

Short communication: Disparities in early luteal phase progesterone concentrations between repeat breeder and fertile Aceh cattle

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Abstract. *Thasmi CN, Hafizuddin UP, Husnurrisal, Siregar TN, Herrialfian, Sutriana A, Rosmaidar, Hanum FF, Gholib, Sayuti A, Yusmadi, Barus RA, Abdelbagi MAE. 2025. Short communication: Disparities in early luteal phase progesterone concentrations between repeat breeder and fertile Aceh cattle. Asian J Agric 9: 854-860.* Repeat breeding remains a major reproductive constraint in cattle, often associated with altered luteal function and inadequate progesterone production during early estrus cycles. Understanding hormonal differences between fertile and repeat breeder cows is essential for improving reproductive efficiency in Aceh cattle (*Bos indicus*). This study aimed to investigate disparities in progesterone concentrations during the early luteal phase between repeat breeder and fertile Aceh cows. Six multiparous Aceh cows, aged 3-5 years and weighing 150-250 kg, were selected as study subjects. The cows were divided into two groups: repeat breeders (n=3) and fertile cows (n=3). To ensure synchronized estrus timing, all cows received 5 ml PGF2 α (Enzaprost®-T, Dinoprost 5 mg) using the by double injection method. Estrus detection was performed following PGF2 α injection in the morning and afternoon. Blood samples were collected on days 3 and 6 post-estrus for progesterone analysis. Progesterone concentrations were determined measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method, and the data were analyzed using the Mann-Whitney U test. Results indicated that progesterone concentrations on days 3 and 6 were 5.68 \pm 4.98 and 6.61 \pm 3.04 ng/mL in fertile cows, whereas in repeat breeder cows, the concentrations were only 0.35 \pm 0.07 and 2.74 \pm 0.58 ng/mL, respectively. A significant difference in progesterone concentration was observed on day 3 between the two groups (p < 0.05). The conclusion indicates that repeat breeder Aceh cows exhibit lower progesterone concentrations during the early luteal phase compared to fertile cows. These findings contribute significantly to the understanding of hormonal differences between the two groups, which may serve as a foundation for developing more effective and sustainable breeding strategies.

Keywords: Indigenous cattle genetic resources, luteal function, progesterone deficiency, reproductive efficiency, sustainable livestock production

INTRODUCTION

Aceh cattle (*Bos indicus* Linnaeus, 1758) are one of the principal local cattle breeds in Indonesia, alongside other breeds such as Bali, Pesisir, Sumba Ongole, Madura, Grati, Ongole Grade, Katingan, Sumbawa, Pasundan, Jabres, and Galekan. These breeds represent an important genetic resource for livestock and must be conserved for sustainable meat production, labor, energy, fertilizer, and

economic purposes (Agung et al. 2019; Sutarno et al. 2019). Geographically, Aceh cattle are native to the Aceh Province, where they have been cultivated for generations and play a vital role in the economic, social, and cultural aspects of the local community. The population of cattle in Aceh is predominantly composed of Aceh cattle, comprising 84.93% of the total cattle population in smallholder farms (Budisatria et al. 2018, 2019).

The management system for Aceh cattle remains traditional, with simple housing structures built near homes or yards. Most farmers raise cattle as a supplementary income alongside their primary occupations. Nevertheless, local beef cattle offer distinct advantages, such as demonstrating optimal productivity and economic efficiency under various constrained conditions (Armansyah et al. 2017). Therefore, local beef cattle are more suitably and economically developed under smallholder farming patterns. Although Aceh cattle remain primarily managed through traditional farming systems, recent decades have seen the introduction of appropriate reproductive technologies (Siregar et al. 2016). These technologies have been applied to cows and Aceh bulls with the aim of improving reproductive performance and addressing reproductive disorders (Rosmaidar et al. 2021; Thasmi et al. 2021, 2024).

Currently, the most adaptive reproductive technologies implemented for Aceh cattle include estrus synchronization and artificial insemination (Baliarti et al. 2021; Hafizuddin et al. 2024), and studies addressing the reproductive performance of Aceh bulls (Hafizuddin et al. 2024; Panjaitan et al. 2024). However, a major barrier to the application of artificial insemination technology in Aceh cattle is the low conception rate following the procedure (Syafuruddin et al. 2021). Cows from smallholder farms often experience repeat breeding, requiring more than three artificial insemination attempts on average to achieve pregnancy (Thasmi et al. 2021). This results in extended intervals between calvings and significantly increased costs associated with breeding (Syafuruddin et al. 2021; Thasmi et al. 2021). Poorly managed repeat breeding conditions have led Aceh Province to experience a deficit, particularly concerning cattle commodities, as evidenced by higher livestock imports and production yields compared to outflows (Sofyan et al. 2020).

