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Research article

Superovulatory responses based on ovarian sizes after superstimulation in Thai-Holstein crossbred dairy cows

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Abstract

This study was designed (1) to examine the relationship between ovary sides/sizes after superstimulation treatment and ovulatory responses in terms of large follicles, corpora lutea (CLs) numbers, and ovulation rate; and (2) to evaluate the coefficient of determination (R^2) as a tool to predict the subsequent superovulatory responses by ovary sizes after superstimulation treatment in the Thai-Holstein crossbreed dairy cows. Data included 33 records from 12 superovulated Thai Holstein crossbreds. Cows were estrus synchronized on day 0 and superstimulated with 400 mg of FSH with decreasing doses twice daily for 4 days. After superovulatory treatment (day 9), the sizes of ovaries were measured and divided into 3 groups by quarters according to the ovarian sizes. Group A (< 816 mm^2) ovaries were 25% smaller and group C (> 1449 mm2) ovaries were 25% larger than group B ovaries (816–1449 mm^2). On day 9 and 16, there were no significant differences in the average ovary area (p > 0.05). The numbers of large follicles and CLs of group B and C were greater than those of group A (p < 0.05). The ovulation rate did not differ among groups (p > 0.05). The moderate R^2 score between ovary size after superovulatory treatment and the numbers of dominant follicles and CLs were calculated (R^2 = 0.445 and 0.370, p < 0.05) while the beta coefficient (b-value) was positive for both observation parameters. In conclusion, the numbers of large follicles and CLs related to the size of ovaries after superovulation treatment. The moderate R^2 score obtained in this study could be indicative of the limited possibility for using ovary size after superovulatory treatment for predicting superovulatory responses.

Keywords: Cow, Dominant follicle, Superovulation, Ovulation rate

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INTRODUCTION

Most dairy cattle in Thailand are crossbred between Holstein and Sahiwal or native Thai breeds which aims to get greater milk production from the former together with more tolerant to heat from the latter (Chanvijit et al., 2005). However, with the increase of Holstein proportion, those population are more susceptible to heat stress and subsequently resulted in lower reproductive performance in terms of greater day open (Boonkum et al., 2011). To improve pregnancy rate, the application of assisted reproductive technology, besides artificial insemination (AI), has drastically improved and been applied.

Multiple ovulation and embryo transfer (MOET) is generally used in the dairy industry to increase the reproductive rate of superior females (Gaddis et al., 2017). MOET has been used in place of AI to improve pregnancy rates during the summer season, largely due to those embryos (blastocyst stage) being more heat resistance in comparison to ovulated oocytes and earlier stage embryos (Sartori et al., 2006). The outcome of superovulation procedures in dairy cows are dependent on the efficiency of ovarian responses such as a greater number of ovulations as well as high number of transferable embryos. Several extrinsic and intrinsic factors including breed (Baruselli et al., 2006), age (Burns et al., 2005; Malhi et al., 2005), gonadotropin preparation (Sumretprasong et al., 2008; Chasombat et al., 2013), route of administration (Bó et al., 2010; Sakaguchi et al., 2018; Ratsiri et al., 2021), and dose used (Nilchuen et al., 2012) affecting superovulation responses have been researched over the last few decades. Regardless of those researches, tremendous variation of superstimulatory response continues to be a critical issue in the profitable and efficient implementation of MOET programs (Kafi and Mcgowan, 1997).

It has been established that one of major sources of variability of superovulatory response is the status of ovarian follicles at the time of initiation of FSH treatment. When a superovulatory treatment is applied, cows with a lower number of growing small antral follicles have a low ovulatory response to treatment (Kawamata, 1994; Kohram and Poorhamdollah, 2012). Moreover, larger right-side ovaries at the beginning of superovulation is significantly related to a higher superovulatory response (Palubinskas et al., 2016). However, it is unknown whether ovary size after superstimulation continues to affect the ovulatory responses.

