Delayed Post-ovulatory Progesterone Rise in Thai-Holstein Dairy Heifers: Association with Endocrine Events around Oestrus, Effects on Conception Rate and Possibility for Progesterone Supplement Post-insemination

Sudsaijai Kornmatitsuk [1]* Bunlue Kornmatitsuk [1] Yindee Kitiyanant [2]

Abstract

The current research aimed to further study the delayed post-ovulatory progesterone (P4) rise in Thai-Holstein dairy heifers in the points of an association with endocrine events around oestrus (experiment 1), effects on conception rate and possibility for P4 supplement post-insemination (experiment 2). In experiment 1, the P4 levels in the delayed P4 heifers reached 1 ng/ml later than in the normal ones (6.8±0.4 vs 3.3±1.5 days after oestrus, resp.) (p = 0.053). At ovulation the pre-ovulatory follicles of the delayed P4 were smaller than of the normal P4 heifers (11.8±0.5 vs 13.1±0.2 mm, resp.) (p = 0.013). The levels of LH at their surges in the delayed P4 were lower than in the normal P4 animals (1.1±0.1 vs 1.8±0.2 ng/l, resp.) (p = 0.044), while the OE2 levels on the day of oestrus were not different (30.5±1.5 vs 28.8±1.3 pmol/l, resp.) ($p \geq$ 0.05). In experiment 2, within the control group (no exogenous P4 supplement), the delayed P4 heifers yielded lower conception rate compared to the normal P4 ones (40% vs 68.75%, resp.) (p < 0.001). In the treatment group, at 8 hours following P4 supplement, the levels of P4 significantly increased from 0.73±0.92 to 2.57±0.73 ng/ml (p < 0.001) and at 48 hours after cessation of P4 supplement, the P4 levels declined from 3.01±0.79 to 2.56±1.39 ng/ml (p = 0.0996). The heifers in the treatment group had higher conception rate (62.85%) compared with the delayed P4 control heifers (40%, p < 0.001) and with the overall control ones (52.43%, p = 0.0416), but not different if compare to the normal P4 control heifers (68.5%, $p \geq$ 0.05). In conclusion, the delayed post-ovulatory P4 rise in Thai-Holstein dairy heifers showed a strong connection with the smaller pre-ovulatory follicle and the lower levels of LH at their surges. The negative effects of the delayed post-ovulatory P4 rise on pregnancy were clearly confirmed. Moreover, supplementation with P4 on day 3 to day 7 after oestrus enhanced conception rates.

Keywords: conception rate, delayed post-ovulatory progesterone rise, progesterone supplement, Thai-Holstein dairy heifers

บทคัดย่อ

สภาวะระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ในโคนมสาวลูกผสมไทย-โฮลสไตน์: สาเหตุเชิงลึก ผลกระทบต่อการตั้งท้องและการอยู่รอดของตัวอ่อนระยะต้น และความเป็นไปได้ในการลดผล กระทบด้วยการให้ฮอร์โมนโปรเจสเตอโรนโดยตรง ณ ระยะต่าง ๆ หลังการเป็นสัด

