OVARIAN RESPONSE OF DAIRY COWS TO PROGESTERONE COMBINED ON ESTRUS SYNCHRONIZATION USING GnRH-PGF2α BASED PROTOCOL

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi respon ovarium melalui perlakuan sinkronisasi berahi dengan menggunakan kombinasi GnRH dan PGF2α dengan progesteron pada sapi perah dengan sistim perkandangan diikat dalam kandang. Sebanyak 20 ekor induk FH dengan tahapan siklus berahi yang berbeda digunakan dalam penelitian ini. Ternak secara acak dibagi dalam dua kelompok perlakuan dengan dan tanpa CIDR (Controlled internal drug release). Semua ternak diinjeksi 10 mg GnRH i.m pada awal perlakuan (H0) tanpa memperhatikan tahapan siklus berahi, diikuti penyuntikan 25 mg PGF2α i.m 7 hari kemudian. CIDR dimasukkan secara intravagina pada kelompok perlakuan CIDR saat injeksi GnRH dan dilepas pada saat injeksi PGF2α. Tanda-tanda berahi diamati dua kali sehari dimulai pada hari kedua setelah perlakuan kecuali pada kelompok CIDR, tanda-tanda berahi mulai diamati pada hari ke-8. Sampel darah diambil pada semua ternak pada hari perlakuan GnRH, PGF2α dan hari ke-10 pelaksanaan perlakuan untuk mengetahui konsentrasi hormon progesteron. Hasil penelitian ini menunjukkan bahwa 30% ternak pada perlakuan tanpa CIDR menunjukkaan tanda-tanda berahi sebelum penyuntikan PGF2α. Pemberian CIDR dalam sinkronisasi berahi dengan GnRH-PGF2α mencegah terjadinnya berahi dini dan mempertahankan konsentrasi progesteron yang tinggi pada saat injeksi PGF2α. Dapat disimpulkan bahwa pemberian CIDR dalam sinkronisasi berahi khususnya dengan GnRH-PGF2α efektif menyinkronkan berahi pada ternak sapi perah dan mencegah terjadinya berahi dini.

Kata kunci: Sapi perah, CIDR, Sinkronisasi berahi, Ovarium, Berahi dini

ABSTRACT

The objective of this study was to evaluate the response of ovaries in estrus synchronization protocol using GnRH-PGF2α based protocol combined progestin in a herd with tie-stall housing system. A total of 20 Holstein Friesian cows at different stages of estrus cycle in a dairy herd were enrolled in the present study. The cows were randomly allocated into two treatment groups; with and without CIDR (Controlled internal drug release) insertion. All cows received 100 µg of GnRH I.M. at the beginning of the treatment (d 0) without regard to the stages of the estrus cycle followed by 25 mg PGF2α IM 7days later. Cows in CIDR group were inserted CIDR into the vagina at the time of GnRH administration and were removed on the day of PGF2α administration. estrus signs were checked twice daily starting on day-2 after initiation of the protocol, except cows in CIDR group the estrus signs were checked starting on day-8 of the protocol. The animals showing estrus signs were noted. Blood samples were collected from all animals on the days of GnRH and PGF2α treatments and on day-10 after initiation of protocol for progesterone concentration. The results of this study showed that 30% animals in cows without CIDR insertion showed estrus signs prior to PGF2α injection. Involving CIDR to synchronized estrus with GnRH-PGF2α based protocol avoided the occurrence of premature estrus and maintained high progesterone concentration on the day of PGF2α administration. It is concluded that ovarian response after involving CIDR in the protocol for estrus synchronization especially using combination of GnRH and PGF2α were much effective to synchronize the initiation of estrus in dairy cows. Likewise, the use of progesterone avoided premature estrus.

Keywords: Dairy cows, CIDR, Synchronization of estrus, Ovaries, Premature estrus.

