

Investigation into the effect of prostaglandin F2a, GnRH analogue and hCG on induction of ovulation in mares

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Abstract

In order to prove the effect of “fixed time service”, this study aimed to determine reproductive efficiency of hormone manipulation to induce ovulation and improve pregnancy rate. Estrus cycles of fifty mares were monitored every other day until ovulation by ultrasound (control group). Six days after ovulation, all mares received 5 mg prostaglandin F2a in order to synchronize estrus cycle. Reproductive tract monitoring was continued until the follicle reached the size of 35 mm and uterine edema was scored 2. Then, the mares were randomly assigned into four groups and treated with different ovulation stimulation methods: T1, spontaneous ovulation (n=10); T2, single dose of 40 µg Buserelin (n=15); T3, single dose of 1500 IU hCG (n=15); and T4, single dose of 3000 IU hCG (n=10). The ultrasonography monitoring was conducted daily in each mare, and the sizes of follicles were recorded. Results showed that interovulatory intervals in the control group and the prostaglandin F2a-treated group (T2) were 20.87 and 14.83 days, respectively. The growth of follicle per day was lowest in the control group when compared to the treatment groups, of which the group treated with GnRH analogue (T3) gave the highest growth rate (6.5 mm). The duration from treatment to ovulation date in T3 (1.53 days) and T2 (1.83 days) was significantly earlier than that of T1 (3.2 days) and T4 (2.1 days). For all mares that were treated with 1500 IU hCG (T3), the ovulation occurred within 48 h and 93.33% of them were pregnant. In conclusion, the treatment with 1500 IU hCG, iv provided the shortest mean number of days from administration until ovulation and the highest pregnancy rate (93.33%).

Keywords: prostaglandin, GnRH analogue, hCG, induced ovulation, mare

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Introduction

Estimating ovulation time in mare is more difficult compared to other species because of the mare's unique prolonged luteinizing hormone (LH) surge (Alexander and Irvine, 2011). Hormone manipulation is commonly used in mares for timed breeding, shipped semen, frozen semen, or embryo transfer program to operate the timing of estrous cycle and induce ovulation (Figueiredo et al., 2011). Moreover, in susceptible mares with a history of post-breeding endometritis, the induction of ovulation would have more benefit of reduction in breeding number (Troedsson, 2006). Human chorionic gonadotropin (hCG), prostaglandin F2 alpha (prostaglandin F2a) (and analogues), and gonadotropin releasing hormone (GnRH) have been used in cyclic mares to control the time of ovulation.

In non-pregnant mare, the uterus normally produces adequate level of prostaglandin to terminate the corpus luteum from previous cycle and the mare returns to heat. In practice, administration of synthetic prostaglandin is used to shorten the cycle, estrous synchronization, treatment of a persistent corpus luteum, and termination of an unwanted pregnancy. Moreover, synthetic prostaglandin and analogues can act indirectly on the hypophyseal-gonadal axis, resulting in reduction in ovulation time (Savage and Liptrap, 1987).

Human chorionic gonadotropin (hCG) is a glycoprotein hormone of which biological activity is similar to LH. The effects of hCG in hastening ovulation were previously studied, and results revealed that the duration of estrus and interval treatment to ovulation was significantly reduced (Rosdale and Lambercht, 1998; Barbacini et al., 2000). hCG is the first ovulatory agent to be used in mares and has dominantly been used by many equine practitioners to date. However, the failure of ovulation induction has been reported, due to the formation of anti-hCG antibodies (Roser et al., 1979; Wilson et al., 1990; McCue et al., 2004).

Gonadotropin releasing hormone is a decapeptide that is released in a pulsatile fashion with a short half-life. Gonadotropin releasing hormone and its analogues are valuable in controlling estrous cycles and timing of ovulation in many mammalian species (Voss, 1993; Squires et al., 1994; Mumford et al., 1995; Bergfelt, 2000). GnRH and GnRH analogue's products, including deslorelin (OvuplantTM), gonadorelin diacetate tetrahydrate (CystorelinTM), are available on the market for horse breeders, but less than bovine industry. OvuplantTM is deslorelin, the first approved agent for use in hastening ovulation in mares of which ovulation is predictable (Meinert et al., 1993). However, the implant needs to be withdrawn within two days after ovulation, thus causing inconvenience to mare practitioners. Therefore, injectable deslorelin has been developed and proved successful in inducing ovulation within 48 h, making it a possible alternative to hCG and OvuplantTM. Buserelin (GnRH analogue) has also shown the capacity to induce ovulation and terminate an estrus period. However, it is not commonly used in practice due to the major limitation

of short-lasting effect, which results in the need for repeated doses.