สุดสายใจ กรมาทิตย์สุข [1] บรรลือ กรมาทิตย์สุข [1] ยินดี กิติยานันท์ [2]*

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาสาเหตุเชิงลึกของสภาวะระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ (การวิจัยย่อยที่ 1) ประเมินผลกระทบของระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าต่อการผสมติดและตั้งท้อง และศึกษาความเป็นไปได้ในการลดผลกระทบดังกล่าว ด้วยการให้ฮอร์โมนโปรเจสเตอโรนโดยตรง ณ วันที่ 3-7 หลังการเป็นสัด (การวิจัยย่อยที่ 2) การวิจัยย่อยที่ 1 ในโคสาวที่มีสภาวะโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ วันแรกที่ระดับโปรเจสเตอโรนสูงกว่า 1 นาโนกรัมต่อมล. ช้ากว่ากลุ่มปกติ (6.8±0.4 เปรียบเทียบกับ 3.3±1.5 วันหลังเป็นสัด) (p = 0.053) ฟอลลิเคิลตกไข่ของกลุ่มโปรเจสเตอโรนเพิ่มช้า มีขนาดเล็กกว่าของกลุ่มปกติ (11.8±0.5 เปรียบเทียบกับ 13.1±0.2 มิลลิเมตร) (p = 0.013) และระดับสูงสุดของฮอร์โมนลูทิไนซิงของกลุ่มโปรเจสเตอโรนเพิ่มช้า มีค่าต่ำกว่าของกลุ่มปกติ (1.1±0.1 เปรียบเทียบกับ 1.8±0.2 นาโนกรัมต่อมล.) (p = 0.044) อย่างไรก็ตาม ระดับสูงสุดของฮอร์โมนเอสตร้าไดออล 17 เบต้า ช่วงระยะเป็นสัดไม่แตกต่างกัน (30.5±1.5 และ 28.8±10.3 พิโคโมลต่อลิตร) ($p \geq$ 0.05) การวิจัยย่อยที่ 2 ในกลุ่มควบคุม โคสาวที่มีสภาวะโปรเจสเตอโรนเพิ่มช้า มีอัตราการผสมติดและตั้งท้องต่ำกว่าโคสาวปกติ (40% เปรียบเทียบกับ 68.75%) (p < 0.001) ในกลุ่มที่ได้รับการปรับเสริมโปรเจสเตอโรน แปดชั่วโมงหลังการปรับเสริมฮอร์โมน ค่าเฉลี่ยของระดับโปรเจสเตอโรนเพิ่มจาก 0.73±0.92 เป็น 2.57±0.73 นาโนกรัมต่อมล. (p < 0.001) และ 48 ชั่วโมง หลังสิ้นสุดการปรับเสริมฮอร์โมน ลดลงจาก 3.01±0.79 เป็น 2.56±1.39 นาโนกรัมต่อมล. (p = 0.0996) โคสาวที่ได้รับการปรับเสริมด้วยโปรเจสเตอโรน มีอัตราการผสมติดและตั้งท้องเพิ่ม (62.85%) เปรียบเทียบกับกลุ่มควบคุมที่มีสภาวะโปรเจสเตอโรนเพิ่มช้า และกลุ่มควบคุมรวม (40%, p < 0.001 และ 52.43%, p = 0.0416 ตามลำดับ) แต่ไม่แตกต่างจากกลุ่มควบคุมที่มีฮอร์โมนโปรเจสเตอโรนปกติหลังตกไข่ ($p \geq$ 0.05) จากการวิจัยสรุปได้ว่า 1) สภาวะระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ เกี่ยวข้องโดยตรงกับขนาดของฟอลลิเคิลตกไข่ที่เล็กกว่า และระดับสูงสุดของฮอร์โมนลูทิไนซิงที่ต่ำกว่า 2) สภาวะระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ มีผลกระทบด้านลบต่อการผสมติดและตั้งท้อง และ 3) การปรับเสริมฮอร์โมนโปรเจสเตอโรนโดยตรง ณ วันที่ 3-7 หลังการเป็นสัด ช่วยลดผลกระทบดังกล่าวได้

คำสำคัญ: อัตราการผสมติดและตั้งท้อง สภาวะระดับฮอร์โมนโปรเจสเตอโรนเพิ่มช้าหลังตกไข่ การปรับเสริมฮอร์โมนโปรเจสเตอโรน โคนมสาวลูกผสมไทย-โฮลสไตน์

[1] *ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล พุทธมณฑล นครปฐม 73170*

[2] *คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล และสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยมหิดล พุทธมณฑล นครปฐม 73170*

*ผู้รับผิดชอบบทความ E-mail: Sudsaijai.kor@mahidol.ac.th

Introduction

Progesterone (P4) is among the key hormones in the control of embryo survival and conception status. Low P4 has been well linked to subtle conception rate (see López-Gatius et al., 2004, for review) according to insufficient embryo growth (Vanroose et al., 2000).