INTRODUCTION

One of the problems in reproductive management for the cows in tie-stall housing system is a difficulty in detecting estrus accurately. This due to that estrus detection based on solely secondary estrus signs without any interaction among the animals to show primary estrus sign particularly standing estrus. On the other hand, to achieve high pregnancy rate in a dairy herd, it is necessary to detect estrus accurately and inseminated the cows timely with proven high quality sire. Esslemont and Kossaibati (2000) stated that to be a good fertility management, 95% cows in the herd after calving must be served by keeping the average calving to first service interval to less than 70 days, more than 55% overall heat detection rate, and 50% or more of pregnancy rate. Requirements to achieve this target or high reproductive efficiency in the dairy herd are a disease-free transition period, high submission rates to AI, and high conception rate per service (Roche *et al*., 2000).

To avoid the difficulty of detecting estrus in the herd, estrus synchronization or estrus induction is a reproductive technology that commonly used to facilitate artificial insemination (AI). This technology has widely been used as an important tool for increasing AI submission rate in beef and dairy herds (Macmillan and Peterson, 1993; Xu and Burton, 1999; Lucy *et al*., 2001) and to reduce the need for estrus detection (DeJarnette *et al*., 2001).

Recently, the most commonly protocol used for synchronization of estrus is GnRH-PGF2α based-protocol. Synchronization protocols that regulate follicular development with a GnRH injection 7 days prior to a luteolytic dose of PGF2α not only improve estrus detection rates and synchrony of estrus (Wolfenson *et al*., 1994; Twagiramungu *et al*., 1995), but also induce fertile estrus cycles in both cyclic and anestrus bovine females (Stevenson *et al*., 2000). However, GnRH-PGF2α based-protocol for synchronizing estrus sometimes does not work properly resulting in premature estrus. This may be because of the increased odds of administering the first GnRH in the absence of a dominant follicle (Haugian and Wiltbank, 2002), leading to a low follicle turnover success and failure to induce a new follicular wave. As a consequence, corpus luteum (CL) is absent at the day of prostaglandin F2α (PGF2α) injection and estrus (premature estrus) and ovulation occurs around the day of PGF2α injection (DeJarnette *et al*., 2001).

To avoid this consequence, involving progestin in the protocol have often been used to increase the synchronized estrus (Ambrosse *et al*., 2005; Cavalieri *et al*., 2006; Yusuf *et al*., 2010). Therefore, the objective of this study was to evaluate the response of ovaries in estrus synchronization protocol using GnRH-PGF2α based protocol combined progestin (CIDR=controlled internal drug release) in a herd with tie-stall housing system.

MATERIALS AND METHODS

Dairy cows and herd management

This study was conducted in a dairy farm with a herd size of 44 cows and 14 of them were in lactation. Cows in the herd were housed in tie-stall barns. The cows were milked once daily in the morning time at approximately from 6:00 to 7:00 am. Feedstuffs consisted of Napier grass or rice straw, concentrate, and mineral supplements. The parity of the cows ranged from one to two. Cows detected in estrus were artificially inseminated (AI) by inseminator/technician approximately 2-6 hours later using frozen/thawed semen from proven Holstein Friesian sires. The voluntary waiting period (VWP) of the herd was determined 40 days after calving.

Clinical Examination and Selection of Cows

Prior to initiate the estrus synchronization, clinical examination was implemented by the authors with the help of local technician and/or management staffs of the herd. All cows were subjected to trans-rectal palpation for pregnancy status and/or the genitalia to asses uterine conditions and ovarian structures. Trans-rectal palpation of the uterine was performed to determine the consistency of uterine including contraction, elasticity, tonicity, symmetry of uterine horns, and the presence of any fluid in the uterus (Gautam *et al*., 2010). The presence of any palpable ovarian structures, ovarian cysts was defined as one or more follicle-like structures >25 mm in diameter without a concurrent corpus luteum (CL). Ovaries without palpable structures (i.e. ovarian follicles > 10 mm in diameter and/or a functional CL) were considered inactive (Yusuf *et al*., 2010), otherwise were considered active. Cows that did not become pregnant during clinical examination and had not suffering from

any type of ovarian cysts were selected to the protocol for synchronization of estrus.