The aim of the current study was to determine the reproductive efficiency of prostaglandin F2a, hCG and Buserelin in order to induce ovulation and improve pregnancy rate.

Materials and Methods

Thoroughbred mares, aged 4-10 years, ranging in body condition scores of 2-4 (on a scale of 0-5), were examined for reproductive status for two consecutive estrous cycles by trans-rectal ultrasound. As standard practice, the mares were examined every other day throughout the estrous cycle until ovulation. Follicle size (Gastal et al., 1997) and uterine edema score (Squires et al., 2014) from the first estrous cycle were recorded and used as control data (control group). In the second estrous cycle, all mares received 5 mg prostaglandin F2a (lutalyse®; Pfizer Animal Health), intramuscularly (im), on D6 after ovulation. When the dominant follicle reached 35 mm in diameter, the mares were randomly assigned to: T2, treated with 40 µg Buserelin (Receptal®; MSD Animal Health), im; T3, treated with 1500 IU hCG (Chorulon®; MSD Animal Health), iv; T4, treated with 3000 IU hCG, iv; and spontaneous ovulation group was recorded as T1. Twenty-four h after treatment, fresh semen from a stallion with normal fertility was inseminated to each mare one time, and then ovulation was daily monitored by trans-rectal ultrasonography. The ovulation time and pregnancy rate at D15 were monitored and recorded.

Rate of dominant growth (mm/day)

$$= \frac{(\text{Diameter of the dominant follicle on the day before ovulation} - 3.5)}{\text{Duration of the day at 35 mm diameter of the dominant follicle until the day before ovulation}}$$

Interval treatment to ovulation (day)

$$= \text{The ovulated date} - \text{The date the dominant follicle reached 35 mm in diameter}$$

% Ovulation within 48 h =

$$\frac{(\text{Number of mares ovulated within 48 h})}{\text{Overall mares in each group}} \times 100$$

Pregnancy rate at D15 (%) =

$$\frac{(\text{Number of mares found conceptus in uterus at D15})}{\text{Number of serviced mares in each group}} \times 100$$

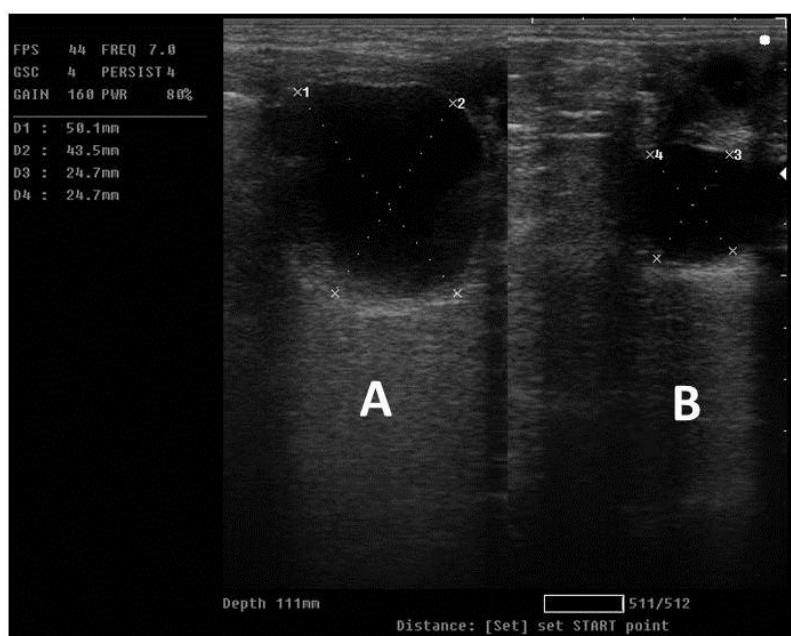
Statistical Analysis: Data were analyzed by using student *t* test to determine the effect of treatment on ovulation.