Our study on characteristics of oestrous cycles in Thai-Holstein dairy heifers (Kornmatitsuk et al., 2009) demonstrated a 'spontaneous' delayed post-ovulatory P4 rise, which one way or another is connected to the most classical problem of our dairy industry, infertility. Concentration of P4 during early conception is now believed to keep embryos survive in their mothers' uterus. This is confirmed by a number of studies which humble P4 concentration was shown to strongly correlate with failure to conceive (see López-Gatius et al., 2004, for review). In combination, Mann and Lamming (2001) suggested that as little as a one-day delay in the rise of P4 post-ovulation might cause embryos to fail to elongate and secrete interferon *tau* by day 16 of life. There has been evidence that diameter and steroid capability of the ovulatory dominant follicle affects corpus luteum afterwards (Mann et al., 2001; Vasconcelos et al., 2001). Kornmatitsuk et al. (2009) also observed a loop of 'smaller ovulatory follicle–CL with cavity–delayed rise in P4–(delayed CL regression)–quick growing but smaller ovulatory follicle and hence poor CL'. It is, hence, of challenge to figure out an (or a combined) underlying cause of, and a precise manner to undo, the loop of the delayed rise in post-ovulatory P4, either at endocrine or at cell levels.

Many studies attempted to ensure the effects of P4 supplementation on conception rate in dairy heifers and cows. Supplementing animals with P4 has been showed to enhance conception rate. Different

treatments, however, have yielded different results (for review, see Mann et al., 2006). Hence, the aim of our current study was to further focus on a delayed post-ovulatory P4 rise in Thai-Holstein dairy heifers, in the points of an association with endocrine events around oestrus, effects on conception rate and possibility for P4 supplement post-insemination.

Materials and Methods

Animals and experimental designs: The current study was divided into 2 experiments in which a total of 86 dairy heifers were included, 16 animals for experiment 1 and 70 animals for experiment 2. The animals were Thai indigenous and Holstein Frisian cross-breeds (over 75% HF) and about 15 months old at the time of the experiment. The heifers weighted 280-315 kg and their body scores were 3.0–3.5. The animals were housed at a commercial farm in Saraburi province with a free-stall system and were fed 4 times a day total mixed ration (TMR, 12% protein, 15% crude fiber and 64% total digestible nutrients), at least 14 kg DMI. The experimental protocol was reviewed and approved by the local animal ethics committee at Mahidol University, following the procedure of the National Research Council, Bangkok, Thailand.

Experiment 1 aimed to study an association of the delayed post-ovulatory progesterone (P4) rise with endocrine events around oestrus. Sixteen animals were studied for one complete oestrous cycle, during which the heifers' ovaries were examined and blood, for assessment of progesterone, was collected once a day. For a period from first sight of strong oestrous signs until ovulation, at every 4th hour, the ovaries were scanned for timing of ovulation and blood was collected in order to illustrate oestradiol 17 beta and luteinising hormones' profiles.

Experiment 2, the effects of the delayed post-ovulatory P4 rise on conception rate and possibility for P4 supplement post-insemination were scrutinised. A total of 70 heifers which failed to conceive after 1st insemination were divided into control group (n = 35) and treatment group (n = 35). They were all artificially inseminated, but contrary to the control group, the animals in the treatment group were supplemented with exogenous P4 3-7 days after oestrus. Then, blood was collected at days 0, 3 (0800 h and 1600 h for the treatment group), 5, 7 and 9 after oestrus and pregnancy was examined at 30 and 60 days subsequently.

Both experiments, progesterone levels that exceeded 1 ng/ml later than 5 days after oestrus or later than 4 days after ovulation were classified as the delayed post-ovulatory P4 rises.

Clinical observation: The heifers were observed twice daily, in experiments 1 and 2, for signs of oestrus: excitement, vocalisation, licking, lowering of the back, vulvar edema and redness, mucous discharge, discharge color and uterine tone. These parameters were scored and summarized to give a total impression of the oestrous signs: 0: not in heat, 1: uncertain, 2: weak oestrous signs, 3: strong oestrous signs.