Protocols for Synchronization of Estrus

Of 44 cows were examined in a herd, 20 cows at different stages of estrus cycle were selected and allocated into two treatment groups. All cows received 100 µg of GnRH-analogue (fertirelin acetate, Conceral®, Schering-Plough Animal Health, Tokyo, Japan) IM at the beginning of the treatment (d 0) without regard to the stages of the estrus cycle followed by 25 mg PGF2α (Dinoprost; Norbrook Laboratories Limited, Newry, BT35 6JP) IM 7 days later (Figure 1 and 2). The animals were randomly allocated into two treated groups (CIDR and without CIDR). At the time of GnRH administration, the CIDR group cows, a CIDR (EAZI-BREED CIDR®, Livestock Improvement Association of Japan, Tokyo, Japan) was inserted into the vagina of each cow. The CIDR was removed on the day of PGF2α administration (Figure 2).

estrus signs were checked twice daily starting on day-2 after initiation of the protocol for the group of cows without CIDR insertion (Figure 1) and starting on day-8 for the group of cows with CIDR insertion until five days after PGF2α injection (Figure 2). The animals showing estrus signs were noted. Those cows showing clear uterine contraction were confirmed to have been in estrus.

Blood Sampling

Blood samples were collected from all animals on the days of GnRH and PGF2α treatments and on day-10 after initiation of protocol via jugular vein or coccygeal venipuncture into heparinized vacuum tubes (Figure 1 and 2). After collection, the samples were centrifuged within 2 hours at 1500 x g for 15 minutes to collect plasma. The plasma was stored at -20ºC until assayed for progesterone concentration.

Hormone Assay and Case Definition

Plasma progesterone concentration was determined using radioimmunoassay (RIA) technique (IZOTOPE, Institute of Isotopes, Ltd. 1535 Budafest, Pf.: 851).

Effect of treatment was evaluated based on plasma progesterone concentrations at the days of GnRH treatment (day-0), PGF2α treatment (day-7), and day 10. Progesterone concentrations below 1.0 ng/ml were considered low and indicative of the absence of CL, while progesterone concentrations of 1.0 ng/ml or higher were considered as an indicative of functional CL and were referred as high (Cordoba and Fricke, 2002; Rivera *et al.,* 2004; Yusuf *et al.,* 2010). Cows showed high progesterone concentrations on day-7 were considered to have positive response to GnRH treatment, and cows showed high progesterone concentrations on day-7 and low on day-10 were considered to have positive response to GnRH and PGF2α treatments.

Statistical Analysis

All calculations were carried out using the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in percentages of luteal phase and follicular phase or inactive ovaries in each group of cows with and without CIDR insertion, high concentration of progesterone on day-7, and high concentration of progesterone on day-7 and low in day-10 among the groups were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Stage of Estrus Cycle prior to Estrus Synchronization

In the present study, the animals were allocated into two different treatment groups; with and without CIDR for estrus synchronization. This division was conducted randomly to each group for their stages of estrus cycle. The stages of estrus cycle at different treatment at the initiation of estrus synchronization are shown in Table 1.

Table 1 shows that after randomly allocated the cows at different treatment, cows treated with CIDR in the protocol had 70% in luteal phase at the initiation of estrus synchronization. This was indicated by high progesterone concentration (≥1 ng/ml). The remaining 30% of the cows in this treatment were in follicular phase or inactive ovaries at the initiation of estrus synchronization; indicated by low progesterone concentration (<1 ng/ml). Chi-Square analysis showed that CIDR treated cows with luteal phase had significantly higher ($P<0.01$) than in follicular phase or inactive ovaries. For the group of cows treated without CIDR in the protocol, comparison between cows in luteal phase and in follicular phase or inactive ovaries was similar (50% each). Totally, out of 20 animals used in the present study, 12 or 60% cows were in luteal phase,

