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A SHORT PRESYNCHRONIZATION WITH PGF2α AND GNRH IMPROVES OVARIAN RESPONSE AND FERTILITY IN LACTATING HOLSTEIN COWS SUBJECTED TO A HEATSYNCH PROTOCOL

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Abstract

The objective of this study was to assess the efficacy of a single injection of Prostaglandin F2α (PGF2α) combined with or without GnRH before initiation of a Heatsynch protocol (GnRH-7d-PGF2α-2d-EB-1d-AI) on ovarian response and fertility in lactating Holstein cows. A total of 252 cows with a corpus luteum (CL; ≥10 mm) were assigned to one of three synchronization groups; 1 – Control (n=84), cows received two injections of PGF2α 14 days apart; 2 – Heatsynch with GnRH (PGH, n=88) the cows received PGF2α followed by GnRH four days later and then a Heatsynch protocol was initiated eight days after GnRH treatment; and 3 – Heatsynch without GnRH (PH, n=80) cows received a single injection of PGF2α followed by a Heatsynch protocol initiated 12 days after PGF2α. Cows detected in estrus were inseminated approximately 12 h after onset of estrus. Ovarian response and pregnancy diagnosis were determined by ultrasonography during the synchronization protocol and 30 days after AI, respectively. Results showed the percentage of cows with plasma P4≤0.4 ng/mL at AI were greater in PGH group compared to other groups (90.3 vs. 75.0%, P=0.03). The percentage of cows that ovulated in response to the GnRH injection of Heatsynch was affected (P<0.01) by synchronization protocol, because PGH cows were more likely to ovulate (77.2%) than PH (56.0%) cows. The proportion of cows displaying estrus was greater in PGH (70.4%) and PH (72.5%) groups compared with control (47.0%, P=0.04). Percentage of cows pregnant at 30 days after AI was (P=0.01) affected by synchronization treatment and was greater in PGH (45.16%) compared to control (25.0%) and PH (34.48%) groups. In summary, a short presynchronization that includes an injection of PGF2α and four days later GnRH increased fertility in Holstein cows subjected to a Heatsynch protocol. The enhanced fertility was due to a greater ovulatory response to GnRH of Heatsynch, more adequate plasma P4 concentrations during follicle development and a greater luteal regression following PGF2α prior to AI.

Key words: short presynchronization, Heatsynch, functional corpus luteum, Holstein dairy cow

Protocols that synchronize follicular dynamics, corpus luteum (CL) regression and ovulation have been developed and are currently available for dairy producers (Colazo and Mapletoft, 2014). Although the Ovsynch program (Pursley et al., 1995) has been adopted in many dairy herds, it has its limitations because of the effects of stage of follicle development on response to gonadotropin-releasing hormone (GnRH). Hence, new strategies have been developed to maximize the response to GnRH-based protocols in lactating dairy cows. A program called "Presynch--Ovsynch" involves the application of two PGF injections (14 days apart) and the initiation of Ovsynch 12 days after the second PGF treatment (Moreira et al., 2001). The goal is to have most of the animals between days 5 and 12 of the estrous cycle when starting the Ovsynch. While presynchronization with PGF is effective in cyclic cows, it would appear to have no benefits in acyclic cows as they do not have a CL. Therefore, other presynchronization protocols that include the use of GnRH would be beneficial in dairy herds with a high incidence of acyclic cows. Recently, a presynchronization protocol that combines 2 PGF2α 14 days apart and a single GnRH treatment given 4 days after second PGF2α decreased the percentage of acyclic cows and increased fertility over the Presynch protocol in heat stressed multiparous lactating dairy cows (Dirandeh et al., 2015 b). However, a short protocol that requires animals to be handled fewer times would be more acceptable to producers. In this regard, Bello et al. (2006) developed a short presynchronization protocol that consists of a single administration of PGF2α and GnRH 2 days later 6 days before Ovsynch (G6G). Moreover, a new ovulation synchronization protocol (Estradoublesynch) that utilizes 1 mg of estradiol benzoate (EB) in lieu of 100 μg GnRH for synchronizing ovulation in a Doublesynch protocol improved pregnancy rate in both cycling and anestrus buffaloes (Mirmahmoudi et al., 2014).

