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Comparison of the effectiveness of pregnancy diagnosis in Aceh cow through measurement of interferon-tau and progesterone concentrations

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Abstract. *Panjaitan B, Siregar TN, Hafizuddin, Sayuti A, Adam M, Armansyah T, Syafruddin. 2021. Comparison of the effectiveness of pregnancy diagnosis in Aceh cow through measurement of interferon-tau and progesterone concentrations. Biodiversitas 22: 1712-1716.* This study aims to determine the effectiveness of IFN-τ and progesterone measurement as the basis for pregnancy diagnosis in Aceh cows. The study used twelve cows aged 3-5 years, weighed 150-250 kg, clinically healthy, and having normal reproduction. The estrous cycle was synchronized using prostaglandin hormone (LutalyseTM, Pharmacia & Upjohn Company, Pfizer Inc., 25 mg/cow, i.m.) with multiple injection protocol (10-day interval). Heat detection was conducted in the morning (08.00 a.m.) and evening (16.00 p.m). Cows were inseminated 12-18 hours after standing heat. Blood was collected on day 4, 5, 6, 7, 14, 15, 16, 17, and 18 post-insemination. Concentrations of IFN-τ and progesterone were determined by the enzyme-linked immunosorbent assay (ELISA) method. Cows with interferon concentration above six pg/mL and progesterone concentration greater than two ng/mL were considered indicative of pregnancy. Pregnancy data were then compared with transrectal ultrasound at day 25 as the gold standard. Concentrations of IFN-τ in pregnant and non-pregnant cows were significantly different on day 18 post-AI, while progesterone concentration was significantly different on day 15. The accuracy of IFN-τ pregnancy diagnosis at peak IFN-τ expression was obtained on day 18 post-insemination, with an accuracy rate of 66.7%, a sensitivity rate of 60%, and a specificity rate of 71.4%. Meanwhile, progesterone-based pregnancy diagnosis accuracy was obtained on day 17 post-insemination, with an accuracy rate of 91.6%, a sensitivity rate of 83.3%, and a specificity rate of 100%. It was concluded that the diagnosis of pregnancy using progesterone measurement was more effective than IFN-τ measurement.

Keywords: Aceh cattle, IFN-τ, pregnancy diagnosis, progesterone

INTRODUCTION

Interferon-tau (IFN-τ), is a type I interferon secreted by trophectoderm cells in pregnant ruminants and is involved in pregnancy recognition (Forde and Lonergan 2017). IFN-τ functions as an anti-inflammatory response and promotes immunological tolerance to the presence of embryos (Yaginuma et al. 2019). IFN-τ induces corpora lutea (CL) resistance to PGF2 alpha, allowing CL to survive and maintain pregnancy (Hansen et al. 2017; Peter et al. 2017). This pregnancy signal is required for implantation on day 16 after fertilization (Spencer et al. 2013). IFN-τ is present from day 14 to day 21 of pregnancy, at its highest expression on day 16, or on day 15 to day 16 after ovulation (Senger 2005; Sheikh et al. 2018). In cattle, IFN-τ mRNA is produced from day 12 to day 19 of pregnancy, with the peak being on day 15/16, decreasing to day 19 of gestation (Farin et al. 1990). IFN-τ gene expression (IFN-τ mRNA) occurs simultaneously with the presence of IFN-τ in the body (Matsuyama et al. 2012).

