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Research Article

Serum Progesterone Levels Post-Cosynch Treatment in Pesisir Cattle as a Genetic Resource in West Sumatra

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Abstract | This study assessed the correlation between blood progesterone levels and age in Pesisir cattle post-Cosynch therapy. Eighteen Pesisir cows were classified into three age categories: under 3 years (A1), 3 to 5 years (A2), and above 5 years (A3). Blood samples were obtained at various intervals throughout three estrous cycles following treatment and assessed by Enzyme-Linked Immunosorbent Assay to quantify blood progesterone concentrations. A chi-square test was conducted to evaluate differences among age groups. The findings indicated marked variations in progesterone levels throughout the estrous cycles, with the minimum concentrations noted on day 11 (A1: 3.08±1.37 ng/ml, A2: 5.63±2.30 ng/ml, A3: 5.39±0.59 ng/ml) and maximum levels recorded during the luteal phase (A1: 14.39±2.25 ng/ml, A2: 16.40±6.56 ng/ml, A3: 23.59±16.38 ng/ml). Older cows (A3) demonstrated elevated progesterone levels relative to younger cows, indicating improved luteal function. Nonetheless, despite synchronization, no pregnancies were observed in either group following artificial insemination. This may be ascribed to heightened progesterone levels during insemination, signifying an insufficient estrous synchronization response. The data indicate that progesterone levels are affected by age, with older cows exhibiting a more significant hormonal profile. The unsuccessful development of pregnancy underscores the necessity for enhanced optimization of estrous synchronization techniques to augment reproductive success in Pesisir cattle.

Keywords | Pesisir cattle, Progesterone levels, Cosynch procedure, Estrous synchronization

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INTRODUCTION

On a rural farm in West Sumatra, local Pesisir cattle serve as genetic resources for beef production. The population tends to decrease over ten years due to high demand for beef, negative selection, and crossing caused by poor management, reproduction, and breeding systems. Characterization of Pesisir cattle as native cattle in Indonesia was a high adaptation to the environment and resistance to parasite diseases (Wahyuni and Dewi, 2018). Reproductive disruption is induced by insufficient progesterone levels during gestation, resulting in abortion. The reproductive capacity of cows is contingent upon the hormonal regulation during the estrous cycle, particularly the progesterone levels. Multiple factors influence progesterone levels, including age, season, and the physiological condition of the cow. Furthermore, the primary role of progesterone is to regulate the fertility and reproductive cycle of the cow. Fertility enhancement can be achieved by evaluating the progesterone levels of cows at various ages. Udin

et al. (2017a) assert that fertility is comparatively favorable, characterized by modest follicle size. Progesterone is essential in the reproductive cycle associated with the maintenance of pregnancy. Assessment of progesterone concentration is a management tool to characterize and detect estrus, ovarian cyclicity, and pregnancy (Banu *et al.*, 2012).

Progesterone is a steroid released by the corpus luteum and placenta to sustain animal fertility (Hafez *et al.*, 2013). The progesterone levels are contingent upon the estrous cycle status, peaking during the luteal phase and reaching their nadir in the follicular phase (Hafez *et al.*, 2013). Besides estrus and pregnancy, the multifunctional steroid hormone played several functions in addressing reproductive issues, such as persistent corpus luteum and cysts (Kim *et al.*, 2012). The measurement of plasma progesterone levels is more accurate than the other traditional methods of estrus detection and can be determined using Enzyme Immuno Assay Kits. Furthermore, the progesterone hormone is used to monitor ovarium activities postpartum, gestation diagnosis, and reproduction problems. Mann and Lamming (2001) indicated this is associated with low luteal phase progesterone concentration. Typically, GnRH and PGF2alpha hormone administration is employed to assay the progesterone concentration, facilitate timed artificial insemination (TAI), and enhance reproductive efficiency (Pang *et al.*, 2009). Multiple estrus detections have been established through the Ovsynch and Cosynch protocols to synchronize ovulation and TAI in dairy and beef cows in previous studies1-3 (Bisinotto *et al.*, 2010). Recently, postpartum dairy and beef cows were utilized to evaluate various iterations of the Ovsynch protocol, including double Ovsynch, Cosynch, and presynchronization-Ovsynch4 (Ayres *et al.*, 2013).

