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Additional Growth of Dominant Follicle During Synchronized Ovulatory Cycle Affects Ovulatory Follicle Size, Corpus Luteum Parameters, and Progesterone Level in Northern Thai Native Cattle

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ABSTRACT

The aim of this research was to evaluate the additional growth rate of dominant follicle (DF) using a pregnant mare's serum gonadotropin (PMSG) in a 7-day progesterone (P_4)-gonadotropin releasing hormone (GnRH)-based program on follicular development and luteal function in White Lamphun cows. Cows in the PMSG-treated group (n=10) received PMSG on day of a P_4 device removal (on Day 7) in 7-day P_4-GnRH-based program, while cows in the PMSG-untreated group (n=10) did not receive PMSG treatment. Ultrasonography was used to scan ovaries to determine development of ovulatory follicle (OF) and formation of corpus luteum (CL). Blood samples were collected to examine P_4 concentration. The OF diameter and CL parameters (size, area, and volume) were greater in the PMSG-treated cows than in the PMSG-untreated cows ($P < 0.05$). Ten days after ovulation, the greatest P_4 concentration was found in the PMSG-treated group ($P < 0.05$). In conclusion, additional growth rate of DF using PMSG in 7-day P_4-GnRH-based program results in increase in OF development, CL parameters, and greater P_4 concentration in White Lamphun cows.

Keywords: Beef cows; Follicular growth; Luteal function; Sex hormone.

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1. Introduction

The White Lamphun breed (*Bos indicus*) that is reared in the upper north of Thailand is a native cattle breed [1]. This breed is often well adapted to tropical environments and climates, as it is able to use poor quality feeds and to cope with parasitic diseases [2]. Nevertheless, changes in the agricultural system and increased demand of meat consumption have subsequently resulted in a decreased number of White Lamphun cattle [3].

Hormonal treatments for synchronization of estrus and ovulation represent the best solution to enhance the utilization of estrous and ovulation synchronization in beef cattle herds [4-6]. Moreover, the success of the ovulation-synchronization program is dependent on synchrony of ovulatory follicle (OF) and the corpus luteum (CL) that has been reported in associations with follicular dynamics, CL, and OF functions following the ovulation-synchronization regimes. Nevertheless, many aspects of ovulation-synchronization protocols require additional investigation, particularly the supplementary hormonal treatment [7], ovarian follicular characteristics [8] as well as breeds (*Bos indicus* or *Bos taurus*) [9]. In beef cows, the 7-day Co-Synch protocol that is normally adjusted to synchronize ovulation combines with exogenous hormones so as to produce gonadotropin releasing hormone (GnRH), controlled internal drug release (CIDR) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Ovulation of an early-stage dominant follicle (DF) that is synchronized by GnRH-based protocol results in a decreased size of ovulatory follicle and fertility in dairy and beef cattle [10]. A pregnant mare's serum gonadotropin (PMSG) is generally used to induce ovulation with improved hormonal protocol of ovulatory and pregnancy rates in beef cows [11]. The PMSG treatment characteristics are also employed in veterinary medicine, as well as for the management of the reproductive function in cattle [11]. The PMSG treatment 2 days before exogenous progesterone (P_4) withdrawal might be a more rational management to further enhance DF size at the time of ovulation in anestrous beef cows [12]. However, addition of PMSG to the 7-day GnRH-based program to induce ovulation with improved DF size and to improve characteristics of CL has not been evaluated in beef cows. Moreover, although the 7-day GnRH-based program has been mostly used in beef cattle of *Bos taurus* breed, little information is available in *Bos indicus* cattle of Asian origin concerning dynamics of follicular growth and characteristics of CL, following addition of PMSG in a 7-day P_4-GnRH-based protocol. For this reason, the present experiment was to increase growth rate of ovarian DF by the addition of PMSG to the 7-day P_4-GnRH-based regime in White Lamphun cows.

2. Materials and Methods

2.1 Animals

The present study was conducted at a dairy and beef cattle farm, in the Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand. The experiment was approved by the Animal Ethic Committee of Chiang Mai University (No. AG0 03/2560 [02/2560-10-12]). Twenty non-pregnant White Lamphun cows (3 to 5 years of age; 200 to 250 kg of body weight) were used for this study. Before experiments, all cows were managed in individual pens and received sweet corn stover silage as roughage *ad libitum* and concentrate supplementation (4% of body weight). Clean water and mineralized salt licks were available *ad libitum*.

