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The Relationship Between Plasma Progesterone Concentration on Day 6 After Artificial Insemination and Pregnancy Rate of Dairy Cows in Vietnam

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ABSTRACT

The study was conducted to determine the relationship between plasma progesterone (P4) concentration on day 6 following artificial insemination (AI) and the pregnancy rate to provide recommendations for measuring P4 concentration before embryo transfer in cows. A total of 85 cows were double AI (day 0) after being detected in natural heat by visual observation. On day 6 after AI, the blood sample was collected from the coccygeal vein to measure plasma P4 concentration by ELISA method. Pregnancy was diagnosed on the 60th day after AI by rectal palpation. The results showed that the plasma P4 concentration at day 6 after AI of the pregnant cow group (4.262±2.195 ng/mL) was significantly higher than that of the non-pregnant cow group (3.146±2.377 ng/mL) (p<0.05). The pregnancy rate was lowest in the group of cows with plasma P4 concentration <2ng/mL, then gradually increased with P4 concentration. The pregnancy rate of cows tended to be stable when P4 concentration ≥4.0 ng/mL. The pregnancy rate of cows was highest when the P4 concentration was from 3-4 ng/mL and 5-6 ng/mL. Additionally, the pregnancy rate of cows is positively correlated with the plasma P4 concentration according to the formula P= 26.356 + 6.612* P4. In conclusion, the plasma concentration of P4 at day 6 after AI has a positive relationship with the pregnancy rate of cows.

Keywords: AI; dairy cows; embryo transfer; pregnancy rate; progesterone

INTRODUCTION

Progesterone is a hormone that plays a vital role in the events involved in the formation and maintenance of pregnancy in cattle. Circulating progesterone levels following fertilization are related to embryonic growth rate, embryo survival, pregnancy prolongation, uterine environment regulation, and pregnancy rates in animals (Lonergan *et al.*, 2013; Lonergan, 2015). In addition, circulating progesterone levels also showed a relationship between embryo viability and early embryo loss (Morris & Diskin, 2008; Lonergan & Sánchez, 2020). The effects of plasma P4 progesterone concentration at the time of AI and after AI on the fertility of cows were reported (Wiltbank *et al.*, 2014; Bisinotto *et al.*, 2015; Pereira *et al.*, 2014; Yan *et al.*, 2016). The relationship between plasma progesterone levels during embryo transfer and pregnancy rates in recipient cows has been investigated (Rao & Yelisetti, 2013; Lestari *et al.*, 2019). Besides, progesterone level is also considered to increase embryo transfer success (Budiyanto *et al.*, 2022).

The embryo transfer technology has recently been applied in dairy farming in Vietnam. The synchronization of estrus in recipient cows utilizing the hormones gonadotropin-releasing hormone (GnRH), controlled intravaginal drug release (CIDR), and prostaglandin F2alpha (PGF2) was initially investigated before the transfer of Blanc-Blue- Belge (BBB) cow embryos to Vietnam (Long *et al.*, 2016). However, research to optimize cow pregnancy rates after embryo transfer has been limited to a few in Vietnam, while importing embryos is costly. The objective of this study was to evaluate the relationship between plasma progesterone levels at day 6 after AI and pregnancy rate, thereby determining the level of progesterone with a high pregnancy rate and applying it in the quantification of plasma P4 concentration before embryo transfer to ensure high pregnancy rate in recipient cows.

MATERIALS AND METHODS

Animals

The experiment was carried out using 85 Holstein Friesian (HF) crossbred dairy cows (over 87.5% of pure HF) raised in small-scale farms with a size of 10 to 30 cows in Vinh Phuc province, Vietnam, from January 2019 to May 2020. The age of cows ranged from 12 to 96 months old, including heifers and calving with parity from 1 to 5. Cows had BCS ranging from 2.5 to 3.75 and no reproductive problems. Cows were vaccinated with Foot-and-mouth disease (FMD) and Pasteurellosis periodically. The feed for cows was grass, beer residue, molasses, cornmeal, and concentrated pellets from CJ company, Korea. Cows were kept in barns with solid corrugated iron roofs made of brick, sprinklers for summer cooling, and frequent cleanings averaging 4-6 times daily. The research process complied with the Animal Welfare agreement in Vietnam (No. 2019-02/QĐ-VAWA).

Methodology

After natural estrus, dairy cows were given a double AI with frozen semen 12 hours apart (day 0). Cows were detected in estrus by conventional observation based on signs of estrus, such as mounting or attempting to mount other cattle, standing to be mounted by other cattle, smelling other females, trailing other females, bellowing, depressed appetite, nervous and excitable behavior, mud on hindquarters and sides of cattle, roughed up tail hair, vulva swelling, and reddening, clear vaginal mucous discharge, and mucous smeared on the rump. Then, the reproductive tract was examined to check uterine muscle tone (strong, weak) by rectal palpation. Artificial insemination was performed by a highly skilled veterinarian with over 10 years of experience and regularly attended advanced training courses on artificial insemination at the Vietnam National University of Agriculture. On day 6, a blood sample was taken to measure plasma progesterone levels. The pregnancy was diagnosed on day 60 after AI by rectal palpation by the veterinarian performing AI (Figure 1).

