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ผลลัพธ์โครงการ (Output)

1. องค์ความรู้ใหม่และการนำไปปรับใช้ (รายละเอียดใน ‘เนื้อหางานวิจัย’ หัวข้อ สรุปผลวิจัย ข้อ 2 องค์ความรู้ใหม่และการนำไปปรับใช้)

2. ผลงานวิจัยที่คาดว่าจะตีพิมพ์

2.1.1 Genuine figures for characteristics of oestrous cycles in ‘Thai-Holstein’ cross-bred dairy heifers with special reference to follicular dynamics and certain hormonal changes (Manuscript 1; ส่งเพื่อตีพิมพ์ในวารสาร Reproduction in Domestic Animals)

2.1.2 A simple technique for minimal dose DIUI (deep intra-uterine insemination) studying in PMSG multiple-ovulating dairy heifers (Manuscript 2; ส่งเพื่อตีพิมพ์ในวารสารสัตวแพทยสาร)

2.1.3 บทความวิชาการภาษาไทยในหัวข้อ 1) คลื่นฟอลลิเคิล: ความสำคัญ ลักษณะเปรียบเทียบ และการนำไปปรับใช้ และ 2) การใช้อัลตราซาวนด์ช่วยในการตรวจระบบสืบพันธุ์โคนม (อยู่ในระหว่างการจัดเตรียม; ส่งเพื่อตีพิมพ์ในวารสารสัตวแพทยสาร)

3. กิจกรรมอื่นที่เกี่ยวข้อง

3.1.1 ผลงานอื่น

3.1.1.1 ได้รับเชิญเป็นวิทยากรในการอบรมเชิงปฏิบัติการดังนี้

3.1.1.1.1 The International Training Course of Swamp Buffalo Reproduction 2 ครั้ง วันที่ 28 เมษายน 2548 และ 26 มีนาคม 2549 ณ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

3.1.1.1.2 เทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม ครั้งที่ 1 เรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ และการตรวจการตั้งครรภ์ระยะต้น (The Use of Ultrasonography in an Examination of Reproductive Tracts and Early Pregnancy Diagnosis) ในระหว่างวันที่ 18–28 พฤษภาคม 2548 ณ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล และโรงพยาบาลสุทัศน์และสัตว์ป่า วิทยาเขตไพรโยค จังหวัดกาญจนบุรี

3.1.1.2 ได้รับเชิญเป็นผู้วิจัยร่วมในโครงการวิจัยเรื่อง การพัฒนาของฟอลลิเคิลในช่วงวงจรการเป็นสัดในกระบือปลัก (Follicular Dynamics during Estrous Cycle in Swamp Buffalo) หัวหน้าโครงการคือ ศาสตราจารย์ ดร. นายสัตวแพทย์มงคล เดชะกัมพู ภาควิชาสัตวศาสตร์ ฐานเวชวิทยา และวิทยาการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

3.1.2 ความเชื่อมโยงทางวิชาการกับนักวิชาการอื่น

3.1.2.1 ศาสตราจารย์ ดร. นายสัตวแพทย์มงคล เดชะกัมพู ภาควิชาสัตวศาสตร์ ฐานเวชวิทยา และวิทยาการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โดยเป็นผู้วิจัยร่วม (รายละเอียดในหัวข้อกิจกรรมอื่นที่เกี่ยวข้อง ผลงานอื่น ข้อ 3.1.1.2)

3.1.2.2 Associate Professor Paul M. Fricke มหาวิทยาลัย Wisconsin-Madison ประเทศสหรัฐอเมริกา โดยเป็นวิทยากรร่วมในการอบรมเชิงปฏิบัติการ เทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม ครั้งที่ 1 ณ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล (รายละเอียดในหัวข้อกิจกรรมอื่นที่เกี่ยวข้อง ผลงานอื่น ข้อ 3.1.1.1.2)

ภาคผนวก

Title

Genuine figures for characteristics of oestrous cycles in 'Thai-Holstein' cross-bred dairy heifers with special reference to follicular dynamics and certain hormonal changes.

Introduction

Through a decade, many studies have been made on characteristics of oestrous cycles (i.e., Rajakoski, 1960; Sirois and Fortune, 1988; Ginther et al., 1989; Duchens et al., 1995; Braw-Tal et al., 2004). Summing up, during the oestrous cycles, ovarian follicles grow in a manner that can be characterised by waves, most commonly 2 or 3 of which include the growth of the ovulatory follicles. Follicular wave, each, is characterised by the simultaneous emergence of 2–4 mm follicles that begin to increase in size. One, or sometimes more, follicles become dominant and continue to grow while the others undergo atresia. The dominant follicle, whose size can vary from 13 to 19 mm in diameter, remains dominant for a few days until it becomes atretic and is replaced by a new dominant follicle from the following follicular wave. If regression of the corpus luteum takes place during its growth phase, the dominant follicle continues to grow and eventually, ovulates. To our knowledge, however, no study has been scrutinised figures for characteristics of oestrous cycles in 'Thai-Holstein' cross-breed.

Precise information on oestrous cyclicity is of beneficial to optimise and yet enhance an efficiency of our breeding programme, either by precisely regulating ovarian follicular and/or corpus luteum or with implication of finest embryo-biotechnology. Hence, the aims of the current study were to illustrate genuine figures (in-housed normal) for the characteristics of oestrous cycles in our crossbred-Holstein dairy heifers. Characteristics of oestrous cycles were drawn especially on, and temporal associations within and between; follicular dynamics, corpus luteum and changes in progesterone and prostaglandin F2alpha.

Materials and methods

1. Definitions

Definitions for technical terms referred in the results part are, by order of present, as follows. **Follicle:** *Dominant follicle* of a wave (an-ovulatory or ovulatory) was a follicle that grew to at least 11 mm in diameter and exceeded the diameter of other follicles in the wave. *Detectable* (day) was the first sonically detection of a 4- or 5-mm follicle retrospectively identified as a dominant follicle. *Size* or follicular diameter (mm) was achieved by averaging of the length (l) and width (w) measurements or by a formula $(l + w)/2$. *Growth rate* (mm per day) was calculated by means of linear regression (slope) using day as independent variable and diameter, dependent variable. *Onset of atresia* (day) was the first day that a dominant follicle began a progressive decrease in diameter. *Number of emerged follicle* was an absolute number of follicles that emerged to the same pool with a dominant follicle of the wave. *Subordinate follicle* was a follicle that originated from the same follicular pool as, and grew in parallel with, a dominant follicle of the wave. **Corpus luteum:** *Volume* was luteal tissue volume of the CL (mm³) calculated on a basis of CL dimension (length, l and width, w). Firstly, radius (r) was calculated by the formula $R = (L/2 + W/2)/2$, and the volume by the formula $4/3 \times \pi \times r^3$. For CL with a cavity, the volume of the cavity was calculated and subtracted from the volume of the CL. *Onset of regression* (day)

was the first day that luteal volume (or dimension) was less than for each of the previous 3 days and continued to decrease after. *Lifespan* was a period where the CL was sonically detectable. *Luteal active period* was a period where concentrations of progesterone exceeded 1 ng/ml, and *mid-luteal period*, 2 ng/ml.

2. Animals

Twenty six healthy and sexual-mature virgin heifers were included in the study. They were of crossbred Holstein Frisian (over 75% HF) and about 15 months of age at the time of the experiment. The heifers had body weight at range 280–315 kilograms and body score 3.0–3.5. They were housed at a commercial farm, Saraburi province with a free-stall system and fed 4 times a day with total mixed ration (TMR, protein at 12%, crude fiber at 15% and total digestible nutrients at 64%), 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.

3. Clinical observation

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

4. Ovarian examination

The heifers' ovaries were examined using transrectal-ultrasonographical technique. A real time B-mode ultrasound scanner (Falco Vet[®], Esoata-Pie Medical, Italy) equipped with a 6-/8-MHz rectal linear-array transducer, by which each examination was recorded on VCD/ DVD recorder for retrospective analyses, was used. The numbers and the sizes of the follicles as well as of the corpus luteum (CL) were documented according to Petyim et al. (2000). Throughout the experiment, the heifers were examined once a day except for a period from first sight of strong oestrous signs until ovulation, every 4th hour.