2.2 Experiment design and ovulation-synchronization protocols

At a random stage of the estrous cycle, a total of 20 Thai native cows were randomly assigned to 7-day GnRH-based protocols to undergo one of two treatments,

as follows: the PMSG-treated group (n = 10) and the PMSG-untreated group (n = 10). At the first day of synchronization treatment (designated Day 0), beef cows in both groups received an intravaginal CIDR® insert (Eazi-Breed™ CIDR®; Zoetis Animal Health, Florham Park, NJ, USA) containing 1.38 g of progesterone (P_4) for 7 days (Day 0 to Day 7) and an intramuscular injection of first 10 µg dose of GnRH (Receptal®, Intervet, Thailand) (Fig. 1). On Day 7, cows in the PMSG-treated group received an intramuscular injection of 500 IU of PMSG (Folligon®, Intervet Limited, Thailand), while the cows in the PMSG-untreated group did not receive PMSG (Fig. 1). Cows in both groups were injected with two 500 µg doses of $PGF_{2\alpha}$ (Estrumate®, Intervet Limited, Thailand), the first given at the time of CIDR withdrawal (Day 7) and the second injection 12 h later (Fig. 1). Three days later (Day 10), all cows were given a second 10 µg dose of GnRH (Fig. 1).

2.3 Transrectal ultrasonography

An ovarian evaluation was performed using transrectal ultrasonography with a 7.5 MHz linear-array transducer (HS-1600V; Honda electronics, Japan) once daily from Day 0 to Day 7 as well as twice daily from Day 7 until ovulation after the second injection of GnRH to determine ovarian structure (Fig. 1). The diameter of follicle ≥ 2 mm and CL were evaluated on ovarian charts. Follicular growth rates were calculated utilizing the diameter of DF at its detection and diameter on day of evaluation, divided by the time of the growth period [13]. Following the synchronization protocols, ovulation was considered by the disappearance of a preovulatory follicle (POF) ≥ 7.0 mm in diameter [14]. After a second dose of GnRH injection, ultrasound determinations were performed every 12 h to assess the ovulation time. Ultrasound evaluations were managed in all cows from 6 days to 10 days after ovulation to determine formation and parameters of a CL (size, cross-sectional area, and volume). Ovarian ultrasonograms were recorded on the saving image system of an HS-1600V ultrasonic scanner for retrospective analysis of follicular and CL data using the measurement function (distance, circumference, area and volume) of this scanner system.

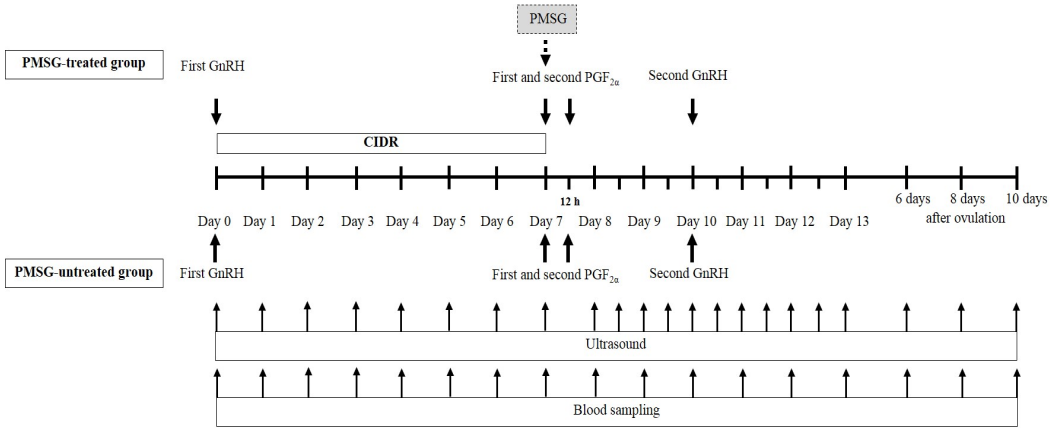

Fig. 1. Description of timing of hormonal injection, ultrasound, and blood sampling for the PMSG-untreated group and the PMSG-treated group.

