Comparison of progesterone implant and GnRH administration after insemination on pregnancy rate in dairy cows exposed to summer heat stress in Thailand

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Abstract

Objective - This study was conducted to investigate the effects of exogenous P_4 ear implant and gonadotropin releasing hormone (GnRH) administration after artificial insemination (AI) on serum P_4 concentrations and pregnancy rates in lactating cows during summer.

Materials and Methods - A total of ninety crossbred (93% Holstein Friesian) lactating cows were randomly allocated into three groups (n=30 per group): (i) received a P_4 ear implant (Crestar® without injection of norgestomet and estradiol valerate) for 14 days (day 5 to day 19 after AI; (ii) were injected with GnRH (Dalmarelin®) on day 5 after AI; (iii) were injected with physiological saline (placebo) on day 5 after AI. Blood samples were collected on day 12 and day 19 after AI. Serum samples from twenty cows were randomly selected in each group for P_4 analysis. Ambient air temperature and relative humidity were recorded three times a day inside the housing for the temperature humidity index (THI) calculation.

Results - The average daily THI throughout the study period was 81.0±2.3 (73.9-85.9), sixty two percentage of average THI values were higher than 80 which was moderate stress condition for dairy cows. The treatment groups had a tendency of higher concentrations of P_4 than the control group on day 12. However, no significant difference of P_4 among the groups was found on day 12 and 19. The pregnancy rates of P_4, GnRH, and control groups were 6.7%, 20.0% and 23.3%, respectively. The result revealed no significant different on pregnancy rate (p>0.05) among the groups.

Conclusion - The treatment of P_4 and GnRH on day 5 after AI had no effect on P_4 concentrations and also could not increase pregnancy rate in dairy cows exposed to summer heat stress in this study.

Keywords: dairy cows, GnRH, pregnancy rate, progesterone, summer

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เปรียบเทียบโปรเจสเตอโรนชนิดฝังหูและจีเอนอาร์เอช หลังการผสมเทียม ต่ออัตราการตั้งท้องในโคนมในช่วงฤดูร้อนในประเทศไทย

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บทคัดย่อ

วัตถุประสงค์ ศึกษาผลของการเสริมโปรเจสเตอโรนชนิดฝังหู และการให้จีเอนอาร์เอช หลังการผสมเทียม ต่อความเข้มข้นของโปรเจสเตอโรนและอัตราการตั้งท้องในโคนมในช่วงฤดูร้อน

วัสดุ อุปกรณ์ และวิธีการ โคนมลูกผสม 90 ตัวถูกสุ่มจัด 3 กลุ่มกลุ่มละ 30 ตัว โดยในวันที่ 5 หลังผสมเทียม กลุ่มที่ 1 ได้รับโปรเจสเตอโรนแบบฝังหู เครสต้าร์ โดยไม่ฉีดนอร์เจสโตเมทและเอสตร้าไดออลวาลีเรท นาน 14 วัน (วันที่ 5-19 หลังผสม) กลุ่มที่ 2 ได้รับจีเอนอาร์เอช (ดาลมารีลิน) และกลุ่มที่ 3 ได้รับน้ำเกลือ โคทุกตัวถูกเก็บเลือดในวันที่ 12 และ 19 หลังผสมเทียม แต่ละกลุ่มถูกสุ่ม 20 ตัว เพื่อตรวจความเข้มข้นโปรเจสเตอโรน ทำการบันทึกอุณหภูมิและความชื้นในโรงเรือนวันละ 3 ครั้ง เพื่อคำนวณดัชนีอุณหภูมิความชื้น (ทีเอชไอ)

ผลการศึกษา พบว่าค่าเฉลี่ยทีเอชไอรายวันตลอดการศึกษาเป็น 81.0±2.3 (73.9-85.9) โดยที่ 62% สูงกว่า 80 ซึ่งเป็นภาวะความเครียดปานกลางในโคนม ความเข้มข้นโปรเจสเตอโรนในกลุ่มทดลองมีแนวโน้มสูงกว่ากลุ่มควบคุมในวันที่ 12 แต่ไม่แตกต่างในวันที่ 19 อัตราการตั้งท้องในกลุ่มโปรเจสเตอโรน กลุ่มจีเอนอาเอช และกลุ่มควบคุมเป็น 6.7%, 20.0% และ 23.3% ตามลำดับ โดยไม่พบความแตกต่างระหว่างกลุ่มทดลอง ($p>0.05$)