5. Blood samplings and hormonal analyses

At experiment, blood samples were drawn from the heifers' tail vein using venipuncture as the same frequency as ovarian examination. Progesterone was determined by means of EIA (Enzyme-linked immunoassay) as described by Kornmatitsuk (accepted for publication in *Reproduction in Domestic Animals*). The main metabolite of PGF_{2α} (15-ketodihydro- PGF_{2α} [PG-metabolite]) was analysed by RIA, according to Granström and Kindahl (1982).

6. Statistical analysis

The values are presented as mean $\pm$ standard deviation (SD). Correlation between two set of data was estimated by Pearson's correlation coefficient (r). Unless otherwise stated, unpaired t -test and F-test were 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

1. Characteristics of oestrous cycles

1.1 Characteristics of oestrous cycle

Of the 26 oestrous cycles, 11 (42.3%) had 2 waves of follicular dynamics and 15 (57.7%), 3 waves ($P > 0.05$) (Table 1). The 2-wave (2-w) cycles represented shorter inter-oestrous intervals (19.8 ± 1.2 days) than the 3-wave (3-w) ones (22.5 ± 1.1 days) ($P < 0.001$). Seasons' change did not significantly affect on either the ratio of the cycles with 2 and 3 follicle dynamics or their characteristics ($P > 0.05$).

Table 1. Oestrous cycle characteristics (Mean $\pm$ SD), comparing 2- versus 3-wave cycles during cold (October – November) and hot season (March – May).

Characteristics	Overight		Cold season		Hot season	
	2-w cycle	3-w cycle	2-w cycle	3-w cycle	2-w cycle	3-w cycle
Percentages of 2- or 3-waves *	42.3	57.7	27.3	72.7	53.3	46.7
Inter-oestrous interval **	19.8 ± 1.2^a	22.5 ± 1.1^b	20.8 ± 0.7	22.2 ± 1.0	19.5 ± 1.2	22.8 ± 1.3
Oestrous length ***	18.4 ± 5.2	16.0 ± 11.3	–	–	–	–
Score of oestrous signs	+3	+3	–	–	–	–
Post-oestrous bleeding ****	54.2	41.1	–	–	–	–

Notes * Percentages of the oestrous cycles with 2- or 3-follicular waves.

** Interval between the day of oestrus of two consecutive oestrous cycles (days).

*** From the onset to the end of oestrus considering all oestrous signs (hours).

**** Percentages of the oestrous cycles with post-oestrous bleeding.

^{a-b} Different superscripts between columns denote significant differences ($P < 0.05$).

1.2 Oestrous behaviours

Oestrous length and summed score of oestrous signs are showed in Table 1. There was no difference on the duration of oestrus as well as the scored intensity of oestrous behaviours, comparing the 2-w or 3-w cycle ($P > 0.05$). Combining the data from the 2-w and the 3-w cycles, characteristics on estrous behaviours are described as follows. Oestrous signs were showed in all heifers. Post-oestrous bleeding, however, was manifested in approximate during half of the cycles studied. Separately, an excitement, vocalisation as well as mucous discharge were not one hundred-percent exposed at oestrus. The duration (referred to standing oestrus) and the end points (referred to ovulation) of oestrous signs were calculated. Vulva oedema, vulva redness and uterine tone were of longest duration (36.6 ± 5.5 , 37.7 ± 15.5 and 32.7 ± 12.5 hours, respectively). Regardless to the intensity, vocalisation, excitement, lowering of the back, mucous discharge and standing oestrus were ended at certain hours prior to ovulation (-20.8 ± 13.0 , -10.4 ± 15.3 , -9.8 ± 18.0 , -7.5 ± 18.9 and -1.4 ± 15.2 hours, respectively). Tone of the uterus, vulva oedema as well as vulva redness continued 6.1 ± 19.5 , 9.9 ± 15.1 , 11.1 ± 14.7 hours, respectively, after ovulation. The duration of standing oestrus highly correlated to of lowering of the back ($r = 0.98$, $P < 0.001$), of excitement ($r = 0.95$, $P < 0.02$) and of mucous discharge ($r = 0.94$, $P < 0.01$).

1.3 Ovarian dynamics and hormonal changes during oestrous cycles

There was no effect (P values range 0.21 – 0.94) of seasons' change on either ovarian dynamics or hormonal changes during oestrous cycles. Hence, following information is given with regardless to different (cold and hot) seasons.

Table 2. Quantitative characteristics of follicular dynamics which are presented as Mean $\pm$ SD (range), comparing 2- versus 3-wave cycles.

Characteristics *	2-wave cycle	3-wave cycle
Number of emerged follicles **		
1st wave	10.7 $\pm$ 1.0 ^a (10–12)	11.0 $\pm$ 2.8 ^a (6–14)
2nd wave	5.0 $\pm$ 1.4 ^b (3–6)	4.6 $\pm$ 1.0 ^b (3–6)
3rd wave	–	5.5 $\pm$ 1.8 ^b (2–7)
Number of subordinate follicles ***		
1st wave	0.6 $\pm$ 0.5 (0–1)	0.2 $\pm$ 0.4 (0–1)
2nd wave	0.9 $\pm$ 0.6 (0–2)	0.3 $\pm$ 0.5 (0–1)
3rd wave	–	0.8 $\pm$ 0.9 (0–2)

Notes * Characteristics on a basis of morphological viewpoints; pooled data regardless to seasons' change.

** and *** For descriptions, see under 'definitions' described elsewhere.

^{a-b} Different superscripts between columns denote significant differences ($P < 0.05$).

1.3.1 Follicular dynamics and characteristics

Characteristics of follicular dynamics during the 2-w and 3-w oestrous cycles are summarised in Tables 2 and 3. Between the 2-w and 3-w cycles, the number of emerged follicles did not differ ($P > 0.05$) comparing within the numerically matching (e.g. the 1st to the 1st) waves. Within the same patterned cycle but between the different waves, the number of emerged follicles was highest in the 1st one ($P < 0.001$) (Table 2).

Table 3. Characteristics of follicular dynamics during oestrous cyclicities, comparing 2- versus 3-wave cycles, (Mean $\pm$ SD).

Characteristics *	2-wave cycle	3-wave cycle
An-ovulatory dominant follicles **		
Detectable (day ***)	2.4 $\pm$ 0.5	2.6 $\pm$ 0.7, 9.9 $\pm$ 1.0
Reaching dominant size (day ***)	5.6 $\pm$ 0.7	6.3 $\pm$ 0.7, 13.2 $\pm$ 1.1
Maximum size (diameter, mm)	12.4 $\pm$ 1.5	12.1 $\pm$ 0.9 ^e , 10.2 $\pm$ 1.5 ^f
Growth rate (mm per day)	2.4 $\pm$ 0.4	1.8 $\pm$ 0.5 ^e , 1.2 $\pm$ 0.7 ^f
Onset of atresia (day ***)	13.3 $\pm$ 1.6	12.5 $\pm$ 1.2, 17.7 $\pm$ 2.0
Ovulatory dominant follicle		
Detectable (day ***)	12.1 $\pm$ 1.3 ^g	16.8 $\pm$ 0.9 ^h
Reaching dominant size (day ***)	16.6 $\pm$ 1.7 ^g	20.6 $\pm$ 1.3 ^h
Growth rate (mm per day)	1.5 $\pm$ 0.3	1.7 $\pm$ 0.3
Interval from onset of oestrus to ovulation (hrs)	22.0 $\pm$ 14.1	28.5 $\pm$ 8.7
Size at ovulation (diameter, mm)	12.5 $\pm$ 0.9	12.4 $\pm$ 1.1

Notes * Characteristics on a basis of morphological viewpoints; pooled data regardless to seasons' change.

** a+b, c+d represents the characteristics of the dominant follicles of the 1st and the 2nd wave, resp.

*** day 0 is the day of oestrus.

^{e-f} and ^{g-h} Different superscripts denote significant differences ($P < 0.05$) within and between columns, resp.