The Ovsynch protocol was developed for timed-AI (TAI) without the necessity for estrus detection, hence, in dairy herds that utilize natural service or estrus detection as a component of their breeding program cows are subjected to different synchronization protocols. Indeed, most of those herds use the "Heatsynch" program, which is a slight modification of Ovsynch (Pancarci et al., 2002). An injection of estradiol (cypionate or benzoate) 48 hours after the injection of PGF2α is given in lieu of the second injection of GnRH. Presynchronization is not commonly done in cows subjected to the Heatsynch, however, a short presynchronization would be incorporated as part of the synchronization protocol whether it is demonstrated that its use improve synchronization and fertility in lactating dairy cows.

We hypothesized that Holstein cows subjected to Heatsynch with their estrous cycle presynchronized with GnRH and PGF2α have greater synchronization and enhanced fertility than those cows with their estrous cycle presynchronized with only PGF2α. The objective of this study was to compare ovarian response, plasma progesterone concentrations, conception and pregnancy rates in lactating Holstein cows subjected to three different synchronization protocols.

Material and methods

Animals, housing, and feeding

A total of 252 cycling and clinically normal lactating Holstein cows during the winter of 2014 and from a commercial dairy farm located in central Iran (Karaj) were used in this study. Cows averaged 2.7±1.2 lactations (mean±SEM) and had a body condition score (BCS) ranging from 2.75 to 3.75 (1 = emaciated and 5 = obese; Edmonson et al., 1989). Cows were housed in free-stall barns equipped with fans for evaporative cooling. Cows from all synchronization treatments were kept together in the same pens throughout the entire period of the study. Cows had free access to water and were fed twice daily with a total mix ration (TMR) that meets or exceeds the nutrient requirements established by NRC (2001) for lactating Holstein cows producing 43 kg/d of milk with 3.5% fat.

Body condition score (BCS) evaluation and milk yield

Body condition score of all cows was evaluated on the day of initiation of the synchronization protocols using a 5-point scale with 0.25 increments (Edmonson et al., 1989). The same individual performed all BCS evaluations during the study. Cows with BCS of less than 2 or greater than 4 were removed from the experiment. Cows were milked three times daily at 8 h intervals. Milk weights were recorded at each milking time and saved in the on-farm computer software program (DairyComp 305, Valley Agricultural Software, Tulare, CA, USA). Average weekly milk weights were extracted from the software program and used to calculate average milk production from calving to the first pregnancy diagnosis.

Synchronization protocols and insemination

The ovaries of 524 Holstein cows (34±3 d postpartum) were examined by transrectal ultrasonography to determine the presence of a corpus luteum (CL). A total 252 cows with a CL (≥10 mm) were randomly assigned to one of three synchronization groups. 1 – Control (n=84), the cows received two injections of PGF2α (25 mg of dinoprost, ENZAPROST®, Ceva Santé Animale, Libourne, France) 14 days apart; 2 – Heatsynch with GnRH (PGH, n=88) the cows received PGF2α followed by GnRH (100 mg of gonadorelin, CYSTORELINE®, Gonadorelin, Ceva Santé Animale) four days later and then Heatsynch protocol initiated eight days after GnRH treatment. 3 – Heatsynch without GnRH (PH, n=80) the cows received PGF2α and then Heatsynch protocol initiated 12 days later (Figure 1). The Heatsynch protocol consisted of administration of GnRH followed by PGF2α 7 days later and 2 mg of estradiol benzoate (EB; Vetastrol®, Aboureyhan Co., Karaj, Iran) administered 2 days after PGF2α. Cows were monitored (three times per day for 30 minutes each, starting at 24 h after PGF2α injection) for signs of estrus. Cows that showed signs of estrus (i.e vaginal mucous discharge, walking the fence line, swelling and reddening of the vulva, mounting other cows, or observed in standing estrus) were inseminated approximately 12 hours after onset of estrus (Dirandeh et al., 2009). Inseminations were performed by two technicians with semen from three commercially available sires that equally balanced among the four synchronization groups.