According to data from the Central Statistics Agency of Indonesia, the demand for beef still exceeds market supply. The Indonesian government has launched policies to support self-sufficiency in beef production; however, this goal has yet to be achieved. Field problems include the prevalence of reproductive disorders in cattle (Yusuf et al. 2019). Reproductive disorders related to infertility, such as inactive ovaries, ovarian hypofunction, cystic ovaries, and endometritis, contribute to low reproductive efficiency (Sutriana et al. 2021). Additionally, repeat breeding is a subfertility condition that indicates reproductive disorders and decreases reproductive performance (Salman et al. 2021). Repeat breeding is defined as the failure of a cow to conceive after three or more services either by natural mating with a fertile bull or artificial insemination using fertile semen despite the absence of any detectable clinical abnormalities (Islam et al. 2017). Generally, the causes of repeat breeding can be categorized into two groups: fertilization failure and early embryonic death (Maulana et al. 2022). However, as noted by Barui et al. (2015) and Matubber et al. (2018), the occurrence of repeat breeding in cattle is influenced by various factors, including management practices, nutrition, infections, and hormonal imbalances. One hormonal disorder associated with repeat

breeding is the inadequacy of progesterone during the luteal phase. Dar et al. (2017) pointed out that hormonal imbalances, particularly low progesterone levels, can lead to embryo implantation failures and early embryonic death. This view is supported by Ghanem and Nishibori (2015) and Wolfenson and Roth (2019), who noted that disrupted progesterone levels (low concentrations) can alter the uterine environment.

Husnurrizal et al. (2021) reported that Aceh cows that successfully conceived had higher progesterone levels during the early luteal phase compared to those that experienced early embryonic loss. However, data regarding progesterone levels during the early luteal phase in Aceh cattle remains limited. Therefore, further research is needed to understand the relationship between progesterone levels and the occurrence of repeat breeding in Aceh cattle.

MATERIALS AND METHODS

Animal samples

This study involved six Aceh cows, each having been pregnant at least once, aged between 3 and 5 years, and weighing between 150 and 250 kg. To assess reproductive performance, the cows were categorized into two distinct groups: repeat breeders ($n=3$) and fertile cows ($n=3$). Repeat breeder cows were defined as cows that had failed to conceive after at least three consecutive artificial insemination attempts, despite exhibiting normal estrous cycles, having no detectable anatomical abnormalities of the reproductive tract, and showing no clinical signs of reproductive disease. Fertile cows were defined as cows with a normal reproductive history, characterized by successful conception within one or two insemination attempts during previous breeding cycles, and no recorded reproductive disorders.

All procedures and sample collection for this study were conducted in accordance with ethical standards, receiving prior approval from the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Universitas Syiah Kuala, Banda Aceh, Indonesia. The study was granted ethical clearance under approval number 274/KEPH/III/2021, ensuring adherence to humane treatment and welfare guidelines throughout the research process. This ethical oversight underscores the commitment to responsible research practices while investigating the reproductive characteristics of the Aceh cattle breed. All cows were maintained under uniform farm management conditions. They were housed in individual pens to eliminate the potential influence of dominance behavior on reproductive performance. Each animal received a standardized diet consisting of fresh forage and commercial concentrates provided twice daily, with unrestricted access to clean drinking water. Comprehensive health management practices, including routine clinical examinations, were consistently implemented across all individuals. Because all cows were multiparous and managed under comparable nutritional and environmental conditions, the observed differences in reproductive status between the repeat-breeder and fertile groups were unlikely

to be attributable to husbandry or other management factors.

Estrus synchronization

Estrous synchronization was performed by administering 5 mL of PGF2 α (Enzaprost®-T, containing 5 mg of Dinoprost) via intramuscular injection, using a double injection protocol. Before starting synchronization, rectal palpation was performed to evaluate the reproductive status of each cow, to ensure that only cows in appropriate physiological conditions were included in the study. After the second injection of 5 ml of PGF2 α , estrus observation was initiated (Husnurrizal et al. 2024).

Estrous observation was carried out systematically in the morning and evening to maximize the opportunity to detect estrus behavior. Cows were considered to be in estrus when they exhibited specific signs, including a red, swollen vulva and clear cervical mucus (Rosmaidar et al. 2021). The most reliable indicator of true estrus standing heat was used as the final confirmation criterion. A cow was considered to be in standing heat when cow remained immobile while being mounted by another animal. Estrus was further validated using a bull teaser; an individual was classified as truly in estrus if cow stood still when mounted by the bull, following the procedure described by Hafizuddin et al. (2024).

Blood sampling

Blood samples were collected on days 3 and 6 after the onset of estrus. A total of 5 mL of blood was drawn from the jugular vein of each cow using a Vacuum Tube to ensure proper collection and minimize contamination. To maintain the integrity of the sample, the tubes were stored in a thermos containing ice gel immediately after collection. After allowing the blood samples to stand for several hours to facilitate clot formation, serum separation was performed. This involved centrifugation at 3000 rpm for 10 minutes, which effectively separated the serum from the cellular components of the blood. After centrifugation was complete, the serum was carefully transferred to a microtube to prevent sample disturbance. The collected serum samples were then stored in a freezer at -20°C until further analysis for hormone levels.