The assessment of progesterone levels is frequently employed on farms to ascertain cattle pregnancy and assess their reproductive health (Sangsritavong *et al.*, 2002). Progesterone levels are typically interpreted to evaluate reproductive cycles and assess standard estrous cycles. This method can also assess estrus quality by analyzing the progesterone profile and determining if the cow is in estrus (Lucy, 2001). Multiple factors influence cow fertility, including breed, age, and reproductive hormone levels. Consequently, it is essential to undertake this research to evaluate the progesterone profile at different ages and the impact of the Cosynch regimen in Pesisir cattle. To facilitate the optimization of enhanced estrus synchronization procedures for the improvement of reproductive success in Pesisir cattle.

MATERIALS AND METHODS

All procedures utilized in this investigation have been reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Indonesia, under approval letter Number B/120/UN14.2.9/PT.01.04/2024. The research subjects comprised native Pesisir cattle at the Faculty of Animal Husbandry, Andalas University, Indonesia, categorized into three groups: less than 3 years (A1), 3-5 years (A2), and over 5 years (A3), with six cattle in each group. The criteria for the cows stipulated that they were non-pregnant and healthy, designated for blood samples in the medical laboratory of the Faculty of Medicine, Andalas University. The co-synch procedure, utilizing GnRH and PGF-2-alpha hormones, was used to induce an estrus cycle and insemination.

Co-synch Protocol

The co-synch protocol was aimed at synchronizing ovulation and assessing the 6estrus cycle of cows and AI without estrus detection. This protocol has two kinds of hormones, with three types of injections. The cows received co-synch protocol at random stage of the estrus cycle (day 0) with GnRH (Fertagyl, MSD Animal Health, Merck and Co., Inc., USA) 2 ml/ cow. On day 7, the cows were treated with PGF2alpha (Lutalyze, MSD Animal Health, Merck and Co., Inc., USA) 5 ml/cow, and on day nine, the cows were treated with GnRH 2 and inseminated at the time (Figure 1).

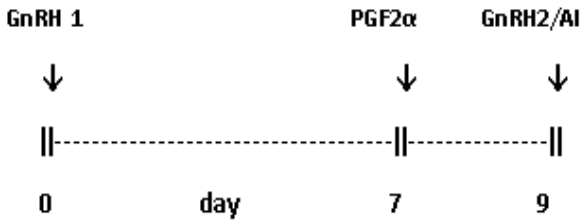

Figure 1: Co- Synch protocol.

Blood Collection

The cows are placed in individual households in 3 groups of age A1(<3 (2 to 2.5 years old), A2 (3 and 4 years old), and A3 group (7 and 8 years old). The blood sample is at two days during the three estrus cycles or 60 days. Blood is collected in the jugular vein by a 10 ml syringe and put in for 2-4 hours to get the serum. Blood samples were collected 14 days before treatment co-synch protocol and 60 days or three estrus cycle lengths to determine the progesterone role.

Measurement of Progesterone Levels

The serum was put in a test tube of 10 ml and stored in the freezer (-20º) before evaluating the progesterone hormone by Enzyme-Linked Immunosorbent Assay (ELISA) in the laboratory. The ELISA test procedure is carried out according to laboratory standards. Absorbance is measured using a spectrophotometer at a wavelength of 450 nm. The ELISA Kit used is the Bovine Progesterone ELISA Kit

(Cusabio, Wuhan, Hubei, China), and the results were read using the Mindray MR-96A Microplate Reader (Mindray, Shenzen, China).

Data Analysis

The obtained data were analyzed using descriptions and graphics based on the age of the cows and chi-squared to determine the difference among the groups of cows.

RESULTS AND DISCUSSIONS

The assessment of serum progesterone concentrations across three age categories of Pesisir cattle following Co-synch therapy demonstrated unique hormonal profiles during the estrous cycles. The youngest group (A1) exhibited the lowest progesterone concentration at day 11 (3.08±1.37 ng/ml), whereas the highest levels were recorded at the luteal phase (14.39±2.25 ng/ml) (Figure 2). The progesterone levels of cows in A2 and A3 followed similar fluctuation patterns but at higher concentrations; in A2, the peak reached 16.40±6.56 ng/ml (Figure 3), and particularly in A3, where the peak reached 23.59±16.38 ng/ml (Figure 4).

