RESEARCH ARTICLE | MAY 25 2021

Comparison of interferon-tau levels in pregnant and repeat breeding Aceh cows

Juli Melia; Widia Fitri; Husnurrizal; Sri Wahyuni ✉; Budianto Panjaitan; Teuku Armansyah; Tongku Nizwan Siregar

AIP Conf. Proc. 2353, 030077 (2021)
https://doi.org/10.1063/5.0052733

Comparison of Interferon-tau Levels in Pregnant and Repeat Breeding Aceh Cows

Juli Melia[1], Widia Fitri[2], Husnurrizal[1], Sri Wahyuni[3,a], Budianto Panjaitan[4], Teuku Armansyah[5], and Tongku Nizwan Siregar[1]

[1]*Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia*
[2]*Study Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia*
[3]*Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia.*
[4]*Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia*
[5]*Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia*

[a]Corresponding author: sriwahyuni@unsyiah.ac.id

Abstract. This study aims to determine whether IFN-τ concentrations differed in pregnant cows versus cows with repeat breeding syndrome. This study used six adult Aceh cows with aged 3 - 5 years old and 50 - 250 kg of body weight. All Aceh cows were divided into two groups, namely RB1 (pregnant) and RB2 (failed pregnancy) of Aceh cow groups where each group consist of three cows. A 5 mL dose of PGF2α was used to synchronize the cow reproductive cycles, consisting of a double injection pattern of 11 days interval, before artificial insemination. Evidence of pregnancy and/or embryo mortality was evaluated using trans rectal ultrasonography on the 25th day following artificial insemination. The concentration of IFN-τ was measured with an enzyme-linked immunoassay (ELISA) method using a bovine interferon ELISA kit. The IFN-τ concentration of RB1 vs RB2 was 12.52 ± 0.69 vs 32.76 ± 2.31 pg/mL. Furthermore, IFN-t concentrations of RB1 vs RB2 on day 14, 15, 16, 17, and 18 were 11.37 ± 1.02 vs. 29.66 ± 3.77; 14.58 ± 0.56 vs. 25.07 ± 3.37; 11.71 ± 0.56 vs. 24.05 ± 3.00; 13.07 ± 1.01 vs. 58.51 ± 6.24; and 11.90 ± 0.95 vs. 26.53 ± 3.80 pg/mL, respectively. Based on the results of the study, IFN-τ levels were shown to have no significant impact on the success of pregnancy and/or embryo mortality in RB1 and RB2 with RB.

INTRODUCTION

Repeat breeding (RB) is a condition in which a cow experiences a normal estrous cycle and period but has not achieved pregnancy despite mating three or more times with a fertile male. Repeat breeding occurs despite no known abnormalities [1]. Generally, cows with RB show a longer calving interval (18 - 24 months) with a low number of conceptions (< 40%), and higher services per conception (> 3) [2, 3]. The incidence of RB in cows worldwide ranges from 5.5 - 33.3%. The detrimental impact on farmers resulting from increases in RB is a problem in the field of animal husbandry that needs to be addressed immediately [1].

The incidence of RB is caused by several factors, including nutrition, infection of the reproductive organs, hormonal disorders, and maintenance management [4]. Maintenance management errors result from breeding factors such as poor housing management [5], food management, and environmental cleanliness [6]. These conditions may lead to both a failure of fertilization and early embryonic death, the main causes of RB disorders.

Early embryonic death is defined as the loss of a fertile embryo at any point up until the end of the implantation period [7]. Early embryonic death occurs until day 27 and late embryonic death occurs from days 28 - 42 [8]. Generally, early embryonic death is caused by genetic disorders, infectious diseases, reproductive tract abnormalities, and hormonal disorders [9]. A low concentration of the hormone progesterone occurs due to lysis of the corpus luteum (CL) [10]. Progesterone plays an important role in maintaining pregnancy, particularly in the early stages. During the initial pregnancy period, an injection of PGF2α results in its termination. Therefore, to prevent luteolysis caused by

International Conference on Life Sciences and Technology (ICoLiST 2020)
AIP Conf. Proc. 2353, 030077-1–030077-5; https://doi.org/10.1063/5.0052733
Published by AIP Publishing. 978-0-7354-4096-8/$30.00

PGF2α, the embryo signifies its viability within the maternal system through a biochemical process known as the maternal recognition of pregnancy [11].