Blood samples were collected from the coccygeal veins of cows using a 5 mL syringe and an 18-gauge needle and immediately transferred to a plasma blood collection tube containing heparin. Blood samples were stored in a cool box containing ice packs and brought to the laboratory immediately, centrifuged at 3000 RPM for 10 minutes at room temperature (Hermle Z216M, Germany). The collected plasma was transferred into a 1.5 mL Eppendorf tube and sent to Medlatec Hospital, No. 42 Nghia Dung street, Phuc Xa ward, Ba Dinh District, Hanoi to measure P4 concentrations. Plasma progesterone concentration (ng/mL) was quantified using a competitive immunoassay with electroluminescence methodology in the Roche Cobas E602 Immunology Analyzer provided by Roche Diagnostics (Germany). The procedure was carried out according to the guidelines of the biochemically technical process specialized in the Ministry of Health (Vietnam).

Serum progesterone quantification results were divided into the following groups: N02 was Progesterone ≤ 2 ng/mL; N23 was 2 ng/mL < Progesterone ≤ 3 ng/mL; N34 was 3 ng/mL < Progesterone ≤ 4 ng/mL; N45 was 4 ng/mL < Progesterone ≤ 5 ng/mL; and N56 was 5 ng/mL < Progesterone ≤ 6 ng/mL.

Data Analysis

Data were analyzed using Minitab version 16 software using the Two Proportions Test. The probability level of less than 0.05 was treated as a statistically significant difference.

RESULTS

A total of 85 experimental cows, including 44, were pregnant (51.8%), and 41 were non-pregnant (48.2%). The progesterone concentration of the pregnant cow group (4.262 ±2.195 ng/mL) was higher than that of the non-pregnant group (3.146±2.377 ng/mL) ($p<0.05$) (Table 1).

When the progesterone concentration changed from 2.0-3.5 ng/mL at specific levels of progesterone concentration, the percentage of pregnant cows below the level was lower than that of above the level ($p<0.05$). However, when the progesterone level reached 4.0 ng/mL, there was no difference in the pregnancy rate above and below the level ($p>0.05$) (Table 2).

Table 3 showed more clearly the relationship between plasma concentration of progesterone at day 6 after AI and pregnancy rate. The pregnancy rate of cows after AI tended to increase with the increased plasma concentration of progesterone. The pregnancy rates were lowest in the group of cows with progesterone concentration ≤ 2 ng/mL and the highest in the group of cows with plasma concentrations of progesterone ranging from 3-4 ng/mL to 5-6 ng/mL ($p<0.05$).

When evaluating the correlation by statistical analysis of one-variable linear regression, it showed that the pregnancy rate of cows tended to be proportional to the plasma concentration of progesterone at day 6 after

Table 1. The plasma progesterone concentrations (ng/mL) in two groups of pregnant and non-pregnant cows

Results	No. of cows	Mean ± SD (ng/mL)
Pregnant	44	4.262 ± 2.195^{a}
Non-pregnant	41	3.146 ± 2.377^{b}

Note: Means in the same row with different superscripts differ significantly ($p<0.05$); SD = standard deviation.

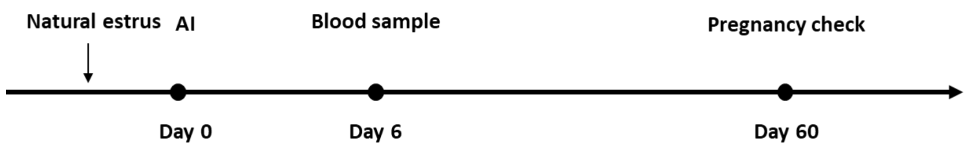

Figure 1. Experiment design. AI= Artificial insemination.