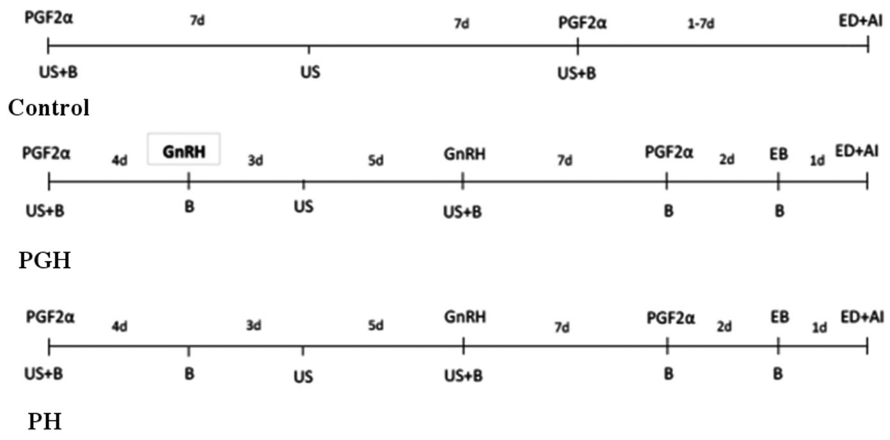

Figure 1. Diagram of activities and treatments during the study. Lactating Holstein cows (n=252) were randomly assigned to one of three synchronization groups based on ultrasound examination. Transrectal ultrasonography (US) and blood sampling (B) were performed to determine ovarian response to treatments and plasma progesterone (P4) concentrations, respectively

Progesterone assay

Blood samples were taken from subset of cows (n=40 per treatment) at each hormonal treatment to determine plasma P4 concentrations. All blood samples were withdrawn approximately 2 h after the morning meal by coccigeal venipuncture into evacuated tubes containing EDTA (10.5 mg, Monoject, Sherwood Medical, St. Louis, MO, USA). The samples were maintained on ice until plasma was separated by centrifugation (1,500 × g for 20 min at 4°C temperature) within 1 h of collection. Plasma was harvested and stored at −20°C until P4 determination. Plasma P4 concentrations were analyzed by ELISA, following the manufacturer's instructions (DRG, Marburg, Germany). Inter- and intra-assay coefficients of variation were 4.5 and 4.9%, respectively.

Ultrasonographic examinations

Transrectal ultrasonography (Agroscan AL ECM, equipped with a 7.5 MHz linear transducer; Ultrasound Australas, Victoria, France) was performed in all cows at 33±3 d postpartum to determine the presence of CL and during the Heatsynch protocol (at GnRH and PGF2α administration of Heatsynch) to determine ovulatory response to GnRH of Heatsynch. Ovarian maps were drawn for each cow, which included the diameter and location of follicles and CL recorded as previously described by Pierson and Ginther (1984). Ovulation to the GnRH treatment of Heatstynch was characterized by the presence of at least one follicle ≥ 10 mm in diameter at the time of GnRH, which was replaced by at least one CL at the time of PGF2α administration (Dirandeh, 2014; Dirandeh et al., 2015 b). Pregnancy diagnosis was also done by ultrasonography at 30 days after AI. Pregnancy was characterized by the presence of fluid, an embryo, and a heartbeat.

Statistical analyses

All data were analyzed using SAS (version 9.1 for Windows; SAS Institute Inc., Cary, NC, USA). Binomial data were analyzed using the LOGISTIC procedure. Data on milk yield, BCS, and plasma concentrations of progesterone were analyzed as repeated measurements, using PROC MIXED of SAS (SAS Institute Inc.) as previously described by Jahani-Moghadam et al. (2015). In addition to the main effect of synchronization group the full model included parity (1, 2, or ≥3), DIM at AI, Sire, Technician, BCS and milk production also were tested. Covariates that were not significant at P<0.1 (parity, DIM, sire, BCS, milk) were removed from the model.

Results

The average milk yield did not differ across synchronization programs (mean±SEM; 41.9±1.14, 41.6±1.27, 42.4±1.2 kg/d for Control, PGH and PH, respectively). Similarly, the average BCS at enrollment did not differ (P=0.48) among synchronization programs (mean±SEM; 3.25±0.25, 3.00±0.50 and 2.75±0.50 for Control, PGH and PH, respectively).

The percentage of cows with plasma P4≤0.4 ng/mL at AI (P=0.03) were greater in PGH group compared to other groups (Table 1). The percentage of cows that ovulated in response to the GnRH injection of Heatsynch was affected (P<0.01) by synchronization protocol, because PGH cows were more likely to ovulate (77.2%) than PH (56.0%) cows.