Maintenance of the pregnancy is dependent on maternal recognition [1], and is known to be affected by interferon-tau (IFN-τ) produced by the developing embryo [12, 13]. It accomplishes this by decreasing oxytocin receptors in the endometrium, thereby inhibiting the secretion of PGF2α [14]. This results in an intact CL which functions to maintain the pregnancy [15]. In cows, the incidence of pregnancy failure is 10-15% caused by low levels of IFN-τ, which is needed to prevent luteolysis [16].

According to Dorniak *et al.*, IFN-τ also functions to assist the CL in secreting progesterone, on which the survival of the embryo is dependent [17]. In cows, IFN-t is produced by trophoblastic tissue during the 15th through 24th days of pregnancy [18]. More specifically, that IFN-τ is a protein produced by the trophectoderm from the 10th to the 12th day of pregnancy with peak secretions occurring from the 15th to the 17th day of pregnancy [19]. Most early embryonic deaths in cows occur during the first three weeks after conception, particularly during the implantation period [20]. Interferon-tau, acting as an anti-luteolytic cytokine is secreted by trophoblastic during implantation [16]. Embryo deaths that occur due to low IFN-t levels are characterized by inhibition and disruption of developing chorioallantois placentomes (or embryo) [21], therefore causing CL regression. Low IFN-τ concentration greatly affects the number of early embryonic deaths reported in cows [15,22]. However, the data related to the comparison of IFN-τ concentrations level in the pregnant and RB Aceh cow are not available. Therefore, the objective of this study was to compare the IFN-τ concentration between pregnant Aceh cows and those Aceh cows that are experiencing RB syndrome.

EXPERIMENTAL DETAILS

The research was conducted at BPTU Indrapuri. IFN-t concentration was measured at the Integrated Research Laboratory, Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam, in Banda Aceh, Indonesia. Research for this study was conducted from December 2018 until April 2019. Six adult female Aceh cows, aged 3-5 years with a bodyweight of 150 - 250 kg were studied. The criteria for cows used were in accordance with the Minister of Agriculture Regulation Number 2907/Kpts/OT.140/6/2011. Cows were placed into two groups of three cows each and named the RB1 Aceh cow group (pregnant) and the RB2 Aceh cow group (pregnancy failure).

Heat Synchronization and Heat Detection

The reproductive cycles of the cows were synchronized with 5 mL of PGF2α hormone (Lutalyse™, Pharmacia & Upjohn Company, Pfizer Inc.) injected intramuscularly with a double injection pattern of 11 days interval. Estrus detection was carried out for 30 min twice a day at 8 A.M. and 4 P.M. through observation of secondary signs such as riding on other cows, restlessness, a red and swollen vulva, cervical mucous discharge, and decreased appetite.

Artificial Insemination (AI)

The AI procedure started with equipment preparation. An AI straw was taken from a nitrogen (liquid N_2) container using tweezers and placed in warm water (37 ^{o}C) for 30 sec. The straw was then lifted and dried using tissue paper and a straw plug was inserted into the hole of an AI gun. Next, the straw tip clamped outside the AI gun was cut out and put on a plastic sheet and tightened with a locking ring so that the tip of the straw was completely covered. The AI gun was inserted through the vagina and the tip was directed to the opening of the cervix. Upon nearing the third cervical ring, the semen was deposited slowly and at which point, the AI gun was removed slowly [22].

Blood Collection

Blood samples were taken on the 14th to 18th days following insemination. Up to 10 mL of blood was drawn from the jugular vein into a tube, stored in an ice-filled thermos and brought to the laboratory. The blood was incubated for several hours until serum appeared, at which point it was centrifuged at 300 rpm for 10 min. The serum was then transferred to a microtube and stored in a freezer at -20 ^{o}C prior to analysis [22].

