

The Comparison Study on Response of Estrus Induction in Dairy Cows by Intramuscular, Intraischiorectal fossa and Intravulvosubmucosa Injection of Prostaglandin $F_{2\alpha}$

Jatuporn Kajaysri^{1*}, Kittisak Sangsakul² and Chiirut Ratanadheb²

¹Department of Clinic for Obstetrics and Gynecology Andrology and Artificial Insemination of Domestic animals, Faculty of Veterinary medicine, Mahanakorn University of Technology, Nong-Chock, Bangkok 10530 Tel. 0 2988 3655 ext. 247, 107, Fax. 0 2988 4040,

²Chonburi Artificial Insemination and Biotechnology Research Center, Banbueng, Chonburi 20220

*Corresponding author Email: jakajaysri@hotmail.com

Abstract

The experiments were carried out in sixty crossbred Holstein Fresian of postpartum cycling dairy cows with Corpus luteum on the one side of ovary. The animals were divided in to three groups of twenty cows. Every cow in the first group was induced estrus with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (Cloprostenol) by intramuscular (IM) injection with 500 μ g (2 ml/cow, full dose) Cloprostenol. In the second group every cow was induced estrus by injecting into intraischiorectal fossa (IRF) with 300 μ g (1.2 ml/cow, 60% of full dose) Cloprostenol. And every cow in the third group was injected intravulvosubmucosal (IVSM) on the same side of finding persistent Corpus luteum in the ovary with 125 μ g (0.5 ml/cow, 25% of full dose) Cloprostenol. The signs of estrus were detected in all of 60 cows on day 3 after $PGF_{2\alpha}$ injection. Every cow presented the sign of estrus with uterine contraction when cows were palpated by rectal palpation. The concentration of serum progesterone (P_4) levels and Estradiol (E_2) levels from each individual cows in all groups were lower than 0.1 ng/ml and above 7.17 pg/ml respectively. The mean and standard deviation values of Estradiol (E_2) levels from 20 cows of group 1, 2 and 3 after $PGF_{2\alpha}$ injection were 27.35 ± 8.09 pg/ml, 26.15 ± 9.07 pg/ml and 27.40 ± 6.61 pg/ml respectively. The mean \pm SD of serum E_2 levels from all groups were not significantly different ($p > 0.05$). The confirmation of successful estrus synchronization after $PGF_{2\alpha}$ treatment with 3 above methods were done by Artificial Insemination (AI). The results showed that the pregnant cows in group 1, 2 and 3 were 75% (15/20 cows), 75% (15/20 cows) and 45% (9/20 cows) respectively. The data from this study indicates that the dose reduction at 40% of the $PGF_{2\alpha}$ full dose, injected via IRF and 75% of the full dose injected via IVSM produced similar estrus induction effectiveness comparable to that of IM full dose.

Keyword : Prostaglandin, Intramuscular, Intraischiorectal fossa, Intravulvosubmucosal, Dairy cow

Introduction

Anestrus cows has been known as the major problems in dairy production industry. Especially in case of anestrus postpartum cows are they prolong day open and calving interval that can cause economic loss (Mwaagna and Janowski, 2000). In Thailand Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogues have been used to produce luteolytic effects of Corpus luteum especially in luteal phase of bovine estrus cycle. $PGF_{2\alpha}$ can regress Corpus luteum and manipulate estrus in through its action by a decrease progesterone hormone and increase estrogen hormone in the blood (De Rensis and Peters, 1999). Several products of $PGF_{2\alpha}$ have been used in Thailand normally for inducing estrus with single dose ≥ 25 mg/cow or double doses 15 and 10 mg/cow (24 hours interval period of injection) (Atthapinit *et al.*, 2001) or with usually single dose of $PGF_{2\alpha}$ analogue (Cloprostenol) 500 μ g/cow, 2 ml (Virakul *et al.*, 2001). Because of the cost of $PGF_{2\alpha}$ is considerably expensive and for the highest benefit of farmer to be worthwhile using $PGF_{2\alpha}$, clinicians and scientists try to reduce effective dose of $PGF_{2\alpha}$ to the minimum. Some studies in Thailand reported that the decreasing dose of $PGF_{2\alpha}$ has been administrated in dairy cows to intravulvosubmucosal (IVSM) or intrauterine (IU) at 25% of full dose (Wenkoff, 1984, Virakul *et al.*, 2001) and inтраischiorectal fossa (IRF) at 60% of full dose (Punyapornwithaya *et al.*, 2005). Those results were presented that the dose of $PGF_{2\alpha}$ can be reduced when administrated by IVSM, IU and IRF methods. However, it is quite difficult to use IU methods as administration route in dairy cows in the field when compared to IVSM and IRF methods, but IRF method must use dose of $PGF_{2\alpha}$ higher than decreasing dose of $PGF_{2\alpha}$ by IVSM administration methods.