Understanding/predicting the outcome of hormonal superstimulation by the characterization of ovary types associated with superstimulatory responses will provide fundamental knowledge on ovarian responses and may expand the application of superovulation in reproductive research in bovine species. We hypothesized that the ovarian sides/sizes after superstimulation affected to the ovulatory responses. Therefore, the present research was designed (1) to examine the relationship between ovarian sides/sizes after superstimulation treatment and ovulatory responses in terms of large follicles and corpora lutea (CLs) numbers and ovulation rate, and (2) to evaluate the coefficient of determination (R^2) as a tool to predict the subsequent superovulatory responses by ovary sizes after superstimulation treatment in the Thai-Holstein crossbreed.

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MATERIALS AND METHODS

Animals

Data included 33 records from 12 superovulated Thai Holstein crossbreds. All were non-lactating, between 3 and 6 years of age, and had a good body condition score between 3 and 3.5 (1–5 scale). They were housed in comfortable living conditions, provided fresh water, and fed optimized nutritional food. This studied has been reviewed and approved by the institutional animal care, based on the ethic of animal experimentation of national research council of Thailand (Record No. IACUC-KKU-87/64, Reference No. 660201.2.11/426).

Superovulation treatment

The superovulation treatment was induced according to Figure 1. Cows at random stages of the estrous cycle were subjected to estrus synchronization with an intravaginal device impregnated with 1.56 g of progesterone (Eazi-Breed CIDR-B®, Zoestis) and an intramuscularly (IM) dose of 5 mg estradiol-17β plus 50 mg progesterone (SRC Animal Health, Pak Chong, Nakhon Ratchasima, Thailand) to induce follicular regression and emergence of a new follicular wave on day 0. Superovulatory treatment was initiated on day 4 by twice daily IM injections of 400 mg FSH (Folltropin®-V; Bioniche Animal Health, Belleville, ON, Canada) with decreasing doses (80, 80, 60, 60, 40, 40, 20, 20 mg, respectively) over 4 days. All cows were administered 25 mg prostaglandin $F_{2\alpha}$ (Lutalyze®, Zoestis) and IM injections twice a day on day 6 (12-hour intervals), with CIDR-Bs being removed on the morning of day 7. On the evening of day 8, all cows received 0.01 mg of GnRH (Receptal®, MSD, Unterschleissheim, Germany) to induce ovulation. About three replications per animal were preformed to induce superstimulation.
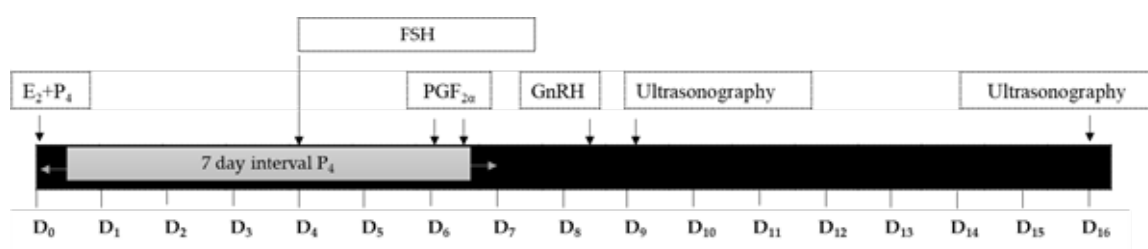

Figure 1 Estrus synchronization and superovulation treatment. E_2, 5 mg of estradiol-17β. P_4, 50 mg of progesterone. Interval P_4, intravaginal device impregnated with 1.56 g progesterone. FSH, 400 mg of FSH in eight decreasing doses administered IM at 12-hour intervals over a 4-day period. $PGF_{2\alpha}$, 25 mg of $PGF_{2\alpha}$. GnRH, 0.01 mg of GnRH.

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Experimental Design

To examine the relationship between ovarian sizes after superstimulation treatment and ovulatory responses, on the morning of day 9 (after superovulatory treatment) and day 16 (6–7 days after ovulation), the ovary sizes (length x width) and the number of ovarian structures (large follicles and CLs) were examined by transrectal ultrasonography (HS-2000 ultrasound scanner; Honda Electronics Co., Toyohashi, Japan). The size of the ovaries was calculated by multiplying the width by length. The ovaries were arranged in increasing order and grouped by quarters according to ovarian sizes on day 9. The quartile divided data into three groups: a lower quartile, median, and upper quartile. Group A (< 816 mm^2) ovaries were 25% smaller and group C (> 1449 mm^2) ovaries were 25% larger than group B ovaries (816–1449 mm^2) (Table 1). The area of the ovaries on both the left and right sides of the body were recorded separately.