Interferon-tau Measurement

The concentration IFN-τ was measured using enzyme-linked immunosorbent assay (ELISA) by preparing an IFN-τ ELISA kit (Cusabio Technology LLC All, USA) consisting of antibody IFN-τ, standard solution and serum samples. In brief, 100 µL of standard solution and the serum sample was inserted into each microplate well and then incubated for 2 h at 37 °C. After incubation, the solution was removed from the wells and then 100 µl of biotin-antibody was added prior to an hour incubation at 37 °C. All contents of the microplate were aspirated and the process was repeated twice. Washing was performed three times with a 200 µL washing buffer. Afterward, 100 µL of HRP-avidin was added to each microplate well. The microplate was incubated for 1 hour at 37 °C and then aspirated and washed five times. After washing, 90 µL of TMB substrate was added to the microplate wells and subsequently incubated for 15 - 30 min at 37 °C. Finally, the enzymatic reaction was stopped by adding 50 µL stop solution to the entire microplate. The results were read using ELISA reader at a wavelength of 450 nm.

Pregnancy Examination

Pregnancy examination was performed by transrectal ultrasonography (USG) (Shenzhen Mindray Bio-Medical Electronics CO., LTD.) on the 25th day following AI. Cows were considered pregnant based on the presence of anechoic fluid, and visualization of an embryo and heart rate in the uterine cornua [24]. The data obtained from the results of IFN-t concentration measurements in the two treatment groups were analyzed using a split plot test.

RESULTS AND DISCUSSION

Following AI, three cows were detected as pregnant (RB1), while the other three were not pregnant (RB2). According to measurement results, INF-t concentration are presented in Table 1 where no significant differences in IFN-τ concentrations between RB1 and RB2 ($p > 0.05$). Based on the concentration of IFN- τ obtained, all cows had successful fertilization and viable embryos because IFN-τ is only produced by developing embryos [11,13]. However, not all cows that maintained pregnancy as seen from the three cows (RB2) with negative results at the time of the USG examination on the 25th day.

TABLE 1. Mean ± SEM of IFN-τ concentration in RB1 and RB2

Treatment Cows	Interferon-tau Concentration (pg/mL)					Mean ± SEM
	14th day	15th day	16th day	17th day	18th day	
RB1	11.37 ± 1.02	14.58 ± 0.56	11.71 ± 0.56	13.07 ± 1.01	11.90 ± 0.95	12.52 ± 0.69
RB2	29.66 ± 3.77	25.07 ± 3.37	24.05 ± 3.00	58.51 ± 6.24	26.53 ± 3.80	32.76 ± 2.31

RB: repeat breeding

The mean total concentration of IFN-t of RB1 vs RB2 were 12.52 ± 0.69 vs. 32.76 ± 2.31 pg/mL. The IFN-τ concentration was not significantly affected by the sampling time ($p > 0.05$). Furthermore, IFN-τ concentrations of RB1 vs RB2 on the 14th, 15th, 16th, 17th, and 18th days were 11.37 ± 1.02 vs. 29.66 ± 3.77; 14.58 ± 0.56 vs. 25.07 ± 3.37; 11.71 ± 0.56 vs. 24.05 ± 3.00; 13.07 ± 1.01 vs. 58.51 ± 6.24; and 11.90 ± 0.95 vs. 26.53 ± 3.80 pg/mL, respectively. The peak secretion of IFN-τ concentration from RB1 was observed on the 15th day (14.58 ± 3.70 pg/mL), while the peak secretion from RB2 was found on the 17th day (58.51 pg/mL). These results differ from results that reported by Sheikh *et al.*, that the increase of INF-τ secretion in pregnant cows on the 16th day was 136.09 pg/mL, followed by the 14th, 18th, and 21st days [25]. The differences between these results is likely influenced by the number of samples used, cow breed, and different sampling pattern. For example, Sheikh et al. [25] used 16 Karan fries (KF) cows. In this study, IFN-τ concentration secreted by RB1 on the 15th day after AI (14.58 ± 0.56 pg/mL) was able to maintain the pregnancy until the 25th day, while in RB2, the IFN-τ concentration secreted on the 17th day after AI (58.51 ± 6.24 pg/mL) could not maintain the pregnancy.