The primary objective of this study is to compare the efficiency of decreasing dose of prostaglandin between inтраischiorectal fossa (IRF) and intravulvosubmucosal (IVSM) to intramuscular (IM) methods base on estrus response and concentration of serum estrogen hormone (Estradiol, E_2) levels during estrus period of crossbred Holstein Friesian dairy cows under field condition in Thailand.

Materials and Methods

Experimental animals

Sixty crossbred Holstein Fresian of postpartum cycling dairy cows varying in ages between 3-10 years old from three farms in Chonburi and Ratchaburi provinces were studied. These farms were similar in general and reproductive management. All of experimental cows with body condition score 2.5-3.5 (5-scales; Edmonson *et al.*, 1989) were chosen to examine by rectal palpation by one fixed veterinarian. The cows were housed in individual stall barns and could be free in grazing field near their barns area. This study was conducted during November 2004 to February 2005.

Estrus induction

All sixty postpartum cycling cows with Corpus luteum on ovary without other abnormality of reproductive tract to find out by rectal palpation were divided in 3 groups (each 20 cows/group) and induced estrus with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue. First control group was injected intramuscular (IM) with 500 μ g (2 ml/cow, full dose) Cloprostenol. Second experimental group was injected inraischiorectal fossa (IRF) with 300 μ g (1.2 ml/cow, 60% of full dose) Cloprostenol. Third experimental group was injected intravulvosubmucosal (IVSM) on the same side of finding persistent Corpus luteum in the ovary with 125 μ g (0.5 ml/cow, 25% of full dose) Cloprostenol.

Estrus detection

Estrus detection was done on day 3 after $PGF_{2\alpha}$ injection in all groups of cows by visual observation of signs by the fixed veterinarian. The signs of estrus were detected by the presence of standing heat, mounting and riding other cows, vaginal mucous or uterine contraction when cows were palpated by rectal palpation.

Serum progesterone and estrogen hormonal assay

The experiment was designed to measure serum progesterone (P_4) and estrogen hormone (Estradiol, E_2) on day 3 after $PGF_{2\alpha}$ injection to find out the estrus cows by comparing the levels of hormones in Secondly experimental group and thirdly experimental group with Firstly control group. 20 ml of blood samples were collected by jugular vein puncture from each cow. The blood samples were allowed to clot overnight in a refrigerator. The serum was separated by centrifugation at 3,000 rpm for 15 minutes. Each serum sample was pipetted, collected and stored at -20°C until E_2 Microparticle Enzyme Immunoassay (MEIA) as previously described of Central Laboratory co., Ltd., Maung district Nonthaburi province.

Artificial Insemination and Pregnancy detection

Artificial Insemination was done with all of estrus cow in 3 groups at the time of estrus after $PGF_{2\alpha}$ injection by the same skillful veterinarian throughout the experiment. The frozen semen (0.25 ml/dose) of Holstein Fresian used in AI was single dose/estrus cow which prepared from Lamphayaklang AI Bull Center, Department of Livestock Development at Lopburi provinces. Pregnancy detection was preformed with the same skillful veterinarian by rectal palpation method on day 60 after AI.