Table 1 Groups of ovaries based on area (width x length) after superovulatory treatment (day 9).

Ovary Groups (n)	Ovary Size After Superovulatory Treatment (mm^2)
A (18)	<816
B (32)	816–1449
C (16)	>1449

(n)=numbers of ovaries

The efficiency of superovulatory response based on ovarian sizes after superovulation treatment was determined by the number of large follicles (≥ 10 mm), number of CLs, and ovulation rate. The ovulation rate was calculated by dividing the number of CLs by the number of large follicles.

Statistical Analysis

Statistical analyses data has been performed using the one-way ANOVA test using Randomized Complete Block Design (RCBD) separated by ovary sizes on day 9 (after superovulatory treatment) and day 16 (6–7 days after ovulation). Duncan's New Multiple Range Test was used to test the differences in the ovary sides/sizes groups affecting the superovulatory response parameters, including ovary area, number of dominant follicles, number of CLs, ovulation rate, and $p < 0.05$ statistically significant. The results were analyzed using SAS 9.0 program. The full statistical model was as follows:

$$Y_{ij} = \mu + Block_i + Treatment_j + \varepsilon_j$$

Where

Y_{ij} = observation values (superovulatory response parameters) from block i (i = 1 to 2) and treatment j (j = 1 to 3)

μ = overall mean

$Block_i$ = the effect of ovary location (left and right) i (i = 1 to 2)

$Treatment_j$ = the effect of ovary size groups (A, B, C) /j (j = 1 to 3)

ε_j = experimental error

The effect of ovary size groups affecting the superovulatory response parameters and create a predictive equation using a simple linear regression method. The equation format is as follows:

$$Y = \beta_0 + \beta_1 X + \varepsilon$$

Where

Y = the dependent variable (Y) for any given value of the independent variable (X), β_0 = the intercept, β_1 = the regression coefficient (slope = b), X = the independent variable, ε =error The accuracy of the prediction equation depends on the R^2, which can be calculated from the equation: $R^2 = 1 - \dfrac{SSE}{SST}$ where SSE = sum of squares error, SST = sum of squares total.

RESULTS

All cows showed signs of estrus, however, two did not respond to the superstimulation (number of ovulations less than two). The ovarian responses in terms of total number of follicles, small follicles (2–5 mm), medium follicles (6–9 mm), and large follicles ($\geq$ 10 mm) are demonstrated in Table 2.

Table 2 The ovarian response of Thai-Holstein dairy cows subjected to superstimulation treatment.

Parameters	Values
No. of records	33
No. of cows in estrus (%)	100%
No. of cows with > 2 ovulations	31(93.9%)
Total no. of follicles	17.8 ± 5.9
- Small follicles (2–5 mm)	3.4 ± 2.2
- Medium follicles (6–9 mm)	4.0 ± 3.2
- Large follicles (>10 mm)	14.5 ± 4.9

Table 3 shows the size of ovaries for both left and right side of body on day 9 (after superovulatory treatment) and day 16 (6–7 days after ovulation). Overall, ovary sizes on day 16 were greater than those of day 9. On day 9, there were no significant differences in size between left and right side (p > 0.05). The size of the left ovary was different than that of the right ovary on day 16 (p < 0.05), but the average ovary area was not significant (p > 0.05).

Table 3 Ovary area (mm^2) dependent on location side on day 9 (after superovulatory treatment) and day 16 (six to seven days after ovulation) average + SD (n).

