Short Communication

Low Pregnancy Rate in Dairy Cattle after Fixed time Artificial Insemination using Norgestomet + PGF2α + eCG Program During the Hot and Humid Months in Thailand

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Abstract

The present study was conducted to synchronize estrus and fixed time insemination in dairy cattle during the hot and humid months (July to November) in Thailand. Thirty-four cows and heifers were treated by using norgestomet+PGF2α+equine chorionic gonadotropin (eCG) program. Norgestomet was implanted subcutaneously at the back of the ear for 10 days. PGF2α was administered IM on day 8 and one injection of eCG was given on the day of implant removal. Fixed time artificial insemination(AI) were performed twice, at about 54-60 and 70-74 h, after the eCG injection. Blood samples were collected at 22 days after AI for progesterone (P4) assay. Real time B-mode ultrasonography was used to determine the pregnancy between 27-30 days after AI and then pregnancy was confirmed by rectal palpation between 60-90 days after AI. Of 34 cows, 16 cows (47%) had serum P4 higher than 1 ng/ml, while only 5 cows (15%) were positive for pregnancy detection using ultrasounography and rectal palpation. Cows with Body Condition Score (BCS) of 3 and non-repeat breeder cows tended to have a higher pregnancy rate than cows with BCS of 2.5 and repeat breeder cows. In conclusion, low pregnancy rate was found after using hormonal treatments during the hot and humid months in Thailand.

Keywords : estrus synchronization, dairy cattle, norgestomet, pregnancy

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บทคัดย่อ

อัตราการตั้งท้องต่ำในแม่โคที่ถูกเหนี่ยวนำให้เป็นสัดด้วยโปรแกรม norgestomet + PGF2α + eCG และผสมเทียมแบบกำหนดเวลา ในเดือนที่ร้อนชื้นของประเทศไทย

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การศึกษาครั้งนี้มีจุดประสงค์เพื่อศึกษาการตั้งท้องในแม่โคนมที่เหนี่ยวนำให้เป็นสัดและผสมเทียมแบบกำหนดเวลาในช่วงเดือนที่มีอากาศร้อนและความชื้นสูง (กรกฎาคม-พฤศจิกายน) โดยเหนี่ยวนำการเป็นสัดและตกไข่ด้วยโปรแกรม norgestomet+ PGF2α+eCG และผสมเทียมแบบกำหนดเวลา แม่โคนม 34 ตัว ถูกคัดเลือกและเหนี่ยวนำให้เป็นสัดโดยมีรายละเอียดของโปรแกรม คือ ทำการฝังฮอร์โมน norgestomet ใต้ผิวหนังบริเวณใบหูเป็นเวลา 10 วัน ในวันที่ 8 ฉีด PGF2α ทำการถอดแท่งฮอร์โมนและฉีด eCG ในวันที่ 10 โคทั้งหมดได้รับการผสมเทียม 2 ครั้งที่ 54- 60 และ 70-74 ชั่วโมง หลังฉีด eCG เก็บตัวอย่างเลือดในวันที่ 22 หลังการผสมครั้งที่ 1 เพื่อทำการตรวจวัดระดับฮอร์โมนโปรเจสเตอโรน ตรวจการตั้งท้องด้วยเครื่องอัลตราซาวด์ในวันที่ 27-30 หลังการผสม และตรวจยืนยันการตั้งท้องโดยการล้วงคลำผ่านทางทวารหนักในวันที่ 60-90 หลังผสม ผลการตรวจวัดระดับฮอร์โมนโปรเจสเตอโรนพบว่าโค 47.10% (16/34) มีโปรเจสเตอโรนสูงกว่า 1 นก./มล.ในวันที่ 22 หลังผสม ในจำนวนนี้เหลือเพียง 14.70% (5/34) ที่ตั้งท้องเมื่อตรวจด้วยเครื่องอัลตราซาวด์และตรวจการตั้งท้องด้วยการล้วงผ่านทางทวารหนัก การศึกษาครั้งนี้พบว่าการมีอัตราการตั้งท้องอยู่ในระดับที่ต่ำ เมื่อใช้โปรแกรมฮอร์โมนเพื่อทำการเหนี่ยวนำการเป็นสัด และการผสมเทียมแบบกำหนดเวลาเพื่อแก้ไขปัญหาความไม่สมบูรณ์พันธุ์ในโคนมระหว่างเดือนที่ร้อน และมีความชื้นสูง