In RB1 IFN-τ concentration measured was lower than RB2. This result was inconsistent with results reported by Sheikh *et al.* showing higher IFN-τ concentrations in pregnant cows compared to cows experiencing embryo death [25]. The occurrence of embryo death in RB2 was not affected by the IFN-τ concentration but is suspected to be due

to other factors, such as hormonal disturbance, nutrition, environment, and infection [26]. Several diseases resulting from genital infections could cause early embryonic death and/or abortion and cause infertility [27]. One such infectious disease known to occur in cows is endometritis, an inflammation of the uterine mucosa, which causes temporary infertility and/or permanent reproduction disturbance [28]. Bacteria entering through the vagina and reaching the cervix during delivery causes endometritis. Bacteria such as *Escherichia coli*, *Arcanobacterium pyogenes* and viral infections are known to cause endometritis and can lead to infertility by causing premature embryo death secondary to bacterial infection or disruption of embryonic attachment on the uterine wall [29]. A poor postpartum uterine environment facilitates the entry of microbes into the uterine lumen, ultimately contaminating the uterine environment, and causing embryo death [30].

Embryo death due to low IFN-τ levels produced by embryonic trophectoderm was reported by Matsuyama et al. [15]. In this study, IFN-τ concentrations of as much as 14.58 ± 3.70 pg/mL was found on the 15th day post-AI can maintain pregnancies until the 25th day. Interferon-τ is a cytokine with anti-luteolytic properties and plays a role in maintaining pregnancy by inhibiting endometrial luteolysis [16]. These anti-luteolytic maintain CL function and progesterone secretion where this hormone is needed by the embryo for a successful implantation until birth [31]. Furthermore, IFN-τ acts within the uterine endometrium to inhibit oxytocin receptor production, therefore oxytocin is not able to synthesize PGF2α [32]. Additionally, the IFN-τ also has a role in preparing the uterus for embryonic development by regulating complementary genes in the cow's endometrium. Interferon-τ is secreted in early pregnancy during embryo elongation and the increasing size of the embryo results in even more progesterone being produced which maintains the pregnancy [25] and importantly contributes to the growth and development of the embryo, rendering the pregnancy viable until delivery.

Embryo death that occurred in RB2 was diagnosed with USG on the 25th day following AI which showed no positive signs of pregnancy. Meanwhile, RB1 was assumed pregnant on the 25th day after AI that proven with the presence of embryonic vesicles. These results differed from reports of Siregar *et al.*, that cows are assumed to be pregnant on the 25th day after insemination based on the presence of anechoic fluid and an embryonic visualization with a heartbeat in one of the uterine horns (uterine cornua) [23]. This was attributed to the difference in cow breeds used, particularly the dairy cows used by Chaudhary and Purohit that is different from Aceh cows used in this study [24]. In Aceh cows, embryo and heartbeat in uterine cornua were not visualized on the 25th day of pregnancy. The results of pregnancy examinations using ultrasonography are presented in Figure 1.