Ovary Groups	Day 9			Day 16		
	Left Ovary (n)	Right Ovary (n)	Average in Total	Left Ovary (n)	Right Ovary (n)	Average in Total
A	587.02 ± 151.63[a] (9)	600.09 ± 215.26[a] (9)	593.56 ± 183.45	1419.24 ± 298.38[b] (9)	1835.93 ± 929.97[a] (9)	1627.59 ± 614.18
B	1133.22 ± 209.74[a] (14)	1124.21 ± 180.42[a] (18)	1128.72 ± 195.08	2877.29 ± 964.61[a] (14)	2266.29 ± 863.19[b] (18)	2571.79 ± 913.90
C	1753.04 ± 242.10[a] (10)	1859.91 ± 352.89[a] (6)	1806.48 ± 297.50	2558.03 ± 881.62[b] (10)	3169.56 ± 475.27[a] (6)	2863.80 ± 678.45
Average	1190.36 ± 488.06[a]	1115.03 ± 476.43[a]	1152.12 ± 479.91	2440.32 ± 989.21[a]	2292.87 ± 900.31[a]	2358.83 ± 931.03

[a,b] rows with superscripts differ significantly between left and right ovaries within groups on the same day ($p < 0.05$)
(n)=numbers of ovaries

The results summarizing the superovulatory responses for large follicles, CLs, and ovulation rate in relation to groups of after superovulatory treatment (day 9) are summarized in Table 4. The number of large follicles and CLs of group B and C were greater than those of group A ($p < 0.05$). Meanwhile, the ovulation rate did not differ among groups ($p > 0.05$). The ovary sizes did not affect ovulation rates in any group ($p > 0.05$).

Table 4 Statistical results of superovulation in relation to groups of ovaries prior insemination average ± SD.

Ovary Groups	Left Ovary				Right Ovary		
	F Number (n)	CLs Number (n)	Ovulation Rate (%)		F Number (n)	CLs Number (n)	Ovulation Rate (%)
A	2.88 ± 1.92 [b]	2.14 ± 1.79 [b]	90.83 [a]		3.29 ± 2.05 [b]	2.71 ± 2.11 [b]	92.95 [a]
B	7.00 ± 2.32 [a]	6.37 ± 2.13 [a]	90.48 [a]		7.09 ± 2.33 [a]	6.38 ± 2.15 [a]	91.07 [a]
C	8.50 ± 2.55 [a]	6.65 ± 2.85 [a]	89.62 [a]		8.53 ± 2.72 [a]	6.93 ± 2.60 [a]	93.49 [a]

[a,b] columns with superscripts differ significantly between ovary groups ($p < 0.05$)
F= large follicles; CLs = corpora lutea; (n) = numbers