คำสำคัญ : การเหนี่ยวนำการเป็นสัด โคนม นอร์เจสโตเมท การตั้งท้อง

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Introduction

Low reproductive performance is one of the major reproductive problems that cause a significant economic loss in dairy farming (Hall et al., 2004). Virakul et al. (2001) investigated the reproductive performance in dairy cattle across Thailand and found that reproductive performance in dairy cattle in Thailand was relatively poor with an average 183 days open in cows and an average age at first conception of 31.9 months in heifers. Department of Livestock Development (DLD, 2004) found for four reproductive parameters for dairy cow in Thailand that: 1) the conception rate at first service was 45.7%; 2) the total conception rate was 38.5%; 3) days open was an average 196 days; 4) age of first calving was 33.8±6.6 months. To achieve a high reproductive efficiency in dairy cows, the standard inter-calving interval of 365 days is necessary (Stevenson, 2001).

Heat stress is a major contributing factor to the low fertility of dairy cows inseminated during the hot and humid month (Wolfenson et al., 2000). This is especially true in tropical areas like Thailand, where the temperature exceeds 30°C for most months of the year (Suadsong et al., 2001). Researchers found that veterinary supervision combined with a program of estrus synchronization and fixed time insemination can improve fertility of cows suffering heat stress (De Rensis et al., 2002).

Norgestomet-based programs have been developed to synchronize estrus in cattle (Stevenson et al., 2003; Colazo et al., 2005). Duffy et al. (2004) found that the injection of 5 mg estradiol valerate and 3 mg norgestromet at the time of insertion of a 3 mg norgestomet implant resulted in new follicle wave emergence in 1-7 days later in post-partum nursing calves beef cows. Equine chorionic gonadotropin (eCG) was effective in inducing estrus after implant removal. This increased ovulation rate in cows treated before dominant follicle selection. Suadsong et al.(2001) reported a significantly higher pregnancy rate (29%) in cows synchronized with progesterone and estradiol than that of the control (18%). Our present study was aimed to investigate the pregnancy rate in dairy cattle after using norgestomet+PGF2α+eCG program and fixed time artificial insemination during the hot and humid months.

Materials and Method

Animals: This study was conducted from July to November, 2006, in a commercial dairy farm with 335 heads located in Chonburi province. During the study period, a total of 145 cows were routinely checked for pregnancy at 2 months after AI by rectal palpation. Body condition scoring used a rating scale of 1 to 5 where 1: extremely thin, 5: extremely obese (Ferguson et al., 1994). Screening for metritis used the Metricheck® device (Intervet, Thailand; McDougall et al., 2006) was performed in non-pregnant cows. A total of 36 cows with body condition score (BCS) of at least 2.5 and clear mucus discharge were included in the study.

Treatment and Artificial Insemination (AI):

A group of non pregnant cows (n=34); repeat breeders (n=12) and non-repeat breeders (n=22) were implanted subcutaneously with 3 mg norgestomet at the back of the ear. This was followed immediately by IM administration of 3 mg norgestomet and 5 mg estradiol valeratr (Crestar®, Intervet Int. B.V., The Netherlands). Repeat breeder cows were defined as a cow failing to conceive after at least three inseminations performed during apparently normal estrus (Rizzo et al., 2007). The date of implant was assigned as Day 1. On Day 8, 0.98 mg of a synthetic prostaglandin $F_{2\alpha}$, tiaprost trometamol (Illiren®, Intervet Int. B.V., The Netherlands) was intramuscularly administered. The implant was removed on Day 10 and 400 I.U. of eCG (Folligon®, Intervet Int. B.V., The Netherlands) was given at the time of implant removal. Two artificial inseminations were performed between 54 to 60 hours and between 70 to74 hours after the eCG injection. The program of treatment was described in figure 1.