Progesterone concentration measurements

The concentration of progesterone was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) following the detailed protocol provided in the ELISA progesterone kit manual (DRG, GmbH Instrument, Germany). This methodology is well-established for its sensitivity and specificity in quantifying hormone levels. The procedure commenced with the preparation of the microplate wells, into which 25 μ L of standard solution, sample, and control were dispensed. These samples were then incubated for 30 minutes at room temperature, maintained at 20-23°C. This initial incubation step allowed for the binding of progesterone to the antibodies coated on the microplate wells, facilitating the subsequent detection process. Following this, 200 μ L of the enzyme conjugate

progesterone was added to each well. This enzyme conjugate is crucial as it catalyzes the reaction that produces a measurable signal. The contents of each well were mixed thoroughly by stirring for 10 seconds, ensuring that the enzyme conjugate uniformly interacted with the bound progesterone. The wells were then incubated for an additional 60 minutes at room temperature, allowing sufficient time for the enzyme to react with the progesterone. After the incubation, the solution in each well was shaken rapidly to dislodge unbound substances, and the wells were washed three times with 400 μ L of wash solution. This washing step is essential for minimizing background noise and enhancing the accuracy of the results by removing non-specifically bound materials. Following the washes, the microplate was tapped on absorbent paper to eliminate any residual droplets, ensuring that only the bound enzyme-progesterone complex remained in the wells. Subsequently, 200 μ L of substrate solution was introduced into each well and incubated for 15 minutes at room temperature. This substrate is converted by the enzyme into a colored product, which is directly proportional to the concentration of progesterone present. The enzymatic reaction was halted by adding 100 μ L of stop solution to each well, effectively stabilizing the color development. Finally, the absorbance values were measured using a microtiter plate reader set to a wavelength of 450 \pm 10 nm within 10 minutes of adding the stop solution. This measurement is crucial for quantifying the progesterone concentration, as the intensity of the color produced is indicative of the hormone levels in the samples. The resulting absorbance data were analyzed to determine progesterone concentrations relative to the standard curve established from known concentrations, thereby providing accurate and reliable quantification of progesterone levels in the samples tested (Adam et al. 2017).

Data analysis

The research data regarding progesterone levels in Aceh cattle obtained in this study were analyzed using the Mann Whitney U test with the statistical software program SPSS for Windows version 22.0.

RESULTS AND DISCUSSION

The average progesterone concentrations of Aceh RB and fertile cattle during the early luteal phase are presented in Table 1. Table 1 shows the mean progesterone concentration and standard error in Aceh repeat breeder cows and fertile cows. On day 3 after estrus, progesterone concentration in repeat breeder cows (0.35 \pm 0.07 ng/mL) was significantly lower ($p = 0.05$) compared to fertile cows (5.68 \pm 4.98 ng/mL). There was no significant difference in progesterone concentration between the two groups on day 6 after estrus ($p = 0.27$) (2.74 \pm 0.58 vs. 6.61 \pm 3.04 ng/mL).

Table 1. Mean (\pm SE) progesterone concentration in the early luteal phase of Aceh cattle RB and fertile

Observation time (after estrus)	Progesterone (ng/mL)	
	Aceh repeat breeder cattle	Aceh fertile cattle
Day 3	0.35 \pm 0.07 ^a	5.68 \pm 4.98 ^b
Day 6	2.74 \pm 0.58 ^a	6.61 \pm 3.04 ^a

Note: ^{a,b}: Different superscripts in the same row indicate significant differences ($P < 0.05$)

This study has provided evidence information that Aceh repeat breeder cows have significantly lower progesterone concentrations on day 3 post-after estrus compared to fertile cows. The lower progesterone concentration in repeat breeder Aceh cows during the early luteal phase indicates potential luteal dysfunction. According to Dar et al. (2017), luteal dysfunction is a cause of repeated breeding in cattle and buffalo (*Bubalus bubalis*), which causes decreased reproductive efficiency and early embryonic death. Luteal dysfunction can interfere with progesterone hormone production or result in luteal regression, which clinically can cause delayed ovulation with a prolonged follicular phase. This results in a prolonged interval between luteolysis and subsequent ovulation.

Progesterone concentration on day 6 did not show significant differences. However, the results showed that the concentration was lower in Aceh cows that experienced repeated mating compared to fertile Aceh cows (2.74 \pm 0.58 vs. 6.61 \pm 3.04 ng/mL). This finding reinforces previous studies that highlighted a positive correlation between postovulatory progesterone concentration and embryo survival rate. Higher progesterone levels were associated with better embryo survival, whereas cows with low postovulatory progesterone concentrations faced an increased risk of embryo death, a major factor contributing to repeated mating. In addition, the persistence of low progesterone concentrations during the early luteal phase can recur throughout the next cycle (Diskin et al. 2016; Wiltbank et al. 2018).