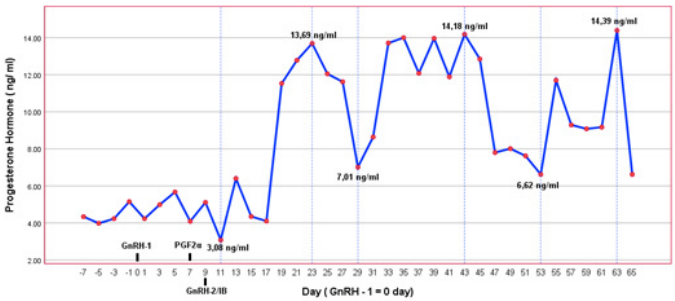

Figure 2: Progesterone concentration in age < 3 years old.

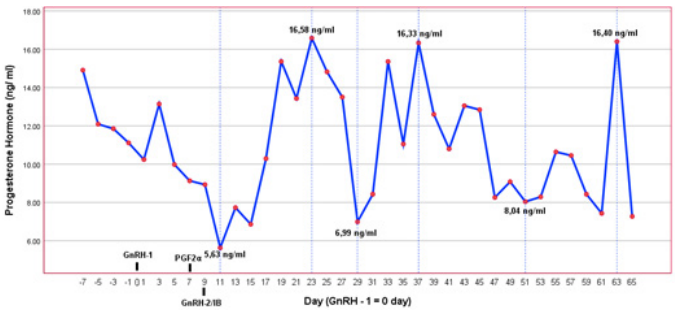

Figure 3: Progesterone concentration in age 3-5 years old.

Progesterone concentrations fluctuate in accordance with the estrous cycle, exhibiting an increase following GnRH-2/IB injection and a decrease in non-pregnant cows (Canadas *et al.*, 2021). This result was supported by Walker *et al.* (2008), who found that low progesterone exposure before estrus was associated with low intensity of sexual behavior during estrus in chronically stressed lame cows. A lower progesterone concentration during diestrus was associated with lower fertility (Herlihy *et al.*, 2012). The size of the corpus luteum is directly proportional to progesterone production, especially in older cows (Moore *et al.*, 2014). Furthermore, the progesterone concentration in blood serum in nonpregnant cows was low on day 21 after AI (Vukovic *et al.*, 2016; Crist *et al.*, 2021).

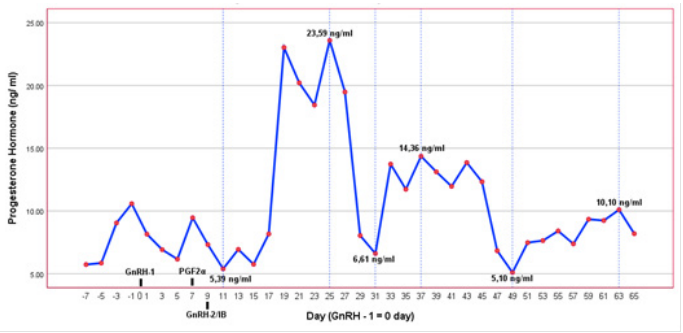

Figure 4: Progesterone concentration in age 6-8 years old.

The progesterone profile at A1 fluctuated more than group A2 and A3 in the three estrus cycles, and the higher progesterone concentration ranged from 14 to 15 days of the estrus cycle. According to Alvares *et al.* (2000), the progesterone concentration reached the highest levels during days 11 to 17 after estrus. The fluctuation of progesterone depends on reproductive status, and the sample is used for assessment (Padilha *et al.*, 2024). The progesterone concentration in the group is lower than that of group A2, and group A3 is caused at puberty, and the reproductive process is intense. According to Hafez *et al.* 2013, the secretion of progesterone hormone depends on estrus cycle status; the highest level is in the luteal phase, which causes the function of the corpus as a source of progesterone hormone. Besides that, it is also essential to manage the reproduction cycle of cows to optimize fertility. Estrous behavior positively correlates with age and parity (Atkins *et al.*, 2013). A previous study by Udin *et al.* (2017b) showed that at parity 3, a low progesterone concentration at day 11 after estrus is 5.63±2.3 ng/ml under pasture grazing. Older cows exhibit elevated progesterone levels compared to younger cows, as evidenced by larger corpus luteum size and improved nutritional status (Hayati *et al.*, 2021; Moore *et al.*, 2014; Valchuck *et al.*, 2023). This result showed that the highest progesterone concentration ranged from day 6 to day 7 of the estrus cycle. This finding that older cows produce a higher concentration of progesterone was supported by Hayati *et al.* (2021). The progesterone concentration in cows at A3 (7 and 8 years old) was higher than that in cows more senior than three years, although it was lower than in cows aged 3-5 years. This showed that younger cows had strong fertility and older cows had decreased levels of progesterone due to the reproduction physiology of cattle related to the development of the reproductive organs, nutrient status, age, and disease (Dipastina *et al.*, 2024). Alvarez *et al.* (2000) state that the progesterone hormone may result from variations in the sampling frequency and the animal's age. This result is supported by Liu and Shi (2015), who state that