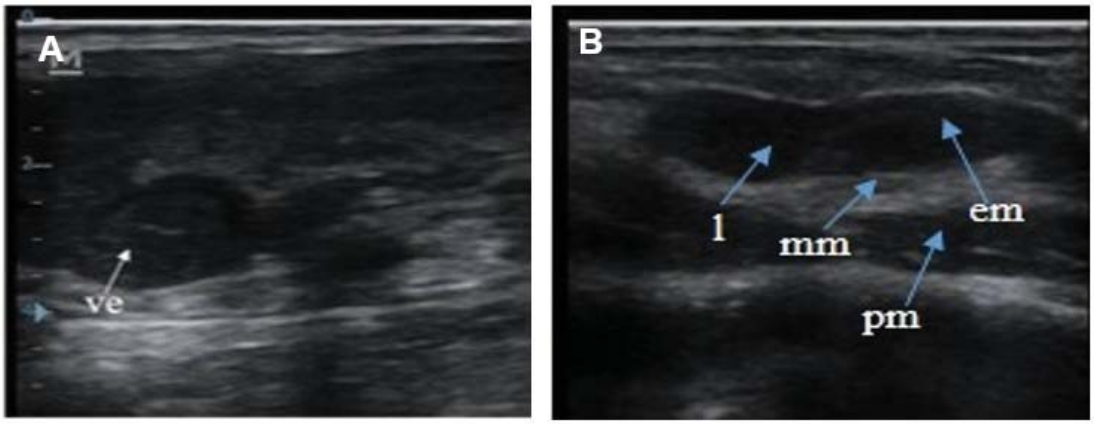

FIGURE 1. Ultrasonographic display of the uterus of RB1 and RB2 cows on the 25th day after insemination. A. Uterine of RB1 cow shows an embryonic vesicle (ve). B. Uterine of RB2 cow experiencing early embryonic death with endometrium (em), lumen (l), myometrium (mm), and perimetrium (pm).

Figure 1A shows that pregnancy in RB1 was diagnosed based on the presence of an embryonic vesicle. The vesicle of an embryo is characterized by USG that shows an isoechoic to hyperechoic appearance encircling hypoechoic (embryonic) fluid [32]. The embryonic vesicle resembles a circle in the endometrial wall. Figure 1B shows an image alteration of the uterus. The alteration seen in the lumen was characterized by an anechoic appearance, which resembled liquid colliding with the lumen, and both the endometrial wall and myometrium with a hyperechoic appearance.

SUMMARY

Based on results according to the comparison of the level of IFN-τ between repeat breeding and non-repeat breeding Aceh cows, it can be concluded that IFN-τ levels had no impact on the success of pregnancy and/or embryo mortality in Aceh cows with repeat breeding.

