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EFFECT OF PRESYNCHRONIZATION PRIOR TO OVSYNCH ON OVULATORY RESPONSE TO FIRST GNRH, OVULATORY FOLLICLE DIAMETER AND PREGNANCY PER AI IN MULTIPAROUS HOLSTEIN COWS DURING SUMMER IN IRAN

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Abstract

The aim was to evaluate the effect of presynchronization with GnRH and PGF$_{2\alpha}$ prior to Ovsynch on ovulatory response to first GnRH, diameter of largest follicle at TAI and pregnancy per AI (P/AI) in multiparous dairy cows during summer. Cows (n=1069) were randomly assigned to one of three timed-AI (TAI) protocols. The TAI protocols were: 1) Ovsynch (O; n=425), GnRH-7d-PGF$_{2\alpha}$-56h-GnRH-16h-TAI), 2) double-Ovsynch (DO; n=302), GnRH-7d-PGF$_{2\alpha}$-3d-GnRH and Ovsynch was initiated 7 days later, and 3) G7G-Ovsynch (G7G; n=342), PGF$_{2\alpha}$-2d-GnRH and Ovsynch was initiated 7 days later. Ovarian examinations were performed by transrectal ultrasonography during Ovsynch to determine ovulatory response to first GnRH and diameter of largest follicle at TAI. Presynchronization increased ovulatory response after first GnRH of Ovsynch (P=0.001), which was greater in DO (74.0%) and G7G (76.0%) groups compared to O group (50.0%). Means (±SEM) diameter (mm) of largest follicle at TAI was smaller in cows presynchronized before Ovsynch (DO and G7G, overall 15.7±0.3) compared to that in cows subjected to a standard Ovsynch without presynchronization (18.5±0.42). P/AI at 32 d after Al was greater (P=0.001) in G7G (32.7%) and DO (31.1%) groups compared to Ovsynch (19.7%) group. Presynchronization prior to Ovsynch also affected P/AI at 60 and 150 d after AI (P<0.05). In conclusion, DO and G7G protocols resulted in greater ovulatory response to first GnRH, smaller ovulatory follicles and greater P/AI compared to a standard Ovsynch protocol. Therefore, TAI protocols that include a presynchronization with GnRH and PGF$_{2\alpha}$ prior to Ovsynch should be used in multiparous cows during summer to achieve acceptable reproductive performance.

Key words: Ovsynch, pregnancy rate, secondary sex ratio, ultrasonography, Holstein cows

Heat stress is associated to poor fertility and reduced milk production in lactating dairy cows (De Vries, 2004; Astiz and Fargas, 2013). In this regard, De Vries (2004) reported a loss in milk production of 15% and a reduction in pregnancy per AI (P/AI) of 53% in summer (July and September) compared to February in Florida. It has been demonstrated that during heat stress impaired ovarian follicular dynamics is the main factor associated to decreased fertility in dairy cows in northern Iran (Dirandeh, 2014; Dirandeh et al., 2015 a). Moreover, the negative carry-over effect of heat stress on follicular steroidogenesis and oocyte quality was still manifest during early autumn (Roth et al., 2001). One of approaches to decrease the severity of heat stress on fertility in dairy cows would be the implementation of ovulation synchronization protocols (Dirandeh, 2014; Ansari et al., 2016). These protocols were developed to synchronize ovarian follicular dynamics and facilitate insemination at a predetermined (fixed) time without the need for estrus detection (Colazo and Mapletoft, 2014; Dirandeh et al., 2015 a, b).

The standard 7-d Ovsynch protocol has been incorporated as part of the reproductive management in dairy herds for several years with P/AI varying from 17 to 25% (Colazo and Mapletoft, 2014). However, previous studies have demonstrated that the stage of estrous cycle at initiation of the Ovsynch affects the overall response to the protocol (Dirandeh, 2014). Initiation of the Ovsynch too early (e.g., 1–4 days after ovulation) or too late (e.g., 13–20 days after ovulation) during the estrous cycle resulted in reduced ovarian synchrony (Vasconcelos et al., 1999). Cattle will respond most consistently when first GnRH is given between days 5 and 12 of the estrous cycle (Moreira et al., 2001). Consequently, several presynchronization protocols have been developed to align cows at days 5–12 of the estrous cycle prior to the initiation of Ovsynch (Galvao et al., 2007). One of these protocols, the G7G included the administration of $PGF_{2\alpha}$, which is intended to induce luteolysis of functional corpora lutea (CL), and 2 d later a GnRH, intended to induce ovulation (Dirandeh et al., 2015 a). Then, the Ovsynch is initiated 7 d after the GnRH treatment when majority of animals are expected to have a dominant follicle capable of ovulating in response to the first GnRH (Pursley et al., 1997). Presynchronization protocols that induce the formation of a CL prior to Ovsynch are also likely to improve fertility in acyclic cows (Herlihy et al., 2012). Another protocol that includes a presynchronization that combines GnRH and $PGF_{2\alpha}$ treatments is the so-called Double-Ovsynch (Souza et al., 2008). Dirandeh et al. (2015) reported that cows subjected to the Double-Ovsynch had greater ovulation response to the first GnRH, synchronization rate, and P/AI compared to cows subjected to Presynch-Ovsynch during heat stress.