The low progesterone levels observed in Aceh cows that experienced repeated mating in this study were influenced by low blood cholesterol levels. This is in line with the statement consistent with the findings of Thasmi et al. (2017), who noted that low blood cholesterol can affect progesterone levels. Acconcia and Marino (2018) and Chatuphonprasert et al. (2018) added that cholesterol functions as a precursor for the biosynthesis of steroid hormones, including estradiol and progesterone.

Progesterone is a crucial hormone for maintaining pregnancy in cattle. Increased concentrations of progesterone during early pregnancy are associated with embryo development, enhanced production of interferon- τ , and higher pregnancy rates (Sponchiado et al. 2017; Lonergan and Sánchez 2020; Michael et al. 2020). Low progesterone levels up to day 5 post-ovulation can lead to decreased pregnancy rates (Kim et al. 2017; Thasmi et al. 2017), which can result in repeat breeding (Parr et al. 2010).

These findings support previous studies showing a positive correlation between postovulatory progesterone concentrations and embryo survival rates. Higher progesterone levels are associated with increased embryo viability (Jaśkowski et al. 2022). Cows with low postovulatory progesterone concentrations are more likely to experience embryonic death, which is a major cause of repeat breeding. Persistent low progesterone levels during the early luteal phase can recur from one cycle to the next (Parr et al. 2010).

This is in line with previous studies on Ongole cattle that experienced repeated mating, which showed lower progesterone concentrations in the early luteal phase compared to fertile cows (1.17 \pm 0.39 ng/mL vs. 3.07 \pm 0.33 ng/mL) (Widayati et al. 2019). Prihatno et al. (2013) also reported that progesterone levels in Holstein Friesian cows that experiencing repeated mating were lower (5.7 \pm 0.8 ng/mL) compared to those of fertile Holstein Friesian cows (7.4 \pm 0.7 ng/mL).

Ghanem and Nishibori (2015) found that total progesterone concentrations in pregnant cows with normal luteal function were significantly higher than in non-pregnant cows with abnormal luteal function during the estrus cycle in summer. However, no significant differences were observed between pregnant and non-pregnant cows with normal luteal function in both seasons. The seasonal difference reported by Ghanem and Nishibori (2015) and Borakhatariya et al. (2024) is primarily attributed to heat stress. During summer, elevated ambient temperatures can impair ovarian activity, reduce luteal function, and suppress progesterone synthesis. As a result, cows with abnormal luteal function exhibit more pronounced reductions in progesterone during heat stress, making the difference between pregnant and non-pregnant cows more evident in summer. In contrast, during cooler seasons, the absence of heat stress minimizes physiological variation, resulting in no significant difference between groups with normal luteal function. The study also showed that abnormalities in the luteal phase can affect pregnancy rates in dairy cows with reproductive disorders.

Suboptimal progesterone levels in the early luteal phase can interfere with embryo implantation and cause pregnancy failure. Previous reports have shown that low progesterone levels in the early luteal phase cause impaired embryo development and reduce pregnancy rates (Panjaitan et al. 2021). Low progesterone levels in the early luteal phase can interfere with endometrial development, inhibit embryo implantation, cause pregnancy failure, and cause cows to experience repeat breeding.

Progesterone plays an important role in preparing the uterus to receive embryos by stimulating the secretion of factors that are important essential for embryo growth and development. Suboptimal progesterone levels can create a uterine environment that does not support implantation. According to Parr et al. (2010), higher embryo survival rates correlate with higher progesterone levels. One of the causes of rebreeding in cows is low progesterone levels after ovulation, which causes embryo death. Low progesterone concentrations in the early luteal phase will repeat from one cycle to the next.

According to Asaduzzaman et al. (2016) and Nasution et al. (2021), reproductive disorders such as repeat breeding are mainly caused by various factors such as fertilization failure, embryo death (Nasution et al. 2021; Maulana et al. 2022), and other factors related to the cow, bull, and its management (Armelia et al. 2019). These problems are generally caused by factors such as uterine infection, hormonal imbalance, environmental conditions, nutrition, and management practices. Management errors, including the type of cage, flooring, and environmental cleanliness (Begum 2020; Sutriana et al. 2021; Wahyuni et al. 2021), lack of understanding of estrus, inaccurate estrus detection, mating time, low nutrition, and poor environmental conditions. Prihatno et al. (2013) can also contribute to these problems. Several studies have shown that endocrine disorders involving estradiol (E2), progesterone (P4), or Luteinizing Hormone (LH) can be potential causes of repeat breeding disorders (Sood et al. 2015; Dar et al. 2017; Gad et al. 2022).