Ovarian examination: In experiment 1, the heifers' ovaries were examined using transrectal-ultrasonical technique. A real time B-mode ultrasound scanner (Falco Vet®, Esoata-Pie Medical, Italy) equipped with a 6-/ 8-MHz rectal linear-array transducer was used. Examinations were recorded on VCD/ DVD recorder for retrospective analyses. Appearances of the follicles as well as of the corpus luteum were documented according to Petyim et al. (2000).

Blood samplings and hormonal analyses: In experiments 1 and 2, blood samples were drawn from the heifers' tail vein into heparinised blood collection tubes. The samples were centrifuged at 3000 rpm for 10 minutes and the extracted plasma was stored at -20^{0}C until analysis. Progesterone was determined by means of EIA (enzyme-linked immunoassay) as described by Kornmatitsuk et al. (2007). Analysis for oestradiol 17 beta was performed using RIA (radio-immunoassay) with oestradiol double antibody validated for bovine plasma (Sirois and Fortune, 1999). Luteinizing hormone was validated by RIA but with a monoclonal antibody and a human tracer as described by Forsberg et al. (1993).

Artificial insemination and pregnancy diagnosis: In experiment 2, the heifers were artificially inseminated under a.m.-p.m. rule or about 12 hours after onset of standing oestrus. The insemination was taken care by the same veterinarian throughout the study and by using randomized batches of frozen semen. On day 30 and 60 after insemination, the animals were examined for pregnancy using a real time B-mode ultrasound scanner (Falco Vet®, Esoata-Pie Medical, Italy) equipped with a 6-/ 8-MHz rectal linear-array transducer. The heifers were diagnosed as pregnant when viable embryo proper was visualized.

Supplementation of hormone progesterone: Of the treatment group in experiment 2, the heifers were continuously subjected to P4 supplementation from days 3 to 7 after oestrus. Supplemented P4 was applied by using an intravaginal CIDR-B device containing 1.9 g progesterone (CIDR®, Pfizer (Thailand) Limited) which was inserted at 0800 h on day 3 and withdrawn at 0800 h on day 7 after oestrus.

Statistical analysis: Values are presented as mean ± standard deviation (SD). Unpaired *t*-test as well as chi-square test was used in order to test significant differences between means and between standard variations, respectively. Probability values (*p*) < 0.05 were considered to be significant.

Results

Experiment 1

Association of delayed post-ovulatory progesterone (P4) rise with characteristics of corpus luteum, pre-ovulatory follicle development and endocrine events around oestrus

According to the retrospective analysis of daily progesterone during one oestrous cycle, 10 (out of 16) heifers showed normal figures of P4 and the others (6 animals) were diagnosed with delayed post-ovulatory P4 rises. The levels of progesterone in the

delayed P4 heifers reached 1 ng/ml at 6.8±0.4 days after oestrus, which was significantly later than in the normal P4 ones (3.3±1.5 days post-oestrus, $p = 0.053$) (Table 1).

Association of delayed post-ovulatory progesterone rise with characteristics of the corpus luteum was presented in Table 1. Characteristics of the corpus luteum (CL) as detected by ultrasonical means appeared to be indifferent between the normal P4 and delayed P4 heifers. CL volumes (calculated by the formula $4/3 \times \pi \times r^3$, r (radius) = (Length/2 + Width/2)/2) on a detectable day were lower in the delayed P4 compared with the normal P4 heifers (435.7±190.0 versus 1100.3±221.3 mm³, respectively) ($p = 0.015$) but their diameters were not different (10.1±1.8 vs 11.9±2.1 mm, respectively) ($p \geq 0.05$). CL central cavity had the highest volume (2352.1 mm³ equal to 16 mm in diameter) in the delayed P4 heifers although their averages did not differ ($p \geq 0.05$). Similarly, an interval from CL detectable day to the first day that P4 reached 1 ng/ml was longer in the delayed P4 than in the normal P4 heifers (3.8±0.8 vs 0.5±2.1 days, respectively) ($p = 0.044$).

