

Effects of Equine Chorionic Gonadotropin on Progesterone-based Estrus Synchronization in Holstein Friesian x Brahman Recipient Cows

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ABSTRACT

Many estrous synchronization protocols have been developed to control the reproductive cycle in embryo transfer recipient cattle. Various breeds of cattle, such as *Bos taurus*, *Bos indicus*, or *Bos taurus* x *Bos indicus* respond to each protocol differently. The objective of this study was to seek an appropriate protocol for estrus synchronization in Holstein Friesian x Brahman crossbred recipients. Two synchronization protocols were tested: group 1) progesterone and prostaglandin (PG); and group 2) progesterone and PG plus equine chorionic gonadotropin (eCG). The addition of eCG reduced the time between progesterone withdrawal and appearance of estrous behavior from 60.8 ± 2.8 (mean $\pm$ SEM) h in group 1 to 31.5 ± 2.5 h in group 2 and improved synchronization (range 22-69 h in group 2 compared to 34-106 h in group 1). Furthermore, cows treated with eCG had better quality corpora lutea and more cows from this group were selected as recipients (62.8% versus 48%). The protocol using progesterone and PG plus 200 IU eCG in these crossbred cattle was more cost effective than the conventional protocol using progesterone and PG.

Key words: estrus synchronization, embryo transfer, recipient, crossbred, cattle

INTRODUCTION

A major hurdle to the genetic improvement of dairy cattle by embryo transfer technology in Thailand is the shortage of recipients. The high cost of maintaining a recipient herd and the ineffectiveness of synchronization limits the success in using this technology to reproduce desirable genetics.

Estrous synchronization is the manipulation of the reproductive cycle so that the majority of cows can be scheduled as recipients during a short, predictable period. Many synchronization protocols have been developed to control the estrus and ovulation in cattle. Variability in the response to the hormonal treatment of the cows results in poor synchrony. Besides individual variations and the stage of the

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estrous cycle when synchronization is started, variations are also encountered for different cattle breeds (Bo *et al.*, 2003; Day and Geary, 2005; Mapletoft *et al.*, 2009). *Bos indicus* (Brahman) cattle tend to be more sensitive to steroid hormones than *Bos taurus* breed (Mapletoft *et al.*, 2009). Prostaglandin F_{2α} has been most commonly used for synchronization of estrus in cattle (Bo *et al.*, 2002; Mapletoft *et al.*, 2009). However, this zebu breed responded poorly to the prostaglandin F_{2α} despite the confirmation of a corpus luteum (CL) at the time of treatment (Bo *et al.*, 2003). In Thailand, Brahmans have made significant contributions to the cattle industry over a long period, while Holstein Friesian x Brahman crossbred cows have been commonly used as recipients in the embryo-transfer program.

Progesterone and prostaglandin (PG) have been generally available and thus commonly used in Thailand for estrous synchronization of recipients. It has also been documented that *Bos indicus* cattle respond well to the progestin and PG systems (Day and Geary, 2005). However, unpublished accumulated data of the authors of the current study on the synchronization over the past two years using progesterone and PG showed only 51.1% (311/609) of treated animals had CL of good quality.

Alternative protocols, such as the Ovsynch program, involving synchronization of follicular-wave emergence and ovulation, can help to overcome the problem in the accuracy of heat detection, thus permitting a fixed-time embryo transfer (Mapletoft *et al.*, 2009). However, the cost of synchronization using a GnRH-based protocol is higher than progesterone and PG treatment. Estradiol can be used to synchronize follicular wave emergence by administration at the time of insertion of a progestin device (Lane *et al.*, 2008; Perea *et al.*, 2008; Mapletoft *et al.*, 2009). The ban of the use of estradiol and its related products for estrous synchronization due to the possible harmful residues in animal products has limited

the use of such regimes in many countries (Lane *et al.*, 2008).

In order to maximize the recipient usage and economize on the cost of the embryo-transfer operation, there is a need for a simple cost-effective protocol of estrous synchronization that will result in a more tightly synchronized estrus and ovulation in the recipient herd. Incorporation of equine chorionic gonadotropin (eCG) at the end of a progestagen synchrony treatment may be a way of inducing an earlier onset and a tighter synchrony of estrus (Baruselli *et al.*, 2004; Duffy *et al.*, 2004; Lane *et al.*, 2008; Pinheiro *et al.*, 2009). The present study aimed to assess the effect of adding eCG to the progesterone-based estrus synchronization protocol in a herd of crossbred Holstein Friesian x Brahman recipients.

MATERIALS AND METHODS

Experiments were conducted at the Dairy Production Research and Development Project experimental farm in Lopburi province. In these experiments, 93 Holstein Friesian x Brahman cycling cows that were between 120 and 180 days *postpartum* were used. The animals were kept under routine feedlot management conditions. Rectal palpation was undertaken to determine cyclicity of the cows based on the presence of a CL. Two synchronization protocols were used: group 1) progesterone and PG (PROG+PG); and group 2) progesterone and PG plus eCG (PROG+PG+eCG). Animals were randomly assigned to one of these protocols.