2.4 Blood sampling and P_4 analysis

Blood samples (3 mL) were collected from the coccygeal vein once daily in the morning (08.00 a.m.) from Day 0 until ovulation and 6 days to 8 days after ovulation (Fig. 1). The samples were immediately centrifuged at 1200 × *g* at least 10 min. The blood plasma was harvested and stored at –20 ºC until P_4 analysis.

The P_4 levels were determined by competitive Enzyme-linked Immunosorbent Assay (ELISA). The P_4 from plasma that was extracted by petroleum ether was detected with anti-P_4 monoclonal antibody (National Center for Genetic Engineering and Biotechnology, Thailand). In duplicate plasma samples, the intra-assay coefficient of variation was 13.01% and assay sensitivity was 0.02 ng/mL.

2.5 Statistical analysis

The continuous data were demonstrated as mean ± SEM. The data were analyzed with ANOVA using the general linear model (GLM) procedure of SAS (SAS Institute Inc, Cary, NC, USA). The differences between the means were evaluated by Student's *t*-test [15]. The proportions of cows with luteolysis and ovulation were analyzed using chi-square analysis [15]. Differences with $P < 0.05$ were considered significant, and those with $0.05 < P < 0.10$ were considered a tendency [16].

3. Results and Discussion

3.1 Daily DF diameter

On the day of exogenous P_4 (CIDR) insertion (Day 0), there was no significant difference ($P > 0.050$) in the diameter of DF between the experimental groups (Fig. 2, Fig. 3A and 3B). However, the diameters of the DF tended to be larger in the PMSG-treated cows than the PMSG-untreated cows on Day 1 ($P = 0.073$) and Day 2 ($P = 0.093$) (Fig. 2). On Day 7, there was no significant difference ($P > 0.050$) in the diameter of DF between the animal groups (Fig. 2, Fig. 3C and 3D). During the 3 days after exogenous P_4 removal (Day 8 to Day 10), beef cows that did receive PMSG in addition to the 7-day GnRH-based protocol were found to have DF with bigger diameter than the beef cows in the PMSG-untreated group ($P < 0.05$) (Fig. 2, Fig. 3E and 3F).

It has to be taken into account that treatment with PMSG on the day of exogenous P_4 removal stimulated the DF diameter in White Lamphun cows; this was possibly due to the proliferation of follicular cells [17], resulting in a large optimal size of the OF on the day before ovulation.

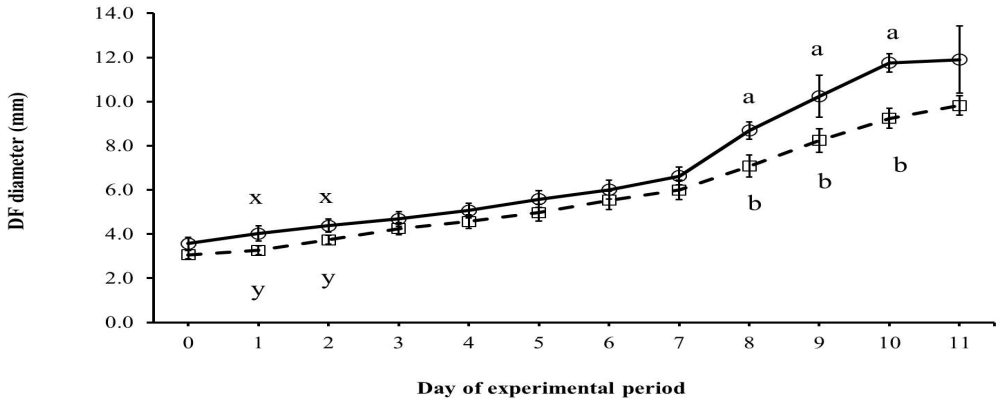

Fig. 2. Daily DF diameter of ovulated White Lamphun cows in the PMSG-untreated group (n = 8; dashed line) and the PMSG-treated group (n = 9; solid line). Within each day of experimental period, means with different superscripts (^{a,b}) denotes significant difference ($P < 0.050$) between groups, while values with different superscripts (^{x,y}) tended to differ ($0.05 < P < 0.100$) between groups.