REFERENCES

1. G.S. Amiridis, T.H. Tsiligianni, E. Dovolou, C. Rekkas, D. Vouzaras, and I. Menegatos, Theriogenology. 72, 542-548 (2009).
2. R. Rustamadji, A. Ahmadi, K. Kustono, and S. Sutarno, Kinerja Usaha Peternakan Sapi Perah Rakyat sebagai Tulang Punggung Pembangunan Persusuan Nasional (Dies 38 Fakultas Peternakan UGM, Yogyakarta, 2007)
3. H. Gustafsson and U. Emanuelson, J. Acta Vet. Scand. 43, 115-125 (2002).
4. D. Ramandani and A. Nururrozi, J. Sain Vet. 33, 23-28 (2015).
5. J.H. Britt, R.G. Scott, J.D. Armstrong, and M.D. Whitacre, J. Dairy Sci. 69, 2195-2202 (1986).
6. J.J. Windig, M.P.L. Calus, and R.F. Veerkamp, J. Dairy Sci. 88, 335-347 (2005).
7. E.S.E. Hafez and B. Hafez, Reproduction in Farm Animals (Lippincott Williams and Wilkins, Baltimore, 2000).
8. P. Humblot, Theriogenology, 56,1417-1433 (2001).
9. A.A. Wawo, Pengaruh Pejantan Terhadap Tingkat Kebuntingan dan Berat Lahir pada Sapi yang Dipelihara secara Semi-Intensif (Thesis Fapet Unhas 2014).
10. N.A. Robinson, K.E. Leslie, and J.S. Walton, J. Dairy Sci. 72, 202-207 (1989).
11. T.D. Lestari, Metode Deteksi Kebuntingan pada Ternak Sapi, (Thesis Fakultas Peternakan Universitas Padjajaran, Bandung, 2006).
12. W.W. Thatcher, M.D. Meyer, and G. Danet-Desnoyers. J. Reprod. Fertil. Suppl. 49,15-28 (1995).
13. Hafizuddin, Yusmadi, and Anwar, J. Ilmiah Peternakan, 4, 29-36 (2016).
14. A.P.F. Flint, Reprod. Fert. Dev. 7, 313-318 (1995).
15. S. Matsuyama, T. Kojima, S. Kato, and K. Kimura, Reprod. Biol. Endocrinol, 10, 21-30 (2012).
16. S.N. Depamede, Wartazoa, 19, 166-171 (2009).
17. P. Dorniak, F.W. Bazer, and T.E. Spencer, Biol. Reprod. 84, 1119-1127 (2011).
18. G.L. Winkelman, R.M. Roberts, A.J. Peterson, A.P. Alexenko, and A.D. Ealy, Biol. Reprod, 61, 1592-1600 (1999).
19. R.C. Bott, R.L. Ashley, L.E. Henkes, A.Q. Antoniazzi, J.E. Bruemmer, G.D. Niswender, F.W. Bazer, T.E. Spencer, N.P. Smirnova, R.V. Anthony, and T.R. Hansen, Biol. Reprod. 82, 725-735 (2010).
20. P. Dorniak and T.E. Spencer, Anim. Reprod. 3, 239-251 (2013).
21. M.C. Wiltbank, G.M. Baez, A. Garcia-Guerra, M.Z. Toledo, P.L. Monteiro, L.F. Melo, J.C. Ochoa, J.E. Santos, and R. Sartori, Theriogenology, 86, 239-253 (2016).
22. T.E. Spencer, N.H. Ing, T.L. Ott, J.S. Mayes, W.C. Becker, G.H. Watson, M.A. Mirando, F.W. Brazer, J. Mol. Endocrinol. 15, 203-220 (1995).
23. T.N. Siregar, T. Armansyah, B. Panjaitan, G. Gholib, H. Herialfian, A. Sutriana, M.A. Reynaldi, F. Razak, Y. Artaliani, and Y. Yuswar, Adv. Anim. Vet. Sci. 7, 306-314.
24. A.K. Chaudhary and G.N. Purohit, J. Anim. Sci. Adv. 2, 706-710 (2012).
25. A.A. Sheikh, O.K. Hooda, A. Kalyan, A. Kamboj, S. Mohammed, M. Alhussien, S. Reddi, P.G. Shimray, A. Rautela, S. Pandita, S. Kapila, S. De, A.K. Dang, Theriogenology, 120, 61-67 (2018).
26. S.A. Prihatno, A. Kusumawati, N.W.K. Karja, and B. Sumiarto, J. Vet. 14, 452-461 (2013).
27. I.M.I.P. Nurjayanti, Kasus Kawin Berulang dan Faktor Penyebab pada Sapi Potong di Wilayah Kerja Petugas Teknis Kesehatan Hewan Kecamatan Kedemean Kabupaten Gresik (Thesis FKH Universitas Airlangga, Surabaya, 2015).
28. S. Hardjoprajonto, Ilmu Kemajiran pada Ternak (Airlangga University Press, 1995).
29. J. Melia, Amrozi, and L.I. Tumbelaka. J. Kedokt. Hewan, 8, 111-115 (2014).
30. G.H. Arthur, R.J. Noakes, and H.W. Person, Veterinary Reproduction and Obstetrics (Saunders. 2001).
31. T.E. Spencer and F.W. Bazer, Reprod. Biol. Endocrinol. 2, 1-15 (2004).
32. P.L. Senger, Pathways to Pregnancy and Parturition, (Current Conception, Inc., Pullman, 2005).
33. A. Sayuti, J. Melia, I.K. Marpaung, T.N. Siregar, Syafruddin, Amiruddin, and B. Panjaitan, J. Kedokt. Hewan, 10, 63-67 (2016).