Of the 1st an-ovulatory wave, no significant difference ($P > 0.05$) was denoted on the day of emergence and on individual events comparing 2- and 3-wave cycles. Within either the 2-w or 3-w cycles, the dominant follicle (DF) of the 1st wave grew faster than of the 2nd wave (ovulatory one for the 2-w cycle) ($P < 0.05$ and 0.001 , respectively). However, within the 3-w cycle, the growth rates of the 1st and of the 3rd (ovulatory) DFs were not different ($P > 0.05$). Ovulatory dominant follicles (OF) exhibited and reached dominant size earlier in the 2-w than in the 3-w ones ($P < 0.001$). Unless otherwise stated, no statistical difference was manifested in any characteristics of the OFs of the 2-w and of 3-w cycles, which included the deviation of the hours from oestrus to ovulation ($P = 0.36$; data not showed in the table).

1.3.2 Corpus luteum and changes in hormones progesterone-prostaglandin F_{2α} (in connection with events of ovulatory dominant follicle)

Table 4. Characteristics of corpus luteum in connection with its progesterone, ovulatory dominant follicle and prostaglandin F_{2α} (Mean ± SD), comparing 2- versus 3-wave cycles.

Characteristics *	2-wave cycle	3-wave cycle
Corpus luteum		
Detectable (day **)	3.5±1.3	3.7±0.8
Volume on detectable day (mm ³)	793.6±666.7	775.9±468.9
Onset of regression (day **)	17.9±2.0	19.6±1.4
Lifespan *** (days)	19.1±2.7	20.4±2.3
Corpus luteum versus progesterone		
Onset of luteal active period (day **)	5.6±2.2	6.0±1.7
Detectable day to onset of luteal active period (days)	3.0±1.4	3.8±1.0
Volume at onset of luteal active period (mm ³)	3723.0±1524.5	4088.1±2429.3
Mean concentrations of progesterone during luteal active period (ng/ml)	2.7±0.8	2.5±1.1
Onset of mid-luteal period (day **)	10.5±2.1	11.0±2.9
Volume at onset of mid-luteal period (mm ³)	4128.1±904.5	4057.31±692.8
Mean concentrations of progesterone during mid-luteal period (ng/ml)	3.0±0.9	2.9±1.2
End of luteal active period (day **)	19.0±1.6	20.0±1.7
Duration of luteal active period (days)	12.5±1.7	13.7±1.2
Progesterone versus ovulatory dominant follicle		
Interval from ovulatory dominant follicle detectable to end of luteal active period (days)	7.4±2.0 ^a	3.8±1.0 ^b
Size of ovulatory dominant follicle at end of luteal active period (diameter, mm)	11.2±1.3	9.9±1.7
Interval from end of luteal active period to oestrus (days)	0.8±0.5	1.8±1.0
Concentrations of progesterone at oestrus (ng/ml)	0.5±0.2	0.3±0.2
Prostaglandin F_{2α}		
Interval from surge of prostaglandin F _{2α} to end of luteal active period (days)	1.2±1.0	1.0±0.7
Interval from surge of prostaglandin F _{2α} to oestrus (days)	2.0±0.6	2.0±0.7
Interval from surge of prostaglandin F _{2α} to ovulation (days)	3.5±0.7	3.0±1.0

Notes * Pooled data regardless to seasons change.

** day 0 is the day of oestrus.

*** For descriptions, see under 'definition' described elsewhere.

^{a-b} Different superscripts between columns denote significant differences ($P < 0.05$).

Information on characteristics of the corpus luteum (CL) and its progesterone (P4) in connection with events of the OFs and changes in prostaglandin F2alpha (PGF_{2α}) are showed in Table 4. There was no difference on morphological as well as P4-connected characteristics of CL comparing the 2- and 3-wave cycles. The CL regression, however, commenced earlier during the 2- than during the 3-w cycles ($P = 0.1$). For the 2-w and 3-w cycles, at 5.6 ± 2.2 and 6.0 ± 1.7 days (respectively) after oestrus or at 3.0 ± 1.4 and 3.8 ± 1.0 days (respectively) later than sonically CL detection, P4 began to rise to exceed 1 ng/ml and continued increasing to 2 ng/ml at 3.60 ± 1.52 and 3.80 ± 2.49 days (respectively) afterwards (data was not showed). Close to the set-off day of CL regression, P4 declined to less than 1 ng/ml, numerically earlier during the 2-w than during the 3-w cycles ($P = 0.46$), in concert to the shorter duration of the CL active periods ($P = 0.33$). Regardless to different-patterned cycles, there were no correlations between the volume of the CL and concentrations of P4 either during the active or the mid-periods ($r = -0.10$; $P > 0.05$ and $r = -0.68$; $P < 0.05$, respectively).

In connection with the events of the OFs, single statistical difference was showed on the interval from OF detectable to the end of active period of the CL, of which was longer in the 3-w than in the 2-w cycles ($P = 0.01$). Referred to the day P4 began to decline, mean-diameter of the OFs was smaller and after-period to oestrus, longer in the 3-w than in the 2-w cycles ($P > 0.05$). At oestrus, certain concentrations of P4 were detected (range 0.15 to 0.60 ng/ml.) during both the different-patterned cycles. The interacted days of PGF_{2α} to P4 as well as to oestrus and ovulation did not significantly differ between the 2-w and 3-w cycles.

Discussion and conclusions

Our crossbred-Holstein dairy heifers did not show difference in ratio of the 2-waves and 3-waves patterned cycle. Seasons' change did not affect on characteristics oestrous cycles as well as on dynamics of follicles and corpus luteum. Unless otherwise stated, pattern of oestrous cycles, follicle-corpus luteum dynamics and changes in progesterone mimic harmonically considering the data from many researchers (i.e., Rajakoski, 1960; Sirois and Fortune, 1988; Ginther et al., 1989; Duchens et al., 1995; Wolfenson et al., 2004). Certain diversities, however, were drawn as follows: the 1st an-ovulatory dominant follicles showed higher growth rate and hence earlier exceeded dominant diameter. This may be in accordance to the delayed post-ovulatory progesterone rise (Robinson et al., 2006) which was prominently showed in our study – to be discussed. Looking on the ovulatory dominant follicle of either of the 2- or of the 3-wave cycles, the follicle tended to quicker ovulate but(or and) with a smaller diameter at ovulation. The corpus luteum developed after ovulation and seemed to have corresponding characteristics except for more than 80 percent exhibited 4 to 16.5 mm in diameter of central cavity. In connection to the levels of progesterone, the corpus luteum turned into active, as well as mid-luteal, period quite late. The duration of the active period of the corpus luteum was shorter. At the end of the cycle –around the day of oestrus, progesterone remained certain low but significant levels. To discuss, our crossbred-Holstein dairy heifers are facing the problem of delayed post-ovulatory progesterone rise which one way or another connected to the most classical problem of our dairy industry 'infertility'. Concentration of progesterone during early conception is now believed to keep embryos survive in their mothers' uterus. This was confirmed by a number of studies where humble progesterone concentration has been showed to strongly correlate to failure to conceive (Lukaszewska and Hansel, 1980; Mann and Lamming, 1995; Larson et al., 1997). In combination, Mann and Lamming (2001) suggested that as little as a one-day delay in the rise of

progesterone post-ovulation may cause embryos 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). This is supported by our results from which the loop 'smaller ovulatory follicle-CL with cavity-delayed rise in progesterone-(delayed CL regression)-quick growing but smaller ovulatory follicle and hence poor CL' was emerged. 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 progesterone, either at endocrine or at cell levels.

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A simple technique for minimal dose DIUI (deep intra-uterine insemination) studying in PMSG multiple-ovulating dairy heifers

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Abstract

The aim of our study was to crisis an accomplishment of minimal-spermatozoa deep intra-uterine insemination (DIUI) using a simple embryo transfer (ET) pistollet, in connection to relative fertilisation rates and early embryo qualities on day 7 post-service, studying in PMSG multiple-ovulating dairy heifers. Ten heifers of crossbred Holstein Frisian were included. They were characterised for their oestrous cycles, afterwards subjected to the superovulatory programme using PMSG/ PGF_{2α} protocol, and fixed-time inseminated: Gr. 1 (n=3), inseminated with 20×10^6 sperm cells at body of uterus; Gr. 2 (n=4), inseminated with 10×10^6 sperm cells at tip of the uterine horns and Gr. 3 (n=3), inseminated with 10×10^6 sperm cells at body of the uterus. Embryos were non-surgically recovered and categorised on 7 days subsequent to the insemination. On the day of each treatment, the ovaries of the heifers were ultrasonographically examined. The heifers, all represented superovulatory response (CLs ≥ 2). The number of recovered embryos/ ovas was numerically highest in Gr. 2, compared to the others ($P \geq 0.05$). The number of fertilised embryos and of transferable embryos did not differ among 3 groups. To conclude, it is substantial to accomplish deep intra-uterine insemination –placing a reduced number of sperms closely to the isthmus area, applying a simplest embryo transfer pistollet, with superior, or at least equal, end results to an ordinary artificial insemination (AI), and seemingly without life-threatening effects.

