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Original Article

Superovulation with different doses of follicle stimulating hormone in Kamphaeng Saen beef cattle

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Abstract

An experiment was conducted to investigate the effect of different doses of follicle stimulating hormone (FSH) used in a superovulation program on numbers of corpora lutea, total ova/embryos and transferable embryos in Kamphaeng Saen beef cattle. Cyclic cows (n=3) and heifers (n=3) of Kamphaeng Saen beef breed were assigned to two levels of FSH (200 and 250 mg. NIH-FSH-P1) in Crossover Design by which two changes 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 in 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 three times at 12 h intervals. 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 seven 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 versus 250 mg) in either cows or heifers.

Keywords: Kamphaeng Saen beef cattle, cow, heifer, superovulation, follicle stimulating hormone

1. Introduction

Kamphaeng Saen beef breed, one type of beef cattle breed 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 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 was introduced by the Vetrepharm company Pty. Ltd. (Australia), which suggests the dose of 400 mg FSH. It has been reported that different breeds of cattle respond to superovulation regimes differently. Currently, researchers are attemption to reduce the doses of FSH for inducing superovulation in cattle. There are several reports indicating that *Bos indicus* breeds are more sensitive to exogenous gonadotropins than *Bos taurus* cattle (Randel, 1984) and that lower doses of FSH for induced superovulation could be applied in *Bos indicus* compared with *Bos taurus*. (Lewis, 1992; Barros *et al.*, 2003; Barati *et al.*, 2006). Additionally, Lewis (1992) recommended

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superovulatory doses for *Bos indicus* breeds was of between 250 and 280 mg. However, Krininger III *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 reports in Thailand concerning superovulation in cattle. Sophon *et al.* (2003) reported that superovulation in Brahman cattle using FSH of 400 mg per dose affected the superovulatory response in regard is 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 induced a superovulatory response of the same magnitude as in the previous report (Leingcharoen *et al.*, 2007), and Brahman cattle responsed to FSH not exceeding 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 of applying a lower dose of FSH that recommended by the company.

2. Materials and Methods

2.1 Animals

Cyclic cows (n=3) and heifers (n=3) 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 conditions at the 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.

2.2 Treatments

All animals were assigned randomly into two levels of FSH (200 and 250 mg) (Folltropin®-V ,Vetrepharm, Canada; 1 mg of Folltropin®-V is equivalent to 1 mg of NIH-FSH-P1 reference standard) in Crossover Design by which two change over treatments were studied over two periods in all animals with a rest period of two months. All animals went through normal estrous for 2 cycles before being treated with the subsequent treatment.

2.3 Estrus synchronization and superovulation

All animals were estrous synchronized 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 9 after the onset of standing estrus, all animals were treated with FSH twice daily for 4 days in 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 three times at 12 h intervals. 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.

2.4 Embryo recovery and evaluation

On day 7 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 three or four 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 was 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 Lindner and Wright (1983). Excellent and good quality embryos were considered as transferable embryos.

2.5 Statistical analyses

Data were analyzed by analysis of variance (ANOVA) (effect of treatments on numbers of corpora lutea) using Crossover models procedure and the differences between two treatments were then compared by *Student*'s t-test. Differences in proportions between treatments of the total ova/embryos, transferable embryos and stages of embryo development were tested by Chi-square analysis ($P<0.05$). Results are expressed as means and the standard errors of the means (SEM).

3. Results

No significant differences were found between groups of donor cows in numbers of corpora lutea (FSH 200 mg: 8.67±0.89 versus 250 mg: 7.33±0.89; mean±S.E.M.), percentages of total ova/embryos (FSH 200 mg: 76.92% versus 250 mg: 72.73%) and percentages of transferable embryos (FSH 200 mg: 60.00% versus 250 mg: 75.00%) (Table 1.).

Numbers of corpora lutea (FSH 200 mg: 10.33±0.89 versus 250 mg: 13.00±0.89), percentages of total ova/embryos (FSH 200 mg: 87.10% versus 250 mg: 87.18%) and percentages of transferable embryos (FSH 200 mg: 62.96% versus 250 mg: 64.71%) were not significantly different in heifers (Table 2.).