The profiles of P4 around mid and late oestrous cycles did not differ between the delayed P4 and the normal P4 patterns ($p \geq 0.05$). Those included the levels of P4 around mid di-oestrus (2.5±0.6 vs 3.6±1.2 ng/ml, respectively.), the accumulated days that P4 levels were higher than 1 ng/ml (13.0±1.9 vs 14.8±2.1 days, respectively.) and the interval from the last day of 1-ng/ml P4 level to the day of oestrus (1.0±0.7 vs 1.5±1.0 days, respectively.).

Association of delayed post-ovulatory progesterone rise with pre-ovulatory follicle development and endocrine events around oestrus was shown in Table 2. On the day of luteolysis or the last day that P4 levels were higher than 1 ng/ml, the diameter of a pre-ovulatory follicle did not differ for the delayed P4 and the normal P4 heifers ($p \geq 0.05$). At ovulation the pre-ovulatory follicles of the delayed P4 were smaller than of the normal P4 ones (11.8±0.5 vs 13.1±0.2 mm, respectively) ($p = 0.013$). However, the interval from an onset of oestrus to ovulation did not differ compared between two P4 patterns ($p \geq 0.05$).

The profiles of oestradiol 17 beta (OE2) and luteinising (LH) hormones were drawn based on 4-hour interval blood samplings. A peak of OE2 at oestrus was not different between the delayed P4 and the normal P4 heifers (30.5±1.5 vs 28.8±10.3 pmol/l, respectively) ($p \geq 0.05$). Unlikely, the levels of LH when surged were lower in the delayed P4 than in the normal P4 ones (1.1±0.1 vs 1.8±0.2 ng/l, respectively) ($p = 0.044$).

Table 1 Characteristics of corpus luteum and changes in progesterone (Mean±SD) of the delayed P4 heifers compared to of the normal heifers

	Delayed P4	Normal
Characteristics of corpus luteum		
Detectable (day *)	3.3±0.8	3.5±1.3
Volume on detectable day (mm³)	435.7±190.0 [a]	1100.3±221.3 [b]
Onset of regression (day *)	19.0±2.5	18.3±0.5
Lifespan (days)	21.0±2.4	18.8±0.5
Minimum – maximum volume of central cavity (mm³)	33.5 – 2352.1	33.5 – 268.1

Corpus luteum versus progesterone

	Delayed P4	Normal
The first day P4 ≥ 1 ng/ml*	6.8±0.4 [a]	3.3±1.5 [b]
Detectable day to the first day P4 ≥ 1 ng/ml (days)	3.8±0.8 [a]	0.5±2.1 [b]
Volume on the first day P4 ≥ 1 ng/ml (mm³)	4304.8±2040.4	1932.5±1398.3
Mean concentrations of progesterone during mid oestrous cycle (ng/ml)	2.5±0.6	3.6±1.2
The last day P4 ≥ 1 ng/ml *	19.8±2.9	17.3±2.9
Interval from the first to the last day P4 ≥ 1 ng/ml (days)	13.0±1.9	14.8±2.1
Interval from the last day P4 ≥ 1 ng/ml to the day of oestrus (days)	1.0±0.7	1.5±1.0
Concentrations of progesterone on the day of oestrus (ng/ml)	0.3±0.1	0.5±0.2

* day 0 is the day of oestrus.
[a-b] Different superscripts between columns denote significant differences ($p < 0.05$).

Table 2 Changes of pre-ovulatory follicles and profiles of hormone oestradiol 17 beta (OE2) and luteinising hormone (LH) around oestrus (Mean±SD) compared between delayed P4 heifers and normal heifers.

	Delayed P4	Normal
Pre-ovulatory follicles		
Diameter on the last day P4 ≥ 1 ng/ml (mm)	10.9±1.9	11.0±2.0
Diameter at ovulation (mm)	11.8±0.5 [a]	13.1±0.2 [b]
Interval from onset of oestrus to ovulation (hrs)	25.2±10.2	24.8±9.5
Oestradiol 17 beta and luteinising hormones		
Peak of OE2 on the day of oestrus (pmol/L)	30.5±1.5	28.8±10.3
Peak of LH prior to ovulation (ng/ml)	1.1±0.1 [a]	1.8±0.2 [b]
Interval from LH peak to ovulation (hrs)	23.8±3.2	23.0±1.4

* day 0 is the day of oestrus.
[a-b] Different superscripts between columns denote significant differences ($p < 0.05$).