Previous reports stated that IFN-τ stimulated genes (ISGs), resulting from IFN-τ induction, can be used to diagnose pregnancy in cows (Han et al. 2006). Previously, pregnancies have not been tested with ISG indicators because the measurement of IFN-τ has no sensitivity if the concentration is below ng/mL (Hansen and Rueda 1991). Thus, IFN-τ presence cannot be determined directly in the blood (Lucy and Pooks 2012). However, other studies state that IFN-τ can be detected at a concentration of 0.910 ng/mL± 0.55 using radioimmunoassay (RIA) technique the uterine vein of sheep as early as day 15 of pregnancy and then on day 16 of pregnancy, IFN-τ is most highly expressed (Antoniazzi et al. 2012). The IFN-τ concentration in cows can be measured by enzyme-linked immunosorbent assay (ELISA) technique using an ELISA kit (Cusabio, CSB-E16948B), which the kit can detect IFN-τ concentrations below 1.56 pg/mL. Our preliminary study also demonstrates that IFN-τ can be detected in the blood serum of Aceh cow from day 14 of pregnancy (Amri et al. 2021). Although not correlated, studies show that Aceh cows with premature embryo mortality also produce IFN-τ (Melia et al. 2020). This indicates IFN-τ level measurement after AI can be used to diagnose pregnancy in Aceh cows.

Pregnancy in a cow has been diagnosed through several methods with varying degrees of accuracy and examination time. The current method using ELISA to measure pregnancy-specific protein B (PSPB) and pregnancy-associated glycoprotein (PAG), yields high accuracy. However, it must be conducted on post-day 26 (Piechotta et al. 2011). Additionally, pregnancy diagnosis using rectal palpation and ultrasonography USG is highly accurate results from day 90 to day 100, with an accuracy of 85-

100%, respectively (Al-Watar et al. 2017). Another study found that diagnosis using USG would be more effective if followed by PAG examination on day 28 and measurement of progesterone levels on day 36, post-insemination (Rashmi et al. 2020). Highly accurate and early identification of pregnancy ensures the cow's reproductive effectiveness. It is crucial to shorten the calving interval through appropriate therapy and to rebreed non-pregnant cows as soon as possible.

Until now, progesterone testing is considered the earliest method to diagnose pregnancy in cows. This method is 80% effective from day 21-24 post-insemination (Sah et al. 2017; Srilatha et al. 2017). Related research indicates examination time can be accelerated (Abdullah et al. 2017; Siregar et al. 2017; Srilatha et al. 2017; Thasmi et al. 2017; Widayati et al. 2019). On day 15, post-insemination progesterone concentrations are significantly higher among pregnant cows than non-pregnant cows (Abdullah et al. 2017). At the peak of progesterone production, Aceh cows that experience repeat breeding and embryo mortality also show lower progesterone concentrations than fertile cows (Siregar et al. 2017; Thasmi et al. 2017). Recent reports have shown that cows with low progesterone concentrations at luteal onset cause impaired embryo development and decrease pregnancy percentage. Thus, this study investigates an alternative method of early-pregnancy diagnosis by measuring the concentration of IFN-τ and progesterone. Particularly for progesterone examination, the examination window (i.e., initial luteal and luteal peak phases) widened so that the best time for diagnosis could be obtained through progesterone analysis, comparing its diagnostic accuracy with IFN-τ measurement.

MATERIALS AND METHODS

This research was conducted at BPTU-HPT (Center for Superior Animal Breeding and Forage Animal Feed) Indrapuri, Aceh Besar, Indonesia. IFN-τ concentration was measured at the Integrated Research Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia. This research was conducted from November 2018 to April 2019 on twelve cows aged 3-5 years, bodyweight of 150-250 kg, clinically healthy, and having normal reproductive status.

The estrous cycle was synchronized using prostaglandin hormone (Lutalyse™, Pharmacia & Upjohn Company, Pfizer Inc., 25 mg/cow, i.m.) with multiple injection protocol (10-day interval). Heat detection was conducted in the morning (08.00 a.m.) and evening (16.00 p.m.). Cows were inseminated 12-18 hours after standing heat. Blood was collected on days 4, 5, 6, 7, 14, 15, 16, 17, and 18 post-insemination. Ten mL of blood samples were taken through the jugular vein, then stored in a blood tube and chilled in a refrigerator. The serum was separated by centrifugation at 3000 rpm for 10 minutes. Blood serum was transferred to a microtube and stored in a freezer at -20°C. Pregnancy was determined on day 25 using transrectal ultrasound (Mindray DP 10 Vet linear probe transrectal 50L60EAV, Shenzhen Mindray Bio-Medical Electronics CO., LTD.)

