Original Article

Effect of IVPRD, estradiol and GnRH for resynchronization on fertility of non-pregnant Holstein dairy cows

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Abstract

The aim of this study was to evaluate the effect of intra vaginal progesterone releasing device (IVPRD), estradiol and GnRH for resynchronization on fertility of non-pregnant Holstein dairy cows. Non-pregnant cows (n = 117) were divided into three groups. The first group (n = 38) was treated with Ovsynch [GnRH, day 0; PGF2α, day 7; GnRH, day 9; timed artificial insemination (TAI), day 10]. The second group (n = 39) was treated with cue-mate-estradiol benzoate (EB) (cue-mate intra vaginal insertion and first EB, day 0; PGF2α, day 7; cue-mate withdrawn and second EB, day 8; TAI, day 10). The third group (n = 40) was treated with PG (PGF2α, day 0; AI after heat expression). The trial was conducted from September 2014 to March 2015. Milk sampling was done for each cow 14, 21 and 24 days after resynchronized AI for analysis of progesterone concentration. Conception rate was significantly decreased ($P < 0.05$) with IVPRD and EB compared to with PG. In contrast, in the Ovsynch and cue-mate-EB treatment groups, return to estrus tended to be higher than in the PG group ($P = 0.05$). In conclusion, the reproductive performance in the PG group was better than in the cue-mate-EB group, but was similar to that in the Ovsynch group.

Keywords: estradiol, GnRH, IVPRD, non-pregnant dairy cows, resynchronization

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Introduction

The reproductive efficiency in dairy herds around the world has declined for several decades (Lucy, 2001). The improvement in reproductive performance can be partially explained by the use of systematic reproductive management programs, including programs for synchronization of ovulation TAI (Wiltbank and Pursley, 2014). Large percentage of cows remains non-pregnant after AI or TAI. Detection of spontaneous return to estrus is a substantial challenge for dairy herds, with approximately 40 to 60% of non-pregnant cows not re-inseminated before an examination to diagnose pregnancy (Sani et al., 2011; Chenault et al., 2003; Chebel et al., 2006; Galvao et al., 2007). Therefore, cows diagnosed as non-pregnant should be submitted to ovulation synchronization protocols and timed AI to assure re-insemination in a timely manner and resynchronization.

The strategy for resynchronization of ovulation on most dairy farms often relies on the timing of initiation of the Ovsynch protocol or variations of the Ovsynch protocol after insemination (Fricke et al., 2003; Bartolome et al., 2005; Sterry et al., 2006). In most experiments, there are usually fewer pregnancies per AI (P/AI) to TAI after resynchronization compared with TAI at first service (Thompson et al., 2015; Galvao et al., 2007; Silva et al., 2009), indicating that fertility to TAI after resynchronization is not optimized.

One reason for the poor fertility to resynch services is that between 15 to 26% of cows lack a corpus luteum (CL) or have low progesterone (P4) at initiation of resynchronization (Fricke et al., 2003; Sterry et al., 2006; Silva et al., 2009). This is problematic because initiating a resynchronization protocol in a low P4 environment reduces fertility compared with that in ovular cows (Fricke et al., 2003; Silva et al., 2007).

To date, resynchronization rates during estradiol-progesterone-based programs in lactating dairy cows have not been adequately evaluated. Early studies that established the physiological basis for these programs utilized beef heifers and found that treatment with various forms of estradiol led to suppression of gonadotropins, regression of growing follicles, and emergence of a new follicular wave (Bo et al., 1993; Bo et al., 1994; Bo et al., 1995). Furthermore, a reduced dose of estradiol cypionate (ECP) at progestin withdrawal enhanced manifestation of estrous behavior and synchronous luteinizing hormone (LH) release and ovulation (Colazo et al., 2003).