Table 2. Pregnancy rates of cows at different levels of plasma concentrations of progesterone

Level of P4 (ng/mL)	Variables	Below level (ng/mL)	Above level (ng/mL)
2.0		C <2.0	C ≥2.0
	No. of pregnant cows	5	39
	Total number of AI cows	19	66
	Pregnancy rate (%)	26.32[a]	59.09[b]
2.5		C <2.5	C ≥2.5
	No. of pregnant cows	7	37
	Total number of AI cows	24	61
	Pregnancy rate (%)	29.17[a]	60.66[b]
3.0		C <3.0	C ≥3.0
	No. of pregnant cows	12	32
	Total number of AI cows	34	51
	Pregnancy rate (%)	35.29[a]	62.75[b]
3.5		C <3.5	C ≥3.5
	No. of pregnant cows	17	27
	Total number of AI cows	43	42
	Pregnancy rate (%)	39.53[a]	64.29[b]
4.0		C <4.0	C ≥4.0
	No. of pregnant cows	25	19
	Total number of AI cows	55	30
	Pregnancy rate (%)	45.45[a]	63.33[b]

Note: AI= Artificial Insemination; P4= Progesterone; C= Concentrations of progesterone. Means in the same row with different superscripts differ significantly (p<0.05).

Table 3. Pregnancy rates of cows at different P4 concentrations

P4 Levels	P4 concentration (Mean, ng/mL)	No of pregnant cows	Total number of AI cows	Pregnancy rate (%)
N02	1.00	5	19	26.32[a]
N23	2.61	7	15	46.67[ab]
N34	3.53	13	21	61.90[b]
N45	4.53	5	10	50.00[ab]
N56	6.94	14	20	70.00[b]

Note: N02= Progesterone ≤ 2 ng/mL; N23= 2 ng/mL < Progesterone ≤ 3 ng/mL; N34= 3 ng/mL < Progesterone ≤ 4 ng/mL; N45= 4 ng/mL < Progesterone ≤ 5 ng/mL; N56= 5 ng/mL < Progesterone ≤ 6 ng/mL; AI= Artificial Insemination; P4= Progesterone; C= Concentrations of progesterone. Means in the same column with different superscripts differ significantly (p<0.05).

Table 4. Regression analysis of pregnancy rates by progesterone levels

Predictor	Coef	SE Coef	T	p-value
Constant	26.356	8.627	3.06	0.055
P4 concentration (ng/mL)	6.612	2.044	3.23	0.048

Note: Coef= Coefficient; SE Coef= Standard Error of Coefficient; T= T values.

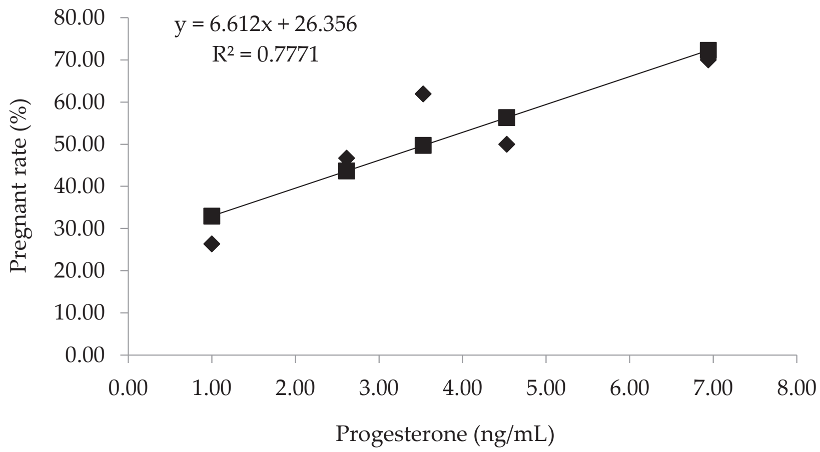

Figure 2. The relationship between progesterone concentration in serum of sixth day and pregnancy rate (PR); PR= ♦; Predicted PR= ■; Linear (PR)= ——

AI (R^2=0.777) (Figure 2). This means that for every 1 ng/mL of plasma concentration of progesterone increase, the pregnancy rate tended to increase by 6.61%.

DISCUSSION

This study found that plasma progesterone levels were significantly greater in the pregnant and non-pregnant groups on day 6 following AI. The difference in P4 levels between the pregnant and non-pregnant groups following artificial insemination or at the time of embryo transfer has been reported in previous studies (Wiltbank *et al.*, 2014; Colazo *et al.*, 2017; Lestari *et al.*, 2019). The results from this research resembled the findings from Madureira *et al.* (2021) that cows with greater concentrations of P4 at day 7 post-timed AI had greater P/AI compared with cows with a lower concentration of P4. According to Rao & Elisetti (2013), the cow with higher progesterone levels in the serum on days 7, 14, and 21 following embryo transfer (ET) have a greater possibility of becoming pregnant. The serum progesterone concentration of pregnant cows was significantly higher than that of non-pregnant cows on day 7 after the estrus at the time of the embryo transfer (Lestari *et al.*, 2019).