Statistical analysis

Estrus response of each group was defined: % of estrous cows that the cows showed signs or symptoms of estrus, serum progesterone (P_4) levels < 0.1 ng/ml (Virakul *et al.*, 2001) and serum estrogen hormone (Estradiol, E_2) levels ≥ 7.17 pg/ml when the cows were in estrus (Pavasuthipaisit *et al.*, 1986). All values of Estradiol level from each individual cows in the same group were expressed as the mean and standard deviation. Statistical analysis were performed with Statistical package for the social sciences (SPSS) program version 11 by one way ANOVA method.

Results

The signs of estrus were detected in all of 60 cows on day 3 after $PGF_{2\alpha}$ injection. Every cow presented the sign of estrus with uterine contraction when cows were palpated by rectal palpation. The least of estrus cows showed the sign of mounting and riding other cows after $PGF_{2\alpha}$ injection. The sign of estrus response were expressed in table 1.

Table 1 The sign of estrus response of all estrus cows in 3 groups after $PGF_{2\alpha}$ injection at IM, IRF and IVSM

Parameter	Value (no./total) of estrus cows after $PGF_{2\alpha}$ injection at IM	Value (no./total) estrus cows after $PGF_{2\alpha}$ injection at IRF	Value (no./total) estrus cows after $PGF_{2\alpha}$ injection at IVSM
-Percentage cows showed the sign of standing heat	25% (5/20)	45% (9/20)	15% (3/20)
-Percentage cows showed the sign of mounting and riding other cows	15% (3/20)	10% (2/20)	20% (4/20)
-Percentage cows showed the sign of vaginal mucous	40% (8/20)	45% (9/20)	50% (10/20)
-Percentage cows showed the sign of uterine contraction	100% (20/20)	100% (20/20)	100% (20/20)

The concentration of serum progesterone (P_4) levels and serum Estradiol (E_2) levels from each individual cows in all of 3 groups < 0.1 ng/ml and > 7.17 pg/ml respectively. The mean and standard deviation values of Estradiol (E_2) levels from 20 cows of group 1 after $PGF_{2\alpha}$ injection at IM, from 20 cows of group 2 after $PGF_{2\alpha}$ injection at IRF and from 20 cows of group 3 after $PGF_{2\alpha}$ injection at IVSM were 27.35 ± 8.09 pg/ml, 26.15 ± 9.07 pg/ml and 27.40 ± 6.61 pg/ml respectively. The mean \pm SD of serum E_2 levels from all of 3 groups were not significant different ($p > 0.05$) and expressed in table 2.

Table 2 The mean \pm SD of serum E_2 levels from all cows of each group on day 3 after $PGF_{2\alpha}$ injection.

Group no.	Parameter	Value (pg/ml)
1	Mean \pm SD of serum E_2 levels from 20 cows after $PGF_{2\alpha}$ injection at IM	$27.35 \pm 8.09^*$
2	Mean \pm SD of serum E_2 levels from 20 cows after $PGF_{2\alpha}$ injection at IRF	$26.15 \pm 9.07^*$
3	Mean \pm SD of serum E_2 levels from 20 cows after $PGF_{2\alpha}$ injection at IVSM	$27.40 \pm 6.61^*$

* No differential significant ($p > 0.05$)

The result of pregnancy detection from all cows of 3 groups by rectal palpation method showed in table 3. The number of pregnant cows from the first and the second group were similar but the least number of pregnant cows was found from the third group.

Table 3 The result of pregnancy detection from all cows of 3 groups by rectal palpation method on day 60 after AI.

Group no.	Parameter	Value (no./total)
1	Percentage of pregnant cows	75% (15/20)
2	Percentage of pregnant cows	75% (15/20)
3	Percentage of pregnant cows	45% (9/20)