The objective of this study was to compare two TAI protocols (Double-Ovsynch and G7G) that include a presynchronization with GnRH and $PGF_{2\alpha}$ prior to Ovsynch versus a standard Ovsynch on ovulatory response to first GnRH, diameter of largest follicle at TAI and pregnancy per AI (P/AI) in multiparous lactating Holstein cows during summer. It was hypothesized that Double-Ovsynch and G7G protocols would result in greater response to treatments and improve P/AI compared to a standard Ovsynch protocol.

Material and methods

Animals, housing, and diets

This experiment was conducted in a commercial dairy herd in northern Iran from June to September in 2014 and 2015 (the average maximum Temperature Humidity Index (THI) during the study period was 87). Cows were housed in free-stall facilities bedded with sand and had *ad libitum* access to fresh feed and water. All cows received the same diet twice daily as a TMR allowing ≤5% feed refusals daily. Diets were formulated to meet NRC (2001) recommendations for lactating mature cows producing >35 kg of 3.7% FCM/d. Main ingredients were silage (corn and alfalfa), grain (barley or corn), hay (alfalfa or grass), and mineral supplements. Cows were milked 3 times daily at approximately 8-h intervals and monitored daily for signs of diseases. If any health issues occurred, animals were moved to hospital pens and appropriate treatment was performed following established SOPs until total recovery.

Treatments

Multiparous Holstein cows (n = 1069) were randomly assigned weekly to one of three TAI protocols over a two-year period (Figure 1). The TAI protocols were 1) Ovsynch (O; n = 425), cows received GnRH-7d-$PGF_{2\alpha}$-56h-GnRH-16h-AI, Dirandeh, 2014), 2) Double-Ovsynch (DO; n = 302), cows received GnRH-7d- $PGF_{2\alpha}$ -3d-GnRH and Ovsynch was initiated 7 days later (Dirandeh et al., 2015 a); and 3) G7G (n = 342), cows received $PGF_{2\alpha}$-2d-GnRH and Ovsynch was initiated 7 days later (Dirandeh et al., 2015 b). Mean (±SD) days in milk (DIM) at initiation of the protocol were 40±4, 23±2, and 24±2 days for O, DO, and G7G, respectively. The voluntary waiting period (VWP) was 45 days in milk (DIM). Estrus detection (clear mucus from vagina, swollen vulva, mounting other cows, and standing to be mounted by other cows by visual observation (thrice daily for at least 30 min each time) was performed starting 1 d after the last $PGF_{2\alpha}$ injection of Ovsynch. Three professional AI technicians performed all inseminations, with frozen-thawed semen from five commercially available sires equally balanced among the experimental groups. Cows that showed signs of estrus were inseminated approximately 12 h after onset of estrus and were considered to have an early AI.

Ultrasonographic examinations

Ovarian examinations were performed in a subset of cows (n = 100 per treatment) by transrectal ultrasonography (BCF equipped with a 6-8 MHz linear transducer; Ultrasound Australas, Victoria, Australia) at first GnRH and $PGF_{2\alpha}$ of Ovsynch and at TAI to evaluate response to first GnRH of Ovsynch (Dirandeh, 2014) and determine the diameter of largest follicle (Dirandeh et al., 2009). Pregnancy diagnosis was performed by ultrasonography at 32 d after AI. Pregnancy was characterized by the presence of fluid, an embryo, and a heartbeat. Cows diagnosed pregnant at 32 d were re-examined at 60 ± 4 d and 150 ± 5 after AI to confirm pregnancy. Pregnancy loss was considered to have occurred when a cow was diagnosed pregnant at 32 d after TAI and not pregnant at 60 d or 150 d.

