregnancy detection is the examination of the content of the hormone progesterone in the blood or milk in the first oestrus cycle after AI. This method also has a weakness because non-pregnant cattle sometimes show symptoms of estrus less than 21 days after insemination and show an increase in the concentration of progesterone from the corpus luteum (CL) at the time of examination (Amiruddin *et al*., 2001), so they are diagnosed as pregnant.

One alternative method for early pregnancy detection is to check the level of maternal recognition of pregnancy in the blood using an enzyme-linked immunosorbent assay (ELISA). Maternal recognition of pregnancy is an early recognition stage that is needed to signal the mother to accept the presence of the embryo during early pregnancy (Hafizuddin, Yusmadi, and Anwar, 2016). In cattle, maternal recognition of pregnancy is called interferon tau (IFN-τ) or bovine trophoblastic protein 1 (b-TP1). Interferon tau in ruminants is a critical factor in ensuring the success of pregnancy.

Interferon tau is a pregnancy recognition signal released by the embryo that affects the endometrium to inhibit the luteolytic mechanism (Bazer *et al*., 2009). According to Depamede (2009), IFN-τ acts as an antiluteolytic cytokine secreted by trophoblasts during implantation. In cattle, about 10-15% of pregnancy failures are caused by insufficient production of IFN-τ to maintain CL. Interferon tau has a function to maintain CL so that progesterone production continues to affect the growth and survival of the embryo (Dorniak, Bazer, and Spencer, 2011).

The presence of IFN-τ in cattle occurs between the 15th and 17th days of gestation (Bott *et al*., 2010). However, according to Winkelman *et al*. (1999), IFN-τ was produced on the 15th to 24th pregnancy. In sheep, IFN-τ is secreted during pregnancy between day ten and day 21 post-conception (Fleming *et al*., 2001). The effect of IFN-τ on the endometrium causes decreased secretion and the effect of prostaglandins on CL thereby inhibiting luteolytic activity (Spencer *et al*., 2013). Although there are reports that IFN-τ cannot be examined directly in the blood (Lucy and Pooks 2012),

the opposite reports state that IFN-τ can be detected in the uterine veins of sheep on the 15th day of gestation (Antoniazzi *et al*., 2012). Because the mechanism of IFN-τ occurs in a paracrine and endocrine manner, it is suspected that IFN-τ can be detected in peripheral blood vessels. Our initial research also proved that IFN-τ could be detected in the blood serum of Aceh cattle from the 14th day of gestation.

MATERIALS AND METHODS

In this study, eight adult female cows were used, aged 3-7 years, bodyweight of 150-250 kg, clinically healthy, and had normal reproduction (already calved and had at least two regular cycles. All cows belonged to UPT Experimental Animals Faculty of Veterinary Medicine Syiah Kuala University. The cattle studied were Aceh cattle that have criteria following the Decree of the Minister of Agriculture Number 2907/Kpts/OT.140/6/2011.

Research procedure

Synchronization of estrus and artificial insemination

All cows were synchronized using prostaglandin hormone injected intramuscularly with a volume of 5 ml (LutalyseTM, Pharmacia & Upjohn Company, Pfiz-er Inc.), with a double injection pattern ten intervals. Oestrus detection was carried out twice daily, in the morning (08.00) and afternoon (16.00) for 30 minutes.

Cows were considered in an estrous circle with signs of secondary heat such as riding on another cow, restlessness, red and swollen vulva, cervical mucus discharge, and decreased appetite. Artificial insemination was carried out 12-18 hours after the appearance of estrus symptoms.

Blood Sampling

The examination of the concentration of interferon tau

Blood sampling for IFN-τ examination was carried out from the 14th to the 18th day after insemination. The collected serum was examined using the enzyme-linked immunosorbent assay (ELISA) method using the DRG trophoblast ELISA kit (DRG International Inc., USA).

Pregnancy Examination with Ultrasound

Pregnancy detection by ultrasound was performed on the 25th day. Cows were considered pregnant on the 25th post-insemination day based on the presence of anechoic fluid with visualization of the embryo and a heartbeat in one of the uterine horns.

Data analysis

The data obtained from the measurement of the concentration of interferon-tau (IFN-τ) calculated the sensitivity, specificity, and accuracy values. The values of sensitivity, specificity, and accuracy were calculated using the formula used by Broaddus and de Vries (2005).