Progesterone assay and pregnancy diagnosis:

Blood samples were collected from the coccygeal vessels at 22 days after the first AI using vacuum tubes and allowed to clot at room temperature. Sera were separated and then stored at -20°C prior to progesterone assays. Serum progesterone concentrations were determined by radioimmunoassay (RIA)(Coat-A-Count progesterone®,DPC, USA). A progesterone concentration at the level of ≥1 ng/ml indicated the presence of a functional corpus luteum. Pregnancy diagnosis was performed at about 27-30 day after the first AI using a real time B-mode ultrasound scanner (Agroscan L, S.E.C.

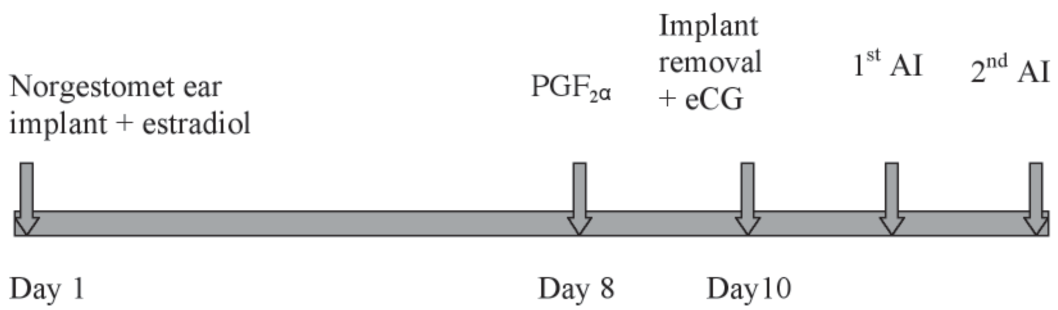

Figure 1. Schematic Representation of Estrus Synchronization Program.

Repro Inc., Ange-Gardien-de-Rouville, Québec, Canada, equipped with a 7.5 MHz linear-array transducer) and between 60 to 90 days by rectal palpation.

Climate data:

Climate data such as daily mean and maximum temperature mean and mean relative humidity were obtained from the Chonburi Meteorological Station located less than 50 km from the herd. Mean Temperature-Humidity index (THI) was calculated and was fitted to the following equation (Garcia-Ispierto et al., 2007):

Mean THI = (0.8 x mean T + (mean RH (%)/100) x (mean T - 14.4) + 46.4) Where T is the temperature, and RH is relative humidity.

Statistical analyses:

Pregnancy rates were analyzed using logistic regression. The GENMOD procedure of the Statistical Analysis Systems (SAS Institute Inc., version 9.0, Cary, NC, USA) was used. Three statistical models were conducted. Pregnancy rate at 20, 30 and 60 days of gestation were assigned as dependent variables for each statistical model. The statistical models included lactation number (<3 and >3), BCS (2.5 and 3) and number of insemination in each cow(< 3 and >3) as fixed effects. Data are presented as percent and proportion and the differences were considered significant when the probability value for equality between least squares means was less than 5%.

Results and Discussion

The overall results of the norgestomet+PGF2α+ eCG treatment was shown in Table 1 and climate data were shown in Table 2. Cows were categorized by lactation number, BCS and repeat or non-repeat breeder.

The conception rate in treated cows on Days 22 was 47%. However, the pregnancy rate decreased at Day 27-30 and Day 60-90. This study shown that norgestomet-PGF2α-eCG program combination with fixed time AI can be used in dairy cattle with an acceptable fertilization rate. This result is consistent with previous report (Humblot et al., 2001, Santos et al., 2004). Only 15 % of the cows became pregnant on days 60-90 post AI. This may due to embryonic mortality during the first month of pregnancy. It is possible that some of cows in the study were not normal and had repeat breeding problems. Seasonal effects can be one another cause.