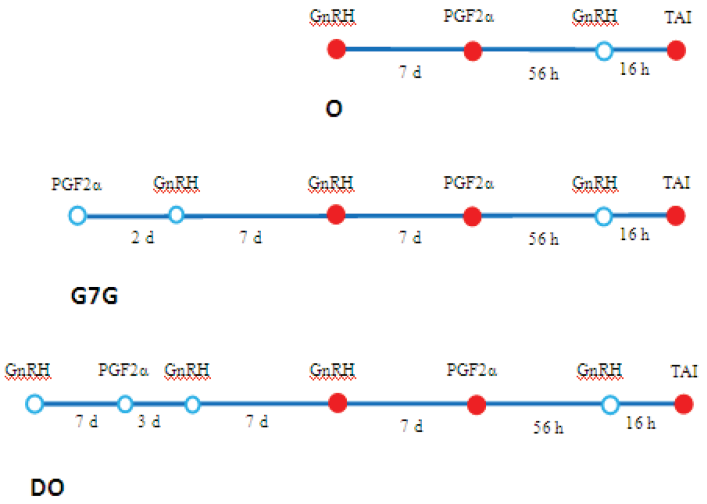

Figure 1. Diagram of activities and treatments during the study. Holstein cows (n= 1069) were randomly assigned to one of three timed-AI protocols; Ovsynch (O), G7G-Ovsynch (G7G) or Double-Ovsynch (DO). Mean ± SD days in milk at initiation of the protocol were 40±4, 23±2, and 24±2 for O, DO, and G7G, respectively. Transrectal ultrasonography was performed during the Ovsynch56 to determine ovarian response to treatments

Statistical analyses

All data were analyzed using SAS (version 9.1 for Windows; SAS Institute Inc., Cary, NC). Binomial data were analyzed using generalized estimating equations (GEE) of the GENMOD procedure, as described (Dirandeh et al., 2015 a). For analysis of pregnancy status at 32, 60 and 150 d after TAI, the model included the effects of TAI protocol, response to first GnRH of Ovsynch, technician effect, sire effect and their interactions in all cow. For analysis of ovarian responses to Ovsynch, the model considered TAI protocol, and their interactions. The effects of technician, sire, parity and their interactions were not significant therefore removed from model. Probability values ≤0.05 were considered significant, whereas those between 0.051 and 0.10 were considered trends. Diameter of the largest follicle was analyzed using PROC MIXED. Secondary sex ratios were analyzed by logistic regression using the LOGISTIC procedure. Differences between means were tested by the PDIFF option and adjusted using the Tukey procedure. Probability values ≤0.05 were considered significant, whereas those from 0.051 to 0.1 were considered trends.

Results

The average maximum THI during the study period was 87, indicating a high degree of heat stress. In north part of Iran, THI can reach 90 from June to September

(Yari et al., 2010). For the purposes of this study, THI values of ≥79 were considered as heat stress condition, respectively, for dairy cattle (Coleman et al., 1996). Number of inseminations per month by TAI treatments are shown in Table 1.

Table 1. Number of inseminations per month by treatments in multiparous lactating Holstein cows subjected to three diffrent timed-AI (TAI) protocols during summer

Inseminations per treatments	Month									Total
	September			August			July			
	TAI protocols			TAI protocols			TAI protocols			
	G7G	DO	O	G7G	DO	O	G7G	DO	O	
Year 2014	83	80	99	72	67	85	61	54	68	669
Year 2015	49	37	62	44	34	63	33	30	48	400

Timed-AI (TAI) protocols: O = GnRH-7d- PGF2α -56 h-GnRH-16 h-TAI (Ovsynch); DO = GnRH-7d-PGF2α -3d-GnRH-7d-Ovsynch (Double-Ovsynch); G7G =PGF2α-2d-GnRH-7d- Ovsynch (G7G-Ovsynch).

The proportion of cows that ovulated after first GnRH of Ovsynch was affected by presynchronization (P = 0.001), it was greater in cows subjected to DO (74.0%) and G7G (76.0%) protocols compared to that in cows receiving the Ovsynch (54.0%, Table 2).

Table 2. Ovulation to first GnRH of Ovsynch (%), diameter of ovulatory follicle (mm) and proportion of male calves at birth (%) in multiparous lactating Holstein cows subjected to three different timed-AI (TAI) protocols during summer

Item	TAI protocols			P-Value
	G7G	DO	O	
Ovulation to first GnRH of Ovsynch [% (no. / total no.)]	76.0 (76/100) a	74.0 (74/100) a	54.0 (54/100) b	0.001
Proportion of male calves at birth [% (no. / total no.)]	58.0 (91/342) b	58.0 (79/302) b	52.0 (68/425) a	0.75
Diameter of ovulatory follicle (mm)	15.9±0.42 b	16.1±0.31 b	19.2±0.52 a	0.001

a, b, c – different letters within a row differ (P<0.05).