RESULT AND DISCUSSION

Based on the study results, of the eight cows, used, four cows (50%) were diagnosed as pregnant and four (50%) were diagnosed as not pregnant. The measurement of the concentration of IFN-τ on days 14-18 showed the peak of secretion of IFN-τ concentrations occurred on the 15th day of 16.74±5.28 pg/ml, then successively on the 14th, 16th, and 18th and 17th days. The peak concentrations of IFN-τ in this study were different from those reported by Sheikh *et al*. (2018) in their study on dairy cows in which the peak of secretion of IFN-τ concentrations occurred on day 16, followed by days 14, 18, 21. This difference may be due to differences in sampling time patterns and different types of cattle. IFN-τ concentrations between pregnant and non-pregnant cows are presented in Table 1.

Of the four non-pregnant cows, there was one non-pregnant cow had an IFN-τ concentration of 0, while the other three non-pregnant cows had a relatively high IFN-τ concentration on day 14 (6.14±5, 54 pg/ml) then fluctuated on the 15th, 16th to 18th day. The 0 concentration in this study was probably because the measured IFN-τ concentration was relatively deficient below the kit's sensitivity. The high concentration of IFN-τ in the non-pregnant cows was thought to have been fertilized. However,

early embryo mortality occurred before the 25th day after AI, so that at the time of ultrasound, the females were diagnosed not to be pregnant. This is in line with Santos *et al*. (2004) cited by Suprihatin, Tumbelaka, and Setiadi (2016) that embryo death can occur from days 0-7 after AI, which is called very early embryo mortality/VEEM. Embryo death occurs at the time of cell division from two cells into a morula. Embryo death can also occur on days 7-24, called early embryo mortality (EEM). Humblot (2001) stated that EEM occurred until the 27th day of pregnancy. During this time, the embryonic death process is in the blastocyst phase until implantation.

Table 1. The average concentration of IFN-τ in pregnant and non-pregnant Aceh cattle 14-18 days after artificial insemination

Diagnostic results	IFN-τ concentrations on day- (pg/ml)					Avg.
	14	15	16	17	18	
Pregnant (n=4)	14,96±8,65	16,74±5,28	14,33±5,90	13,87±5,42	13,93±6,16	14,77±1,19
Not Pregnant (n=4)	6,14±5,54	4,44±3,51	5,78±5,20	4,38±3,76	3,24±2,63	4,80± 2,0

In this study, the most negligible average concentration of IFN-τ in pregnant cows was 7.88±1.84 pg/ml. Therefore, the concentration was determined as the standard concentration of IFN-τ that guaranteed pregnancy. Furthermore, the standard was used as a reference for determining the accuracy of pregnancy diagnostic calculations. The percentage of pregnancy diagnostic accuracy with interferon-tau measurement is presented in Table 2.

Based on Table 2, the best time to diagnose pregnancy based on IFN-τ concentration was day 15. On the 15th and 16th days after AI, the average accuracy of early pregnancy diagnosis was 87.5%. This value was obtained from the accuracy of pregnant cows by 100% and non-pregnant cows by 75%. This indicates that the fastest time with high accuracy for pregnancy diagnosis is the 15th day after AI. Although the use of IFN-τ for pregnancy diagnosis shows high accuracy, this study also found false-positive diagnoses. This happened because the cow had concentrations of IFN-τ above the standard to identify it as pregnant. However, after being examined by ultrasound, the result was negative. This condition can occur caused by VEEM or EEM. When compared with progesterone assays' accuracy, as reported by Lee *et al*. (1996), this method's accuracy was higher in diagnosing pregnant cows at 100% vs. 87%, but relatively lower in diagnosing non-pregnant cows at 75% vs. 91%. The lower accuracy of this method in diagnosing non-pregnant cows compared to other studies could be due to the relatively small number of samples compared to those used by other researchers, so it is necessary to increase the number of samples for further research.

Based on the results of this study, the method for diagnosing early pregnancy can be done using the method of measuring the concentration of IFN-τ. Interferon-tau is a pregnancy marker (maternal recognition of pregnancy/MRP) which functions to keep the corpus luteum from continuing to secrete progesterone so that it can maintain embryonic growth (Bazer *et al*., 2009). Interferon-tau is produced by embryonic trophoblast tissue that acts in a paracrine manner that inhibits the action of oxytocin receptors (OTRs) on the endometrial epithelium, thereby inhibiting the release of prostaglandins. Interferon-tau plays an essential role in embryonic growth and maintaining a pregnancy (Spencer and Hansen, 2015).