Results

The diameter of follicles measured with calipers on frozen sonogram is shown in Figure 1. In this study, the uterine edema was scored in 4 degrees (0-3) (Figure2). The diameter of preovulatory follicle and uterine edema score 6 days before ovulation in the control group is shown in Table 1. The dominant follicle (more than 30 mm in diameter) was present since day 6 before ovulation and continued to increase

in diameter until spontaneous ovulation. However, during 6 days before ovulation (D-6) and 4 days before ovulation (D-4), the highest rate of follicular growth was found. The biggest size was found on day 2 before ovulation and the average diameter of follicle was 40.49 ± 2.91 , ranging from 35.8 to 45.2 mm. The largest degree of uterine edema was on 2 days before ovulation and the average uterine edema score was 1.92, ranging from 0 to 3. Close to ovulation, uterine edema score should be decreased, however, in this study, on the days after ovulation, the uterine edema scores of 6% of the mares (3/50) were found to be the same as those detected on the ovulation day. Table 2 shows the largest follicular diameter, rate of follicular growth, 35 mm diameter to ovulation interval and percentage of pregnancy in each treatment. The mares showed estrous behavioral symptom in all cycles during this study period. None of the overall incidence of hemorrhagic anovulatory follicle development after hormone administration was observed, and all of the dominant follicles ovulated. Interovulatory intervals in

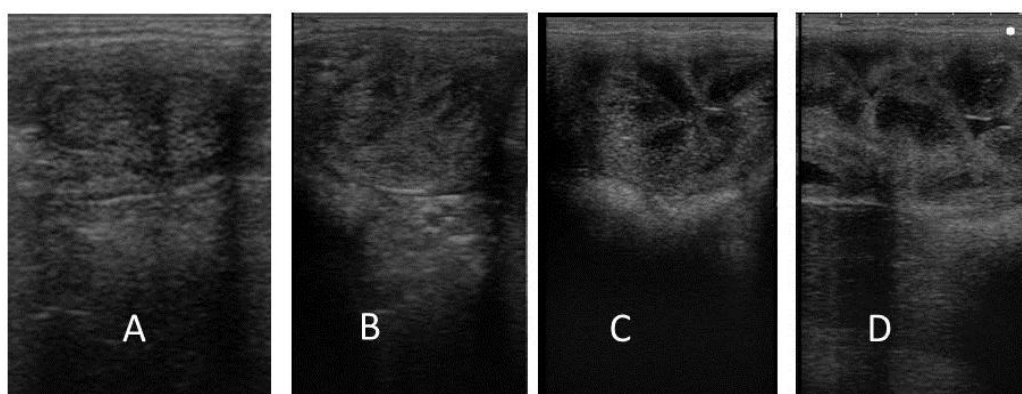
the mares treated with and without prostaglandin F2a were 14.83 and 20.87 days, respectively. The rate of dominant growth was significantly different between the groups. T2 had the largest and T1 had the smallest diameter of dominant follicles on the day before ovulation and rate of dominant growth. The interval treatment to ovulation was also significantly different between the groups, except T1 and T2. T3 had the shortest interval treatment to ovulation, while the control group had the longest. The percentages of ovulation occurring within 24 h after treatment were 0%, 40%, 46.67% and 0% of the cases (T1-T4, respectively). The ovulation occurred between 24-48 h after treatment in 30%, 40%, 53.33% and 60% of the cases (T1-T4, respectively). For the control group, the ovulation rate of mares increased after 48 h. After insemination once at 24 h after treatment, the pregnancy rates of the mares treated with hCG and GnRH were higher than 70%, while that of the mares treated with prostaglandin F2a was lower than 50%.



A: follicle diameter is $(50.1 + 43.5) / 2 = 46.65$ millimeters.

B: follicle diameter is $(24.7 + 24.7) / 2 = 24.7$ millimeters.