The findings of this study underline the importance of progesterone, especially in the early luteal phase, in the reproduction of Aceh cattle. Low progesterone levels on the 3rd day post-after estrus in repeat breeder Aceh cows indicate potential luteal dysfunction. Luteal dysfunction can cause a sub less than optimal uterine environment for embryo development and implantation, thereby increasing the risk of pregnancy failure. In addition to the factors that have been mentioned that cause repeat breeding, nutritional factors are factors that need to be managed properly.

In addition to the factors previously mentioned that contribute to repeat breeding, nutritional factors also need to be properly managed (Villar et al. 2025).

Nutritional deficiencies, which can affect impact ovarian performance and cause hormonal disturbances (Regmi and Dhakal 2020; Thasmi et al. 2020), are factors that need to must be considered in the context when addressing of luteal dysfunction in repeat breeder Aceh cattle. Therefore, effective management strategies are essential for improving the reproductive performance of beef cattle (Ratnawati et al. 2018). The findings of this study contribute important insights toward identifying progesterone-based biomarkers that may help distinguish fertile cows from repeat breeders, thereby supporting more accurate selection of breeding females for sustainable herd development. However, repeat breeding is a multifactorial condition, and progesterone deficiency represents only one component of its physiological basis. Further research is urgently needed to investigate additional factors that influence progesterone dynamics in Aceh cattle, including

nutritional status, energy balance, metabolic stress, and environmental conditions. Moreover, targeted interventions such as nutritional optimization (e.g., lipid or precursor supplementation to support steroidogenesis), hormonal therapies to improve luteal function, and enhanced reproductive monitoring programs should be explored to reduce the prevalence of repeat breeding (Bishop et al. 2022; Zeng et al. 2023). Integrating these strategies may offer a more comprehensive and sustainable approach to improving reproductive efficiency in Aceh cattle under smallholder farming conditions. In conclusion, the results of this study reveal important hormonal differences between repeat breeder and fertile Aceh cattle, highlighting the critical role of progesterone dynamics during the early luteal phase in determining reproductive success. These findings support the potential development of targeted progesterone-based biomarkers for identifying fertile cows, thereby contributing to more sustainable and efficient livestock breeding practices. Further research is needed to explore the multifactorial factors that influence progesterone levels in repeat breeders of Aceh cattle, including nutritional status and management conditions. Such advancements will be essential for addressing repeat breeding problems and improving reproductive performance in this indigenous cattle breed.

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REFERENCES

- Acconcia F, Marino M. 2018. Steroid hormones: Synthesis, secretion, and transport. In: Belfiore A, LeRoith D (eds). *Principles of Endocrinology and Hormone Action*. Springer, Cham. DOI: 10.1007/978-3-319-27318-1_4-1.
- Adam M, Siregar TN, Wahyuni S, Gholib G, Ramadhana CE, Ananda R, Afifuddin A. 2017. Steroid level and pregnancy rate of Aceh cows in response to ovulation induction using presynchovsynch method. *J Kedokteran Hewan* 11 (4): 138-141. DOI: 10.21157/j.ked.hewan.v11i4.8994.