ข้อสรุป การเสริมโปรเจสเตอโรนหรือจีเอนอาเอช หลังผสมเทียมในโคนมไม่มีผลต่อความเข้มข้นของระดับโปรเจสเตอโรน และไม่เพิ่มการตั้งท้อง ในช่วงฤดูร้อนที่แม่โคได้รับความเครียดจากความร้อนชื้น

คำสำคัญ: โคนม จีเอนอาร์เอช อัตราการตั้งท้อง โปรเจสเตอโรน ฤดูร้อน

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Introduction

Heat stress occurs when animals are unable to maintain their body temperature under high environmental temperature conditions [1]. The periods of high ambient air temperature coupled with high relative humidity compromise the ability of cows to dissipate excess body heat. The temperature humidity index (THI) is a value representing the combined effects of air temperature and humidity associated with the level of heat stress on animal. In dairy cows, it is generally accepted that cows are under moderate heat stress at THI higher than 80. The weather conditions of Thailand are characterized as hot and humid with a mean temperature of 29.8°C and mean relative humidity of 74% during summer [2]. which give rise to a THI value of 86.6. The adverse effects of heat stress are the result of either the hyperthermia by itself or the physiological adjustments of the cows to control body temperature [3]. Cows under heat stress exhibit lower dry matter intake, milk yield, and reproductive efficiency [4]. Heat stress can affect several aspects of cow reproduction such as expression of estrus, development of follicles and corpus luteum which may alter the metabolism of P_4, quality of oocytes, protein synthesis of embryos during the morula and blastocysts periods, and endometrial activity [5, 6].

P_4 plays an important role in maintenance of pregnancy. Decreased secretion of luteal P_4 and associated with an embryo development resulted in increase of early embryonic loss. Supplementation of P_4 at different stages of early embryonic development increased peripheral P_4 concentrations and embryo survival rate [7]. A high percentage of embryonic loss was observed in cows bred during summer in Thailand [8]. Therefore, additional reproductive strategies are needed to counteract with the adverse effects of heat stress on cow fertility during summer [9]. Insertion of controlled internal drug release (CIDR) from day 5 to day 10 post insemination improved pregnancy rates in first and second lactation dairy cows [10]. However, intra vaginal device cause vaginitis in some cows which might affect the conception rate [14]. Supplementation of P_4 through ear implant could avoid vaginitis. Administration of GnRH at insemination and/ or 5 days post AI increased pregnancy rate in lactating dairy cows in Thailand [11]. Administration of GnRH or human chorionic gonadotropin (hCG) on day 5 post AI in lactating cows during summer showed tendency to improve conception rate by increasing serum P_4 [12]. Administration of GnRH on day 11 after ovulation also improved pregnancy and calving rates following transfer of *in vitro* produced embryos to heat stress lactating cows [13].

It is hypothesized that heat stress during summer might cause P_4 insufficiency in early pregnancy resulting in embryonic loss. Increased P_4 levels will increase pregnancy rate. Supplementation with exogenous P_4 ear implant and GnRH administration on day 5 after AI would increase circulating P_4 concentrations and also decrease pregnancy loss.

Materials and Methods

Animals, treatments and data collection

The study was conducted on a commercial dairy farm located in Nakhonratchasima province of Thailand during April-September 2013. Ninety Holstein Friesian crossbred (93% HF) lactating cows were included in this study. The cows were at the average age of 3.3±0.7 years, lactating number of 1.3±0.5, day in milk of 88.2±49.0 days, body condition score of 2.77±0.20, and milk yielding of 18.8±4.8 kg per day. The cows were kept in free stall housing with electric fans operated all day. Total mixed ration (TMR) which consisted of approximately 16% crude protein was offered daily in 3 equal portions at 04:00, 09:30, and 15:00.

Cows were visually monitored for standing oestrus based on mucous vaginal discharge and mounting activities and were inseminated at approximately 13.14±3.92 h after the onset of oestrus. One AI for each cow was conducted by two veterinarians using frozen semen from two bulls. The day of AI was considered as day 0 and cows entered the study on Day 5 after AI.