Keywords: minimal dose deep intra-uterine insemination (DIUI), embryo transfer (ET) pistollet, dairy heifers

Introduction

To establish a dairy-breeding programme whose general objective is to maximise the total genetic merits and economic returns of the on-going generation of offspring is a crucial task for a progressive outgrowth of the dairy industry. This, in turn, has been facilitated by parallel enhancements of modern reproductive technologies, particularly artificial insemination (AI), which has been applied in livestock breeding programmes for over 50 years. The use of AI technology has enabled the selection of superior males through progeny testing and large-scale dissemination of their genes throughout the commercial populations (Cunningham, 1998).

Techniques of insemination, however, have not developed significantly since introduction of the Cassou straw, even though there have been minor modifications to the volume of sperm suspension deposited in the uterine body. By contrast, studies on sperm transport and identification of the functional sperm reservoir in the female tract, as which the caudal isthmus of the fallopian tube(s) is considered, have progressed conspicuously (reviewed by Hunter, 2003). In parallel, the techniques of sorting X- and Y-bearing spermatozoa has become of interest in the field of the assisted reproduction of farm animals, though the quality of sperm is still unprivileged (Seidel, 2003). The deposition of semen closer to the oviduct –at the utero-tubal junction (UTJ), however, may be an of use means with lower insemination doses and less fertile semen e.g. sex-sorted ones (reviewed by Hunter, 2003). Lowering the inseminated doses by using female-sorted sperms and placing directly to their reservoir, without a decrease in fertilisation rate, may increase the numbers of insemination and descendents of highly valuable sires.

There have been studies on deep intrauterine insemination or so-called DIUI, especially an establishment of *Ghent device*; a disposable plastic catheter which can easily follow the curvature of the horns of uterus and approach the UTJ (Lopez-Gatius and Camon-Urgel, 1988; Lopez-Gatius, 1996; Verberckmoes *et al.*, 2005). The questions not only on a practical but also on an economic viewpoint have, in a decade, been raised on the device. Hence, the aim of our study was to crisis an accomplishment of minimal-spermatozoa DIUI using a simple embryo transfer (ET) pistollet, in connection to relative fertilisation rates and early embryo qualities on day 7 post-service, studying in PMSG multiple-ovulating dairy heifers.

Materials and Methods

Animals and experimental procedures

Ten* healthy and sexual-mature virgin heifers were included in the study. They were of crossbred Holstein Frisian (over 87.50% HF) and about 15 months of age at the time of the experiment. The heifers had body weight at range 280–315 kilograms and body score 3.0–3.5, and earlier showed a complete-functional oestrous cycle. They were housed at a commercial farm, Saraburi province with a free-stall system and fed 4 times a day with total mixed ration (TMR, protein at 12%, crude fiber at 15% and total digestible nutrients at 64%), 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.

The heifers were randomly divided into 3 groups: Group 1, 3 heifers inseminated with 20×10^6 sperm cells at body of uterus; Group 2, 4 heifers inseminated with 10×10^6 sperm cells at tip of the uterine horns and Group 3, 3 heifers inseminated with 10×10^6 sperm cells at body of the uterus. After characterisation of their normal oestrous cycles**, all animals were subjected to the superovulatory programme using PMSG/ PGF_{2α} protocol, and thereafter fixed-time inseminated, using frozen-thawed semen from a same bull, by manners according to their groups. Embryos were non-surgically recovered, and categorised, on 7 days subsequent to the insemination. On the day of each treatment, the ovaries of the heifers were ultrasonographically examined.

* and ** Twelve heifers were at first included to equally fix 4 heifers per group, however two of those showed abnormal oestrous cycle (prolonged oestrous interval, 26 and 27 days, resp.) and were excluded from the study.

Semen preparation and quality control

One single ejaculation of one single bull was subjected in order to elucidate any individual/ male effect on the experiment. Frozen semen was prepared on a selected healthy pure-bred Holstein Friesian bull at Livestock Semen Production Center-Inthanont Royal Project, Department of Livestock Development (DLD), Doi Inthanont, Chiang Mai. Briefly, ejaculate from the bull was collected using an artificial vagina and evaluated. Mass movement was +4, motility 80% and progressive motility +4. Ejaculated semen was extended in Tris-egg yolk diluter with glycerol concentration of 7% (w/v) and diluted to final concentration of 80 and 40 million spermatozoa/ ml. Diluted semen was filled in 0.25 French straws; sealed at room temperature; placed on a rack at 4°C, and lastly transferred into liquid nitrogen tank at -196°C. Eventually, the frozen semen was prepared in 2 insemination doses, 10×10^6 sperm cells/ straw and 20×10^6 sperm cells/ straw.

On farm, frozen-thawed samples were randomly evaluated before using for insemination. Comparing 0-, 5-, 10- and 15-minute after-thawed samples, there were no differences on motility as well as on progressive motility (range 45 to 55% and +3 to +3.5, resp.).

Superovulatory programme

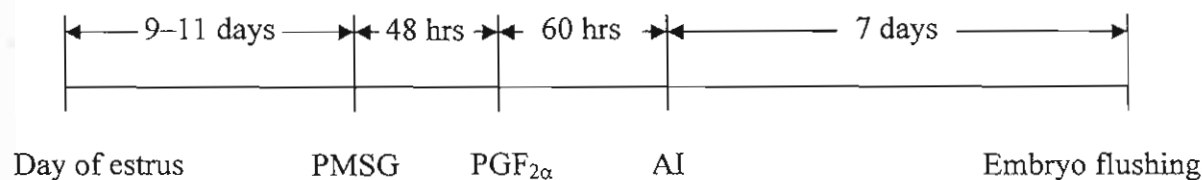


Figure 1: Protocol for superovulatory programme using Pregnant Mare Serum Gonadotropin (PMSG); Prostaglandin F_{2α} (PGF_{2α}) and fixed-time AI.

In correspond to **Figure 1**, superovulation treatment was set off on average day 10 (range 9–11), on a basis of retrospective information on follicular dynamics, of estrous cycle for each animal. The heifers were intramuscularly injected with 3000 IU (single dose) of Pregnant Mare Serum Gonadotropin (PMSG) (Folligon® Intervet Co.Ltd., Thailand) and, at 48 hours after PMSG treatment, with Prostaglandin F_{2α} (PGF_{2α}) (Illiren® Intervet Co.Ltd., Thailand) 25 mg each. At subsequent 60 hours, the animals were inseminated without estrous observation (fixed-time AI), using the technique and/or sperm concentration depending on study groups.

Techniques for insemination

Every artificial insemination sessions was performed by a single AI/ET-experienced veterinarian. In Group 1 and Group 3, the animals were inseminated by means of conventional artificial insemination (AI), depositing thawed semen at uterine body with a DLD (Department of Livestock Development)-standard insemination dose (20×10^6 sperm cells) and with a half dose (10×10^6 sperm cells), respectively.

In Group 2, deep intra-uterine insemination (DIUI) was performed, using an ordinary device for embryo transfer (ET pistol), into which the after-thawed semen straw was loaded in the same manner as into the AI gun, covered with stainless steel-tipped, side-opening sheath (IMV, France). The ET pistol was eventually inserted into the vagina and on into the cervix and the body of the uterus, alike to AI, and further passed through the greater curvature of the uterine

horn as far anterior as safely accomplished. The number of 10×10^6 spermatozoa was deposited close to the ovarian end of the uterine horns, half of a dose left and half of a dose right (approximately 5×10^6 each side).