Starting on day 0, a 1.38 g progesterone intravaginal device, CIDR-B (Pfizer Animal Health, USA) was inserted into all animals. On day 9, 500 mg cloprostenol (PG; Estrumate, Schering Plough Animal Health, USA) was injected intramuscularly into each animal. In addition, group 2 animals received 200 IU of eCG (Folligon, Intervet, Netherland) intramuscularly on the same day as PG. The CIDR-B devices were

removed on day 11 in both groups (Figure 1).

Estrus was detected by observations of animal estrous behavior and its time was recorded. The mean intervals from CIDR-B removal to standing estrus of animals in each group were compared using student's t-test. Rectal palpation to verify the presence of a CL was performed on the transfer date for each animal. The quality of CL was judged by the size of CL compared to the ovary and cows having CL occupying at least half the size of the ovary were selected as recipients.

RESULTS

For group 1, there was a 90% (45/50) success rate in synchronization treatment with the mean interval from the CIDR-B removal to standing estrus of 60.8 ± 2.8 (mean $\pm$ SEM) h. The cows showed signs of estrus between 34-106 h, with 37.8, 42.2, 11.1 and 8.9% observed in standing heat between 34-48, 49-72, 73-95 and

>95 h, respectively, after removal of the CIDR-B. Only 48% (24/50) of animals had good quality CL and these cows were used as embryo transfer recipients.

In contrast, animals in group 2 (PROG+PG+eCG), had more tightly synchronized estrus. All these animals came into estrus with a mean interval from the CIDR-B removal to standing estrus of 31.5 ± 2.5 h (range 22-69 hours). Thirty-seven out of 43 cows (86.0%) showed signs of estrus within 48 h starting from 22 h onward, while the remainder (14%) showed estrus between 49-72 h after withdrawal of the progesterone. Among all treated animals in group 2, 62.8% (27/43 cows) had CL of good quality with 9.3% (4/43 cows) had multiple CLs. The difference between the mean duration from the end of treatment to the sign of standing estrus of group 1 and group 2 was statistically significant ($p < 0.0001$) as shown in Table 1. The distribution of estrus in both groups is shown in Figure 2.

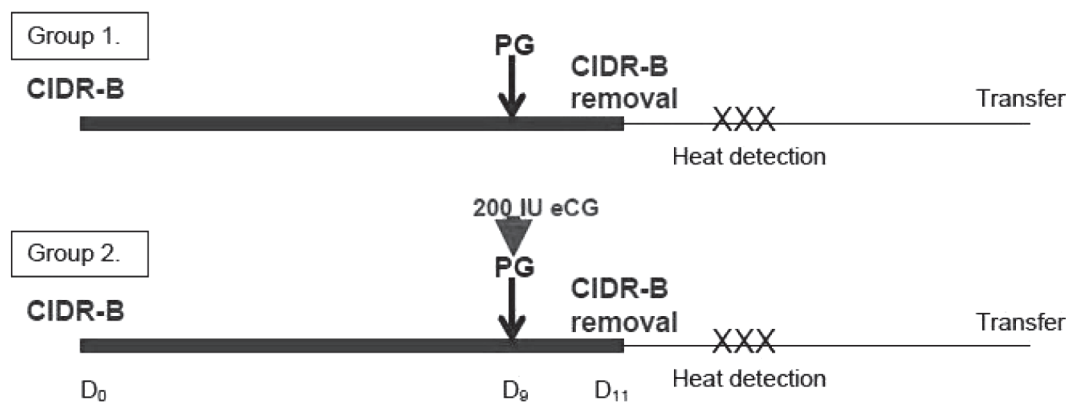


Figure 1 Schematic outline of the synchronization protocols in both groups.

Table 1 The difference between the mean duration from the end of treatment to signs of standing estrus by group and the proportion of the recipients selected for embryo transfer.

	Duration (days)		Number of cows transferred/treated (%)
	Mean $\pm$ SE	Range	
Group 1	60.8 $\pm$ 2.8 ^a	34-106	24/50 (48.0%)
Group 2	31.5 $\pm$ 2.5 ^b	22-69	27/43 (62.8%)

Significant differences ($P < 0.0001$) within a column are indicated by different letters (a, b).

SE = standard error of the mean.