Resynchronization of estrus in cattle must not disrupt pregnancy from a previous insemination. In this regard, the use of estradiol to induce follicular wave emergence or estrus is of some concern about CL regression in cattle with unknown pregnancy status (Colazo et al., 2007). Therefore, the resynchronization protocol in this study was started after pregnancy diagnosis. After negative pregnancy diagnosis, if CL exists, $PGF_2\alpha$ is useful, and after luteolysis, AI can be done according to heat expression. One of the oldest ways to synchronize estrus is by using a luteolytic agent such as Prostaglandin F2α, or an analogue, which causes regression of the corpus luteum (Islam, 2011; Rowson et al., 1972; King and Robertson, 1974; Lauderdale et al., 1974; Louis et al., 1974). Prostaglandin F2a is only effective if administered between days 8 to 17 of the estrous cycle, when functional corpus luteum is available in one of the ovaries. One of the methods used is 'one shot prostaglandin', which means a single injection of prostaglandin is given to cyclic females, and then these females are bred as they express estrus. The disadvantage of this program is that one-third of the females do not respond to the injection, however it is cost-effective because only one injection is given. This method can be used as resynchronization in cows that have corpus luteum.

Therefore, the objective of this study was to evaluate the effects of three AI resynchronization methods on estrus detection rate, luteolysis of a corpus luteum, conception rate and pregnancy loss.

Materials and Methods

Animal and Treatments: Lactating dairy cows (n = 117) from a dairy herd in the suburb of Semnan were enrolled in this experiment after pregnancy diagnosis, 32 ± 3 days after pre-enrollment AI (PAI). The cows were milked thrice daily and were fed a total mixed ratio (TMR) three times daily. TMR was formulated to meet or exceed requirements for lactation. The cows were blocked according to parity (1, 2, or ≥ 3), number of AI (1, 2, or ≥ 3) and milk production (median = 35 kg, range = 12-52 kg). Within each block, the cows were randomly assigned to one of the three resynchronization treatments (Fig 1), i.e. Ovsynch, cue-mate-EB and PG. All cows enrolled in the Ovsynch protocol were examined for pregnancy at 32 ± 3 days after PAI (day 0) by ultrasound examination. Cows that were non-pregnant were submitted to treatment consisting of GnRH injection (250 µg gonadorelin; Gonabreed, Parnell Laboratories, Australia) on day 0, PGF2α injection (500 µg cloprostenol; Estroplan, Parnell Laboratories, Australia) on day 7, and second GnRH injection concomitant with TAI on day 10. For all cows that enrolled in the cue-mate-EB group and were diagnosed as non-pregnant at 32 ± 3 days after PAI (day 0), an intravaginal progesterone device (cue-mate, Bioniche, Australia) containing 1.56 g progesterone was inserted from days 0-8. In addition, the cows in this group underwent treatment consisting of 2 mg EB i.m. (2 mg; Vetastrol, Aburaihan Pharmaceutical Co., Iran) on day 0, PGF2α injection (500 µg cloprostenol; Estroplan, Parnell Laboratories, Australia) on day 7, second EB injection on day 8 and AI with TAI on day 10. For the PG group, cows that were diagnosed as non-pregnant were submitted to treatment consisting of PGF2α injection (500 µg cloprostenol; Estroplan, Parnell Laboratories, Australia) on day 0 and AI at observed estrus.

In all treatments, the cows were observed for estrus thrice daily and those observed in estrus were inseminated according to the AM–PM rule. Three technicians performed the insemination for all cows using semen from different sires randomly assigned to the treatments while maintaining an equal use of sires across treatments. All of the experimental protocols and animal care were approved by the Animal Health Committee of the School of Veterinary Medicine, Semnan University (Number 23).

Milk sampling for progesterone analysis: Milk samples were collected from all cows on days 14, 21 and 24 after resynchronized AI (RAI) into 10 ml Falcon tubes. The milk samples were frozen at -25ºC until later analysis of progesterone concentration. Progesterone concentration was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Progesterone ELISA, DRG Ins. GmbH, Germany). Sensitivity of the assay was 0.05 ng/ml. Intra and inter-assay coefficients of variation were 3.7 and 8.3%, respectively. Validity of the kit for use with cow milk was done as described previously (Sani et al., 2011). Data for progesterone concentrations were later dichotomized as ≥ 2 or < 2 ng/ml (Cavalieri et al., 2003) to evaluate the effect of resynchronization treatments on the presence of an active CL and incidence of luteolysis. Cows having a progesterone concentration ≥ 2 ng/ml at any time point were assumed to have an active CL, and a drop in progesterone concentration from ≥ 2 to < 2 ng/ml between 14 and 21 days or 21 and 24 days after RAI was indicative of luteolysis.