4. Discussion

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). During the normal bovine estrous cycle, a number of small antral follicles are recruited from the ovarian pool and their development presented under the influence of FSH. The selected follicles either become atretic or develop into a single dominant follicle that ovulates. The principle of superovulation involves providing the female with higher than normal levels of FSH, which can stimulate abnormally high numbers of follicles to be recruited and develop and thus a large number to ovulate (Senger, 1997).

Gonzalez *et al.* (1990), Hockley *et al.* (1992) and Mishra et al. (1990) reported that FSH binds to the limited numbers of receptors located on the granulosa cells of the antral follicle in the 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 the 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 any further increase in response because of the limited numbers

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

Superovulatory response	Follicle stimulating hormone (mg)	
	200	250
Number of corpora lutea	8.67 ± 0.89 (6-11)	7.33 ± 0.89 (4-12)
Number of total ova/embryos	6.67 ±1.19 (4-8) (76.92 %)*	5.33 ± 1.19 (2-10) (72.73 %)*
Number of transferable embryos	4.00 ± 2.38 (3-5) (60.00 %)**	4.00 ± 2.38 (0-9) (75.00 %)**

* 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.

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

Superovulatory response	Follicle stimulating hormone (mg)	
	200	250
Number of corpora lutea	10.33 ± 0.89 (7-13)	13.00 ± 0.89 (11-16)
Number of total ova/embryos	9.00 ± 1.19 (6-12) (87.10 %)*	11.33 ± 1.19 (9-15) (87.18 %)*
Number of transferable embryos	5.67 ± 3.27 (1-12) (62.96 %)**	7.33 ± 3.27 (1-12) (64.71 %)**

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

of FSH receptors in the ovary.

Our results showed that cows have a lower superovulatory response than heifers when treated with FSH (at either level) in the 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 lower 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 in each follicular wave (Malhi et al., 2005), and have fewer large follicles after ovarian superstimulation (Malhi *et al.*, 2006).

Some reports have show in a number of breeds that administration of low and high doses of FSH for superovulation elicit similar superovulatory responses. Barati *et al.* (2006) reported that Sistani beef cattle, a native *Bos indicus* breed of Iran, had a similar 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 Brazil, treated with the doses of 100, 133 and 200 mg FSH, demonstrated the similar effect on superovulatory response. In Thailand, Sumretprasong *et al.* (2008) found that the superovulatory responses of Thai dairy cattle were indistinguishable when 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 breed of *Bos indicus*, had a similar 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 any differences in superovulatory response and embryo quality in Thai Black cattle (*Bos taurus* X *Bos indicus*).

Evaluation of superovulatory response in cattle is based on total numbers of ova/embryos and transferable embryos. The present study in Kamphaeng Saen beef cattle found that total ova/embryos and transferable embryos in the group treated with a total doses of 200 mg FSH are 6.67 and 4.00 in cows and 9.00 and 5.67 in heifers, respectively.

The similar study was made by Barati *et al.* (2006) who reported that Sistani beef cattle treated with a dose of 200 mg FSH, demonstrated effect a superovulatory response in which the mean numbers of total ova/embryos was 8.2 and that of transferable embryos 4.3. An experiment conducted in Nelore cattle by Baruselli *et al.* (2006) showed that the cattle treated with the dose of 200 mg FSH, resulted in mean numbers of total ova/embryos of 10.6 and of transferable embryos of 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).

Kamphaeng Saen beef breed has been developed from a cross breed of Thai native, Brahman and Charolais which is *Bos indicus* bred 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 recommended doses of 400 mg FSH without affecting the superovulatory response.

5. Conclusion

Kamphaeng Sean beef cattle can efficiently respond to FSH treatment when the superovulation technique is applied. Superovulatory response in Kamphaeng Saen beef cattle (both cow and heifer) treated with two doses of 200 and 250 mg FSH for superovulation were not different. It is possible to use lower doses of FSH than that recommended by the company for superovulation in Kamphaeng Saen beef cattle.

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