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Superovulation with Different Doses of Follicle Stimulating Hormone in Kamphaeng Saen Beef Cattle

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Abstract: The experiment was conducted to investigate the effect of different doses of Follicle Stimulating Hormone (FSH) using in superovulation program on numbers of corpora lutea, total ova/embryos and transferable embryos in Kamphaeng Saen beef cattle. Cyclic cows (n = 4) and heifers (n = 4) of Kamphaeng Saen beef breed were assigned for two levels of FSH (200 and 250 mg NIH-FSH-P1) in change-over design by which two change over treatments were studied over two periods in all animals. Cows and heifers were estrous synchronized by cloprostenol (500 μg). Estrus detection was performed by teaser bull (Day 0 = Day of the onset of standing estrus). On day 9 after the onset of standing estrus, all animals were treated with FSH twice daily decreasing doses over 4 days. On day 3 of FSH injection each animal was treated with cloprostenol (500 μg). At the first standing estrus, all animals were artificially inseminated 3 times with 12 h interval, two straws of frozen-thawed semen of Kamphaeng Saen bull were used per insemination. All animals were treated with gonadotropin releasing hormone (10 μg of Buserelin) at first insemination. Numbers of corpora lutea were determined by rectal palpation and embryos were flushed 7 days after the onset of standing estrus and classified according to the development stage and quality. The results showed that numbers of corpora lutea, percentages of total ova/embryos and percentages of transferable embryos were not significantly different ($p>0.05$) between treatments (FSH:200 vs. 250 mg) in cow and heifer.

Key words: Kamphaeng Saen beef cattle, cow, heifer, superovulation, follicle stimulating hormone, Thailand

INTRODUCTION

Kamphaeng Saen beef breed, one type of beef cattle breeds in Thailand has been developed from a cross breed of Thai native cattle, Brahman and Charolais. Kamphaeng Saen beef cattle is suitable for tropical environment in Thailand and has good quality of meat. Nevertheless, the genetic improvement and breeding of cattle take a long period therefore, embryo transfer and superovulation technology have been applied in the breeding program to reduce the time of improving the genetics. These advanced techniques are more beneficial than the natural breeding (Barati *et al*., 2006). However, the high cost of superovulation technology has limited its effectiveness in improving the genetics of the beef industry in Thailand. Superovulation with FSH (NIH-FSH-P1) in cattle is introduced by the Vetrepharm Company Pty. Ltd., (Australia) that suggests the dose of 400 mg FSH. There have been reported that different breeds of cattle respond to superovulate regimes differently. Currently, researcher have attemped to reduce the doses of FSH for inducing superovulation in cattle. There are several reports indicated that *Bos indicus* breeds have been shown more sensitivity to exogenous gonadotropins than *Bos taurus* cattle (Randel, 1984) and the lower doses of FSH for induced superovulation could be applied in *Bos indicus* cattle compared with *Bos taurus* cattle (Lewis, 1992; Barros *et al*., 2003; Barati *et al*., 2006). Additionally, Lewis (1992) recommended that superovulatory doses for *Bos indicus* breeds was 250-280 mg. However, Krininger *et al*. (2003) reported the superovulation response in *Bos taurus* was generally similar to that in *Bos indicus* cows when superovulatory doses of 240 mg FSH were used.

There are some technical reported in Thailand concerning superovulation in cattle. Sophon *et al*. (2003) reported that superovulation in Brahman cattle by using FSH of 400 mg per dose affected on superovulatory response in total numbers of ova/embryos (10.4) and transferable embryos (4.3). However, some reports found that the use of FSH in a lower dose than 400 mg in Brahman cattle affected on superovulatory response which showed the same effect as the previous report (Leingcharoen *et al*., 2007) and Brahman cattle responsed

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to FSH not exceed the dose of 200 mg (Leingcharoen *et al*., 2007). However, there are no any reports showing the use of FSH to induce superovulation and the specific concentration of hormone dose used in cow and heifer of Kamphaeng Saen beef breed in Thailand.

The objective of this experiment was to evaluate the superovulatory response of Kamphaeng Saen beef cattle induced with two different doses of FSH and the possibility to apply the lower dose of FSH than the dose recommended by the company.

MATERIALS AND METHODS

Animals: Cyclic cows (n = 4) and heifers (n = 4) of Kamphaeng Saen beef breed, a crossbred cattle (25% Thai native cattle, 25% Brahman breed and 50% Charolais breed) with an average live body weight of 400±73.14 and 326±17.97 kg and the age of 6-7 and 2-3 years, respectively were subjected to superovulation with different doses of FSH. All animals were housed in a dirt lot with an indoor feeding area under good care condition at Buffalo and Beef Production Research and Development Center, Suwanvajokkasikit Animal Research and Development Institute at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. All animals were fed a ration balanced to meet minimum nutritional requirements that consisted of pasture and concentrate and had free access to water.