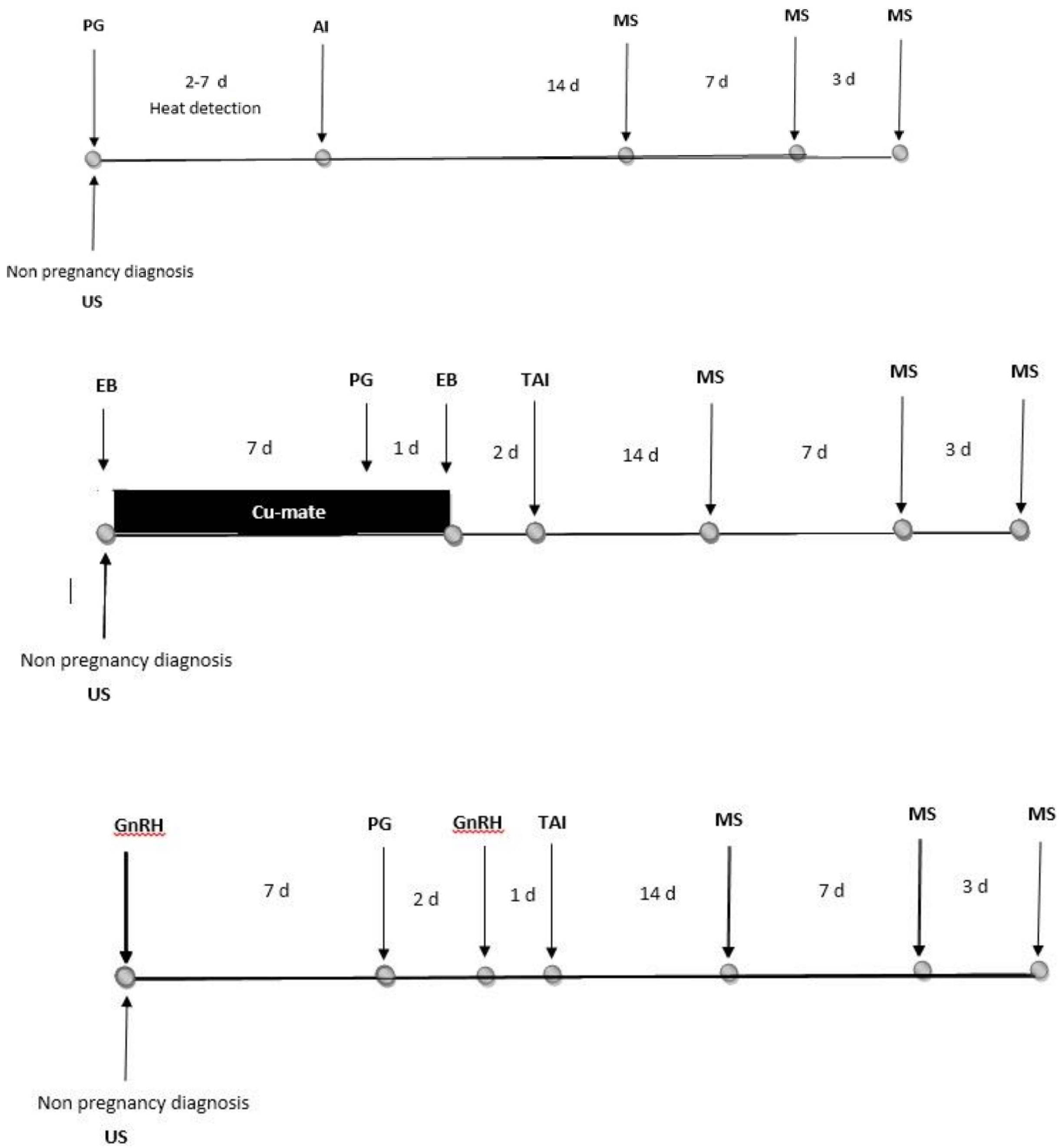

Figure 1 Diagrammatic representation of three treatments used to resynchronize estrus following previous AI for Holstein dairy cows. AI = artificial insemination; Cue-mate = intravaginal progesterone releasing device; GnRH = 250 µg gonadorelin i.m.; PG = 500 µg cloprostenol i.m.; EB = 2 mg i.m. estradiol benzoate; US = ultrasound examination for pregnancy diagnosis; TAI = timed AI; MS = milk sampling for progesterone concentration measurement.

Pregnancy diagnosis, pregnancy loss, and reproductive performance: All cows were examined for pregnancy by ultrasonography on days 32 ± 3 after PAI. Detection of an embryonic vesicle with a viable embryo in which heartbeat was visualized was used as an indicator of pregnancy. Pregnant cows were palpated again per rectum 30 days later (67 ± 3) for confirmation of pregnancy. Conception rate was defined as the number of pregnant cows divided by the total number of cows inseminated in each treatment. Cows diagnosed as pregnant on day 32 and as non-pregnant on day 67 were considered to experience late embryonic or early fetal loss.

Statistical analysis: Data were analysed using SAS statistical software version 8.2. All binary outcomes (luteolysis, defined as progesterone concentrations equal or less than 2 ng/ml, estrus incidence, conception and pregnancy loss) were analysed using a multivariable logistic regression (LOGISTIC procedure in SAS) model that included parity of the cows. The cows were classified into three parity groups (1, 2 and ≥ 3 parity).

Results