Table 1. Effect of synchronization groups on mean (±SEM) plasma progesterone (P4) concentrations (ng/mL) at different times during the protocol

	Synchronization protocols[1]			P
	control	PGH	PH	
Total no. cows	40	40	40	
Presynchronization (P4 concentration, ng/mL)				
at first PGF2α	4.4±0.29	4.6±0.30	4.95±0.30	0.30
at GnRH		1.4±0.32	0.9±0.37	0.66
Synchronization (P4 concentration, ng/mL)				
at GnRH		4.92±0.33a	3.33±0.31 b	0.04
at second PGF2α	4.6±0.34	4.83±0.39	5.11±0.35	0.63
at EB		1.29±0.30	1.25±0.28	0.71
Cows with P4≤0.4 at AI, ng/mL, % (no.)	72.5 (29/40) b	90.3 (36/40) a	77.5 (31/40) b	0.03
Luteal regression after PGF2α, % (no.)[2]	55.0 (11/20) c	83.3 (25/30) a	68.5 (24/35) b	0.04

[1]Synchronization protocols: Control = PGF2α -14d- PGF2α; PGH = PGF2α -4d-GnRH-8d-HeatSynch; and PH = PGF2α-12d-HeatSynch.

[2]Luteal regression was defined to occur when plasma P4 concentration was ≥1 ng/mL immediately before PGF2α treatment and decreased to <1 ng/mL at EB treatment in PGH and PH groups or at AI in control group.

a, b, c – percentages within a row with different superscripts differ.

Table 2. Effect of synchronization protocol on estrus detection rate, conception and pregnancy rate and ovulatory response to GnRH of Heatsynch

	Synchronization protocols[1]			P
	control	PGH	PH	
Estrus detection rate[2], % (no.)	47.0 (40/84) b	70.4 (62/88) a	72.5 (58/80) a	0.04
Conception rate[3], % (no.)	25.0 (10/40) c	45.16 (28/62) a	34.48 (20/58) b	0.01
Pregnancy rate[4], % (no.)	11.9 (10/84) c	31.8 (28/88) a	25.0 (20/80) b	0.01
Response to GnRH of Heatsynch, % (no.)		77.2 (68/88) a	55.0 (44/80) b	0.01

[1]TAI protocols: Control = PGF2α-14d-PGF2α; PGH = PGF2α-4d-GnRH-8d-HeatSynch and PH = PGF2α -12d-HeatSynch.

[2]Percentage of cows observed in estrus divided by the total number of cows included in the synchronization protocol.

[3]Percentage of cows that become pregnant divided by the total number of cows AI.

[4]Percentage of cows that become pregnant divided by the total number of cows included in the synchronization protocol.

a, b, c – percentages within a row with different superscripts differ.

The proportion of cows displaying estrus was greater in PGH (70.4%) and PH (72.5%) groups compared with control (47.0%, P = 0.04). Percentage of cows pregnant at 30 days after AI was affected (P=0.01) by synchronization treatment and was greater in PGH (45.16%) compared to control (25.0%) and PH (34.48%) groups (Table 2).

Discussion

Greater concentrations of progesterone during follicle development prior to ovulation, ovulatory response to first GnRH, or both, may affect the overall pregnancy outcomes in lactating dairy cows (Bello et al., 2006; Colazo et al., 2013; Dirandeh et al., 2015 a, b; Folman et al., 1990). Therefore, the objective of the current study was to improve ovulatory response to GnRH of Heatsynch, consequently increase progesterone concentration during follicle development and fertility in lactating Holstein cows.

In support of our hypothesis, the percentage of cows that ovulated in response to first GnRH of Heatsynch was greater in PGH group compared to cows in the PH group. Galvao et al. (2007) have shown that initiation of a Heatsynch protocol on approximately days 5 to 8 of the estrous cycle compared with days 8 to 11 increased the proportion of lactating dairy cows ovulating to initial GnRH injection (61.4 vs. 40.5%). Moreover, Bello et al. (2006) reported that ovulation rates to initial GnRH injection in dairy cows treated 3, 4, or 5 days after induced ovulation were 56, 67, and 85%, respectively. Although the stage of the estrous cycle at GnRH administration is unknown in our study, we speculate that the additional GnRH treatment given to cows in the PGH group optimizes the stage of follicle development at the initiation of Heatsynch.