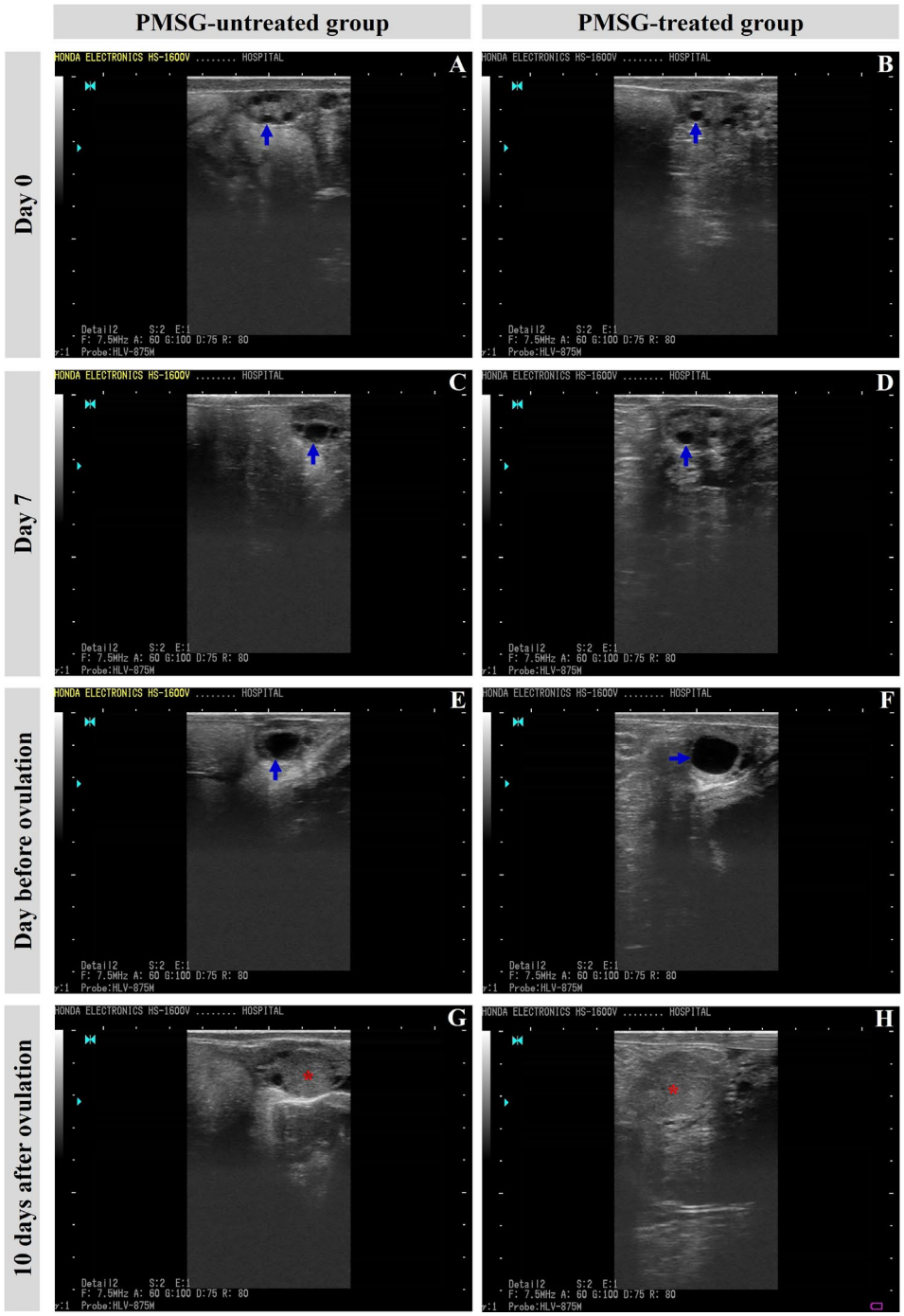

Fig. 3. Sonogram images illustrate DF (arrows) on ovaries of the PMSG-untreated and PMSG-treated cows on Day 0 (A and B), Day 7 (C and D) and day before ovulation (E and F). On Day 20 after ovulation, sonogram images illustrate CL structure (asterisks) on ovaries of the PMSG-untreated cows and PMSG-treated cows (G and H).