Our study also revealed a positive relationship between progesterone levels on day 6 following AI and the pregnancy rate in dairy cows. When P4 concentration was ≥2 ng/mL, the pregnancy rate increased with the increased P4 levels, and the rates tended to be stable when P4 concentration was ≥4 ng/mL ($p<0.05$). According to Niemann *et al.* (1985), the pregnancy rate of cows after embryo transfer by non-surgical methods was the lowest when progesterone levels on day 7 were <2.0 ng/mL and the highest when progesterone levels were between 2-5 ng/mL and decreased when progesterone levels were >5 ng/mL. It is possible that the high levels of progesterone >5 ng/mL in this phase upset the delicate hormonal balance of estrogen and progesterone. The balance between estrogen and progesterone during this phase of reproduction is necessary for adequate nutritional requirements and uterine environment during the free-living state of the embryo in the uterus, explaining why the pregnancy rate decreased when P4 concentration was >5 ng/mL (Remsen *et al.*, 1982). When the progesterone concentration in milk was <8 ng/mL and in serum <3 ng/mL, the success rate of embryo transfer decreases and therefore it is recommended to transfer embryos in cows with serum progesterone level ≥3 ng/mL (Novotný *et al.*, 2005). According to Starbuck *et al.* (2004), 50.0% of cows with serum progesterone levels below 2.8 ng/mL suffered abortions after 9 weeks of pregnancy, whereas 95.0% of pregnant cows had a serum progesterone level of 6 ng/mL at week 5. However, other studies have not shown a relationship between the plasma concentration of progesterone around the time of embryo transfer and pregnancy rates (Smith *et al.*, 1996; Nogueira *et al.*, 2012). Silva *et al.* (2002) suggested that the difference could be due to the problems associated with different blood sampling methods, such as serum or plasma. Factors, such as corpus luteum, milk production, lactation period, etc., might affect circulating progesterone concentrations. Maillo *et al.* (2012) indicated that lactating cows had significantly higher progesterone concentrations from day 9 to 14 following embryo transfer than non-lactating cows, which was consistent with a higher CL weight observed. Nogueira *et al.* (2012) reported that the evaluation of a positive relationship between corpus luteum (CL) size and progesterone production, and the size of corpus luteum may influence pregnancy. During the development phase, P4 concentration was increased more sharply than CL volume, while in the luteal regression phase, CL volume decreased much more quickly than plasma P4 level (Gómez-Seco *et al.*, 2017). Between day 5 and day 8 of the cycle, there is a correlation between CL size and plasma P4 concentration, both corpus luteum size and P4 concentration increased; but after day 8 of the cycle, the corpus luteum size did not affect the levels of circulating progesterone (Man *et al.*, 2009). Jaśkowski *et al.* (2022) indicated that the mean serum P4 concentration was higher in cows with corpus luteum homogeneous than those with corpus luteum cavitary.

Cyclic P4 levels are associated with the loss of expression of P4 receptors (PGR) in the endometrium - a factor associated with receptivity to implantation. When P4 levels are high, PGR loss occurs earlier and uterine receptivity is established earlier in embryo transfer, whereas low or suboptimal P4 levels will lead to a delayed loss of the PGR as well as delaying embryo receptivity of endometrium (Bazer *et al.*, 2010; Okumu *et al.*, 2010). The endometrial epithelium is induced by progesterone to express several genes, which are then further stimulated by factors from the conceptus (such as IFNT and PGs) and the endometrium itself (Brooks *et al.*, 2014; Lonergan *et al.*, 2016). The high circulating progesterone levels after AI might be associated with the increased of interferon-tau (IFNT) production, which is a crucial factor for maternal recognition of pregnancy in ruminants. Early P4 concentrations suggested that maternal luteal factors were not responsible for pregnancy loss, which appeared to be caused by impaired conceptus development (regardless of the kind of embryo), as indicated by low maternal bovine pregnancy-associated glycoprotein-1 (bPAG-1) concentrations before embryonic death (Breukelman *et al.*, 2012). Wiltbank *et al*. (2014) reported that the low P4 before AI was generally related to the increased pregnancy loss following subsequent AI, but this was not due to the differences in the concentration of circulating P4 after AI.

Based on the results of this experiment in combination with previously published studies, we recommend choosing recipient cows with plasma progesterone levels ≥3 ng/mL to ensure successful embryo transfer in Vietnamese conditions. Applying ET on cows with progesterone levels below two ng/mL on day 6 post-estrus is not recommended because of the high cost of imported embryos.

CONCLUSION

The plasma progesterone (P4) concentration at day 6 after AI showed a positive relationship with the pregnancy rate of cows. The P4 concentration of pregnant cows was significantly higher than that of non-pregnant cows. P4 concentration between 3-4 ng/mL or above on day 6 after the AI timepoint should be applied for embryo transfer of dairy cattle in Vietnam.

CONFLICT OF INTEREST

None of the authors in this research has a financial or individual relationship with other persons or organizations that can suitably affect or bias the content of the research.

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