Recovery and categorisation of embryos

Embryos and/or ovas were recovered 7 days after insemination, using standard non-surgical uterine flushing techniques by means of *the body flush* of which, in heifers, the technique was considered to be easier (quicker) and lesser harmed to the endometrium (Eldsen and Seidel, 1995), compared to *the*, well-liked, *horn flush*. In brief, the heifers were received epidural anesthesia with 3–5 ml, 2% lidocaine HCl (Lidocaine 2% injection[®], Union Drug Laboratories Ltd., Thailand). The foley catheter (Krusse, Sweden), equipped with 75- μ mesh embryo filter (Emcon[®] Krusse, Sweden) at the end, was gently inserted under guidance of metal stylet into the vagina and the lumen of the cervix, and straight forwards into the uterus. The balloon of the catheter was inflated exactly at the body of the uterus with 10–15 ml of air, and the catheter pulled back toward the cervix to allow the uterus to be full-filled with fluid. Normal saline solution 0.9% (NaCl) of 150–200 ml was flushed, in and out, at least 5 times (in total 700–1,000 ml) in order to ensure that as many as possible embryos were recovered.

Subsequent to the recovery procedure, the filter which contained all recovered material was rinsed, using NaCl, into a petri-dish and, embryos and/or ovas were recovered under a stereomicroscope. The recovered embryos/ ovas were counted and the relative fertilisation rate calculated by formula “(recovered embryos/ ovas divide by the number of CLs) multiply with 100”. The embryos were afterwards classified, on a basis of developmental stage and morphological characteristics, into 2 categories; transferable and non transferable embryos, according to Eldsen and Seidel (1995).

Ultrasonographical examination of the ovaries

The heifers' ovaries were examined 4 times: 1) on a day of PMSG injection; 2) on a day of PGF_{2 α} injection; 3) on a day of AI (at least 12 hours prior to AI), and 4) on a day of embryo flushing, using transrectal-ultrasonographical technique as described by Pierson *et al.* (1988). A real time B-mode ultrasound scanner (Falco Vet[®], Esoata-Pie Medical, Italy) equipped with a 6-/8-MHz rectal linear-array transducer, by which each examination was recorded on VCD/ DVD recorder for retrospective analyses, was used. The numbers and the sizes of the follicles as well as of the corpus luteum (CL) were documented according to Petyim *et al.* (2000). The heifer was considered to represent superovulatory response or SR, if the number of CLs, on the day of embryo flushing, exceeded 2 (Lopes da Costa *et al.*, 2001).

Statistical analysis

The values are presented as mean $\pm$ standard deviation (SD). Statistical analyses were carried out using a Student's *t*-test in order to test significant differences between means. Probability values < 0.05 were considered to be significant.

Results

Superovulatory responses

The results on PMSG-PGF_{2 α} superovulatory protocol are showed in **Table 1**. On the set-off day of the treatment (the day of PMSG injection), the majorities of the follicles were of small

sizes, after which, increased numerically ($P > 0.05$). Likewise, the numbers of medium follicles increased after treated by PMSG ($P < 0.05$ in Gr. 2; $P = 0.088$ and 0.238 in Gr. 1 and 3, resp.) and after PGF_{2α} ($P = 0.342$, 0.067 and 0.081 in Gr. 1, 2 and 3, resp.), compared to the set-off day. Large follicles, by contrast, were closed to zero either on, or after, the day of PMSG injection, but increased subsequent to the day of PGF_{2α} administration or at 12 hours prior to AI ($P < 0.05$ in Gr. 2; $P = 0.067$ and 0.069 in Gr. 1 and 3, resp.). The heifers, either collectively or individually, all represented SR (CLs ≥ 2 ; ranged 2–13).

Table 1: The number of small- (SF), medium- (MF) and large-sized follicles (LF) documented: on a day of PMSG injection; on a day of PGF_{2α} injection and on a day of AI, and the number of CLs evident on a day of embryo flushing (Mean $\pm$ SD).

Gr.	day of PMSG injection			day of PGF _{2α} injection			day of AI			CL
	SF	MF	LF	SF	MF	LF	SF	MF	LF	
1	7.33 ^a ± 6.03	1.33 ^{c,e} ± 1.15	0.67 ^{b,i} ± 0.58	12.33 ^a ± 11.15	8.0 ^{c*} ± 5.0	1.0 ^g ± 1.0	1.33 ± 1.53	10.0 ^e ± 13.89	10.33 ^{i*} ± 6.68	7.0 ± 4.58
2	7.25 ^a ± 5.06	1.5 ^{c,e} ± 0.58	0.75 ^{b,i} ± 0.5	12.0 ^a ± 9.05	7.25 ^d ± 2.63	0.75 ^g ± 1.5	1.25 ± 0.96	9.75 ^{e*} ± 7.37	12.5 ^j ± 6.81	9.25 ± 2.98
3	9.33 ^a ± 5.13	1.33 ^{c,e} ± 0.58	0 ^{g,i}	18.0 ^a ± 13.53	8.33 ^c ± 8.74	0 ^g	18.67 $\pm$ 16.2	12.0 ^{e*} ± 7.94	4.33 ^{i*} ± 2.08	6.33 $\pm$ 5.86

^{a-b, c-d, e-f, g-h, i-j} Different superscripts between columns denote significant differences ($P < 0.05$); same superscripts but marked with * connote the values tended to be different ($0.05 \leq P < 0.1$).

Embryo recovery, fertilisation rate and embryo qualities

Mean number of CLs and the number of recovered embryos/ ovas, of fertilised embryos and, of transferable embryos documented on a day of embryo flushing were showed in **Table 2**. Not included in the table, values on the relative fertilisation rate varied from zero to extremely high ($\geq 100\%$) in each group and, hence, no statistical analyses was accomplished on the issue. The number of recovered embryos/ ovas was numerically highest in Gr. 2 (range 0–12), compared to the others (range 0–4 in Gr. 1 and 3), but not significantly. Likewise, the number of fertilised embryos and of transferable embryos did not statistically differ among 3 groups.

Table 2: Mean ($\pm$ SD) number of CLs; the numbers of recovered embryos and/or ovas, of fertilised embryos, and of transferable embryos documented on a day of embryo flushing.

Gr.	CLs	Embryos / Ovas*	Fertilised embryos	Transferable embryos			
				Morula	Early blast	Blastocyst	Total
1	7.0 $\pm$ 4.58	2.67 ^a $\pm$ 2.31	2.67 ^b $\pm$ 2.31	0.33 $\pm$ 0.58	0.67 $\pm$ 1.15	0	1.0 ^c $\pm$ 1.73
2	9.25 $\pm$ 2.98	4.25 ^a $\pm$ 5.32	2.75 ^b $\pm$ 4.27	1.0 $\pm$ 2.0	0	0.75 $\pm$ 0.96	1.75 ^c $\pm$ 2.87
3	6.33 $\pm$ 5.86	2.0 ^a $\pm$ 2.83	1.5 ^b $\pm$ 2.12	0	0.5 $\pm$ 0.71	0	0.5 ^c $\pm$ 0.71

* Recovered embryos and/or ovas on the day of flushing regardless to categorisation.

^{a, b, c} Values with the same superscripts within column do not differ significantly ($P > 0.05$).

Discussion

In our study, multiple-ovulation regime was a need, in order to maximise sample sizes or a number of embryos which acted as a main parameter to evaluate an efficiency of DIUI. Usually, superovulation is accomplished by replicated injections of extra follicle stimulating hormone (FSH) to emerge an extra cohort of follicles. Subsequently, corpus luteum (and its progesterone) was prematurely regressed by means of an administration of $\text{PGF}_{2\alpha}$, causing rapid growth, and eventually, ovulation of full-grown follicles (Goulding *et al.*, 1991). However, a very different approach to enhance a number of ovulated follicles has sometimes been used, as well as in the current study. In our treatment protocol, instead of FSH, gonadotropin; PMSG was applied. The PMSG has FSH-like properties and is degraded very slowly in the heifer, so one single injection is sufficient (reviewed by Seidel *et al.*, 2003). Indeed, the other but critical reason for using PMSG in our study was that it was, at the time, only multiple-ovulating chemical available on domestic markets.