higher (P<0.05) than the cows in follicular phase or inactive ovaries (8 cows; 40%). This result was in agreement with the previous study (Galvao and Santos, 2010) using 466 lactating Holstein cows reported that 59.4% of the cows were in luteal phase and the remaining 40.6% were in follicular phase or inactive ovaries. The importance of these reproductive physiological statuses prior to synchronized or induced estrus has been well studied by Bartolome *et al*. (2005) in order to apply estrus synchronization protocols in regard to the stage of estrus cycle at the beginning of the treatment. For example, their study suggests that Heatsynch protocol increased pregnancy rate for cows in metestrus at the time of initiation of the treatment regime, and the Ovsynch protocol was more effective for cows with ovarian cysts. Basically, synchronization of estrus in cattle implies the manipulation of the estrus cycle or induction of estrus to bring a large percentage of cattle in a herd into estrus at predetermined time (Odde, 1990) and to reduce the need for estrus detection (DeJarnette *et al*., 2001). This technique has widely been used as an important tool for increasing AI submission rate in beef and dairy herds (Macmillan and Peterson 1993; Xu and Burton 1999; Lucy *et al.*, 2001). Otherwise, efficient and accurate detection of estrus are fundamental for successful reproductive management when no fixed-time AI protocols are implemented (Heersche and Nebel, 1994; Stevenson *et al*., 2008). This reproductive protocol, however, requires daily observation for signs of estrus and may result in extended interval from puberty to pregnancy depending on estrus detection efficiency and accuracy (Stevenson *et al*., 2008). This implies that since the dairy or beef cattle producers have a difficulty to detect estrus in a certain time especially in tie-stall housing system, estrus synchronization is an alternative way for successful reproductive management.

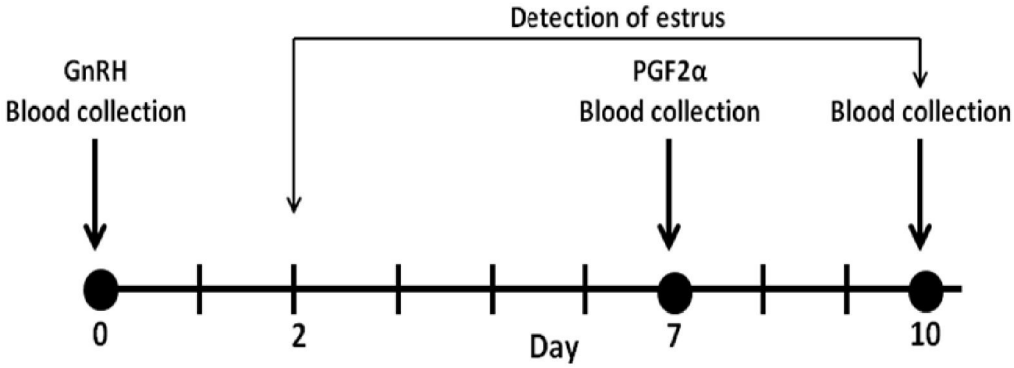

Figure 1. Estrus Synchronization Protocol, Blood Samples Collection, and Detection of Estrus for the Group of Cows Without CIDR Insertion

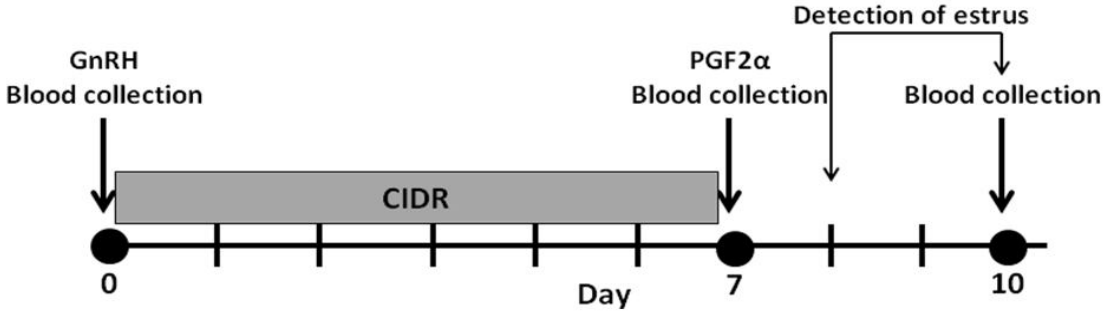

Figure 2. Estrus Synchronization Protocol, Blood Samples Collection, and Detection of Estrus for the Group of Cows with CIDR Insertion