3.2 DF growth rate, OF size, ovulation rate and time, and CL parameters

Overall, cows receiving PMSG in addition to the 7-day GnRH-based protocol had a greater ($P < 0.050$) growth rate of the DF (from exogenous P_4 insertion to ovulation and growth rate from exogenous P_4 removal to ovulation) than cows that did not receive PMSG treatment (Table 1).

In addition, the beef cows that were treated with PMSG injection had greater ($P < 0.050$) size of the final OF on the day before ovulation compared to the beef cows that were not treated with PMSG at the time of exogenous P_4 removal (Fig. 3E and 3F; Table 1). The percentage of beef cows with completed lueolysis did not differ ($P > 0.050$) between the groups (Table 1). There was no significant difference ($P > 0.05$) in the proportion of cows that ovulated after the first and second GnRH injections between the groups (Table 1). The average time intervals from exogenous P_4 removal and the second GnRH injection to ovulation were shorter ($P < 0.050$) in the PMSG-treated cows than in the PMSG-untreated cows (Table 1).

When the ovulating beef cows in the two groups were examined, the cows that received PMSG treatment at the time of exogenous P_4 removal had a greater ($P < 0.050$) diameter, cross-sectional area, and volume of induced CL than the cows that did not receive PMSG treatment (Fig. 3G and 3H; Table 1). Moreover, the cows receiving PMSG treatment were observed to have the induced CL with greater ($P < 0.05$) diameter (Fig. 4A), cross-sectional area (Fig. 4B), and volume (Fig. 4C) than the PMSG-untreated cows from 6 to 10 days after ovulation. Ten days after ovulation, plasma P_4 concentration was higher ($P < 0.05$) in the PMSG-treated cows than the PMSG-untreated cows (Fig. 4D).

Table 1. DF growth rate, OF diameter, luteolysis, ovulation rate and time, and CL characteristics of White Lamphun cows in the PMSG-untreated group and the PMSG-treated group.

	Treatment		P-value
	Untreated group	Treated group	
No. of beef cows (no.)	10	10	
Growth rate of DF (mm/day)			
Growth rate from exogenous P_4 insertion to ovulation	0.62 ± 0.03	0.76 ± 0.04	0.011
Growth rate from exogenous P_4 removal to ovulation	0.84 ± 0.05	1.33 ± 0.13	0.004
OF diameter (mm)	10.04 ± 0.34	11.86 ± 0.44	0.005
No. of cows undergoing luteolysis after $PGF_{2\alpha}$ injections (%)	6/10 (60.0)	7/10 (70.0)	0.832
Ovulation rate (%)			
No. of cows ovulating after first GnRH injection	1/10 (10.0)	3/10 (30.0)	0.370
No. of cows ovulating after second GnRH injection	8/10 (80.0)	9/10 (90.0)	0.542
Ovulation time (h)			
Interval from exogenous P_4 removal to ovulation	115.5 ± 2.95	92.0 ± 4.62	0.001
Interval from second GnRH injection to ovulation	43.5 ± 2.95	20.0 ± 4.62	0.001
CL diameter (mm)	13.85 ± 0.48	15.55 ± 0.42	0.018
CL cross-sectional area (mm^2)	149.20 ± 9.86	188.54 ± 10.66	0.018
CL volume (mm^3)	11479.10 ± 1120.43	16263.08 ± 1282.87	0.013

Abbreviations: CL, corpus luteum; DF, dominant follicle; GnRH, gonadotropin-releasing hormone; OF, ovulatory follicle; P_4, progesterone; PMSG, pregnant mare's serum gonadotropin.