Treatments: All animals were assigned randomly into two levels of FSH (200 and 250 mg) (Folltropin®-V, Vetrepharm, Canada; a mg of Folltropin®-V is equivalent to a mg of NIH-FSH-P1 reference standard) in Change-over design by which two change over treatments were studied over two periods in all animals with a rest period of 2 months. All animals were normally estrous for 2 cycles before each animal was treated with the next treatment.

Estrus synchronization and superovulation: All animals were estrous synchronized by using Cloprostenol via Intramuscular injection (IM) (500 μg; Estrumate®, Schering-Plough Animal Health, USA). Estrus detection was performed by teaser bull (Day 0 = Day of the onset of standing estrus). On day 9th after the onset of standing estrus, all animals were treated with FSH twice daily for 4 days by decreasing doses of 200 mg (T1; 80, 60, 40 and 20 mg) and 250 mg (T2; 100, 70, 50 and 30 mg). On day 3 of FSH injection, all animals were treated with cloprostenol by IM (500 μg; Estrumate®). At the first standing estrus, each animal was artificially inseminated 9 times with 12 h interval. Two straws of frozen-thawed semen of Kamphaeng Saen bull were used per insemination. All animals were treated with Gonadotropin releasing hormone (GnRH) by IM (10 μg of buserelin; Receptal®, Intervet International B.V. Holland) at the first insemination.

Embryo recovery and evaluation: On day 7th after the onset of standing estrus, corpora lutea were determined by trans-rectal palpation and embryos were collected by a standard non-surgical and double uterine body flushing procedure as described by Neto *et al*. (2005). To perform uterine flushings, a two-way Foley catheter was passed through the cervix and the tip was placed in the uterine body, caudal to the external bifurcation of the uterus. The balloon was inflated with air and the uterus was flushed 3-4 times with modified Dulbecco's Phosphate Buffer Saline (mDPBS) containing 1% heat treated estrus cow serum. Then, catheters were left in the same position and mDPBS were infused through the catheter and the plunger was closed with a disposable 3 mL syringe. Donors were allowed to rest in a holding pen for 30 min and the remaining mDPBS was recovered by manipulation of the uterus. After recovery, the embryos were evaluated and classified for different stages of development (i.e., ova, 2-8 cell, 8-16 cell, morula, compacted morula, early blastocyst, blastocyst and expanded blastocyst) and quality (i.e., excellent, good, fair, poor and degenerated) as described by Linder and Wright (1983). Excellent and good quality embryos were considered as transferable embryos.

Statistical analyses: Data were analyzed by Analysis of Variance (ANOVA) (effect of treatments on numbers of corpora lutea) using Change-over models procedure and the differences between two treatments were then compared by Student's t-test. The proportional data sets of the total ova/embryos, transferable embryos and stages of embryo development were tested by Chi-square analysis for the significant difference between the treatments ($p<0.05$). Results are expressed as means and the Standard Errors of the Means (SEM).

RESULTS AND DISCUSSION

The results showed that no significant differences were found between groups of donor cows in numbers of corpora lutea (FSH 200 mg: 15.00±4.28 vs. 250 mg: 7.25±4.28, respectively mean±SEM), percentages of total ova/embryos (FSH 200 mg: 88.33% vs. 250 mg: 72.41%) and percentages of transferable embryos (FSH 200 mg: 64.15% vs. 250 mg: 66.67%) (Table 1).

Table 1: Effect of follicle stimulating hormone doses with superovulation program on superovulatory response in Kamphaeng Saen beef breed cows (mean±SEM (range))

	Follicle stimulating hormone (mg)	
Superovulatory response	200	250
Numbers of corpora lutea	15.00±4.28 (6-34)	7.25±4.28 (4-12)
Numbers of total ova/embryos	13.25±4.30 (4-33) (88.33%)*	5.25±4.30 (2-10) (72.41%)*
Numbers of transferable embryos	8.50±3.48 (3-22) (64.15%)**	3.50±3.48 (0-10) (66.67%)**

Table 2: Effect of follicle stimulating hormone doses with superovulation program on superovulatory response in Kamphaeng Saen beef breed heifers (mean±SEM (range))

	Follicle stimulating hormone (mg)	
Superovulatory response	200	250
Numbers of corpora lutea	15.50±3.40 (7-31)	13.50±3.40 (11-16)
Numbers of total ova/embryos	14.25±3.61 (6-30) (91.94%)*	11.75±3.61 (9-15) (87.04%)*
Numbers of transferable embryos	9.75±3.01 (1-22) (68.42%)**	7.75±3.01 (1-12) (65.96%)**

*The ratio between numbers of total ova/embryos and numbers of corpora lutea; **The ratio between numbers of transferable embryos and numbers of total ova/embryos

Numbers of corpora lutea (FSH 200 mg: 15.50±3.40 vs. 250 mg: 13.50±3.40), percentages of total ova/embryos (FSH 200 mg: 91.94% vs. 250 mg: 87.04%) and percentages of transferable embryos (FSH 200 mg: 68.42% vs. 250 mg: 65.96%) were not significantly different in heifer (Table 2).