Table 3 Conception rates (%) * and effect (+ %) of progesterone supplementation at day 3 – 7 after oestrus on conception rates, compared between treatment and control (delayed P4, normal P4 and overall) groups.

Conception rates of the control group:	Treatment group	Effect	Significance
Delayed P4 40	62.85	+ 22.85	$p < 0.001$
Normal P4 68.5	62.85	- 5.65	$p \geq 0.05$
Overall 52.43	62.85	+ 10.42	($p = 0.0416$)

* Pregnancy diagnosis gave similar results, either at 30 or 60 days after insemination

Experiment 2

In experiment 2, the effects of the delayed post-ovulatory progesterone rise on conception rate were scrutinized based on the control group, which was not subjected to exogenous P4. Out of 35, 16 of them were diagnosed as having delayed rise in P4 and the other 16 were normal. The rest of the control heifers showed an unexpected pattern of P4 and were excluded from the experiment later on.

Figure 1 illustrates the P4 profiles of the control groups, comparing between the delayed P4 and the normal P4 heifers. The P4 levels were not different between them on day 0 (day of oestrus), day 3 and day 9 ($p \geq 0.05$). Of the delayed P4 heifers, the P4 levels were 0.11±0.13, 0.25±0.21 and 2.48±0.87 ng/ml, respectively on days 0, 3 and 9 whilst of the normal P4 heifers were 0.24±0.24, 0.38±0.25 and 2.78±0.63 ng/ml. Significantly, the levels of P4 were lower in the delayed P4 heifers on day 5 and day 7 (0.69±0.19 and 1.52±0.63 ng/ml, respectively) compared to the levels in the normal P4 heifers (1.32±0.32 and 2.49±0.68 ng/ml, respectively) ($p < 0.001$).

Either at 30 days or 60 days after insemination, pregnancy diagnosis gave resembling outcomes. The (overall) control heifers got 51.43% of conception rate. Comparing between the different patterns of P4, the delayed P4 heifers yielded lower conception rate than the normal P4 ones (40% vs 68.75%, respectively) ($p < 0.001$). However, if dividing into pregnant and non-pregnant categories, no difference in the P4 levels was shown on each day of samplings (Fig 2). Exceptionally on day 3, in the delayed P4 heifers, the levels of P4 were lower in the
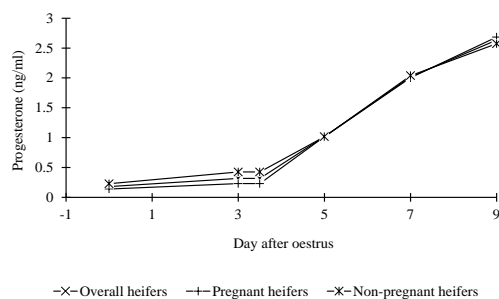
significantly, from 3.01±0.79 to 2.56±1.39 ng/ml ($p = 0.0996$).

Comparing to the control heifers and prior to supplementation, the levels of P4 in the treatment ones did not differ either on day 0 or day 3 (0.18±0.20 and 0.32±0.24 ng/ml, respectively for the treatment group vs 0.14±0.20 and 0.73±0.92 ng/ml, respectively for the control group) ($p \geq 0.05$). After supplementation, the P4 levels were significantly higher ($p < 0.001$) in the treatment group than in the control one. At 8 hours, the levels of P4 were 2.57±0.73 ng/ml in the treatment group and 0.32±0.24 ng/ml in the control group. On day 5, the levels were 2.52±0.76 and 1.02±0.41 ng/ml and on day 7, 3.01±0.79 and 2.02±0.81 ng/ml in the treatment and the control groups, respectively. However, at 48 hours following the end of P4 supplement, the levels of P4 did not differ between the treatment and the control groups (2.56±1.39 vs 2.63±0.75 ng/ml, respectively) ($p \geq 0.05$).