The heifers in groups 1, 2 and 3, all responded to treatment protocols documented by a prominently increase of the number of small follicles after treatment of PMSG as well as the progressively increased numbers of medium and large follicles at the end of a whole protocol. Looking at the other parameter, the least number of corpora lutea clearly countable on the day of flushing was two, of which the values substantially notify that the animals all had superovulatory response (Lopes da Costa *et al.*, 2001). One of the factors significantly affecting response on superovulation was the stage of the follicular dynamics where doses of gonadotropin were given. In our study, the programme was started on approximately day 9 to 11 of estrous cycle or around the day of the 2nd follicular wave emergence, according to retrospective information. The strategy was claimed to enhance superovulatory response (Eldsen and Seidel, 1995). Estimated on evidences of CLs, an average number of ovulation was almost eight, varied from 2 to 13. Not surprisingly, the variation seemed to be large in corresponding to Seidel *et al.* (2003) who reviewed that there was always a huge variation, probably from zero to 50 ovulations per animal. Further, the conversed results showed on a day of AI in group 3; huge numbers of not large but small follicles which had less possibility to ovulate, may owe to a large individual variation. Consequent to the experiment, 9 from 10 of the heifers regained their oestrous cycle –physically showing oestrous signs; being inseminated and pregnant, not later than 45 days after the day of embryo flushing/ $\text{PGF}_{2\alpha}$ injection. The rest (1 of 10) had multiple luteinised-follicular cysts, but which treated and the heifer returned to the cycle at approximate 75 days afterwards. Summing up, multiple-ovulation protocol employing PMSG/ $\text{PGF}_{2\alpha}$ in our study numerically succeeded, in accordance to the precise “set-off” day, and on a basis of retrospective information on each animal’s follicular dynamics. Embryo yields and the qualities, however, must be concerned.

There have been studies on deep intra-uterine insemination with a new *Ghent device* (Lopez-Gatius and Camon-Urgel, 1988; Lopez-Gatius, 1996; Verberckmoes *et al.*, 2005). Not saying about satisfactory outcomes, an insemination technique especially on a thawing procedure seems to be complicated and possibly increases the risk of sperm cold-shock damage (Verberckmoes *et al.*, 2005). To the case, a simple embryo transfer catheter or ET pistol, into which after-thawed semen straw can be loaded directly, is recommended (Kurykin *et al.*, 2003). Looking further on the technical/ practical viewpoints, DIUI using the ET pistol has pretty close similarities to non-surgical procedure of embryo recovery from the upper area of uterine horn, which any AI/ ET well-trained inseminator may progress to a large-scale population. However, one must keep in mind that, unlikely to recovery of embryo under progesterone

priming, in a manner of fully oestrous DIUI, the uterine tissues may be taut and tonic, causing a tight curvature towards the ovarian end of the horn (Hunter, 2003). Corresponding to Kurykin *et al.* (2003) and Andersson *et al.* (2004), Seidel *et al.* (1999), further suggested that DIUI-inseminator should aware on lesser manipulation of the uterus and least injury of its endometrium rather than on depositing semen deeply; in most cases, semen was deposited between the anterior third- and mid-cornua. In our study, the ET pistollet was effortlessly guided through the curvature and the after-thawed semen well deposited close to the tip of the uterine horn and, after which no blood was stuck on the apex of the pistollet. Besides, additional effect on endometrium was disregarded as 9 out of 10 heifers regained cyclicities and turned pregnant afterwards (Srisongchate, personal communication).

Corresponding to the other studies where DIUI, regardless to the techniques, yielded a higher non-return rate compared to classical uterine body insemination (Lopez-Gatius, 1996; Kurykin *et al.*, 2003; Verberckmoes *et al.*, 2005), the heifers in Gr. 2, where DIUI with half of DLD-standard semen concentrations employed, offered satisfactory recovery results. In fact, if excluded one heifer, whom accidentally was injected with an inadequate dose (2000 out of 3000 IU) of PMSG, embryos were retrieved in all heifers in Gr. 2. Further, compared to the other groups, one of them with half-dose DIUI gave the highest number of recovered embryos, numerically, either transferable or non-transferable ones. An explanation for the results is perhaps a new theory of sperm reservoir, which the caudal isthmus is to date deemed to serve as (Larsson and Larsson, 1985; Hunter, 2003). Concomitantly, to minimise the extent of exposure to endometrial tissues and uterine fluids by means of DIUI may result in a higher proportion of cells in the sperm suspension remaining sufficiently viable to establish the isthmus reservoir (Dalton *et al.*, 1999).

The results on relative fertilisation rate, especially of the heifers who had extremely high value ($\geq 100\%$), were not added in. Similarly to Chagas e Silva *et al.* (2002), an excuse was that the number of corpora lutea appeared to be underestimated, though in our study, the ultrasonography was subjected. Owing to a large number of follicle-like structures evident, the other explanation was possibly that some of CLs remained imperfect luteinisation because of an endocrinological imbalance (reviewed by Seidel *et al.*, 2003). In the case, CLs may afterwards wholly luteinise and be sonically documented on the other days (Kornmatitsuk, unpublished data). According to certain necessity of the experimental farm, however, CLs were exogenously regressed directly on the day of flushing. Regardless to the doubted number of CLs, Lopes da Costa *et al.* (2001) suggested the formula “(cleaved ovas/ total ovas recovered) $\times$ 100)” to estimate the fertilisation rate.

The “reduced” number of sperms per insemination dose in our study was yet higher than in the others (i.e., Kurykin *et al.*, 2003; Verberckmoes *et al.*, 2005), in where the smallest doses of DIUI; 8, 4 and 2 million spermatozoa were scrutinised. The authors figured out that neither site of semen deposition –uterine body versus tip of uterine horn, nor sperm dosage affected pregnancy rates. Surprisingly, the conventional AI at uterine body of the half-dose frozen-thawed semen of DLD-standard (10×10^6 sperm cells), compared to of the full-dose (20×10^6 sperm cells), earned a numerical-like number of recovered embryos. The finding presumably says that it has possibility and, yes indeed, benefits, for frozen-semen producing in our country to small the number of spermatozoa per straw. This is to gain additional doses per a bull-ejaculation, especially of a master or genetic-merited sire, to be wider disseminated on a large scale of our country dairy-populations.

priming, in a manner of fully oestrous DIUI, the uterine tissues may be taut and tonic, causing a tight curvature towards the ovarian end of the horn (Hunter, 2003). Corresponding to Kurykin *et al.* (2003) and Andersson *et al.* (2004), Seidel *et al.* (1999), further suggested that DIU-inseminator should aware on lesser manipulation of the uterus and least injury of its endometrium rather than on depositing semen deeply; in most cases, semen was deposited between the anterior third- and mid-cornua. In our study, the ET pistollet was effortlessly guided through the curvature and the after-thawed semen well deposited close to the tip of the uterine horn and, after which no blood was stuck on the apex of the pistollet. Besides, additional effect on endometrium was disregarded as 9 out of 10 heifers regained cyclicities and turned pregnant afterwards (Srisongchate, personal communication).

Corresponding to the other studies where DIUI, regardless to the techniques, yielded a higher non-return rate compared to classical uterine body insemination (Lopez-Gatius, 1996; Kurykin *et al.*, 2003; Verberckmoes *et al.*, 2005), the heifers in Gr. 2, where DIUI with half of DLD-standard semen concentrations employed, offered satisfactory recovery results. In fact, if excluded one heifer, whom accidentally was injected with an inadequate dose (2000 out of 3000 IU) of PMSG, embryos were retrieved in all heifers in Gr. 2. Further, compared to the other groups, one of them with half-dose DIUI gave the highest number of recovered embryos, numerically, either transferable or non-transferable ones. An explanation for the results is perhaps a new theory of sperm reservoir, which the caudal isthmus is to date deemed to serve as (Larsson and Larsson, 1985; Hunter, 2003). Concomitantly, to minimise the extent of exposure to endometrial tissues and uterine fluids by means of DIUI may result in a higher proportion of cells in the sperm suspension remaining sufficiently viable to establish the isthmus reservoir (Dalton *et al.*, 1999).