Interferon-tau concentration analysis

The IFN concentration was determined with ELISA using the Bovine Interferon ELISA Kit (Cusabio Technology LLC AII, USA). The minimum concentration detected by this kit is 1.56 pg/mL. This assay has high sensitivity (15.6 pg/tube) and excellent specificity for detection of IFN-τ. The inter-assay and intra-assay coefficients were <10% and <8%, respectively. Cows with an interferon concentration above 6 pg/mL were considered pregnant, whereas those with an interferon concentration below 6 pg/mL were considered non-pregnant. This interval limit was based on the preliminary study, which found that all cows confirmed to be pregnant by ultrasound examination on day 25 had interferon concentrations greater than 6.04 pg/mL (Amri et al. 2021).

Progesterone analysis

The concentration of progesterone was determined with ELISA using a progesterone ELISA kit (Cat No. 1562, DRG Instrument GmbH Germany). The assay sensitivity was 0.045 ng/mL. Cows with a progesterone concentration >2 ng/mL were considered pregnant, while cows with a progesterone concentration <2 ng/mL were considered non-pregnant (Han et al., 2006).

Data analysis

Pregnancy diagnoses measured by IFN-τ concentration and progesterone in the blood were then compared with transrectal ultrasound data as the gold standard. The difference in pregnancy determinations was evaluated using SAS chi-square analysis. The values for sensitivity, specificity, and accuracy were calculated using the formula used by Broaddus and de Vries (2005).

RESULTS AND DISCUSSION

The ultrasound results on day 25 after AI showed that 41.67% of the cows were pregnant and 58.3% were not pregnant. The mean percentage of pregnancy predicted by IFN-τ on day 18 was 66.7%, while the percentage for non-pregnancy was 33.3%. The chi-square test showed insignificant differences (P >0.05) between this method and the ultrasound-based examination. Similar results were obtained between pregnancy prediction with progesterone on day 17 and ultrasound (P> 0.05). IFN-τ examination on day18 and progesterone examination on day 17 were based on the highest accuracy value at the time of the examination. However, the accuracy of progesterone test results tends to be higher than the results of the IFN-test, as presented in Table 1.

Table 1. Prediction of pregnancy by measurement of IFN-τ or progesterone after ultrasound confirmation (day 25) in Aceh cows

	IFN- τ					Progesterone					
	h-14	**h-15**	**h-16**	**h-17**	**h-18**	**h-5**	**h-6**	**h-7**	**h-15**	**h-16**	**h-17**
The number of cows	12	12	12	12	12	12	12	12	12	12	12
Correct pregnant	3	3	3	3	3	2	2	3	5	5	5
Incorrect pregnant	5	4	4	4	2	3	3	2	0	0	0
Correct non-pregnant	2	3	3	3	5	3	3	5	5	5	6
Incorrect non-pregnant	2	2	2	2	2	4	4	2	2	2	1
Sensitivity (%)[a]	60	60	60	60	60	33.3	33.3	60	71.4	71.4	83.3
Specificity (%)[b]	40	42.9	42.9	42.9	71.4	50.0	50.0	71.4	100	100	100
PPV (%)[c]	37.5	42.9	42.9	42.9	60	40.0	40.0	60.0	100	100	100
NPV (%)[d]	50	60	60	60	71.4	42.9	42.9	71.4	71.4	71.4	85.7
Accuracy (%)[e]	41.7	50	50	50	66.7	41.7	41.7	66.7	83.3	83.3	91.6

Note: [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]PPV (positive predictive value): probability that the cow is truly pregnant if the diagnosis is pregnant; correct pregnant/(correct pregnant + incorrect pregnant) x 100%. [d]NPV (negative predictive value): probability that the cow is truly non-pregnant if the diagnosis is non-pregnant; correct-nonpregnant/(correct non-pregnant + incorrect non-pregnant) x100%. [e]Accuracy: a proportion of correctly classified subjects (correct pregnant + correct non-pregnant)/ (correct pregnant + correct non-pregnant + incorrect pregnant + incorrect non-pregnant)