The data in Table 3 shows that the heifers in the treatment group had higher conception rate (62.85%) compared to the delayed P4 control heifers (40%, $p < 0.001$) and to the overall control

Figure 1 Progesterone profiles of delayed P4 control, normal P4 control and overall control groups at days 0, 3, 5, 7 and 9 after oestrus

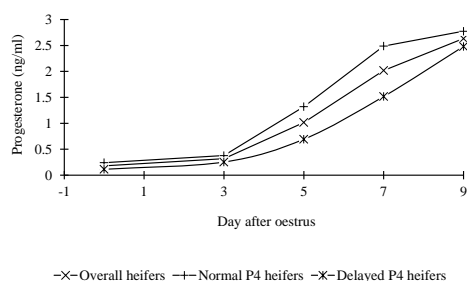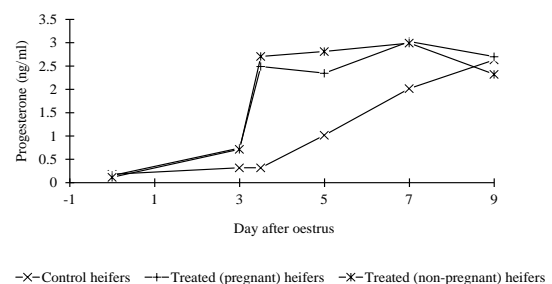**Figure 2** Progesterone profiles of control group divided into pregnant and non-pregnant heifers at days 0, 3, 5, 7 and 9 after oestrus

Figure 3 Progesterone profiles of treatment and control groups at days 0, 3 (0800 h and 1600 h), 5, 7 and 9 after oestrus
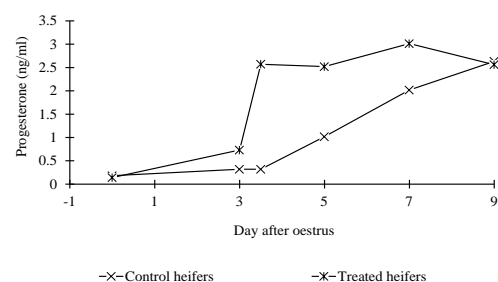

Figure 4 Progesterone profiles of treatment group divided into pregnant and non-pregnant heifers at days 0, 3 (0800 h and 1600 h), 5, 7 and 9 after oestrus

non-pregnant heifers compared to the pregnant ones (0.11±0.09 vs 0.35±0.22 ng/ml) ($p = 0.0138$).

The P4 profiles of the treatment group were drawn in Figure 3. At 8 hours following supplement with exogenous P4, the levels of P4 significantly increased from 0.73±0.92 to 2.57±0.73 ng/ml ($p < 0.001$). Meanwhile, at 48 hours after cessation of P4 supplement, the P4 levels declined, but not

ones (52.43%, $p = 0.0416$), but not different if compared to the normal P4 control heifers (68.5%, $p \geq 0.05$). Figure 4 illustrates the patterns of P4 in the treatment group based on pregnant or non-pregnant categories. No difference in the P4 levels was noted on each day, except at 48 hours following the end of P4 supplement. At that hour, the levels of P4 were slightly higher in the pregnant treatment heifers compared to the non-pregnant ones (2.70±1.51 vs 2.32±1.17 ng/ml) ($p = 0.4136$).

Discussion

Our previous results confirmed the fact that Thai-Holstein dairy heifers are facing the problem of a 'spontaneous' delayed post-ovulatory progesterone (P4) rise (Kornmatitsuk et al., 2009). It is undoubted that a delay in the rise of post-ovulatory P4 leads to less embryo survival in many species including dairy heifers and cows (Mann and Lamming, 2001; Baird et al., 2003). The underlying causes, unfortunately, are rarely documented.