progesterone concentration may cause up- or down-regulation in the hypothalamus of the number of genes involved in estrus behavior through the E2 receptor.

The mean progesterone level in 3 increased on day 13 and decreased up to day 27 to 29 of the estrus cycle, indicating the typical estrus cycle of Pesisir cows. However, this result is higher than Anto *et al.* (2019) reported in Bali cows, which is 0.35 to 0.43 during estrus time. This is supported by Naik *et al.* (2013) that the mean progesterone level in Punganur cows during the estrus cycle increased significantly up to the 15th day of the estrus cycle and gradually decreased after that, with the values ranging from 0.43 on 0 days and 10.66 on day 15th. According to AL-Jabri *et al.* (2019), progesterone levels change continuously during the estrus cycle, and these changes bring about changes in the hypothalamus-pituitary-ovarian relationship which is the basis for endocrine regulation of the reproductive cycle in mammalian females.

Progesterone levels in Pesisir cattle exceeded those in Bali cattle. The decline in progesterone levels following insemination suggests a correlation between lower progesterone and improved estrus expression, as well as increased conception rates (Naik *et al.*, 2013). The variability in progesterone profiles and their morphological distinctions are influenced by the breeding system and nutritional status, as indicated by research examining the correlation between progesterone dynamics and estrous cycles (Blavy *et al.*, 2016; Madoz *et al.*, 2020). The elevated progesterone levels observed during estrus or at the time of artificial insemination in the cosynch protocol may be attributed to variations in sampling frequency, age, and physiological condition (lactating versus non-lactating) among the animals, as noted in studies on dairy cow reproductive performance (Omontese *et al.*, 2020). The Progesterone concentration increased significantly from day 0 to day 15; after that, it decreased. The decrease was significant up to day 19 and insignificant from day 20 to subsequent estrus day 0 in Punganur cow (Naik *et al.*, 2013). This study revealed that the progesterone profile in three groups of cows varied with the estrus cycle, with peak progesterone levels observed at six and seven days, post-estrus in group A3, approximately 23 ng/ml and corroborating findings that post-AI progesterone levels are indicative of pregnancy success (Marques *et al.*, 2023).

The Cosynch protocol treatment in three age groups of Pesisir cows revealed no pregnancies following TAI, and after nine days, elevated progesterone concentration persisted, indicating a delay. This suggests that the cows experienced cycle disorders due to elevated progesterone concentrations at the time of artificial insemination across all three groups. This finding aligns with research suggesting that elevated progesterone levels after artificial insemination can adversely impact fertility by inhibiting ovulation and modifying endometrial receptivity (Omontese *et al.*, 2020). This result was similar to those reported by Dadarwal *et al.* (2013), indicating that diminished progesterone during preovulatory follicle development resulted in augmented corpus luteum size, thereby offsetting the impact of abbreviated proestrus on pregnancy rates. This phenomenon was similarly noted in synchronization protocols where progesterone affects follicular dynamics (Bilbao *et al.*, 2019). The CO-72 without improving fertility as measured by P/AI and pregnancy losses is also in line with research suggesting that variations in synchronization protocol efficiency depend on the hormonal status of the cows (Alnimer *et al.*, 2011).