Table 2. Pregnancy diagnosis accuracy by measuring IFN-τ

	bIFN-T				
	h-14	h-15	h-16	h-17	h-18
Total number of cows	8	8	8	8	8
Correct pregnant	3	4	4	3	3
Incorrect pregnant	2	1	1	1	0
Correct non-pregnant	2	3	3	3	4
Incorrect non-pregnant	1	0	0	1	1
Sensitivity (%)[a]	60.0	80.0	80.0	75.0	100.0
Specificity (%)[b]	66.7	100.0	100.0	75.0	80.0
Accuracy (%)[c]	62.5	87.5	87.5	75.0	87.5

[a] Sensitivity: probability that diagnosis is pregnant among cows which are truly pregnant; correct pregnant/(correct pregnant+ incorrect non-pregnant) x100%

[b] Specificity: probability that diagnosis is non-pregnant among cows which are truly non-pregnant; correct non pregnant/(correct nonpregnant + incorrect pregnant) x100%

[c] Accuracy: a proportion of correctly classified subjects (correct pregnant + correct non-pregnant)/ (correct pregnant + correct non-pregnant + incorrect pregnant + incorrect non-pregnant)

The validation of pregnancy by transrectal ultrasound examination is presented in Figure 1. Figure 1A shows that the uterus of Aceh cattle was diagnosed as pregnant based on the presence of embryonic vesicles. Embryonic vesicles display isoechoic/anechoic to hyperechoic color sonographic images surrounding the hypoechoic fluid of the embryonic fluid (Sayuti *et al*., 2016). The shape of the embryonic vesicle is based on the spherical shape of the endometrial wall of the uterus. Based on research conducted by Amrozi and Setiawan (2011), embryonic vesicles are formed at the beginning of pregnancy. In contrast, Chaudary and Purohit (2012) stated that cows were considered pregnant on the 25th day after insemination based on the presence of anechoic fluid with visualization of the embryo and a heartbeat in one of the uterine horns. This is thought to be caused by differences in the breeds of cattle used. Chaudary and Purohit (2012) used dairy cows, while Aceh cattle were used in this study on the 25th day of pregnancy. Aceh cattle did not show any visualization of the embryo and heartbeat in one of the uterine horns.

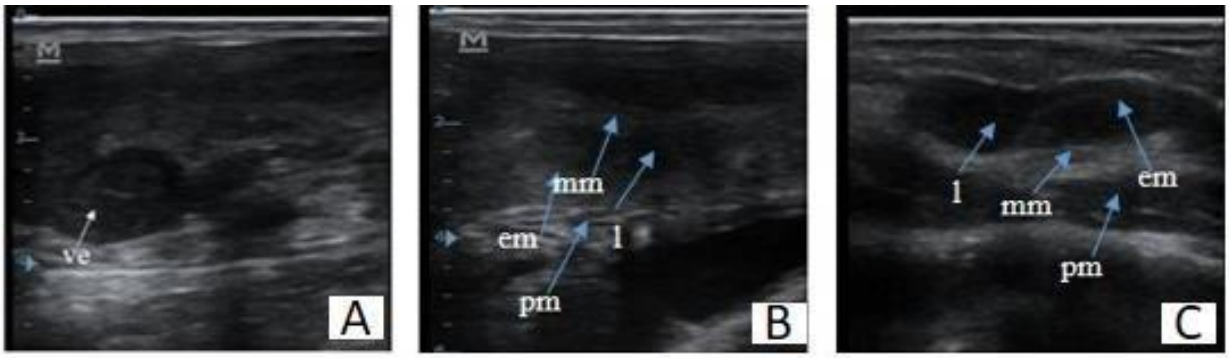

Figure 1. A. Sonographic view of pregnant Aceh cow uterus; B. Aceh cow that was not pregnant, and C. Aceh cow that experienced early embryonic death on the 25th day after AI.

Description: em: endometrium; mm: myometrium; pm: perimetrium; ve: embryonic vesicles; l: lumen.

Figure 1B shows that the uterus of an Aceh cow was diagnosed as not pregnant, there was no change in the endometrial wall. The sonography of the endometrium was hypoechoic to hyperechoic in color with anechoic to hypoechoic lumen and the

myometrium color was hypoechoic. It was in contrast to Figure 1C, which shows an altered uterine appearance. These changes were found in the lumen which was anechoic in color as fluid collided with the lumen and endometrial wall which gave a hyperechoic color and myometrium that was hyperechoic in color.

CONCLUSIONS

The best time for IFN-τ examination in the pregnancy diagnosis through the IFN-τ examination is on the 15th day after AI with an accuracy of 87.5%, specificity of 100%, and sensitivity of 80%.

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