Discussion

The results from this study showed that use of $\text{PGF}_{2\alpha}$ injection with full dose at IM or reduce dose at IRF or IVSM, can synchronize estrus in dairy cows and improve estrus cow by the sign of estrus detection and the concentration of serum progesterone (P_4) levels and serum Estradiol (E_2) levels. From this experiment showed the sign of estrus with uterine contraction when cows were palpated by rectal palpation in all of 60 cows after $\text{PGF}_{2\alpha}$ treatment. But the estrus sign of mounting and riding other cows was found in the least number (9 cows) of estrus cows. It was observed that the sign of estrus was difference in each cow. Because it depended on the individual cow, estrus detection (Pursley *et al.*, 1997) and the stage of follicular growth in ovary at the time of luteal regression (De Rensis and Peters, 1999). For the concentration of serum progesterone (P_4) levels < 0.1 ng/ml and Estradiol (E_2) levels > 7.17 pg/ml were found in all of 60 cows that express all 60 cows were in estrus period (Virakul *et al.* 2001, Pavasuthipaisit *et al.*, 1986). The mean \pm SD of serum E_2 levels from 20 cows in group 2 on day 3 after $\text{PGF}_{2\alpha}$ injection at IRF with the mean \pm SD of serum E_2 levels from 20 cows in group 3 on day 3 after $\text{PGF}_{2\alpha}$ injection at IVSM were similarly ($p > 0.05$) and this both groups had the mean \pm SD of serum E_2 levels not significant difference from the mean \pm SD of serum E_2 levels from 20 cows in group 1 on day 3 after $\text{PGF}_{2\alpha}$ injection at IM ($p > 0.05$). The confirming of successfully estrus synchronization after $\text{PGF}_{2\alpha}$ treatment at IRF and IVSM with IM were pregnancy detection of cow after AI by rectal palpation. They were found, had the pregnant cows in all of 3 groups about on day 60 after estrus synchronization and AI. Although in the third group was found percentage of pregnant cows less than the first and the second groups. Because the conception rate of estrus cow after synchronization might be also depending on many other factors.

Then in this study presented that the using of reduced dose $\text{PGF}_{2\alpha}$ by injection at IRF or IVSM with IM to synchronize estrus cows were more similarly efficiency. It was similar to the previous report of Atthapinit *et al.* (2001), Colazo *et al.* (2002a), Colazo *et al.* (2002b), Horta *et al.* (1986), Punyapornwithaya *et al.* (2005) or Virakul *et al.* (2001).

The success of estrus synchronization by reduced dose $\text{PGF}_{2\alpha}$ injection at IRF was discussed by Colazo *et al.* (2002a). The $\text{PGF}_{2\alpha}$ was deposit in fat tissue of subcutaneous when it was injected into IRF. Its distribution from injection area to blood flow was prolonging. The large number of $\text{PGF}_{2\alpha}$ signals and more effectively CL luteolysis were done from this mechanism. For Atthapinit *et al.* (2001) discussed the success of estrus synchronization by reduced dose $\text{PGF}_{2\alpha}$ injection at IVSM. The $\text{PGF}_{2\alpha}$ would be deposit in fat tissue of vulva-submucosa and absorbed to utero-ovarian vein for effectively CL luteolysis on ovary in the same side of injection area.

Conclusion

We can conclude, based on economic benefit, from this study that the using of reduced 40% PGF_{2α} from full dose injection at IRF and 75% PGF_{2α} from full dose injection at IVSM will produce full effective route for estrus synchronization in dairy cows.

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การศึกษาเปรียบเทียบการตอบสนองต่อการใช้ฮอร์โมน พรอสตาแกลนดิน เหนี่ยวนำการเป็นสัดในแม่โคนม ด้วยวิธีการฉีดเข้ากล้ามเนื้อ การฉีดเข้าแอ่งโคนหาง และการฉีดเข้าเยื่อเมือกปากช่องคลอด

จตุพร กระจายศรี^{1*} กิตติศักดิ์ แสงสกุล² และจิรัฏฐ์ รัตนเทพ²

¹ภาควิชา คลินิกสำหรับสุติ-ไถเน่วิทยา แอนโดรวิทยา และการผสมเทียมของสัตว์เลี้ยง

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเทคโนโลยีมหานคร หนองจอก กรุงเทพฯ 10530