- Agung PP, Saputra F, Zein MSA, Wulandari AS, Putra WPB, Said S, Jakaria J. 2019. Genetic diversity of Indonesian cattle breeds based on microsatellite markers. *Asian-Australas J Anim Sci* 32 (4): 467-476. DOI: 10.5713/ajas.18.0283.
- Armansyah T, Siregar TN, Aliza D, Melia J, Meutia N, Panjaitan B, Hafizuddin H, Adam M, Abrar M. 2017. Aceh cattle follicle dynamic under environmental heat stress. *J Kedokteran Hewan* 11 (3): 86-90. DOI: 10.21157/j.ked.hewan.v11i3.6377.
- Armelia V, Saleh DM, Setianto NA. 2019. Identification of factors contributed to beef cattle reproductive disorders in Ogan Komering Ulu Timur Regency (OKU Timur) of South Sumatra Province in UPSUS SIWAB Program 2018. *Anim Prod* 20 (3): 199-209. DOI: 10.20884/1.jap.2018.20.3.743.
- Asaduzzaman K, Bhuiyan M, Rahman M, Bhattacharjee J. 2016. Prevalence of repeat breeding and its effective treatment in cows at selected areas of Bangladesh. *Bangladesh J Vet Med* 14 (2): 183-190. DOI: 10.3329/bjvm.v14i2.31391.
- Baliarti E, Wardani A, Prabowo B, Meutia N, Bintara S, Widi T, Ngadiyono N. 2021. Comparison of Aceh cow's performance on different mating systems at BPTU HPT Indrapuri, Aceh Province, Indonesia. *IOP Conf Ser Earth Environ Sci* 902 (1): 012040. DOI: 10.1088/1755-1315/902/1/012040.
- Barui A, Batabyal S, Ghosh S, Saha D, Chattopadhyay S. 2015. Plasma mineral profiles and hormonal activities of normal cycling and repeat breeding crossbred cows: A comparative study. *Vet World* 8 (1): 42-45. DOI: 10.14202/vetworld.2015.42-45.
- Begum K. 2020. Repeat Breeding Syndrome of Cows in Bangladesh-A review. *Chattogram Veterinary and Animal Sciences University, Chattogram*.
- Bishop CV, Selvaraj V, Townson DH, Pate JL, Wiltbank MC. 2022. History, insights, and future perspectives on studies into luteal function in cattle. *J Anim Sci* 100 (7): 1-15. DOI: 10.1093/jas/skac143.
- Borakhatariya DN, Singh VK, Karangiya VK, Vijyeta HP, Vala KB, Padodara RJ. 2024. Relationship of the pregnancy, stress biomarkers and embryonic losses in jaffrabadi buffaloes during summer season. *Indian J Vet Biotechnol* 20 (6): 84-88. DOI: 10.48165/ijvst.20.6.16.
- Budisatria IGS, Baliarti E, Widi TSM, Ibrahim A. 2019. Reproductive management and performances of Aceh cows, local Indonesian cattle kept by farmers in a traditional system. *Am-Eur J Sustain Agric* 13 (3): 21-31. DOI: 10.22587/aejsa.2019.13.3.3.
- Budisatria IGS, Baliarti E, Widi TSM, Vierman V, Ibrahim A, Pratama AR, Koesmara H, Atmoko BA. 2018. Body size of Aceh cattle in smallholder farm level and in Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Indrapuri Aceh. *Proceeding of the 1st Int Conf Food Agric*.
- Chatuphonprasert W, Jarukamjorn K, Ellinger I. 2018. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. *Front Pharmacol* 9: 1-29. DOI: 10.3389/fphar.2018.01027.
- Dar RR, Firdous S, Amin BY, Ali A, Narayanan K, Patel M. 2017. Luteal dysfunction: A potential cause of repeat breeding and the strategies to combat it. *Theriogenol Insight-An Intl J Reprod Anim* 7 (2): 105-112. DOI: 10.5958/2277-3371.2017.00026.2.
- Diskin M, Waters S, Parr M, Kenny D. 2016. Pregnancy losses in cattle: Potential for improvement. *Reprod Fertil Dev* 28 (2): 83-93. DOI: 10.1071/RD15366.
- Gad M, Elbaz H, Abdelrazek E, Zagloul A, Genedy T. 2022. Different therapeutic approaches for treatment of repeat breeder cows and its effect on maximization conception rate in holstein dairy cows. *J Curr Vet Res* 4: 168-174. DOI: 10.21608/jcvr.2022.240923.
- Ghanem M, Nishibori M. 2015. Effects of season on plasma progesterone profiles in repeat breeding cows. *Veterinárni Medicina* 60 (5): 227-234. DOI: 10.17221/8174-VETMED.
- Hafizuddin H, Husnurrizal H, Thasmi CN, Herrialifian H, Salsabil NL, Siregar TN, Sutriana A. 2024. Estrous characteristics and steroid concentrations in Aceh cows (*Bos indicus*) after estrus synchronization using prostaglandin F_{2α}. *Biodiversitas* 25 (1): 322-328. DOI: 10.13057/biodiv/d250137.
- Husnurrizal H, Siregar TN, Gholib G, Panjaitan B, Armansyah T, Wahyuni S. 2021. Profiles of progesterone and bovine interferon- τ in repeat breeding and non-repeat breeding Aceh cows. *Vet World* 14 (1): 230. DOI: 10.14202/vetworld.2021.230-236.
- Husnurrizal H, Thasmi CN, Herrialifian H, Ramadhani MR, Hafizuddin H, Sutriana A, Siregar TN. 2024. Accuration of estrous diagnosis based on Electrical Resistance of Vaginal Mucus (ERVM) in Aceh cattle. *AIP Conf Proc* 3082 (1): 040050. DOI: 10.1063/5.0203942.