The IFN-τ pregnancy diagnosis yielded the highest accuracy on day 18, with an accuracy rate of 66.7%, a sensitivity rate of 60%, and a specificity rate of 71.4%. The accuracy of this method was lower than that of progesterone analysis testing at day 17 (66.7% vs. 91.3%). These results support findings by Al-Watar et al. (2017), which stated that the best method to determine early pregnancy in cattle is through progesterone measurement, followed by ultrasound examination. The sensitivity and specificity of progesterone early pregnancy diagnosis (95%) are relatively similar to those achieved by ultrasound examination on days 30-282. However, progesterone-based diagnosis is advantageous because it can diagnose pregnancy as early as day 15. In comparison, ultrasound and transrectal palpation are most accurate (100%) on day 42 (Karthik and Krishnastya 2017).

Pregnancy diagnosis using IFN-τ analysis may not be able to replace progesterone examination, both in terms of accuracy and examination time. It is possible that the IFN-τ measurement method is more effective in predicting non-pregnant cows because the method's specificity value is higher than its sensitivity (71.4 vs. 60.0%). This aligns with research by Han et al. (2006), who found that detection of low levels of ISG15 mRNA from day 17-25 accurately indicates non-pregnant cows. IFN-τ induces ISG expression in peripheral mononuclear blood cells (Oliveira et al. 2008). ISGs expressed during the pre-implantation period can be used as a marker for pregnancy diagnosis in domestic animals (Mishra and Sarkar 2018).

The mean IFN-τ concentrations in pregnant cows and non-pregnant cows showed a significant difference (P <0.05) only on day 18 after AI (10.30±8.29 vs. 5.62±4.49 ng/mL), while the average IFN concentration on day 14-17 post-AI showed insignificant differences (P >0.05), with the respective concentrations of 11.27 ± 10.08 vs. 9.51±4.01; 11.44±8.16 vs. 8.62±6.36; 11.02±7.75 vs. 7.25±4.92 and 9.23±5.88 vs. 6.36±4.27 ng/mL. The average IFN concentrations in pregnant and non-pregnant cows for each day did not show significant differences (Figure 1).

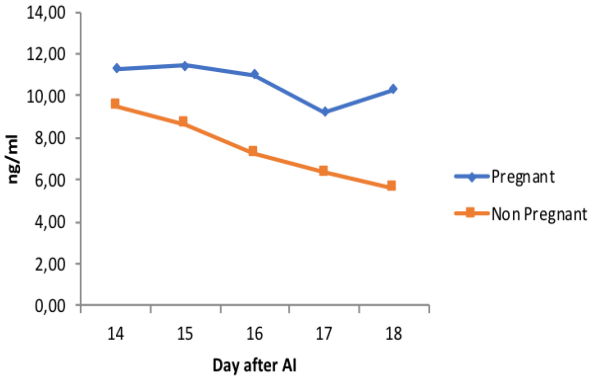

Figure 1. The concentration of IFN-τ in pregnant and non-pregnant Aceh cattle

Measurement of IFN-τ measurement in pregnant cows on day 14-18 showed that peak secretion of IFN-τ, at 11.44 ± 4.16 pg/mL, occurred on day 15, followed by day 14, 16, 18, and 17. This differed from research on dairy cows by Sheikh et al. (2018) that the peak concentration occurred on day 16, followed by day 14, 18, and 21. This variation may be due to differences in time of sampling patterns and breed of cattle.

Based on the results of the study, of the 12 cows used, five were diagnosed as pregnant (41.7%), and seven (58.3%) were non-pregnant. Some non-pregnant cows were then diagnosed as pregnant, as indicated by a high concentration of IFN-τ, especially on days 14 and 15. High IFN-τ concentration at the time of examination, paired with no signs of pregnancy at the time of ultrasound examination, may be caused by early embryo mortality before day 25 post-AI. Embryo mortality can occur from day 0-7, known as very early embryo mortality (VEEM). It may also occur from day 7-24, up until day 27 (Santos et al. 2004 cited by Suprihatin et al. 2016; Humblot 2001), known as early embryo mortality (EEM).