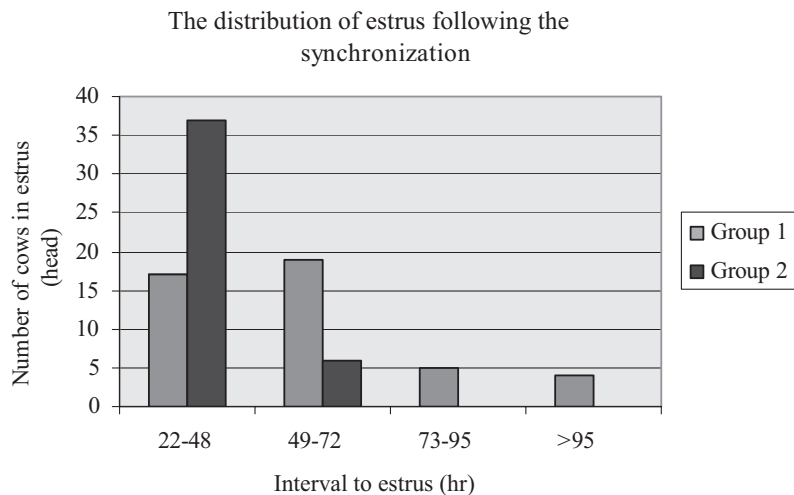


Figure 2 Distribution of estrus after CIDR-B removal in group 1 and group 2.

DISCUSSION

To choose an estrous synchronization program for embryo transfer recipients, it is essential to consider an effective, tight synchrony program. Prostaglandin is only active when a functional CL is present, between day 6 to 16 of the estrous cycle (Bo and Mapletoft, 2004; Day and Geary, 2005; Mapletoft *et al.*, 2009). Using the synchronization protocol in postpartum cows, the estrous response may be improved if the PG is administered following diagnosis of an existing CL (Lane *et al.*, 2008). With a single injection of PG, about 70% of cycling cows would respond and the cows would be expected to show sign of estrus within 2-5 days after the treatment. The interval from PG treatment to the expression of estrus and ovulation is determined by the stage of development of the dominant follicle at the time of treatment (Bo *et al.*, 2002). If PG is given after a dominant follicle is selected, estrus will result, followed by ovulation in 2-3 days, whereas the interval from administration to onset of estrus will be lengthened to 4-5 days, if given before follicular dominance is attained (Bo *et al.*, 2002; Lane *et al.*, 2008).

Progesterone alters the ovarian function in cattle by suppressing estrus and preventing ovulation. Pretreatment with progesterone for 7-10 days caused a group of randomly cycling cows to be responsive when PG was administered to induce regression of CL resulting in more effective synchronization than by using one injection of PG alone (Mapletoft *et al.*, 2003; Day and Geary, 2005).

The injection of eCG during the synchronization protocol may induce multiple ovulations, thus increase circulating progesterone concentrations in recipients (Bo and Mapletoft, 2004). Treatment with 1,000 or 800 IU of eCG, 2.5 days prior to removing the progestin resulted in more recipients suitable for embryo transfer and higher overall pregnancy rates than was the case without eCG treatment (Bo and Mapletoft, 2004). Plasma progesterone concentrations tended to be higher in heifers that had a single ovulation when treated with eCG than those without eCG (Bo *et al.*, 2002). The follow-up study reported that when a rate 400 IU of eCG was used, 1.9% (3/156 cows) had two CLs. The current results indicated that the dose of eCG for Thai crossbred cattle could probably be further reduced, with 9.3% (4/43) of

cows having more than one CL when a rate of 200 IU of eCG was used. The beneficial effect of eCG treatment would seem to be through the stimulation of follicular growth and an increase in the follicle size and the ovulation rate, resulting in increased CL diameters, CL numbers and progesterone concentrations (Bo *et al.*, 2002; Mapletoft *et al.*, 2009; Pinheiro *et al.*, 2009). In the current study, the percentage of animals that had good quality CL, and hence were selected as recipients, was higher in the eCG-treated group compared to the other (62.8 versus 48.0%). Moreover, the treatment with eCG tended to increase pregnancy rates in *Bos indicus* x *Bos taurus* recipients (Bo *et al.*, 2002; Nasser *et al.*, 2004).

The effect of the addition of eCG to the progesterone-based synchronization protocol resulted in increased CL diameters and improved pregnancy rates in beef cattle (Bo *et al.*, 2003; Mapletoft *et al.*, 2009; Small *et al.*, 2009). The beneficial attribution of eCG treatment was considered to have resulted from stimulation of dominant follicle growth and maturation, resulting in increased progesterone production by the subsequent CL (Baruselli *et al.*, 2004). However, the size and the number of the induced CL depended on the day of eCG administration. Treatment with eCG three days before the withdrawal of progesterone produced a greater CL area (Ferreira *et al.*, 2006) and a higher number of CL (Nasser *et al.*, 2004) compared to eCG administration at the withdrawal time. Therefore, at least 2-3 days may be required for eCG to stimulate the development of a dominant follicle before ovulation can take place after the end of progesterone treatment.

CONCLUSION

The data from this study showed that the protocol using progesterone and PG with an additional 200 IU eCG administration produced a

substantial improvement in synchronization of estrus and enabled a greater number of cows to be selected as embryo-transfer recipients than by using the conventional protocol with progesterone and PG. Thus, this resulted in greater ease of use and lower cost of embryo transfer.

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