Pooling entire data from experiment 1, we clearly established certain connection of the delayed post-ovulatory P4 rise with a pre-ovulatory follicle development and endocrine events around oestrus especially 1) smaller pre-ovulatory follicles and 2) lower levels of luteinising hormones at their surges. The follicles belonging to the delayed P4 heifers had smaller sizes at ovulation compared to the normal P4 ones. This is in agreement with Mann and Starbuck (2004) and Starbuck et al. (2006). Robinson et al. (2005), as well, stated that the cows with larger follicles produced CLs with higher P4 producing capacity and, hence, higher plasma P4 during the critical early luteal phase than the cows with smaller follicles. Connecting to conception, Perr et al. (2005) further speculated that the ovulation of follicles that were physiologically immature had a negative impact on embryo development. Surprisingly, but similarly to Robinson et al. (2006), although the diameters of the pre-ovulatory follicles at ovulation were smaller in the delayed P4 heifers, there was no difference in the oestradiol 17 beta concentrations around oestrus and the CL diameters were not different compared to the normal P4 heifers. In the case, Wathes et al. (2003) suggested that the 'quality' of the pre-ovulatory follicles was a determinant of subsequent luteal function, not solely relating to their size. In our study, we also demonstrated the lower levels of LH at their surges, which logically were either caused by or, in turn, led to the inferior development of the pre-ovulatory follicles and subsequent CL (Starbuck et al., 2006; Kornmatitsuk et al., 2009). On the other hand, the connection of CL, i.e. sizes and detection day of the CL, with the probability of the delayed P4 rise could not be drawn. Thus, it seems difficult to predict whether the P4 rise will be delayed or normal based on the characteristics of the CL or even the pre-ovulatory follicles.

Collecting data from experiment 2, the negative effects of the delayed post-ovulatory P4 rise on conception rate were evidently confirmed. This is in agreement with Mann and Lamming (2001) and Mann et al. (2006). Not only the concentration, but the timing of the post-ovulatory P4 rise is critical to maintain an appropriate synchronization between the ovary-endometrium and embryo because any luteal inadequacy might cause dramatic effects. Mann and Lamming (2001) demonstrated that as little as one day delay in the rise in post-ovulatory P4 significantly reduced the subsequent development of the embryo. The reasons behind the negative effect of the delayed P4 rise on pregnancy were described elsewhere. Undoubtedly, P4 plays an important role in stimulating the production of a variety of endometrial secretions necessary for the successful development of embryos. During early pregnancy, embryos need to inhibit the development of the luteolytic mechanism to maintain the secretion of P4 necessary for continuing development. Embryos must secrete, interferon *tau* (IFN-τ), protein which acts locally within the uterus to inhibit luteolytic PGF 2alpha secretion by depressing the development of oxytocin receptors on the luminal epithelium (Robinson et al., 1999) and through the induction of a prostaglandin synthesis inhibitor (Scenna et al., 2005).

Many schedules have been tried on the effects of P4 supplementation on pregnancy rate in dairy heifers and cows. For example, López-Gatius et al. (2004) showed that progesterone supplementation had the potential to reduce the incidence of pregnancy loss during early fetal period. More recently, Mann et al. (2006) demonstrated that early P4 supplementation from day 5 to day 9, but not later supplementation from day 12 to day 16, increased both trophoblastic length fivefold and uterine IFN-τ concentrations. In our study, we began the supplementation of exogenous P4 on day 3 as our aim was to increase P4 concentration up to 1 ng/ml not later than the end of day 4 after oestrus, according to the P4 patterns of the normal P4 heifers (from experiment 1). Our results did confirm the benefit of P4 supplementation early on day 3 and continuing to day 7 after oestrus on pregnancy rate, corresponding to Mann et al. (2006), who concentrated on the timing of P4 supplementation by showing that the treatments given by approximately day 3–5 of pregnancy consistently improve pregnancy rates while later supplementation is much less effective.

In conclusion, the delayed post-ovulatory progesterone rise in Thai-Holstein dairy heifers was well associated with the smaller pre-ovulatory follicle and the lower levels of luteinising hormone at their surges. The negative effects of the delayed post-ovulatory progesterone rise on pregnancy were clearly confirmed. Moreover the supplementation with exogenous progesterone on day 3 to day 7 after oestrus was of benefit to the conception rate.

Acknowledgements

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