Timed-AI (TAI) protocols: O = GnRH-7d- $PGF_{2\alpha}$ -56 h-GnRH-16 h-TAI (Ovsynch); DO = GnRH-7d- $PGF_{2\alpha}$ -3d-GnRH-7d-Ovsynch (Double-Ovsynch); G7G =$PGF_{2\alpha}$-2d-GnRH-7d- Ovsynch (G7G-Ovsynch).

Mean (±SEM) diameter (mm) of largest follicle at TAI was larger in O (18.5±0.42) compared to DO (16.1±0.31) and G7G (15.7±0.21, P = 0.01, Table 2) groups, but sex ratio of offspring did not differ between among treatment groups (P = 0.75, Table 2).

Table 3. Pregnancy per AI (P/AI) at 32, 60 and 150 d after AI, and pregnancy loss in multiparous lactating Holstein cows subjected to three different timed-AI (TAI) protocols during summer

Item	TAI protocols			P-Value
	G7G	DO	O	
P/AI [% (no. / total no.)]				
Based on estrus detection	34.3 (11/32)	33.3 (10/30)	26.6 (4/15)	0.63
At 32 d after AI	32.7 (112/342) b	31.1 (94/302) b	19.7 (84/425) a	0.001
At 60 d after AI	27.7 (95/342) b	26.8 (81/302) b	16.4 (70/425) a	0.001
At 150 d after AI	26.6 (91/342) b	26.1 (79/302) b	16.0 (68/425) a	0.001
Pregnancy loss between d 32 and 60 after AI	15.6 (17/112)	13.8 (13/94)	16.6 (14/84)	0.48
Pregnancy loss between d 60 and 150 after AI	3.1 (4/126)	2.1 (2/94)	2.0 (2/84)	0.63

a, b, c – different letters within a row differ (P<0.05).

Timed-AI (TAI) protocols: O = GnRH-7d- $PGF_{2\alpha}$ -56 h-GnRH-16 h-TAI (Ovsynch); DO = GnRH-7d- $PGF_{2\alpha}$ -3d-GnRH-7d-Ovsynch (Double-Ovsynch); G7G =$PGF_{2\alpha}$-2d-GnRH-7d- Ovsynch (G7G-Ovsynch).

Cows that were presynchronized prior to Ovsynch had greater P/AI at 32, 60 and 150 d after AI compared to cows that received Ovsynch without presynchronization (P = 0.001, Table 3). However pregnancy loss from 32 to 60 d (P = 0.48) and from 60 to 150 d (P = 0.63) after TAI did not differ among TAI protocols and were 19.8 and 13.1% between d 32 and 60 in cows subjected to Ovsynch without presynchronization and those presynchronized before Ovsynch, respectively (Table 3). Pregnancy loss between d 60 and 150 after Al was between 2.0 and 3.6% in all three TAI protocols (Table 3). Parity, AI technician, sire and their interactions did not affect P/AI (P>0.05).

Discussion

The success of GnRH-based ovulation synchronization protocols is highly associated to the day of estrous cycle at the initiation of treatment (Vasconcelos et al., 1999; Dirandeh, 2014), ovulatory response to first and second GnRH treatments (Bello et al., 2006), progesterone concentrations during follicular development prior to AI (Dirandeh et al., 2015 b) and size of the dominant follicle at the time of second GnRH administration (Motavalli et al., 2017). Ovulatory response to the first GnRH of Ovsynch is positively associated to P/AI (Bello et al., 2006), as ovulation to the first GnRH results in the initiation of a new follicular wave approximately 36 h later (Martinez et al., 1999; Dirandeh et al., 2009) ensuring the synchronous development of a healthy preovulatory follicle (Vasconcelos et al., 1999; Bello et al., 2006, Heidari et al., 2017). In this regard Dirandeh (2014) reported that P/AI at 32 d after AI was greater in cows that responded to first GnRH of Ovsynch compared to those that did not respond (40.9 vs 30.6%). Recently, a presynchronization protocol that combines 2 $PGF_{2\alpha}$ 14 d apart and a single GnRH treatment given 4 d after second $PGF_{2\alpha}$ decreased the percentage of cows without a CL and increased P/AI over the Presynch-Ovsynch protocol in heat-stressed multiparous lactating dairy cows (Dirandeh et al., 2015 a). Finding from the previous study also suggested that a presynchronization protocol that includes the administration of GnRH is an acceptable approach for acyclic cows, and further optimize the ovarian response to Ovsynch in cyclic cows (Dirandeh et al., 2015 a). It has been shown that the presence of a CL and a follicle ≥8 mm at first GnRH of Ovsynch improve conception rates in cows subjected to the Presynch-Ovsynch (Galvao et al., 2007). However, when GnRH treatment was given in the presence of a dominant follicle before dominance or during the regression growth phase, ovulation was impaired and synchronous emergence of a new follicular wave did not occur (Martinez et al., 1999; Zareh et al., 2009, Masoumi et al., 2017). Therefore, cattle will respond most consistently when GnRH is administered between days 5 and 12 of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000; Ansari et al., 2016).