Figure 2 shows the R^2 and regression coefficient (slope; b-value) that predicted the number of dominant follicles and CLs using ovary size after superovulatory treatment in Thai-Holstein dairy cows. This analysis showed a moderate, and statistically significant ($p < 0.05$), R^2 between ovary size after superovulatory treatment and the number of dominant follicles and CLs ($R^2 = 0.445$ and 0.370). The b-value was positive for both observation parameters. In other words, the number of large follicles (b = 0.125) and CLs (b = 0.447) increased with the sizes of ovary.
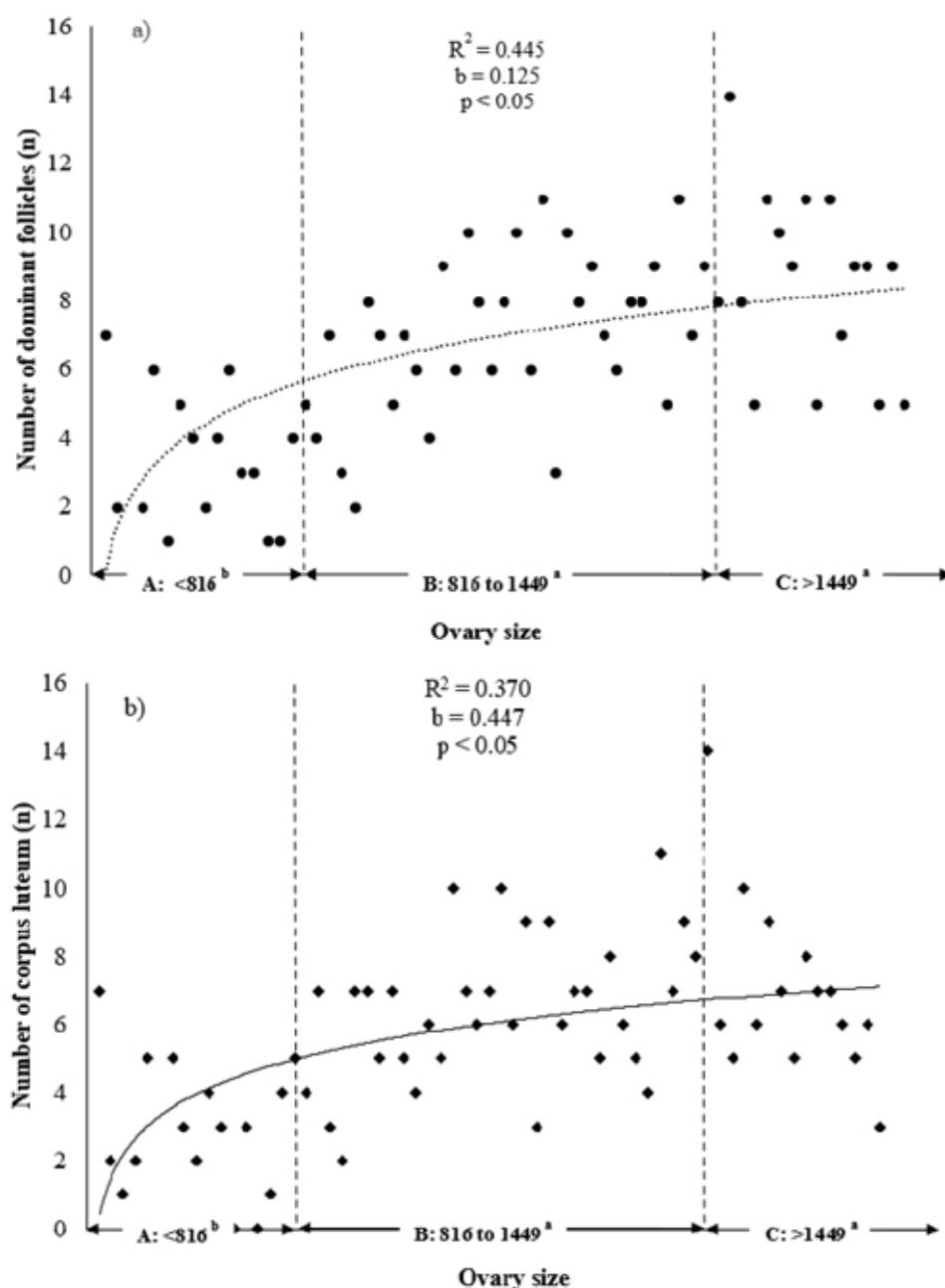
Figure 4 R^2 and b-value that predict the number of dominant follicles (a) and CLs (b) using ovary size after superovulatory treatment in Thai-Holstein dairy cows.

DISCUSSION

In the present study a superovulatory response in terms of large follicles, CLs numbers, and ovulation rate was evaluated and correlated with the ovary sides/sizes after superstimulation treatment. The results are considered satisfactory since 93.9% of the cows responded to the treatment with three or more CLs. The numbers of large follicles and CLs related to the size of ovaries after superovulation treatment while there were no significant differences in size of ovaries between left and right side. The R^2 as a tool to predict the subsequent superovulatory responses by ovary sizes after superstimulation treatment showed moderate while the b-value was positive for both observation parameters.

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During both natural and synchronized estrous cycles, ovulation occurs more frequently in the right than left ovary, varying from 54%–60% in ewes and goats and from 60%–65% in cows (Pineda, 1989; Stevenson, 2019). Similar observations have been reported in superovulation, the right ovary being 27% larger than the left side after treatment, and resulted in greater CLs and embryos yielded from the right (Palubinskas et al., 2016). Explanations for right-frequenting ovulations could potentially be due to the presence of the left ovary being close to the rumen. Therefore, the left ovary is being disrupted by extrinsic factors such as temperature, pressure fluctuations, and mechanical contractions (Pineda, 1989). However, in this study, the mean ovary, left and right ovaries sizes, after superovulatory treatment were not different (Table 3). This finding is similar to others reports (Patel et al., 2009; Mesquita et al., 2016). Although it is unknown why those sizes were similar between left and right side, one of the possibilities is that use of FSH increased those size of ovary after superovulation. Also, significant increase in the size of ovary after ovulation was observed. Therefore, the number of large follicles and CLs between both sides did not differ as well (Table 4).