Three cows were excluded from the study because of their cue-mate loss (no = 1, 2.5%) and culling during treatment (no = 2, 1.4%). Milk yield, AI number and parity did not differ significantly among the cows in different treatment groups (P > 0.05). The percentage of return to estrus between days 14 and 21 was similar among the groups (P > 0.01). Also, the return to estrus rate between days 21 and 24 was similar among all treatment groups (P > 0.01). However, the PG group tended to be re-inseminated (after RAI) at a smaller rate than the other groups (P = 0.05) between days 14 and 24 (Table 1). The cows in the PG group, as expected, were more likely (P < 0.01) to be detected in estrus than the other groups for RAI, and no significant differences were observed between the Ovsynch and cue-mate-EB treatment groups (P > 0.01). Therefore, the incorporation of a Cue-mate insert to the resynchronization protocol did not affect the expression of estrus at AI.

Table 1 Effect of different protocols for resynchronization of estrus in Holstein dairy cows on estrus detection rate at different intervals, luteolysis of corpus luteum established, CR[1] and pregnancy loss, after RAI[2]

Items	Treatments			
	PG (n = 40)	**Ovsynch (n = 38)**	**Cue-mate-EB[3] (n = 39)**	**P-value**
Returned to estrus after RAI				
Days 14-21	%10 (4/40)	%21.05 (8/38)	%23.08 (9/39)	0.26
Days 21-24	%12.5 (5/40)	%26.32 (10/38)	%25.64 (10/39)	0.14
Days 14-24	%22.5 (9/40)	%47.37 (18/38)	%48.72 (19/39)	0.05 Luteolysis after RAI
Days 14-21	%25 (10/40)	%42.11 (16/38)	%33.33 (13/39)	0.28
Days 21-24	%5 (2/40)	%5.26 (2/38)	%17.95 (7/39)	0.08
Days 14-24	% 30 (12/40)	%47.37 (18/38)	%51.28 (20/39)	0.12
P/AI at 32 ± 3 d of RAI	%32.5 (13/40[b])	%39.47 (15/38[b])	%10.26 (4/39[a])	< 0.01
P/AI at 61 ± 3 d of RAI	%100 (13/13)	%100 (15/15)	% 100 (4/4)	> 0.05

[a,b] Values within a row with different superscripts are different (P < 0.05). [1] Conception rate, [2] Resynchronized artificial insemination, [3] Estradiol benzoate.