It is plausible to assume that follicular growth in cows in the DO and G7G was more synchronous with the majority of the cows likely to be on early diestrus at first GnRH of Ovsynch, when GnRH treatment would be more effective in inducing ovulation (Moreira et al., 2000; Dirandeh, 2014). In addition, cows would have a more responsive CL at $PGF_{2\alpha}$ of Ovsynch treatment and a reduced interval to onset of estrus (Rosenberg et al., 1990).

Moreover, in the present study mean diameter of largest follicle at TAI was larger in cows subjected to Ovsynch compared to those in DO and G7G groups. Several studies have investigated the effect of the largest follicle diameter at TAI on subsequent fertility in lactating dairy cows (Bello et al., 2006; Dirandeh, 2014; Dirandeh et al., 2015 a, b). We speculate that the ovulation of larger follicles at second GnRH in cows failing to ovulate at first GnRH may be associated with ovulation of persistent or aged follicles, which may reduce embryo quality (Cerri et al., 2009) and P/AI (Mihm et al., 1994). Extending the length of dominance by using a progesterone device resulted in increased largest follicle size (11.3 vs. 16.2 mm) and reduced first service conception rate (65 vs. 37%) compared to normal follicular growth in Holstein heifers (Savio et al., 1993). Increased P/AI has been reported in cows ovulating follicles between 14 and 18 mm in diameter compared with cows ovulating follicles <14 mm or >19 mm in diameter (Bello et al., 2006; Souza et al., 2008). Others have reported no decrease in P/AI but greater pregnancy loss in lactating dairy cows ovulating follicles >20 mm in diameter (Colazo et al., 2015).

In the present study, the overall greater P/AI obtained in cows subjected to DO and G7G protocol may be attributed to a better ovarian follicular synchrony. Indeed, a greater proportion of cows in the DO and G7G groups ovulated following first GnRH of Ovsynch. Ovulation to first GnRH of Ovsynch induces the formation of a CL, which will be present at the time of the $PGF_{2\alpha}$ treatment of the Ovsynch protocol, thereby increasing responses to the $PGF_{2\alpha}$ and second GnRH of Ovsynch treatments of the protocol (Vasconcelos et al., 1999; Bello et al., 2006). Cows that are in the late stages of the estrous cycle when an Ovsynch protocol is initiated are less likely to be properly synchronized, resulting in fewer P/AI (Vasconcelos et al., 1999). The lower synchronization rate in cows that initiated an Ovsynch protocol during late diestrus might be partly attributed to a low response to the first GnRH of the Ovsynch protocol. Cows in late diestrus that failed to ovulate to the first GnRH injection of the protocol are also more likely to have spontaneous luteolysis before the $PGF_{2α}$ injection (Dirandeh, 2014). This leads to an LH surge before the last GnRH injection and ovulation before TAI thereby decreasing the chances of conception (Vasconcelos et al., 1999; Moreira et al., 2000). A lack of follicle turnover due to failure to respond to the initial GnRH administration might compromise the quality of embryos and consequently reduce P/AI. Cerri et al. (2009) demonstrated that embryo quality but not fertilization rate was compromised when follicle turnover occurred in a very low percentage of cow subjected to a TAI protocol. In addition, embryos from those cows had fewer blastomeres and a lower proportion of live blastomeres (Cerri et al., 2009).

The overall pregnancy loss from 32 to 60 d after TAI was 14.6%. This finding is in agreement with previous studies performing pregnancy diagnosis by transrectal ultrasonography at approximately the same interval after AI that was done in the present study (Chebel et al., 2006; Silva et al., 2009; Dirandeh et al., 2015 a, b).

In conclusion, DO and G7G protocols resulted in greater ovulatory response to first GnRH, smaller ovulatory follicles and greater P/AI compared to a standard Ovsynch protocol. Therefore, TAI protocols that include a presynchronization with GnRH and $PGF_{2α}$ prior to Ovsynch should be used in multiparous cows during summer to achieve acceptable reproductive performance.

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