Figure 1 Follicle diameter measurement by real-time 2D mode with transrectal, 7 MHz linear-array ultrasonography



A: Grade of zero (0), no uterine edema with a typical homogeneous echo-texture characteristic

B: Grade of one (1), smallest degree of readily detectable uterine edema with a fish bone characteristic

C: Grade of two (2), moderate degree of edema with a wheel characteristic

D: Grade of three (3), largest degree of edema throughout the whole uterus, sometimes free fluid also noted

Figure 2 Subjective scoring system (0-3) for degree of endometrial edema

Table 1 Diameter of preovulatory follicle and uterine edema score 6 days before ovulation in the control group

Day before ovulation	Uterine edema score	Number of mare	Preovulatory follicle diameter (mm)
D-6	0	20	29.8±3.46
	1	23	31.9±3.71
	2	7	34.4±1.58
	3	0	-
mean	0.76±0.7	50	31.5±3.70
D-4	0	3	32.9±1.9
	1	28	36.8±2.47
	2	19	38.9±2.75
	3	0	-
mean	1.33±0.58	50	37.4±2.96
D-2	0	1	36.5±0
	1	14	38.4±2.78
	2	23	40.4±2.16
	3	12	43.3±1.92
mean	1.92±0.77	50	40.49±2.90
D-1	0	19	39.5±2.40
	1	30	40.8±2.48
	2	1	38.5±0
	3	0	-
mean	0.62±0.52	50	40.27±2.52

Table 2 Ovarian status and response to treatment with prostaglandin F2a, GnRH and hCG

	Control (normal cycle)	T1 (prostaglandin F2a)	T2 (40 µg GnRH)	T3 (1500 IU hCG)	T4 (3000 IU hCG)
Number of cycles	50	10	15	15	10
diameter of dominant follicle on the day before ovulation (mm)	40.27±2.53 ^a (36.0-45.0)	38.54±1.86 ^b (35.0-41.0)	45.47±3.53 ^c (41.0-51.8)	41.64±3.48 ^a (36.0-48.6)	43.69±1.51 ^c (41.5-46.0)
Rate of dominant growth (mm/day)	2.36±0.34 ^a	4.02±0.58 ^b	6.52±0.60 ^c	6.33±0.59 ^c	5.44±1.02 ^d
Interval treatment to ovulation (day)	3.34±1.08 ^a (2-5)	3.2±0.79 ^a (2-4)	1.83±0.77 ^b (1-4)	1.53±0.53 ^b (1-2)	2.10±0.57 ^c (1-3)
% Ovulation within 48 h	26% (13/50)	30% (3/10)	80% (12/15)	100% (15/15)	60% (6/10)
Pregnancy rate at D15 (%)	-	40% (4/10)	73.33% (11/15)	93.33% (14/15)	70% (7/10)

*measured from the day which the dominant follicle reached 35 mm in diameter

^{a,b,c} Statistical difference between groups (P < 0.05)

Discussion

In routine practice, the size of follicle and uterine edema score are used to predict the day of ovulation (Samper, 2008, Squires et al., 2014). The degree of uterine edema increases in response to estrogen produced from the preovulatory follicle (Watson, 2003) and decreases when the time is close to ovulation (Samper, 2008). Unsurprisingly, in the present study, the same result was also found, therefore it is suggested that two days after detecting the largest diameter of follicle (40 mm) and the largest degree of edematous score (scores 2-3) could be the optimal time to naturally cover mare.

The mares in this study were selected based on the fact that they had never received any ovulation induction agents such as hCG or deslorelin in their life. The sizes of follicles in all groups ranged from 35 to 52 mm in diameter, which are in the ideal range (34 to 70 mm) of pre-ovulatory follicle diameter within 24 h prior to ovulation (Cuervo-Arango and Newcombe, 2008).