On day 15, 16, and 17 post-AI, the average progesterone concentrations in pregnant cows and non-pregnant cows showed significant differences ($P < 0.05$) (13.78±16.50 vs. 4.34±3.10; 15.54±15.10 vs. 2.37±2.25; and 15.36±15.29 vs. 2.11±2.12 ng/mL). Meanwhile, on day 5, 6, and 7 post-AI (luteal onset), the average progesterone concentrations in pregnant cows and non-pregnant cows, showed insignificant differences ($P > 0.05$) with the respective concentrations of 3.83±4.53 vs. 2.45±2.49; 2.67±3.14 vs. 2.78± 2.80; and 5.01±6.44 vs. 2.58±2.00 ng/mL. Starting from day 15 post-AI, the average progesterone concentrations in pregnant cows continued to increase while those in non-pregnant cows began to decrease, as shown in Figure 2.

The average progesterone concentrations in pregnant cows and pregnant cows appeared different ($P<0.05$) from day 15-17 post-AI but showed no significant differences ($P>0.05$) from day 5-7 post-AI. In this study, progesterone levels of both cattle groups at luteal onset did not align with previous reports (Stronge et al. 2005; Parr et al. 2010; Widayati et al. 2019). Cows that failed to get pregnant started to differ significantly in progesterone concentration from day 5, i.e., 3.07±0.33 ng/mL in pregnant Ongole cows and 1.17±0.39 ng/mL in repeat breeding cows that failed to become pregnant (Widayati et al. 2019). This indicates that pregnancy failure in this study was not caused by impaired embryo development. Low progesterone concentrations on day five after ovulation have a negative effect on the uterine preparation for the presence of embryos and impair embryo development (Parr et al. 2010).

Pregnancy detection using progesterone on days 5, 6, and 7 was only 41.7%, 41.7%, and 61.7% accurate, respectively, but this percentage increased on days 15, 16, and 17 with 83.3%, 83.3%, and 91.6% accuracy, respectively. Test accuracy on days 15, 16, and 17 outperformed those reported by Shah et al. (2017), who obtained 80% accuracy of progesterone examination on days 37-40 post-insemination. Relatively similar accuracy was reported by Rashmi et al. (2020), who found 80% accuracy of progesterone examination on day 20-24 post-insemination, and Srilatha et al. (2017), who obtained 85% accuracy on day 21 post-insemination. In this study, progesterone measurement accelerated diagnosis time (Nyman et al. 2018; Rashmi et al. 2020). Nyman et al. reported lower progesterone concentrations in non-pregnant cows than in pregnant cows from days 10, 21, and 30 post-AI. Rashmi et al. (2020) reported that the differences in progesterone concentrations in pregnant cows, non-pregnant cows, and cows that experienced embryo mortality could be detected after day 12 post-insemination. Based on these findings, this study recommends earlier progesterone pregnancy diagnosis in cows.

Cows with high progesterone concentrations from day 15-17 and above-threshold IFN-τ concentrations from day 14-18 were interpreted as pregnant and could have surviving embryos until day 25. Notably, cows with the above-threshold concentration of IFN-τ on day 14 yet decreasing from day 15-18 tend not to lose embryos by day 25. Thus, measurement of IFN-τ concentration may serve as the basis for embryo mortality diagnosis in cattle, although Melia et al. (2020) report the contrary. The embryo-maintaining role of IFN-τ is reinforced by a recent report stating that embryo transfer after artificial insemination increases the conception rate in repeat breeding cattle. Findings show that transferred embryos produce IFN-τ, which is expressed by increased ISGs (Yaginuma et al. 2019). This study concludes that pregnancy diagnosis in Aceh cows is more effective when measuring progesterone compared to IFN-τ.

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