โทรศัพท์ 0 2988 3655 9 ต่อ 247, 107, โทรสาร 0 2988 4040

²ศูนย์วิจัยการผสมเทียม และเทคโนโลยีชีวภาพ ชลบุรี บ้านบึง ชลบุรี 20220

*ผู้เขียนผู้รับผิดชอบ; e-mail: jakajaysri@hotmail.com

บทคัดย่อ

แม่โคนมถูกผสมไฮสไตร์น ฟรีเซียน หลังคลอดลูก มีวงรอบการเป็นสัดปกติ และมีคอร์ปัส ลูเทียม อยู่บนรังไข่ข้างใดข้างหนึ่ง จำนวน 60 ตัว ถูกแบ่งออกเป็น 3 กลุ่ม กลุ่มละ 20 ตัว กลุ่มที่ 1 แม่โคทุกตัว ถูกเหนี่ยวนำให้เป็นสัดด้วยการฉีดฮอร์โมน พรอสตาแกลนดิน ชนิดสังเคราะห์ (Cloprostenol) ขนาด 500 ไมโครกรัม (2 มล./แม่โค 1 ตัว) ของ Cloprostenol เข้ากล้ามเนื้อ กลุ่มที่ 2 แม่โคทุกตัว ถูกเหนี่ยวนำให้เป็นสัดด้วยการฉีด Cloprostenol ขนาด 300 ไมโครกรัม (1.2 มล./แม่โค 1 ตัว) เข้าแอ่งโคนหาง และกลุ่มที่ 3 แม่โคทุกตัวถูกเหนี่ยวนำให้เป็นสัดด้วยการฉีด Cloprostenol ขนาด 125 ไมโครกรัม (0.5 มล./แม่โค 1 ตัว) เข้าเยื่อเมือกปากช่องคลอด พบว่า 3 วันหลังจากฉีด พรอสตาแกลนดิน แม่โคทั้ง 60 ตัวแสดงอาการเป็นสัดโดยมีการบิบทดตัวและเกิดโตนของมดลูก เมื่อถูกคลำผ่านทางทวารหนักของแม่โค นอกจากนี้ยังพบว่าระดับความเข้มข้นของ ฮอร์โมนโปรเจสเตอโรน และเอสตราไดออล ในซีรัมของ แม่โคทุกตัวอยู่ในระดับที่ต่ำกว่า 0.1 นาโนกรัม/มิลลิลิตร และสูงมากกว่า 7.17 พิโคกรัม/มิลลิลิตร ตามลำดับ โดยค่าเฉลี่ยระดับความเข้มข้นของ เอสตราไดออล ในซีรัมของแม่โคในกลุ่มที่ 1, 2 และ 3 คือ 27.35 ± 8.09 พิโคกรัม/มิลลิลิตร, 26.15 ± 9.07 พิโคกรัม/มิลลิลิตร และ 27.40 ± 6.61 พิโคกรัม/มิลลิลิตร ตามลำดับ ซึ่งค่าเฉลี่ยดังกล่าวของทั้ง 3 กลุ่มไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($p > 0.05$) และเพื่อยืนยันว่า การใช้ฮอร์โมน พรอสตาแกลนดิน แบบทั้ง 3 วิธีดังกล่าวข้างต้น สามารถเหนี่ยวนำให้แม่โคเป็นสัดได้จริง จึงทำการผสมเทียมแม่โคทั้ง 60 ตัวนี้ที่เป็นสัดหลังถูกเหนี่ยวนำ ผลปรากฏว่าในกลุ่มที่ 1, 2 และ 3 มีแม่โคตั้งท้อง 75% (15/20 ตัว), 75% (15/20 ตัว) และ 45% (9/20 ตัว) ตามลำดับ จากผลการศึกษา แสดงให้เห็นว่าการใช้ฮอร์โมนพรอสตาแกลนดิน ในขนาดที่ลดลง 40% ของขนาดเต็ม ฉีดเข้าตำแหน่ง แอ่งโคนหาง และในขนาดที่ลดลง 75% ของขนาดเต็ม ฉีดเข้าได้เยื่อเมือกปากช่องคลอด มีประสิทธิภาพ ในการเหนี่ยวนำการเป็นสัดในแม่โคนม ไม่ต่างจากวิธีการฉีดเต็มขนาดเข้ากล้ามเนื้อ

คำสำคัญ : พรอสตาแกลนดิน, เข้ากล้ามเนื้อ, เข้าแอ่งโคนหาง, เข้าเยื่อเมือกปากช่องคลอด, โคนม