The Cosynch treatment has emerged as the primary management strategy for artificial insemination and frequently serves as an alternative approach to enhance reproductive success. Research indicates that the Cosynch program improves pregnancy rates when effectively synced with hormone therapies. Additionally, it serves as a therapeutic method for several ovarian illnesses (Nowicki *et al.*, 2017), especially in the treatment of anovulatory cows and enhancement of corpus luteum activity (Kim *et al.*, 2020). Vlčková *et al.* (2022) indicated that the combination of melatonin, progestogen, and eCG greatly enhances ovulation rates, underscoring the significance of hormonal manipulation in synchronization procedures (Bilbao *et al.*, 2019). This innovative approach stands as a potential enhancement within reproductive management. Various methods, like the Cosynch treatment, have been pivotal in advancing AI and refining reproductive outcomes, particularly in cyclic cows. Wolfenson *et al.* (2000) state that heat stress may have altered ovarian follicle development and steroidogenic capacity, with a decrease in the length and intensity of estrus. Carraviello *et al.* (2006) found that cows with an ovular >12 at GnRH-induced ovulation had higher pregnancy rates. This result was supported by Giordano *et al.* (2013) that the first GnRH, administered at the random stage of the estrus cycle, would induce ovulation in cows with the functional dominant follicle. A new follicular wave would be initiated with the dominant follicle selected during the next seven days. PGF2α would induce luteolysis in a high percentage of cows treated seven days earlier with GNRH. A dominant follicle would continue to grow, increasing circulating E2. Cows would begin to show estrus at 48 hours after PGF2α treatment, and a final GNRH treatment at 48 hours after PGF2α treatment would induce a surge of LH and synchronized ovulation, allowing for proper timing of AI before ovulation. Furthermore, primiparous cows with higher BCS were more likely to express high-intensity estrus, and these cows were also more likely to have a greater concentration of circulating P4 and improve fertility (Alves *et al.*, 2021).

Pregnancy failure in Pesisir cows undergoing the cosynch protocol is likely attributed to elevated progesterone levels during artificial insemination, which impede follicle maturation and diminish conception rates (Pereira *et al*., 2017; Omontese *et al*., 2020). Variations in the protocol that included timed insemination at the same time as the third injection (Co-synch) resulted in lower conception rates compared with insemination 24 hours later, as demonstrated in studies evaluating different AI synchronization protocols. A previous study by Udin *et al*. (2017b) found that co-synch protocol is caused by varies in ovulatory follicle size, clinical signs of estrus, and conception rate of postpartum Simmental cows, factors that are critical determinants of pregnancy outcomes. The application of the synch and co-synch procedure can stimulate ovarian activity, ovulation, and conception rates in postpartum Simmental cows; however, efficacy is contingent upon the reproductive status of each cow (Bilbao *et al*., 2019). There were three significant fertility predictors: the concentration of P4 at PGF2α, the concentration of E2, and follicle size at the final GNRH, with larger preovulatory follicles associated with higher fertility. Fertility correlated with the dimensions and functionality of the pre-ovulatory follicle during the final GnRH administration of the cosynch regimen, as demonstrated in prior research on follicular dynamics (Pereira *et al*., 2017). Greater progesterone concentration at AI was associated with lower intensity and duration of estrus and lower fertility (Bilbao *et al*., 2019). Besides that, none of the groups of Pesisir cows were pregnant after AI, which may have caused the condition to be abnormal or stressful.

CONCLUSIONS AND RECOMMENDATIONS

The primary finding of this study is that progesterone concentration was elevated on days 5 and 7 of the cycle among the three groups of Pesisir cows. Group A3 exhibited the highest levels of progesterone, with profiles demonstrating a characteristic fluctuation during the cycle. All age categories of Pesisir cows experience pregnancy loss following therapy. The Ovsynch procedure resulted in elevated progesterone levels at the time of artificial insemination.

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NOVELITY STATEMENT

This study demonstrates notable differences in progesterone levels correlated with the age of Pesisir calves following Cosynch treatment, indicating that older cattle have elevated progesterone levels, signifying superior luteal function relative to their younger counterparts. Despite the absence of documented pregnancies following artificial insemination, our data illustrate the need to refine estrus synchronization techniques to improve reproductive success in local cattle.

AUTHOR'S CONTRIBUTIONS

All authors contributed to the conception of the experiments.
Tinda Afriani and Zaituni Udin: Planned the study design.
James Hellyward and Endang Purwati: Sample collection and laboratory work.
Jaswandi Jaswandi and I. Made Merdana: Conducted the data analysis.
Tinda Afriani and Zaituni Udin: Wrote the manuscript.
All authors corrected, read, and approved the final manuscript.

Conflict of Interest

The authors state that there is no conflict of interest.

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