The cows were randomly allocated into three groups (30 cows per group): (i) exogenous P_4 supplementation group; cows received an ear implant containing 3 mg of norgestomet (Crestar®, Intervet International b.v. Boxmeer, The Netherlands) for 14 days (day 5 to day 19) without injection of norgestomet and estradiol valerate, (ii) GnRH group; cows received an injection of 50 mcg of lecrielin acetate (Dalmarelin®, FATRO S.P.A. Industrial Pharmaceutical Veterinarian and Bologna, Italy) on day 5 after AI, (iii) control group; 5 ml of physiological saline (placebo) was injected to the cows on day 5 after AI. The ambient air temperature and relative humidity were recorded three times daily (08:00, 12:00 and 15:00) inside the housing. The THI was calculated by using the formula: THI=0.72 (W+D) +40.6; where W= wet temperature in °C and D = dry temperature in °C [15].

On days 35-45 after AI, pregnancy diagnosis was performed using transrectal ultrasonography and reconfirmed by rectal palpation on day 60 after AI

Sampling and P_4 assay

On days 12 and 19 after AI, blood sample (6 ml) was collected from coccygeal vein and centrifuged at 3000 rpm for 10 minutes. The serum samples were collected and stored at -20°C until analysis. Serum samples from twenty cows in each group were randomly selected for P_4 analysis using electrochemiluminescence immunoassay with P_4 kit (Elecsys 1010, COBAS E 411, Roche Diagnostics GmbH, USA). All samples were analyzed in duplicate and values were expressed in ng/ml. The detection limit of the assay was 0.03 ng/ml, the inter-assay coefficient of variation (CV) and the intra-assay coefficient of variation for P_4 concentrations were 5.67% and 4.46%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS program version 19.0. The concentrations of P4 were subjected to analysis of variance (ANOVA). Least Significant Difference (LSD) test was used to identify difference between treatment means. Effect of treatment on pregnancy rate was determined by Chi-square test. Difference among treatments was considered significant when $p<0.05$.

Results

The comparisons of age, lactation number, days in milk, body condition score, and milk yield were conducted by using a One way-ANOVA test. No significant difference in any criteria was found among three groups as well as no difference among two inseminators and two bulls.

Ambient air temperature, relative humidity and THI

The average ambient air temperature, relative humidity, and THI in housing were 30.1±2.3 (24.3-35.3)°C, 70.9±7.3 (53.7-90.0) % and 81.0±2.4 (73.9-85.9), respectively. Sixty two percentage of average daily THI (115 days /183 days) were higher than 80.

Serum P_4 concentration and pregnancy rate

The data of P_4 are presented in Table 1. Treatment of Crestar® and GnRH showed a tendency of higher P_4 on day 12 after AI than the control group (8.53± 4.16, 8.31±3.16, 6.29±3.21 (P=0.07), respectively. No significant difference of P_4 among groups on day 19 after AI was found (4.15±5.45, 7.25±6.92, 6.12±5.22 ng/ml (P=0.25), respectively. In non pregnant cows, P_4 concentrations in Crestar® and GnRH groups were slightly higher than control group on day 12 after AI (p=0.07). However, no significant differences were found on day 19 after AI (p=0.98).

The overall pregnancy rate of cows in this study was 16.6%. The pregnancy results showed no significant difference among groups in which the pregnancy rate of Crestar®, GnRH, and control group were 6.7%, 20.0% and 23.3%, respectively (Table 2).

Table 1. Mean (±SD) serum progesterone concentrations (ng/ml) at day 12 and day 19 form cows treated with Crestar or GnRH on day 5 after AI.

Treatment	Progesterone concentrations (ng /ml)					
	All cows		Pregnant cows		Non pregnant cows	
	(n) Day 12	(n) Day 19	(n) Day 12	(n) Day 19	(n) Day 12	(n) Day 19
Crestar®	(20) 8.53±4.16	(20) 4.15±5.45	(2) 9.49±3.75	(2) 9.26±4.92	(18) 8.42±4.29	(18) 3.59±5.34
GnRH	(20) 8.31±3.16	(20) 7.25±6.92	(6) 7.84±3.01	(6)15.19±3.03	(14)8.51±3.32	(14) 3.85±5.01
Control	(20) 6.29±3.21	(20) 6.12±5.22	(7) 7.36±1.77	(7)10.28±4.45	(13)5.71±2.43	(13) 3.89±4.22
P value	0.070	0.254	0.597	0.084	0.073	0.982

Table 2. The pregnancy rates of cows in Crestar, GnRH and control group using ultrasonography on day 35-45 and rectal palpation on day 60 after AI.

