Dose Response to Superovulation in Thai Dairy Cattle

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Introduction
The high cost of embryo transfer technology has limited its effectiveness in improving the genetics of the dairy industry in Thailand. There has been reported that different breeds of cattle respond to superovulation regimes differently (1). Thai dairy cattle is somewhat a cross breed of Holstein Friesian and Thai native cattle which has been developed for dairy industry for more than 50 years. The dose rates of 370-400 mg of Folltropin-V (Vetrepharm Inc., Canada), follicle stimulating hormone (FSH), has been used in superovulation as a standard protocol in Thailand for more than a decade. Boland, et al. (1991) mentioned that Folltropin when used in the wide range of 10 to 30 mg total dose, gave acceptable ovulation rates and numbers of transferable embryos (2).

To maximize the number of transferable embryos yielded per collection while at the same time minimizing the dose of gonadotropin used will help to lower the cost of superovulation. The objective of the study is to evaluate the response of two different doses of superovulatory drug in Thai dairy cattle.

Materials and Methods
Fifty donor cows of a Thai Friesian cross-breed were included in 97 superovulations and embryo transfer program. They were subjected to superovulatory treatment using a commercially available gonadotropin, Folltropin-V. Two total doses of a conventional 360 mg (group 1, n=34) and a reduced dose 260 mg. (group 2, n=53) were intramuscularly administered to the donors twice daily in a decreasing dose regime (60, 60, 50, 40, 40, 30 and 60, 60, 40, 40, 20, 20, 20 mg respectively), starting on day 5 after progesterone intravaginal (CIDR-B, EAZI-BREED, InterAg, New Zealand) insertion (day 0) and 2 mg of estradiol benzoate injected on the following day (day 1). The prostaglandin analogue (Cloprostilin, Estrumate, Schering-Plough Animal Health, USA) were given at the dose rate of 500 µg, morning and afternoon, on the third day of the superovulation program (Fig. 1).

Fig. 1 Superovulatory program using estradiol to synchronize follicular wave emergence in CIDR-treated donor cows

Artificial inseminations were done 3 times every 12 hrs starting from onset of standing estrus (day 0). Embryo collections were performed on day 7. Superovulatory responses in terms of the numbers of corpus luteum (CL), number of unovulated follicles and the yield of transferable embryos (TE) were recorded.

Data were adjusted by variance of order, season, location of the farm, lactational status, and operator. The differences were then tested by t-test analysis. Chi-square test were used to analyze the difference between the percentage of embryo recovery and usable embryo in each category. A value of \(p<0.05\) was considered significant.

Results and Discussion
Superovulatory responses in terms of number of CLs, unovulated follicles and TE were compared. There were no differences in the numbers of CL, number of unovulated follicles and the yield of TE in both groups. The mean numbers of CL were (mean±SEM) 12.8±0.9, ranged 0-14, and 15.4±1.0, ranged 0-16, in group 1 and group 2 respectively. Group 1 seemed to have higher number of unovulated follicles compared to group 2, 1.7±0.6 vs 0.7±0.9 respectively. The mean numbers of TE were 4.2±0.7 in group 1 and 4.3±0.6 in group 2 (Table 1). There were no difference in the percentage of embryos recovered and usable embryos in both group, 74.2 vs 74.8% and 44.3 vs 37.1% in group 1 and group 2 respectively.
The number of embryos collected ranged from 0-29 in group 1 and 0-40 in group 2. The number of usable embryos ranged from 0-13 vs 0-14 in group 1 vs group 2. The mean number of TE collected per donor were comparable with the report of Hasler (2006) from the accumulated result working in Holstein donor over 10-year span of 4.0-4.2 (3). The season, location, lactational state and operator did not affect the results. The data indicated that the dose rate for superovulation protocol in Thai Friesian cross breed cows can be reduced to at least 260 mg of Folltropin-V without affecting the yield of embryo production.

Table 1 Superovulatory responses (mean±SEM) of donor cows received the total dose of Folltropin-V 360 mg and 260 mg.

<table>
<thead>
<tr>
<th>Gr</th>
<th>Dose (mg)</th>
<th>No. of CL</th>
<th>No. of F</th>
<th>No. of TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>360</td>
<td>12.8±0.9</td>
<td>1.7±0.6</td>
<td>4.2±0.7</td>
</tr>
<tr>
<td>2</td>
<td>260</td>
<td>15.4±1.0</td>
<td>0.7±0.9</td>
<td>4.3±0.6</td>
</tr>
</tbody>
</table>

The ultimate goal of superovulatory treatment in the cow is to obtain a maximum number of fertilized and transferable embryos hence high probabilities of producing pregnancies (4). The breed of the donor cow was a significant source of variation in the results of embryo production, total number of transferable embryos per collection ranged from 2.8 to 6.6 among different breeds (5). Thai dairy cattle has been developed from a cross bred of Bos taurus breed, Holstein Friesian and Thai native cattle which is Bos indicus breed for many generations. It is well accepted that the dose of FSH required for superovulation in Bos indicus cattle is less than that of Bos taurus cattle (6). Lewis (1992) recommended the doses of 360-400 mg Folltropin-V for Bos taurus cattle and 250-280 mg for Bos indicus breed. Bos indicus breeds require 25-30% less than Bos Taurus breeds (7). Barati, et al. (2006) mentioned that superovulatory dose regime of 250 mg Folltropin-V caused an overstimulation and high incidence of unovulatory follicles in Sastani cattle, native Bos indicus breed of Iran (6). In our study, the dose rate of 360 mg gave more number of unovulated follicles than 260 mg. We suggest that Thai dairy cattle require less FSH than pure breed Holstein Frenesian cattle.

Kanitz et al. (2002) (8) mentioned that with increasing doses of FSH the number of ovulations increases significantly until a plateau was reached and it was not possible to increase ovulation number with further increasing FSH doses. Very high FSH doses were followed by a depression in ovulation number. High FSH doses may disturb the ovulation process. Yaakub, et al. (1998) (9) also reported that excess gonadotropin is not beneficial to embryo production. The reduced dose of the superovulatory drug, Folltropin-V, to 260 mg in our study minimized the cost in conducting the embryo transfer in our dairy cattle without compromising the results.

In order to optimize the superovulatory response, gonadotropin treatment must be initiated at the expected time of follicular wave emergence (10, 11). In this study the synchronization of the emergence of the follicular wave using 2 mg of estradiol benzoate (CIDIROL) prior to initiating of superovulatory treatments were used in the protocol. This could reduce the length of time spent in superovulatory treatment from 20 days in the conventional protocol which the superovulation were initiated a during mid cycle or 9 days after CIDR-B insertion to 16 days. Exogenous control of follicle wave emergence offers the advantage of initiating superovulatory treatments at an optimal time for follicle recruitment, regardless of the stage of the estrus cycle (4). This treatment protocol makes the program more adjustable to the convenience of the operators. This can be concluded that the dose rate for superovulation protocol in Thai-Friesian cross-breed cattle can be reduced to at least 260 mg of Folltropin-V without affecting the yield of embryo production. Lower cost of embryo production may stimulate widespread use of embryo transfer technology to increase reproductive rates of selected cows, hence those genetically outstanding cows can contribute more to the breeding program.

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References
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