In this study, the interovulatory interval in the mares treated with prostaglandin F2a was shorter than in the control group. The interval treatment prostaglandin F2a administration (D6 after ovulation) to subsequent ovulation in T2 did not differ from the interval dominant follicle reaching 35 mm in diameter

to ovulation in the control mares. These might be because the rate of dominant growth of T2 was higher than the control, and the diameter of dominant follicle on the day before ovulation of T2 was smaller than the control. This result is in contrast with the study of Schauer et al. (2013) which found that prostaglandin F2a did not have effects on follicle growth or ovulation. The interval treatment to ovulation in the mares treated with prostaglandin F2a from the present study was longer than from previous reports (Savage and Liptrap, 1987). This might be because of the size of the remaining follicles (Lindeberg et al., 2002; Newcombe et al., 2008), the degree of endometrial edema at the time of treatment (Samper, 2008; McCue, 2015), and the dose of prostaglandin F2a (Newcombe et al., 2008). In this study, the administration of a single dose of prostaglandin F2a at 6 days post ovulation had complete luteolysis effect which did not increase in the incidence of hemorrhagic anovulatory follicles (HAFs). However, a single dose of prostaglandin F2a at 3 days post ovulation (Bergfelt et al., 2006; Carluccio et al., 2008) could result in luteolysis effect similar to daily administration of prostaglandin F2a twice in the early diestrus period (Rubio et al., 2008). The pregnancy rate of the mares treated with 5 mg prostaglandin F2a was very low. In accord with the present study, Lindeberg et al. (2002) also found the same result and suggested that this might be due to the unpredicted ovulation

time after treatment with prostaglandin F2a. Therefore, a single dose of 5 mg prostaglandin F2a (lutalyse®; Pfizer Animal Health), im, on D6 after ovulation is recommended to fasten a return to estrous in mares, without hastening ovulation.

Inducing ovulation with hCG could be done with various dosages. Previous studies showed that 1500-3000 IU of hCG could induce ovulation within 48 to 56 h, while 80% of treatment with dosages from 1500 to 5000 IU hCG induced ovulation of animals within 48 h (Barbacini et al., 2000; Bergfelt et al., 2007; Figueiredo et al., 2011). Comparable to the previous results, in the present study the percentages of number of mares that ovulated within 48 h after treatment were 100% and 60% in T3 and T4, respectively. High dose of exogenous hCG may cause downregulation of the hypothalamic-pituitary-ovary pathway. The higher ovulation rate found in this study might be due to the fact that all mares had never been under any hormonal program. Therefore, to predict accurate ovulation time and increase pregnancy rate, 1500 IU of hCG given intravenously 24 h prior to fresh semen insemination is recommended.

Besides hCG, Deslorelin acetate subcutaneous implant (Ovuplant) is used to synchronize ovulation in mares (Michaela and Allen, 2005). Although repeated use of implant is not associated with a reduction in inducing ovulation (Farquhar et al., 2000), non-removal implant prolongs ovulation interval. Buserelin acetate (Receptal; MSD Animal Health) is also licensed for synchronizing ovulation in anestrus mares but is impractically used in cycling mares due to its short-lasting effect and need for multiple doses (Campbell, 2012). The ovulation rate within 2 days of Buserelin in the present study (80%) seems slightly lower than that reported by McCue et al. (2002) in cycling mares treated with 48 h deslorelin implants. The present study showed that ovulation could be induced by using a single shot of 40 µg Buserelin, im, with similar result to multiple treatment of 20 µg Buserelin (Barrier-Battut, 2000). Comparative studies of the effects of deslorelin acetate and hCG on the interval treatment to ovulation showed various results (Voss, 1993; Vanderwall et al., 2001; Samper et al., 2002 and Hemberg et al., 2006), but in the present study, the interval of ovulation with Buserelin was not significantly different from 1500 IU hCG, but more accurate in 48 h ovulation than 3000 IU hCG. This indicates that the single dose of 40 µg Buserelin, im is a suitable alternative protocol for mares which fail to ovulate from the repeated Chorulon protocol.

In conclusion, ovulation could be hastened and normal fertility, in the type of natural cover system, could be accomplished using 1500 IU hCG, iv or 40 µg Buserelin, im in cycling mares. Furthermore, the 1500 IU hCG-treated mares provided the shortest mean number of days from administration until ovulation and the highest pregnancy rate.