Treatment (n)	Ultrasonography day 35-45	Rectal palpation day 60	Pregnancy rate (%)
Crestar® (30)	2	2	6.7%
GnRH (30)	6	6	20.0%
Control (30)	7	7	23.3%
P value			0.186

Discussion

The present study was carried out to compare the post AI administration of P_4 ear implant (Crestar®) and GnRH on serum P_4 concentrations and pregnancy rates in lactating cows under summer heat stress condition. The negative effects of heat stress on dairy cows are multifaceted. Summer heat stress has long been recognized for reducing the reproductive efficacy in dairy cows. The average daily THI in this study (81.0±2.4) was similar to previous studies during summer in Thailand [8,12]. It was shown that the conception rate was lowest when the THI were 79-89 all day [8]. In dairy cows, it is generally accepted that, cows are under mild to moderate heat stress at THI at above 72 and at THI higher than 80, cows are considered to be in moderate heat stress [16]. Our THI indicated that cows in this study were under mild to moderate heat stress throughout the study period. At moderate heat stress (THI= 80-89), saliva production, respiration rate, and body temperature of the cows will increase, feed intake may decrease but water consumption will increase. Milk production and reproduction will be decreased [17,18].

Studies showed that plasma concentrations of oestradial-17β, P_4, and luteinizing hormone were reduced in lactating cows during summer [6,17,19]. Low P_4 concentrations in blood partially explain the low fertility of cows undergone heat stress. A delayed rise in P_4 concentrations have been reported in low fertility and repeat breeder cows on summer [14]. The use of GnRH or hCG after AI increases P_4 secretion due to luteotropic effect and also increased pregnancy rate [23,24,25]. Studies have shown that supplementing cows with exogenous P_4 as CIDR early in post AI can increase pregnancy rates compared to control cows [20,21]. No study reported the use of Crestar as P_4 supplement in dairy cattle. Crestar® is an exogenous P_4 and GnRH had luteotropic effect, both could increase P_4 concentrations in blood and maintain pregnancy. Although the P_4 concentrations in this study were not significantly different among groups on day 12 and day 19, the Crestar® and GnRH groups had a tendency of higher P_4 than control group only on day 12. In GnRH groups, in the pregnant cows, P_4 on day 19 had a tendency of higher than other groups (p=0.08). GnRH could be a tool to increase P_4 than Crestar®. In non pregnant groups, Crestar® and GnRH groups had a tendency of higher P_4 than control group on day 12 (p=0.07). Crestar® could not increase P_4 on day 19 and seemed to be lower levels than on day 12 even though Crestar® was removed from cows on day 19. However, the level of P_4 in pregnant and non pregnant cows on day 12 and 19 were not different among groups.

The overall pregnancy rate of cows in this study was low (16.6%) and did not different among groups. This reflects the effect of summer heat stress on conception rate during the study period. Supplementation of exogenous P_4 ear implant (Crestar®) and GnRH could not increase pregnancy rate in this study. Other studies also reported the failure of exogenous P_4 supplementation via intravaginal device after AI could not improve pregnancy rate in dairy cows

[27,28]. GnRH treatment after AI cannot increase pregnancy rate in cows with body temperature higher than 39.7°C [22]. The failure of GnRH administration on day 5 and day 13 after AI did not improve pregnancy rate in several studies [24,25,26]. However, administration of GnRH or hCG on day 5 after AI have been shown to improve pregnancy rate after insemination during summer in several studies [11,12,23].

In summary, the present study fails to support the hypothesis that supplementation of exogenous P_4 by Crestar® and GnRH administration on day 5 after AI could increase P_4 and pregnancy rate in dairy cows under heat stress.

Conclusion

The treatment of P_4 and GnRH on day 5 after AI had no effect on P_4 concentrations and could not increase pregnancy rate in dairy cows exposed to summer heat stress.

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