The results on relative fertilisation rate, especially of the heifers who had extremely high value ($\geq 100\%$), were not added in. Similarly to Chagas e Silva *et al.* (2002), an excuse was that the number of corpora lutea appeared to be underestimated, though in our study, the ultrasonography was subjected. Owing to a large number of follicle-like structures evident, the other explanation was possibly that some of CLs remained imperfect luteinisation because of an endocrinological imbalance (reviewed by Seidel *et al.*, 2003). In the case, CLs may afterwards wholly luteinise and be sonically documented on the other days (Kornmatitsuk, unpublished data). According to certain necessity of the experimental farm, however, CLs were exogenously regressed directly on the day of flushing. Regardless to the doubted number of CLs, Lopes da Costa *et al.* (2001) suggested the formula “(cleaved ovas/ total ovas recovered) $\times$ 100)” to estimate the fertilisation rate.

The “reduced” number of sperms per insemination dose in our study was yet higher than in the others (i.e., Kurykin *et al.*, 2003; Verberckmoes *et al.*, 2005), in where the smallest doses of DIUI; 8, 4 and 2 million spermatozoa were scrutinised. The authors figured out that neither site of semen deposition –uterine body versus tip of uterine horn, nor sperm dosage affected pregnancy rates. Surprisingly, the conventional AI at uterine body of the half-dose frozen-thawed semen of DLD-standard (10×10^6 sperm cells), compared to of the full-dose (20×10^6 sperm cells), earned a numerical-like number of recovered embryos. The finding presumably says that it has possibility and, yes indeed, benefits, for frozen-semen producing in our country to small the number of spermatozoa per straw. This is to gain additional doses per a bull-ejaculation, especially of a master or genetic-merited sire, to be wider disseminated on a large scale of our country dairy-populations.

Conclusion

As a concluding remark, it is substantial to accomplish deep intra-uterine insemination – placing a reduced number of sperms closely to the isthmus area, applying a simplest embryo transfer pistol, with superior, or at least equivalent, end results to an ordinary artificial insemination, and seemingly without life-threatening effects. However, experiments carried out on a larger number of not only heifers but cows, under standard field conditions (i.e., spontaneous oestrous heifers; well-trained district inseminator), and with more parameters emphasised are required.

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โครงการอบรมเชิงปฏิบัติการ เทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม ครั้งที่ 1

เรื่อง

การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ และการตรวจการตั้งครรภ์ระยะต้น (The Use of Ultrasonography in an Examination of Reproductive Tracts and Early Pregnancy Diagnosis)

1. หลักการและเหตุผล

การใช้เครื่องตรวจคลื่นเสียงความถี่สูง (Ultrasonography) ในการตรวจระบบสืบพันธุ์โคนม เพิ่มเติมจากการล้วงตรวจผ่านทางทวารหนัก โดยเฉพาะในด้านการศึกษาวิจัย ทำให้นายสัตวแพทย์ และผู้ปฏิบัติงานด้านระบบสืบพันธุ์โคนม ได้มีความรู้ ความเข้าใจที่ถูกต้องและชัดเจนมากยิ่งขึ้น เกี่ยวกับลักษณะทางสรีรวิทยาของระบบสืบพันธุ์ในโคนม ในทศวรรษที่ผ่านมา ได้เริ่มมีการนำเครื่องตรวจคลื่นเสียงความถี่สูงมาใช้อย่างกว้างขวางในอุตสาหกรรมการเลี้ยงโคนม โดยเฉพาะอย่างยิ่งในประเทศที่มีความก้าวหน้าด้านเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์ ด้วยเหตุผลหลายประการ อาทิเช่น การประเมินสถานะการตั้งครรภ์และการมีชีวิตรอดของตัวลูกอ่อนได้ตั้งแต่ระยะต้น สามารถบ่งชี้และช่วยในการแก้ไขปัญหาในโคที่ล้มเหลวในการตั้งครรภ์ได้รวดเร็วขึ้น เป็นการลดระยะห่างระหว่างการให้บริการผสมเทียม และเพิ่มอัตราการผลิตผสมเทียม การตรวจพบที่รวดเร็ว ในกรณีโคตั้งครรภ์ลูกแฝด และ/หรือการตรวจแยกเพศของลูก มีประโยชน์ต่อการวางแผนที่เหมาะสมในการดูแลโคทั้งในระยะตั้งครรภ์และการคลอด เป็นการลดการสูญเสียที่จะตามมา การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยให้การวินิจฉัยโรค และ/หรือพยาธิสภาพของรังไข่และมดลูก และการตัดสินใจในการรักษาที่ถูกต้องแม่นยำกว่า เมื่อเปรียบเทียบกับการล้วงตรวจผ่านทางทวารหนักเพียงอย่างเดียว นอกเหนือไปกว่านั้น การใช้เครื่องตรวจคลื่นเสียงความถี่สูง ร่วมกับเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์อื่น ๆ เอื้อให้เกิดการพัฒนาของการจัดการระบบสืบพันธุ์โคนมแบบบูรณาการ และมีประสิทธิภาพมากขึ้น ในประเทศที่กล่าวมาแล้ว การอบรม/เพิ่มพูนทักษะ และความชำนาญ ของนายสัตวแพทย์ปฏิบัติงานด้านระบบสืบพันธุ์โคนม ในการใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ นับเป็นจุดเปลี่ยนแปลงสำคัญ ต่ออุตสาหกรรมการเลี้ยงโคนม โดยเฉพาะในด้านการจัดการระบบสืบพันธุ์

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล และผู้สนับสนุนโครงการ เข้าใจถึงความสำคัญของศาสตร์ดังกล่าว โดยมองเห็นว่าการสร้างความคุ้นเคย และความรู้ ความเข้าใจที่ถูกต้อง ในการใช้เครื่องตรวจคลื่นเสียงความถี่สูง ของนายสัตวแพทย์ปฏิบัติงานด้านวิทยาการสืบพันธุ์โคนม จะเป็นการส่งเสริมให้มีการนำเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์มาใช้ เพื่อประโยชน์สูงสุดในการจัดการด้านระบบสืบพันธุ์โคนมของประเทศไทยต่อไป

2. วัตถุประสงค์

- 2.1 เพื่อสร้างความคุ้นเคย และความรู้ ความเข้าใจที่ถูกต้อง ในการใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ และการตรวจการตั้งครรภ์ระยะต้นในโคนม
- 2.2 เพื่อส่งเสริมให้มีการนำเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์มาใช้ เพื่อประโยชน์สูงสุดในการจัดการด้านระบบสืบพันธุ์โคนมของประเทศไทย
- 2.3 เพื่อแลกเปลี่ยนประสบการณ์ และความร่วมมือระหว่างคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล และสถาบันอื่นกับ Department of Dairy Science, University of Wisconsin-Madison ในการใช้เครื่องตรวจคลื่นเสียงความถี่สูง และเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม

2.4 เพื่อเป็นโครงการนำร่องในโครงการอบรมเชิงปฏิบัติการ เกี่ยวกับเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม
ด้านอื่น ๆ ที่จะจัดให้มีขึ้นต่อไปในอนาคต

3. วิธีการดำเนินงาน

3.1 การบรรยาย 1 วัน ในหัวข้อ

3.1.1 Scanning the future –Ultrasonography as a reproductive management tool for dairy cattle (½ ชั่วโมง)

3.1.2 ความรู้เบื้องต้นเกี่ยวกับเครื่องตรวจคลื่นเสียงความถี่สูง (1 ชั่วโมง)

3.1.3 การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ (1 ชั่วโมง)

3.1.4 การใช้เครื่องตรวจคลื่นเสียงความถี่สูงตรวจการตั้งท้องระยะต้น (1½ ชั่วโมง)

3.1.5 การตรวจแยกเพศลูกด้วยเครื่องตรวจคลื่นเสียงความถี่สูง (1½ ชั่วโมง)

3.2 การปฏิบัติการ 2 วัน หัวข้อ

3.2.1 การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ (3½ ชั่วโมง)

3.2.2 การใช้เครื่องตรวจคลื่นเสียงความถี่สูงในการตรวจการตั้งท้องระยะต้นและแยกเพศลูก (6½ ชั่วโมง)