- Islam MS, Deb G, Nurunnahar T, Ershaduzzaman M, Habib M, Ali MY, Kabir MH, Yousuf MA, Afroz MF, Yeasmin T. 2017. Identification of possible causes of repeat breeding in dairy cows at Baghabari milk shed areas, Sirajgonj, Bangladesh. *Asian J Med Biol Res* 3 (2): 186-190. DOI: 10.3329/ajmbr.v3i2.33567.
- Jaškowski BM, Herudzińska M, Gehrke M, Nizański W. 2022. The impact of the cavitary corpus luteum on the blood progesterone concentration and pregnancy rate of embryo recipient heifers. *Theriogenology* 178: 73-76. DOI: 10.1016/j.theriogenology.2021.11.003.
- Kim JJ, Park KB, Choi EJ, Hyun SH, Kim N-H, Jeong YW, Hwang WS. 2017. Relationship between time post-ovulation and progesterone on oocyte maturation and pregnancy in canine cloning. *Anim Reprod Sci* 185: 75-82. DOI: 10.1016/j.anireprosci.2017.08.004.
- Lonergan P, Sánchez J. 2020. Symposium review: Progesterone effects on early embryo development in cattle. *J Dairy Sci* 103 (9): 8698-8707. DOI: 10.3168/jds.2020-18583.
- Matubber B, Paul AK, Das S. 2018. Evaluation of different parameters in relation to repeat breeding of cows at the coastal areas of Bangladesh. *Res Agric Livest Fish* 5 (1): 49-55. DOI: 10.3329/ralf.v5i1.36552.
- Maulana R, Susetya H, Prihatno SA. 2022. Prevalence and risk factors associated with repeat breeding of beef cattle in Sleman Regency, Indonesia. *Vet World* 15 (4): 870-877. DOI: 10.14202/vetworld.2022.870-877.
- Michael J, Campanile G, Baruselli PS. 2020. Transforming growth factor- β superfamily and interferon- τ in ovarian function and embryo development in female cattle: review of biology and application. *Reprod Fertil Dev* 32 (6): 539-552. DOI: 10.1071/RD19123.
- Nasution M, Siregar TN, Sayuti A, Hafizuddin H, Rosmaidar R, Adam M. 2021. Identification of factors causing reproductive disorders of the cow found in North Labuhanbatu Regency, North Sumatra Province. *Livest Anim Res* 19 (1): 80-86. DOI: 10.20961/lar.v19i1.41766.
- Panjaitan B, Husnurrizal H, Edward V, Nishcaya D, Armansyah T, Siregar TN, Adam M, Sayuti A, Sutriana A, Hafizuddin H. 2024. The effect of time interval differences between the prostaglandin F_{2α} injection and semen collection on the improvement of spermatozoa and testosterone concentrations in Aceh bull. *Adv Anim Vet Sci* 12 (2): 337-341. DOI: 10.17582/journal.aavs/2024/12.2.337.341.
- Panjaitan B, Siregar TN, Hafizuddin H, Sayuti A, Adam M, Armansyah T, Syafruddin S. 2021. Comparison of the effectiveness of pregnancy diagnosis in Aceh cattle through measurement of interferon-tau and progesterone concentrations. *Biodiversitas* 22 (4): 1712-1716. DOI: 10.13057/biodiv/d220414.
- Parr M, Mullen M, Crowe M, Roche J, Lonergan P, Evans A, Diskin M. 2010. The relationship between plasma progesterone concentration during the early luteal phase and embryo survival in dairy heifers. *Adv Anim Biosci* 1 (1): 160-160. DOI: 10.1017/S2040470010003031.
- Prihatno SA, Kusumawati A, Karja NWK, Sumiarto B. 2013. Blood biochemical profile in repeat breeding dairy cows. *J Kedokteran Hewan* 7 (1): 29-31. DOI: 10.21157/j.ked.hewan.v7i1.561.
- Ratnawati D, Indrakusuma D, Affandhy L, Cowley F, Mayberry D, Poppi D. 2018. Management strategies to improve reproductive performance of Brahman cross cattle (*Bos indicus*) in East Java, Indonesia. *Jurnal Ilmu Ternak dan Veteriner* 21 (4): 231-237. DOI: 10.14334/jitv.v21i4.1512.
- Regmi G, Dhakal IP. 2020. Systemic levels of iron, phosphorus, and total protein in normocyclic versus repeat breeder Holstein Friesian crossbred cows of Kesharbag, Chitwan, Nepal. *Vet World* 13 (11): 2353-2357. DOI: 10.14202/vetworld.2020.2353-2357.
- Rosmaidar R, Handayani M, Fadillah F, Armansyah T, Siregar TN, Hafizuddin H, Husnurrizal H. 2021. The effect of red betel leaf (*Piper crocatum*) and moringa leaf extracts on endometritis levels in Aceh cows. *Trad Med J* 26 (3): 161-168. DOI: 10.22146/mot.64626.
- Salman A, Prihatno SA, Sumiarto B. 2021. Reproductive performance of beef cattle with ovarian hypofunction and repeat breeding in Jepara Regency, Central Java, Indonesia. *Vet World* 14 (3): 784-787. DOI: 10.14202/vetworld.2021.784-787.
- Siregar TN, Hafizuddin H, Akmal M, Arman Sayuti DA, Melia J, Armansyah T, Syafruddin S, Panjaitan B, Adani DL. 2016. Follicle dynamics of Aceh cattle during estrous cycle. *Global Vet* 17 (5): 424-429. DOI: 10.5829/idosi.gv.2016.424.429.
- Sofyan H, Sudarnika E, Satyaningtjas AS, Sumantri C, Agungpriyono S. 2020. The economic potential of aceh cattle based on its farmers,