The average proportion of cows with an active CL was 91.4% (107/117) on day 14 of RAI, 64.8% (76/117) on day 21 of RAI and 65.6% (77/117) on day 24 of RAI, and was not significantly different among the treatments (P > 0.01). The incidence of luteolysis, as indicated by a drop in milk progesterone concentration which was 25%, 33.3% and 42.1% from days 14 to 21 in the PG, cue-mate-EB and Ovsynch groups, respectively, ranged from 5 to 17.9% from days 21 to 24, and from 30 to 51.2% from days 14 to 24 (Table 1). Differences between the treatments were not significant, but there was a tendency to significance in luteolysis from days 21 to 24. In this period, the cue-mate-EB group tended to be luteolysis at a higher rate than the other groups (P = 0.08).

The treatment affected (P < 0.05) P/AI on days 32 ± 3 of RAI. The cue-mate-EB cows (10.26%) were (P = 0.01) less likely to be diagnosed as pregnant than the PG (32.5%) and Ovsynch (39.4%) cows, whereas the pregnancy rate of the PG and Ovsynch cows did not differ (P > 0.05). The pregnancy per AI at 61 ± 3 days of RAI was not affected by the treatments, which therefore did not affect pregnancy loss (P > 0.05) (Table 1).

Discussion

In cattle resynchronized with progestin, fertility could be compromised by prolonged growth and dominance of the ovulatory follicle in those that have spontaneous luteal regression prior to progestin withdrawal. In that regard, fertility has been reported to be lower in heifers resynchronized with only a used intravaginal progesterone device than in untreated controls (Stevenson et al., 2003; Colazo et al., 2006). However, this could be overcome by inducing a new follicular wave at the start of progesterone treatment.

In this study EB was used for inducing a new follicular wave and improving fertility, but PAI was decreased in comparison with the other treatments. Our result is in agreement with those of other studies that administered 1 mg of ECP but did not effectively synchronize follicular wave emergence and improve fertility with ECP (Colazo et al., 2007). Different results were observed from studies that used estradiol for resynchronization. In some of them, the pregnancy rate was apparently unaffected when cows were given 1 mg of EB (Stevenson et al., 2003). In contrast, the administration of 1 mg of EB to synchronize follicle wave emergence (Cutaia et al., 2002) or 0.5 mg estradiol-17b to resynchronize estrus (Colazo et al.,

2006) in beef heifers seemed to be associated with reduced pregnancy rates to TAI. It is suggested that estradiol might induce luteolysis because the cue-mate-EB group tended to be luteolysis at a higher rate than the other groups in this study.

Several other studies reported regression of CL after treatment with EB (Araujo et al., 2009) or 17β-estradiol (Ford et al., 1975; Thatcher et al., 1986). Treatment with E_2 could increase PGF production by the uterus (Ford et al., 1975; Thatcher et al., 1986). This effect appears to be mediated by binding of E_2 to estrogen receptor α, subsequent upregulation of oxytocin receptor expression, binding of oxytocin to oxytocin receptor, and subsequent production of PGF pulses due to the pulsatile pattern of oxytocin pulses (McCracken et al., 1999; Fleming et al., 2006; Spencer et al., 2006). Thus, although greater doses of EB might be expected to produce greater suppression of gonadotropins, this effect might be more than counteracted by the decrease in circulating P4 due to increased CL regression with a greater dose of EB (Monteiro et al., 2015).

Another method that was used for resynchronization in the present study was the $PGF_2α$ injection. In this group AI was done after heat expression. All cows in this group presented heat until 7 days after injection. Our results showed that the pregnancy rate in this group was similar to that of the Ovsynch group. Therefore, the use of one $PGF_2α$ injection as a resynchronization method could produce good pregnancy rate compared to that of the Ovsynch group. However, this method is heat dependent and, as a result, if heat detection rate is low in a farm, this method should not be used.