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บทคัดย่อ

การศึกษาผลการเหนี่ยวนำการตกไข่ด้วย prostaglandin F2a, GnRH analogue และ hCG ในแม่ม้า

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เนื่องด้วยปัจจุบันมีการใช้วิธีการผสมแบบระยะเวลาที่แน่นอนเพื่อเพิ่มประสิทธิภาพทางระบบสืบพันธุ์ จึงทำให้มีการศึกษาถึงประสิทธิภาพในการเหนี่ยวนำการตกไข่และอัตราการตั้งท้องของแม่ม้าที่ได้รับฮอร์โมนชนิดต่าง ๆ ทำการเฝ้าติดตามวงรอบการเป็นสัดของแม่ม้าจำนวน 50 ตัว (กลุ่มควบคุม) โดยการตรวจด้วยเครื่องอัลตราซาวด์ทุก 3 วันจนกระทั่งเกิดการตกไข่ จากนั้นทำการฉีดฮอร์โมนโปรสตาแกลนดิน (prostaglandin F2a) 5 มิลลิกรัมเข้ากล้ามเนื้อของแม่ม้าทุกตัวในวันที่ 6 หลังการตกไข่ ทำการตรวจระบบสืบพันธุ์ของแม่ม้าด้วยเครื่องอัลตราซาวด์ทุก 3 วันจนกระทั่งพบฟอลลิเคิลที่มีเส้นผ่าศูนย์กลางขนาดอย่างน้อย 3.5 เซนติเมตรและมดลูกมีระดับการบวม น้ำของผนังมดลูกชั้นในเท่ากับ 2 แบ่งแม่ม้าออกเป็น 4 กลุ่ม โดยกลุ่มที่หนึ่ง คือ แม่ม้าที่ได้รับโปรสตาแกลนดินอย่างเดียว (10 ตัว) กลุ่มที่สองได้รับฮอร์โมนโกนาโดโทรฟิน รีรีซิง อนุลอก (GnRH analogue) (Buserelin) 40 μ g (15 ตัว) กลุ่มที่สามได้รับฮอร์โมนฮิวแมน คอริโอนิก โกนาโดโทรฟิน (human chorionic gonadotropin; hCG) ขนาด 1500 ยูนิต (15 ตัว) และกลุ่มที่สี่ได้รับ hCG ขนาด 3000 ยูนิต (10 ตัว) การศึกษาพบว่า วงรอบการเป็นสัด (ระยะเวลาตกไข่ถึงตกไข่ในวงรอบถัดไป) ในกลุ่มควบคุมและกลุ่มที่ได้รับโปรสตาแกลนดิน (กลุ่มที่ 1) มีค่าเท่ากับ 20.87 วันและ 14.83 วัน ตามลำดับ พบความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของขนาดเส้นผ่าศูนย์กลางของฟอลลิเคิลในวันก่อนตกไข่ระหว่างกลุ่มควบคุม (40.7 มิลลิเมตร) กับกลุ่มที่ 1 (38.54 มิลลิเมตร) กลุ่มที่ 2 (45.47 มิลลิเมตร) และกลุ่มที่ 4 (43.69 มิลลิเมตร) อัตราการเจริญของฟอลลิเคิลต่อวันของกลุ่มควบคุมมีค่าต่ำสุด ขณะที่กลุ่มที่ 3 มีอัตราการเจริญของฟอลลิเคิลสูงสุด (6.5 มิลลิเมตร) ช่วงเวลาระหว่างการได้รับฮอร์โมนถึงการตกไข่ของกลุ่มที่ 3 (1.53 วัน) และกลุ่มที่ 2 (1.83 วัน) มีระยะเวลาสั้นกว่ากลุ่มที่ 1 (3.2 วัน) และกลุ่มที่ 4 (2.1 วัน) อย่างมีนัยสำคัญ แม่ม้าที่ได้รับ hCG ขนาด 1500 ยูนิต (กลุ่มที่ 3) ทุกตัวเกิดการตกไข่ภายใน 48 ชั่วโมง และมีอัตราการตั้งท้องร้อยละ 93.33 สรุปได้ว่า hCG ขนาด 1500 ยูนิตสามารถเหนี่ยวนำการตกไข่ในแม่ม้า โดยให้ระยะเวลาหลังได้รับฮอร์โมนถึงการตกไข่สั้นที่สุดและอัตราการตั้งท้องสูงที่สุด

คำสำคัญ: prostaglandin GnRH analogue hCG เหนี่ยวนำการตกไข่ แม่ม้า

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