- traders, and consumers perspective. *Front Sustain* 1: 546177. DOI: 10.3389/frsus.2020.546177.
- Sood P, Zachut M, Dube H, Moallem U. 2015. Behavioral and hormonal pattern of repeat breeder cows around estrus. *Reproduction* 149 (6): 545-554. DOI: 10.1530/REP-14-0598.
- Sponchiado M, Gomes NS, Fontes PK, Martins T, Del Collado M, Pastore AdA, Pugliesi G, Nogueira MFG, Binelli M. 2017. Pre-hatching embryo-dependent and-independent programming of endometrial function in cattle. *PLoS one* 12 (4): e0175954. DOI: 10.1371/journal.pone.0175954.
- Sutarno S, Zahrah S, Astirin OP, Herawati E, Setyawan AD. 2019. Genetic diversity of Ongole Grade, Aceh, and Sumbawa cattle based on polymorphism on ND-5 fragment mitochondrial DNA using PCR-RFLP technique. *Biodiversitas* 20 (3): 783-788. DOI: 10.13057/biodiv/d200324.
- Sutriana A, Sayuti A, Panjaitan B, TR TA, Tunnisa AF, Melia J, Siregar TN, Hafizuddin H, Aliza D. 2021. The effectiveness of Iugol on the increasing of pregnancy rate in Aceh cow with endometritis. *J Agripet* 21 (2): 187-191. DOI: 10.17969/agripet.v21i2.18513.
- Syafuruddin S, Adam M, Roslizawaty R, Siregar TN, Hafizuddin H, Panjaitan B, Armansyah T, Wahyuni S, Sayuti A. 2021. Utilization 2% Iugol's solution to overcome the repeat breeding case of cows in Cot Madhi Village, Aceh Besar. *Bull Community Serv* 1 (2): 59-62. DOI: 10.24815/bulpengmas.v1i1.20108.
- Thasmi CN, Hafizuddin H, Husnurrisal H, Dasrul D, Sutriana A, Gani BA, Nazar M. 2024. Moringa oleifera as a potential bioactive agent against Gram-positive and negative bacteria: In-silico analysis of 1YN5 and 3RG1 receptor binding. *Biodiversitas* 25 (8): 3411-3421. DOI: 10.13057/biodiv/d250812.
- Thasmi CN, Husnurrisal H, Akmal M, Wahyuni S, Siregar TN. 2020. Detection of serum macro minerals concentration in repeat breeding Aceh cattle. *J Kedokteran Hewan* 14 (2): 25-28. DOI: 10.21157/j.ked.hewan.v14i2.16543.
- Thasmi CN, Husnurrisal H, Wahyuni S, Hafizuddin H, Siregar TN. 2021. Identification of bacteria in Aceh cattle with repeat breeding. *Veterinaria ir Zootechnika* 79 (2): 32-36.
- Thasmi CN, Siregar TN, Wahyuni S, Aliza D, Hamdan H, Panjaitan B, Asmiliana N, Husnurrisal H. 2017. Estrus performance and steroid level of repeat breeding Aceh cattle synchronized with Pgf2a. *Veterinaria* 66 (1): 36-41.
- Villar SL, Pérez-Marín CC, Álvarez J, Yáñez U, Becerra JJ, Peña AL, Herradón PG, Quintela LA. 2025. Evaluation of prevalence, risk factors, and therapeutic approach for subclinical endometritis and oviductal occlusion in repeat breeder Holstein cattle. *Res Vet Sci* 183: 105511. DOI: 10.1016/j.rvsc.2024.105511.
- Wahyuni S, Siregar T, Mazaya A, Thasmi C, Hafizuddin H, Adam M, Sayuti A. 2021. Morphology and morphometry of reproductive organs in the repeat breeder Aceh cows. *Vet Pract* 22 (2): 99-103.
- Widayati D, Darmawan M, da C Freitas J. 2019. Progesterone level of normal cycling and repeat breeding Ongole grade cows. *IOP Conf Ser Earth Environ Sci* 387 (1): 012008. DOI: 10.1088/1755-1315/387/1/012008.
- Wiltbank MC, Mezera MA, Toledo MZ, Drum JN, Baez GM, García-Guerra A, Sartori R. 2018. Physiological mechanisms involved in maintaining the corpus luteum during the first two months of pregnancy. *Anim Reprod* 15 (1): 805-821. DOI: 10.21451/1984-3143-AR2018-0045.
- Wolfenson D, Roth Z. 2019. Impact of heat stress on cow reproduction and fertility. *Anim Front* 9 (1): 32-38. DOI: 10.1093/af/vfy027.
- Yusuf M, Toleng AL, Rahardja DP, Long ST. 2019. The incidence of reproductive disorders in dairy cows under smallholder farms. *Hasanuddin J Anim Sci (HAJAS)* 1 (2): 22-27. DOI: 10.20956/hajas.v1i2.9428.
- Zeng X, Li S, Liu L, Cai S, Ye Q, Xue B, Wang X, Zhang S, Chen F, Cai C. 2023. Role of functional fatty acids in modulation of reproductive potential in livestock. *J Anim Sci Biotechnol* 14 (1): 1-19. DOI: 10.1186/s40104-022-00818-9.