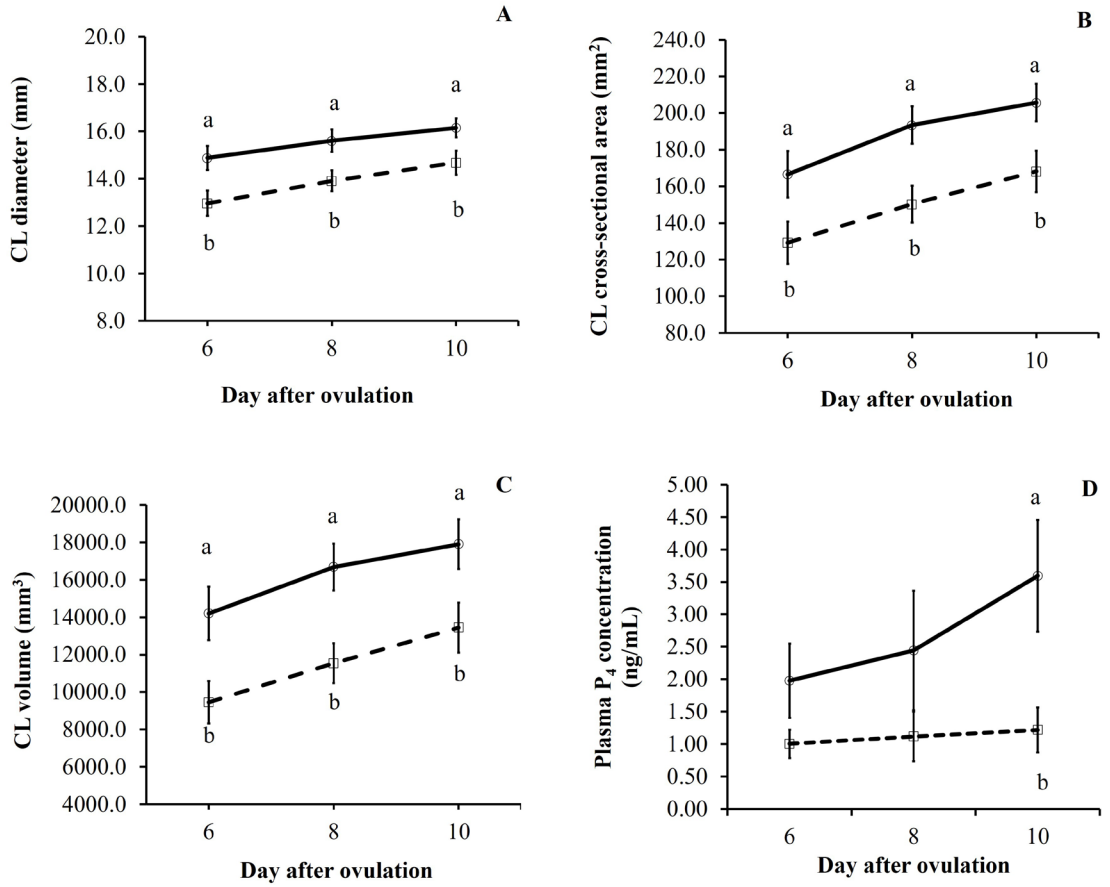

Fig. 4. The CL diameter (A), cross-sectional area (B), volume (C), and plasma P4 concentration (D) of ovulated White Lamphun cows in the PMSG-untreated group (n = 8; dashed line) and the PMSG-treated group (n = 9; solid line). Within each day of experimental period, means with different superscripts (^{a,b}) denotes significant difference ($P < 0.050$) between groups. Abbreviations: CL, corpus luteum; PMSG, pregnant mare's serum gonadotropin.

White Lamphun cows that were not treated with PMSG had lower growth rate of the DF, smaller OF, and formed smaller CL. Similar to the data provided by previous studies, the fixed-time artificial insemination (FTAI) protocol with GnRH induced a surge of luteinizing hormone (LH) and the ovulation of the physiologically immature DF resulted in the formation of a small CL that released inadequate endogenous P_4 [18], whereas adequately circulating P_4 is important for maintenance of bovine pregnancy [18]. The recent results advise that the increase in P_4 secretion of CL induced by PMSG treatment at the day of CIDR removal may improve the CL function. The CL function (P_4 production) is important for uterine function to support embryo development and implantation [19]. Moreover, the administration of 300 to 500 IU PMSG at the time of CIDR removal resulted in the presence of more evident estrous signs in *Bos indicus* cattle under field conditions [20]. In *Bos indicus* cattle (Nelore), diameter of the largest follicle and growth rate of the largest follicle were greater in PMSG-treated cows than in cows that did not receive PMSG on day of a P_4 device removal in a 9-day P_4-estradiol (E_2)-GnRH-