Table 1. Stages of Estrus Cycle in Dairy Cows at the Initiation of Estrus Synchronization

Treatment	No. of cows	Luteal phase (%)	Follicular phase/ inactive ovaries (%)	P-value
CIDR	10	70	30	< 0.01
Without CIDR	10	50	50	1.000
Total	20	60	40	0.046

Table 2. Response of Ovaries to estrus synchronization Protocol in Dairy Cows on Day-7 Treated and Concentration of Progesterone in Dairy cows in which High on Day-7 and Low in Day-10 Treated with or Without CIDR

Variable	Treatment	
	CIDR	Without CIDR
No. of cows examined	10	10
No. of cows with luteal phase (%)	10 (100)[a]	7 (70)[b]
No. of cows with high concentration of progesterone on day-7 and low in day-10 (%)	8 (80)[a]	4 (40)[b]

[a,b] Different superscript in the same row indicate significantly different (P<0.05)

Response of Ovaries after CIDR or Without CIDR based on Plasma Progesterone Concentrations

In the present study, response of ovaries at different protocol was based on high progesterone concentration on day-7 after initiation of estrus synchronization. Table 2 shows that all cows treated with CIDR in estrus synchronization protocol showed high progesterone concentration on day-7. While the group of cows without CIDR insertion were significantly (P<0.05) lower number of cows had high progesterone concentration on day-7 (70%). This indicated that cows treated CIDR in estrus synchronization protocol using combination of GnRH and PGF2α were much effective than without CIDR insertion.

In the group of cows without CIDR insertion, 3 of 10 cows showed signs of estrus before PGF2α administration (day-7). This means that involved CIDR in the protocol avoided premature estrus. Study of Martinez *et al*. (2007) in beef heifers showed that inclusion of an intravaginal progesterone device (CIDR) in a GnRH-based timed-artificial insemination (TAI) regimen prevented premature estrus between the first injection of GnRH and PGF2α treatment and enhanced the pregnancy rate. Likewise, the study of Lucy et al. (2001) showed that treatment of heifers with intravaginal progesterone inserts and PGF2α has resulted in tighter estrus synchrony compared with heifers synchronized with 1 injection of PGF2α alone, with approximately 84 and 57% of heifers displaying signs of estrus during the first 3 d after treatment, respectively.

On the other hand, out of 20 cows used in the present study, 17 cows or 85% were in luteal phase or had high progesterone concentration on day-7, and subsequently implicated the effectively of applying estrus synchronization. Out of 10 cows without CIDR insertion, only 4 or 40% cows with high progesterone concentration on day-7 and low on day-10. While those cows with CIDR insertion, number of cows with high progesterone concentration on day-7 and low on day-10 were 8 cows (80%), or showed significantly (P<0.01) higher than the group of cows without CIDR insertion (Table 2). On day-7 of treatment regimen concurrently with CIDR

removal for the CIDR group cows, injection of PGF2α were implemented to all cows, except those cows showed premature estrus. Injection of PGF2α causes luteolysis of CL present and allows the cows to express estrus because of the reduced progesterone concentration (Stevenson *et al*., 2008).

CONCLUSIONS

It can be concluded that ovarian response after involving CIDR in the protocol for estrus synchronization especially using combination of GnRH and PGF2α were much effective to synchronize the initiation of estrus in dairy cows. Likewise, the use of progesterone avoided premature estrus.

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