Conversely, the number of large follicles and CLs were influenced by the size of the ovaries in all groups. The number of large follicles and CLs of groups B and C, which had a greater surface area, were higher than those of group A (p < 0.05, Table 4). Research has demonstrated that the size of an ovary mostly depends on the number and size of follicles (Sirois and Fortune, 1988; Mesquita et al., 2016). It is possible that small ovaries have a low intraovarian signaling system or insufficient receptors to the gonadotropins, which are dependent required for development of secondary to antral follicles (Kawashima and Kawamura, 2018), which leads to poor response to superovulatory treatment.

GnRH treatment during estrus has been well documented to either prevent ovulation failure or reduce any variation in the ovulation interval by inducing a luteinizing hormone (LH) peak (Kaim et al., 2003; López-Gatius et al., 2006). It is possible that GnRH has potentially been administered to induce ovulation in our superovulation protocols. Therefore, the ovulation rates in all groups were not significantly different (Table 4).

Analysis showed a moderate but statistically significant difference between ovary size after superovulatory treatment and the number of dominant follicles and CLs (R^2 = 0.445 and 0.370). In general, a high R^2 value indicates that the model is a good fit for the data, although interpretations of fit depend on the context of analysis. The R^2 of 0.445 indicated that 44.5 % of the number of dominant follicles has been explained just by predicting the ovary size using the covariates included in the model. Meanwhile, R^2 of 0.370 indicated that 37.0 % of the number of CLs has been explained just by predicting the ovary size. At the same time, the P-value in this study showed statistical differences in the number of dominant follicles and CLs parameters, suggesting that the ovary size after superovulatory factor for predicting superovulatory responses was necessary for the change in numbers. However, other factors besides ovary size (55% and 63%) such as breed (Baruselli et al., 2006), age (Burns et al., 2005; Malhi et al., 2005), gonadotropin preparation (Sumretprasong et al., 2008; Chasombat et al., 2013), route of administration (Bó et al., 2010; Sakaguchi et al., 2018), and dose used (Nilchuen et al., 2012) had an effected ovarian response.

The regression analysis showed a small to moderate positive relationship between X and Y variables means that an increase in ovary size (X-variable) increases the number of dominant follicles (Y-variable) and CLs (Y-variable) parameters accordingly. In particular, the ovary size of groups B and C affected both two parameters more significantly than the ovary size of groups A (P < 0.05). The slope value between ovary size and the number of dominant follicles was 0.125 indicated that the number of dominant follicles increased 0.125 when ovary size increase 1 unit, as well as slope value between ovary size and number of CLs was 0.447 indicated that the number of CLs increase 0.447 when ovary size increase 1 unit.

CONCLUSIONS

In conclusion, this study indicates that the number of large follicles and CLs relate to the size, but not side, of ovaries after superovulation treatment. By increasing the size of the ovaries, a greater number of large follicles and CLs were obtained. Meanwhile, the mean of ovary size from left and right ovaries after superovulation treatment were not different. Therefore, the number of large follicles and CLs between both sides did not differ as well. The moderate R^2 score obtained in this study could be indicative of the limitation of predicting superovulatory responses using ovary size after superovulatory treatment. The b-value was positive for both observation parameters. This is inferred that increasing sizes of ovary, greater number of large follicles and CLs were obtained.

AUTHOR CONTRIBUTIONS

Rujira Chumchai: Methodology, formal analysis, writing original draft, Thanaporn Ratsiri: Methodology, Ruthaiporn Ratchamak: Methodology, Wuttigrai Boonkum: Data curation and formal analysis, and Vibuntita Chantikisakul: Supervision, conceptualization and writing review and editing.

CONFLICT OF INTEREST

There are no conflicts of interest in this study.

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