based protocol (11.6 ± 0.7 mm vs. 9.3 ± 0.9 mm and 1.56 ± 0.2 mm/day vs. 0.40 ± 0.2 mm/day, respectively) [21]. This results in long half-life and high affinity for follicle stimulating hormone (FSH) and LH receptors of PMSG [22]. Exogenous PMSG is used to stimulate the treatment for improving the quality of the DF during the FTAI; administration of PMSG affected the follicular cell and the ensuing CL, and led to a greater CL diameter and higher function as indicated by increased circulating P_4 [23,24].

Intramuscular injection with 500 IU dose of PMSG on the day of exogenous P_4 withdrawal in the 7-day GnRH-based regime, as employed in this study, may stimulate the granulosa cells (GCs) in the DF to secrete more E_2, resulting in increased OF size and increasing characteristics of CL (diameter, cross-sectional area, and volume). One possible reason for the differences in the DF growth during preovulatory period and CL characteristics production after ovulation between the PMSG-treated beef cows and the control beef cows could be that the DF in the PMSG-treated cows had higher growth of follicular cells containing a greater number of proliferating ovarian cells and/or retained more of the GCs after induced ovulation [25]. The mechanism by which a follicle increases in size and surrounding GCs divide to form multiple layers might be the reason for the increase in the number of the GCs [26,27] . In contrast, the smaller OF ability of spontaneous ovulation might have decreased the numbers of the GCs [28]. The results of the present study are consistent with the increase in the diameter, area, and volume of induced CL after ovulation in bovine ruminants reported by Wiltbank et al. [29], who highlighted that high volume and circulating P_4 during the first 7 days of bovine estrous cycle is likely due to rapid enhancement in the P_4 secretion. The acquirement of ovulatory ability and ovulation time are dependent on follicular diameter. Ovulation time, regardless of experimental group, occurred from 20 to 43.5 h after second GnRH administration. Moreover, the complete ovulatory ability has been reported that the DF reaches a size of 10 mm in *Bos indicus* beef cattle [30].

The present data are consistent with the optimal size of the OF in *Bos indicus* cattle, as indicated by Martins et al. [28], who concluded that when Nellore heifers were made to undergo the FTAI regime, the OF size at the FTAI was a major factor that influenced the pregnancy rate. Busch et al. [28] reported that in beef cows, GnRH-influenced ovulation of small OF (≤11.0 mm in diameter) was found to correlate with decreased pregnancy rate, a possible reason being that small OFs decrease the circulating P_4 concentration during early pregnancy. Interestingly, the optimal diameter of the OF in the PMSG-treated cows, which ranged from 10.38 mm to 14.0 mm, confirmed the findings of the previous studies in which the optimal diameters of the OF at AI were 11.1 mm to >14.4 mm (Nellore cows; *Bos indicus*) [23], 10.8 mm to 15.7 mm (Nellore heifers; *Bos indicus*) [31], and 10.7 mm to 15.7 mm (crossbred beef heifers; *Bos taurus*) [32]. On the other hand, White Lamphun cows that did not receive PMSG treatment on the day of exogenous P_4 withdrawal had OF of small size (8.8 mm to 11.5 mm in diameter) on the day before ovulation. The concentration of P_4 decreased dramatically <1 ng/ml in both protocols resulting in the animals demonstrating estrous behavior and ovulation [33]. Moreover, Sumiyoshi et al. [34] reported that level of P_4 changes to low, as approximated by 0.7 ± 0.4 ng/ml, around 2 days before ovulation. Presumably, these results of plasma P_4 concentrations (<1 ng/ml) indicated that cows exhibited complete luteolysis after 2 $PGF_{2\alpha}$ injections [35].

4. Conclusion

Additional growth rate of DF using PMSG on day of CIDR withdrawal in a 7-day P_4-GnRH-based program results in larger size of OF, greater parameters of CL (diameter, cross-sectional area, and volume) on ovary, and subsequent increase in P_4 concentration after ovulation in White Lamphun cows.

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