Cows subjected to GnRH-based protocols that become pregnant following AI had greater plasma progesterone concentrations at first GnRH injection (Zareh et al., 2008; Dirandeh et al., 2015 b), which in turn has been associated with decreased LH pulsatility (Inskeep, 2004) and improved oocyte competence (Mihm et al., 1994). Mirmahmoudi and Prakash (2012) reported LH peak concentrations (ng/mL) were 99.8±28.5 ng/mL and 62.7±11.9 ng/mL after first and second GnRH injection, respectively. In present study, plasma progesterone concentration was greater in PGH cows compared to PH cows at initiation of Heatsynch, indicating that the additional GnRH treatment might have induced accessory CLs or a better synchrony of the estrous cycle. In agreement, other studies have indicated that a presynchronization protocol that includes GnRH reduces the proportion of animals without a CL at onset of Ovsynch (Dirandeh et al., 2015 b). In addition, Stevenson and Pulley (2012) reported that greater percentage of cows subjected to a presynchronization protocol which included a GnRH injection preceded 3 days earlier by PGF2α treatment had P4 ≥1 ng/mL at first GnRH compared with those subjected to a presynchronization with only PGF2α.

Luteal regression after last PGF2α treatment was greater in cows subjected to PGH compared to the other two synchronization groups. Also, the percentage of cows with high plasma P4 concentrations (>0.4 ng/mL) at AI was lower in PGH group compared to the other two groups. Inadequate luteolysis can result in an elevation in circulating P4 near AI and a reduction in fertility that seems to be a problem with some GnRH-based timed-AI programs. Studies have shown that when circulating progesterone concentrations do not decrease to <0.5 ng/mL at 48 h following PGF2α administration the probability of pregnancy would be significantly reduced (Brusveen et al., 2009; Souza et al., 2007). Dirandeh et al. (2015 b) reported that inadequate CL regression and therefore high progesterone concentrations on the day of timed-AI, will result in a reduction in fertility in cattle. However, based on our finding, incomplete luteal regression is clearly a problem even in cows inseminated following estrus detection. Elevated concentrations of circulating progesterone at the time of AI may affect semen transport and fertilization by reducing uterine contractility because progesterone decreases the number of oxytocin, angiotensin II, and estrogen receptors in the uterus and antagonizes estrogen induction of estrogen receptors in the myometrium (Graham and Clarke, 1997). Delayed (prolonged P4 clearance) or incomplete CL regression may also have an indirect negative effect on estradiol-17β production and pulses of LH (Bridges and Fortune, 2003; Inskeep, 2004) that may impair the normal ovulation process.

Our results further indicated that presynchronization with a single injection of PGF2α combined with GnRH enhanced conception rate and pregnancy rate in cows subjected to Heatsynch. The success of ovulation synchronization protocols has been associated to ovulatory response to first GnRH injection of Ovsynch (Bello et al., 2006). In this regard, Dirandeh et al. (2015 a, b) also reported a higher pregnancy rate in cows that had ovulated following first GnRH treatment of Ovsynch. Cows in diestrus at the initiation of an Ovsynch protocol, and those ovulating in response to first GnRH of the Ovsynch protocol were more likely to have higher P4 concentrations at the time of PGF2α treatment (Bello et al., 2006; Moreira et al., 2001). It is plausible to assume that the formation of an accessory CL in PGH cows resulted in increased plasma progesterone concentrations during follicular development prior to AI. The impact of high circulating P4 concentrations prior to AI on subsequent fertility of lactating dairy cows has been demonstrated by Folman et al. (1990) and Colazo et al. (2013).

In conclusion, results from this study support the hypothesis that a short presynchronization protocol, which includes a single injection of PGF2α combined with GnRH would increase fertility in dairy cows subjected to a Heatsynch protocol. The enhanced fertility was due to a greater ovulatory response to GnRH of Heatsynch, perhaps more adequate plasma P4 concentration during follicle development and a greater luteal regression following PGF2α prior to AI.

Conflicts of interest

There is no conflict of interest.

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