In this study, numbers of corpora lutea, total ova/embryos and transferable embryos did not differ between Kamphaeng Saen beef breed groups (both cow and heifer) treated with a total of 200 and 250 mg (NIH-FSH-P1). The hypothesis would involve a number of small antral follicles will develop into dominant follicle at the beginning that use FSH for superovulation. During the bovine estrous cycle, small antral follicles are recruited from the ovarian pool by promoting follicular development from FSH. They are selected and either become atretic or develop into a single dominant follicle that ovulate. However, the principle of superovulation involves providing the female with higher level of FSH than normal. Therefore, this causes abnormally high numbers of follicle which are recruited and selected (Senger, 1997).

Gonzalez *et al.* (1990), Hockley *et al.* (1992) and Mishra *et al.* (1996) reported that FSH binds to the limited numbers of receptors located at granulosa cell of the antral follicle in ovary in order to activate the growth and development of follicles. However, when cattle are treated with low doses of FSH, animals show response to that hormone since, the sufficient numbers of FSH receptors are able to support the reaction. Nevertheless when cattle are induced with high doses of FSH, animals do not express the increasing response because of the limited numbers of FSH receptors in ovary.

However, the results showed that cow has superovulatory response less than heifer when treated with FSH (both two levels) in superovulation program. Several investigators reported that an increase in age of donor had a negative influence on superovulatory response in *Bos taurus* (Lerner *et al.*, 1986; Breuel *et al.*, 1991; Malhi *et al.*, 2007) and *Bos indicus* (Silva *et al.*, 2009) breeds. The lesser superovulatory response from aged cows may be related to follicular and endocrine changes that occur as age increases. Although, the follicular wave pattern in older animals is similar to that in young cows, old cows have fewer small ovarian follicles recruited into a follicular wave (Malhi *et al.*, 2005) and have fewer large follicles after ovarian superstimulation (Malhi *et al.*, 2006).

Some report showed that administration of low or high doses of FSH for superovulation had a similar consequence in superovulatory response in each breed. Barati *et al.* (2006) reported that Sistani beef cattle, a native *Bos indicus* breed of Iran had a similar effect on superovulatory response when treated with the doses of 120, 160 and 200 mg FSH. Baruselli *et al.* (2006) reported that Nelore cattle, a native Bos indicus breed of Brasil, treated with the doses of 100, 133 and 200 mg FSH demonstrated the similar effect on superovulatory response. The report in Thailand suggested by Sumretprasong *et al.* (2008) found that Thai dairy cattle had an indistinguishable effect on superovulatory response when cattle were given in the different doses of 260 and 360 mg FSH. In addition, Leingcharoen *et al.* (2006) reported that Kao Lum Poon cows, a Thai native cattle (*Bos indicus*) had a similar consequence in superovulatory response when treated with the doses of 150 and 200 mg FSH. Furthermore, Leingcharoen *et al.* (2010) demonstrated that doses of 160, 180 or 200 mg FSH did not show different results in superovulatory response and embryo quality in Thai Black cattle (*Bos taurus* x *Bos indicus*).

Evalution of superovulatory response in cattle is demonstrated in total numbers of ova/embryos and transferable embryos. The present study in Kamphaeng Saen beef cattle found that total ova/embryos and transferable embryos between groups treated with a total doses of 200 mg and 250 mg FSH are 13.75, 9.13 and 8.50, 5.63, respectively.

The similar study was investigated by Barati *et al.* (2006) who reported that Sistani beef cattle treated with the doses of 200 mg FSH, demonstrated effect on superovulatory response in numbers of total ova/embryos (8.2) and transferable embryos (4.3). The experiment conducted in Nelore cattle by Baruselli *et al.* (2006)

showed that the cattle treated with the doses of 200 mg FSH, demonstrated an effect on superovulatory response in numbers of total ova/embryos (10.6) and transferable embryos (6.5). Leingcharoen *et al.* (2006) reported that numbers of transferable embryos from Kao Lum Poon cattle treated with doses of 200 mg FSH was 1.82. In Thai Black cattle (*Bos taurus* x *Bos indicus*), numbers of total ova/embryos and transferable embryos from animals treated with doses of 200 mg FSH were 11.14 and 9.79, respectively (Leingcharoen *et al*., 2010).

CONCLUSION

Kamphaeng Saen beef breed has been developed from a cross breed of Thai native, Brahman and Charolais which is *Bos indicus* breed for many generations. It is generally accepted that the dose of FSH for superovulation is less than that suggested in the instructions of the Vetrepharm Company Pty. Ltd., (Australia) which encouraged the recommended doses of 400 mg FSH without affecting the superovulatory response.

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