The stage of estrous cycle in which the first GnRH of the resynchronization protocol was administered influenced subsequent P/AI in some (Fricke et al., 2003; Sterry et al., 2006), but not all (Sani et al., 2011; Silva et al., 2009) studies. In the present experiment, the cows received the first GnRH of the resynchronization protocol 39 d after the previous AI. The length of the estrous cycle in lactating dairy cows averages 22.9 ± 0.7 days (Sartori et al., 2004); therefore, on average, it is expected that most of the cows would be on day 11 of the estrous cycle when the resynchronization protocol was initiated. This is important because approximately 50% of cows treated with GnRH between days 10 and 16 of the estrous cycle failed to ovulate (Vasconcelos et al., 1999), which, in association with spontaneous regression of CL before the injection of PGF2α, resulted in increased premature ovulation at the end of the synchronization program and reduced fertility (Vasconcelos et al., 1999). Therefore, in our study the intra vaginal progesterone releasing device (IVPRD) was used to eliminate the effect of decreased progesterone before the injection of PGF2α and compare it with GnRH as a resynchronization method (Ovsynch). Our results, however, showed that Ovsynch was better than IVPRD + EB. The reason might be the effect of EB on CL. .

Non-pregnant cows that do not return to estrus have been termed "phantom cows". Under traditional reproductive management, a phantom cow is not detected until pregnancy exam; 35-42 days after initial insemination. High rates of both synchronous estrus and conception are needed to optimize pregnancy rates in non-pregnant cattle returning to estrus following AI. Therefore, nowadays resynchronization is done to decrease these cows. Our result showed that the return to estrus rate with cue-mate tended to be higher than with PG, but was similar with Ovsynch. A previous study showed that there was no significant effect of ECP treatment on estrous rate and also on conception rate (Colazo et al., 2007). In another study (Colazo et al., 2006), the use of a previously used IVPRD following TAI to resynchronize return to estrus resulted in a reduced period of estrus detection. The higher return to estrus in the cue-mate group was probably caused by luteolysis.

Conclusion

The fertility of lactating dairy cows subjected to $PGF_2α$ and Ovsynch protocols was not different, but was decreased with the cue-mate-EB protocol, although this protocol tended to increase the return to estrus compared with the others. Moreover, $PGF_2α$ is an effective method for resynchronization if heat detection rate is high.

Acknowledgements

The authors would like to thank Mr. Haji Golikhani and Mr. Dadashpoor for their assistance and collaboration.

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บทคัดย่อ

ผลของ IVPRD, estradiol และ GnRH สำหรับ resynchronization เพื่อการผสมพันธุ์ ในโคนมพันธุ์โฮลสไตล์ที่ไม่ตั้งท้อง

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วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อประเมินผลกระทบของ IVPRD estradiol และ GnRH สำหรับ resynchronization เพื่อการผสมพันธุ์ ในโคนมพันธุ์โฮลสไตล์ ที่ไม่ตั้งท้อง โดยวัวไม่ตั้งท้องได้รับการรักษาด้วย Ovsynch จำนวน 38 ตัว, cue-mate-estradiol benzoate (EB) จำนวน 39 ตัว และ PG จำนวน 40 ตัว โดยศึกษาตั้งแต่กันยายน 2014 ถึงเดือนมีนาคม 2015 (n = 117) และสุ่มตัวอย่างน้ำนมในโคแต่ละตัวที่ 14 วัน 21 วันและ 24 วันหลังจาก resynchronized และผสมเทียม เพื่อวิเคราะห์ของความเข้มข้นของฮอร์โมนโปรเจสเตอโรน ผลการศึกษาพบ อัตราการผสมติดลดลงอย่างมีนัยสำคัญ ($P < 0.05$) ในกลุ่ม IVPRD และ EB เมื่อเปรียบเทียบกับกลุ่มอื่น ในทางตรงกันข้ามในกลุ่ม Ovsynch และกลุ่ม EB ให้ผลการกลับไปเป็นสัดที่มีแนวโน้มสูงกว่ากลุ่ม PG ($p = 0.05$) สรุปผลการดำเนินงาน การรักษาด้วยกลุ่ม PG ดีกว่ากลุ่ม EB แต่ให้ผลคล้ายกับกลุ่ม Ovsynch

คำสำคัญ: Estradiol GnRH IVPRD โคนมไม่ตั้งท้อง Resynchronization

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