In this study, the cows were inseminated during the hot and humid months of July and August with a high Temperature-Humidity Index (Table 2; THI~81-82), which negatively correlated with the herd conception rate. It should be noted that the reliability of progesterone tests to detect pregnant cows is estimated to be around 80%. This gives a false positive rate of 20%. The common reasons for errors in progesterone measurement are: 1) cows with persistent corpus luteum; 2) short estrus intervals and 3) luteal cysts (Shrestha et al., 2004). In this study, the progesterone test should have detected real pregnancy about 13 cows. But our result found only 5 cows were pregnant on days 27-30 and 60-90. This result indicates that high embryonic loss occurred before days 27-30 after insemination. This finding agrees with previous studies(Suadsong et al., 2001). Starbuck et al.(2004) demonstrated that retention of pregnancy varied with age of the cows. Heifers showed the highest pregnancy, followed by younger cows. Older cows had the lowest pregnancy. This study found no statistical difference in pregnancy rates among lactation number. This may be partly explained because the sample size was too small and this was not enough power to test statistical difference regarding lactation number.

Body condition score can affect the pregnancy rate (Starbuck et al., 2004). This study supports this previous finding. Cows with BCS of 2.5 tended to have a lower pregnancy rate than cows with BCS of 3.0. This finding is also similar to Moreira et al.(2000). Body condition score measurements allow producers to follow the evolution of body reserves in cows around calving

Table 1 The overall pregnancy rate categorized by Lactation Number, BCS, and Repeat Breeder.

	Pregnancy rate % (Pregnant cow/ Total cow)			
	N	**Day 22**	**Day 27-30**	**Day 60-90**
Total	**34**	**47(16/34)**	**15(5/34)**	**15 (5/34)**
Lactation number				
<3*	19	47 (9/19)	11 (2/19)	11 (2/19)
≥3*	15	47 (7/15)	20 (3/15)	20 (3/15)
BCS				
2.5**	20	30 (6/20)	10 (2/20)	10 (2/20)
3**	14	71 (10/14)	21 (3/14)	21 (3/14)
Repeat breeder				
No***	22	59 (13/22)	23 (5/22)	23 (5/22)
Yes***	12	25 (3/12)	0 (0/12)	0 (0/12)

$^{*}p > 0.05$; $^{**}p = 0.07$; $^{***}p = 0.01$

Table 2 Mean monthly temperatures and humidity for the study year (2006)

Month	**Mean *T* (°C)**	**Mean RH (%)**	**Mean THI**
January	22.77	64.26	77.21
February	29.34	68.21	80.07
March	29.99	72.17	81.65
April	30.46	72.77	82.46
May	29.98	76.26	82.27
June	30.02	75.00	82.13
July	30.18	72.71	82.01
August	29.49	74.16	81.18
September	28.83	79.10	80.88
October	29.27	75.94	81.10
November	29.17	66.83	79.61
December	27.06	59.77	75.61

T, temperature; RH, relative humidity; THI, temperature-humidity index.

and early lactation. Due to negative energy balance induced by high milk production and lack of food intake, cows often mobilize body reserves and loose weight showing BCS losses-especially during early lactation. This study supports previous research performed in temperate and tropical conditions showing that high BCS losses are associated with impaired reproductive function. Cows in poor condition showed a significant reduction in pregnancy rate at first AI on average 9% compared to cows in intermediate condition (Lopez-Gatius et al, 2003). Repeat breeder cows with more than three AIs tend to have a poor conception rate compared to those cows with less than three AIs. Chebel et al. (2004) reported that increasing the number of AI was associated with reduced conception rate by an average of 7.0%. It is probable that cows with an increased number of AIs have lower fertility, which further compromises conception rate. In conclusion, low pregnancy rate was found after using hormonal treatments during the hot and humid months in Thailand.

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