4. ค่าลงทะเบียน

ค่าลงทะเบียน คนละ 2,450 บาท

หมายเหตุ ค่าลงทะเบียน รวมห้องพัก และอาหาร/ อาหารว่างตลอดการอบรมฯ

5. ผู้เข้าร่วมโครงการ

5.1 อาจารย์ และ/หรือนักศึกษาระดับปริญญาโท-เอก และ/หรือนายสัตวแพทย์ ปฏิบัติงานด้านวิทยาการสืบพันธุ์โคนม
คณะสัตวแพทยศาสตร์ 6 สถาบัน และสถาบันการศึกษาที่เกี่ยวข้อง จำนวน 15 คน

5.2 นายสัตวแพทย์ปฏิบัติงานด้านวิทยาการสืบพันธุ์โคนม กรมปศุสัตว์ จำนวน 5 คน

5.3 นายสัตวแพทย์ปฏิบัติงานด้านวิทยาการสืบพันธุ์โคนม หน่วยงานอื่น ๆ จำนวน 5 คน

6. ผู้เชี่ยวชาญต่างประเทศ

Associate Professor Paul M. Fricke, PhD

Department of Dairy Science, University of Wisconsin-Madison, Madison, WI

7. กำหนดเวลา และสถานที่

ระยะเวลา 3 วัน

วันที่ 18–20 พฤษภาคม 2548

สถานที่ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล วิทยาเขตศาลายา จังหวัดนครปฐม และ
โรงพยาบาลปศุสัตว์และสัตว์ป่า วิทยาเขตไทรโยค จังหวัดกาญจนบุรี

8. ผลที่คาดว่าจะได้รับ

8.1 ผู้เข้าร่วมการอบรมฯ มีความคุ้นเคยและความรู้ ความเข้าใจที่ถูกต้อง ในการใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วย
ในการตรวจระบบสืบพันธุ์ และการตรวจการตั้งท้องระยะต้นในโคนม และสามารถนำไปใช้เพื่อเพิ่มประสิทธิภาพ
ในการจัดการด้านระบบสืบพันธุ์โคนม

8.2 ผู้เข้าร่วมการอบรมฯ คณะจารย์ของคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล และสถาบันอื่น ๆ และผู้เชี่ยวชาญต่างประเทศของ Department of Dairy Science, University of Wisconsin-Madison ได้แลกเปลี่ยนประสบการณ์เกี่ยวกับการใช้เครื่องตรวจคลื่นเสียงความถี่สูง และเทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนมที่เกี่ยวข้อง

8.3 แนวทางในการจัดโครงการอบรมเชิงปฏิบัติการ เทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม ในครั้งต่อไป

9. ผู้รับผิดชอบโครงการ

9.1 คณบดี

ที่ปรึกษา

9.2 ผู้ช่วยคณบดีฝ่ายวิจัยและวิเทศสัมพันธ์

ที่ปรึกษา

9.3 ผู้อำนวยการโรงพยาบาลสุสัตว์และสัตว์ป่า

ที่ปรึกษา

9.4 อาจารย์ ดร. นายสัตวแพทย์ บรรลือ กรมาทิตย์สุข

9.5 อาจารย์ ดร. สัตวแพทย์หญิง สุดสายใจ กรมาทิตย์สุข

9.6 อาจารย์ ดร. นายสัตวแพทย์ จิตรกมล ธนศักดิ์

9.7 อาจารย์ นายสัตวแพทย์ สมเกียรติ ห้วยจันทิก

10. ผู้สนับสนุนโครงการ

10.1 คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

10.2 Department of Dairy Science, University of Wisconsin-Madison

10.3 บริษัท สุพรีมโปรดักส์ จำกัด

10.4 ฟาร์มโคนมในเขตพื้นที่การให้บริการของโรงพยาบาลสุสัตว์และสัตว์ป่า คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

กำหนดการโครงการอบรมเชิงปฏิบัติการ เทคโนโลยีชีวภาพทางวิทยาการสืบพันธุ์โคนม ครั้งที่ 1
เรื่อง

การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ และการตรวจการตั้งครรภ์ระยะต้น (The Use of Ultrasonography in an Examination of Reproductive Tracts and Early Pregnancy Diagnosis)

วันพุธที่ 18 พฤษภาคม 2548

- 08.00–08.30 น. ลงทะเบียน
- 08.30–08.45 น. พิธีเปิดการอบรมฯ โดย รองศาสตราจารย์ นายสัตวแพทย์ ปานเทพ รัตนาก
คณบดีคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
- 08.45–09.15 น. การบรรยายเรื่อง Scanning the future –Ultrasonography as a reproductive
management tool for dairy cattle (Assoc. Prof. Paul M. Fricke)
- 09.15–09.30 น. พักรับประทานอาหารว่าง
- 09.30–10.30 น. การบรรยายเรื่อง ความรู้เบื้องต้นเกี่ยวกับเครื่องตรวจคลื่นเสียงความถี่สูง (อ.ดร.สพญ. สุดสาย
ใจ กรมาทิพย์สุข)
- 10.30–11.30 น. การบรรยายเรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ (อ.ดร.
น.สพ. บรรลือ กรมาทิพย์สุข)
- 11.30–12.30 น. รับประทานอาหารกลางวัน
- 12.30–14.00 น. การบรรยายเรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงตรวจการตั้งครรภ์ระยะต้น (Assoc.
Prof. Paul M. Fricke)
- 14.00–14.15 น. พักรับประทานอาหารว่าง
- 14.15–15.45 น. การบรรยายเรื่อง การตรวจแยกเพศลูกด้วยเครื่องตรวจคลื่นเสียงความถี่สูง (Assoc. Prof.
Paul M. Fricke)
- 16.00 น. เดินทางจากคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ไปยังโรงพยาบาลปศุสัตว์และสัตว์ป่า
วิทยาเขตไทรโยค จังหวัดกาญจนบุรี
- 18.30 น. รับประทานอาหารเย็น

วันพฤหัสบดีที่ 19 พฤษภาคม 2548

- 07.30 น. รับประทานอาหารเช้า
- 08.30–12.00 น. ปฏิบัติการเรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงช่วยในการตรวจระบบสืบพันธุ์ –อาโ
ฟาร์ม (Assoc. Prof. Paul M. Fricke/ Staff)
- 12.00–13.00 น. รับประทานอาหารกลางวัน
- 13.00–16.30 น. ปฏิบัติการเรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงในการตรวจการตั้งครรภ์ระยะต้นและ
แยกเพศลูก I –สตาร์ฟาร์ม (Assoc. Prof. Paul M. Fricke/ Staff)
- 16.30–18.00 น. สรุปการปฏิบัติการและซักถาม
ผู้เข้าร่วมการอบรมฯ แลกเปลี่ยนประสบการณ์/ ความคิดเห็นในการใช้เครื่องตรวจคลื่นเสียง
ความถี่สูงช่วยในการปฏิบัติงานด้านวิทยาการสืบพันธุ์โคนม
- 18.30 น. รับประทานอาหารเย็น

วันศุกร์ที่ 20 พฤษภาคม 2548

07.30 น. รับประทานอาหารเช้า

08.30–11.30 น. ปฏิบัติการเรื่อง การใช้เครื่องตรวจคลื่นเสียงความถี่สูงในการตรวจการตั้งครรภ์ระยะต้นและ
แยกเพศลูก II –กษกรฟาร์ม (Assoc. Prof. Paul M. Fricke/ Staff)

11.30–12.30 น. รับประทานอาหารกลางวัน

12.30–13.30 น. สรุปการปฏิบัติการและซักถาม

13.30–13.45 น. พิธีปิดการอบรมฯ และมอบประกาศนียบัตร โดย Assoc. Prof. Paul M. Fricke

14.15 น. เดินทางกลับจากโรงพยาบาลสุทัศน์และสัตว์ป่า วิทยาเขตไทรโยค จังหวัดกาญจนบุรี

หมายเหตุ Staff: อ.ดร.น.สพ. บรรลือ กรมาทิพย์สุข/ อ.ดร.สพญ. สุดสายใจ กรมาทิพย์สุข/ อ.ดร.น.สพ. จิตรกมล ชนศักดิ์ และ อ.น.สพ. สมเกียรติ ห้วยจันทิก