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Double-Ovsynch, compared with presynch with or without GnRH, improves fertility in heat-stressed lactating dairy cows

E. Dirandeh [a,*], A. Rezaei Roodbari [b], M.G. Colazo [c]

[a] Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran
[b] Department of Animal Science, University of Tehran, Karaj, Alborz, Iran
[c] Livestock Research Branch, Alberta Agriculture and Rural Development, Edmonton, AB, Canada

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ABSTRACT

The objective was to compare 3 timed artificial insemination (TAI) protocols in lactating dairy cows during heat stress. Multiparous Holstein cows yielding (mean ± SEM) 29.4 ± 0.3 kg of milk/d randomly were assigned to 1 of 3 TAI protocols at 34 ± 5.1 days in milk: 1) double-Ovsynch (DO; n = 486): the cows received GnRH-7d-2α-3d-GnRH and Ovsynch56 (GnRH-7d-PGF2α-56h-GnRH-16h-AI) was initiated 7 days later; 2) Presynch-GnRH-Ovsynch (PGO; n = 453): the cows received PGF2α-14d-PGF2α-2d-GnRH and Ovsynch56 was initiated 7 days later; and 3) presynch-Ovsynch (PO; n = 435): the cows received PGF2α-14d-PGF2α and Ovsynch56 was initiated 12 days later. The ovulatory response to the first GnRH of Ovsynch56 was higher in DO (65.0%) compared to PGO (53.2%) and PO (45.5%). Luteolytic response to PGF2α of Ovsynch was similar among TAI protocols (90.1%, 87.1%, and 86.2% for DO, PGO, and PO, respectively). Synchronization rate was greater in DO (86.2%) than in PGO (78.1%) and PO (72.1%) protocols. Irrespective of the TAI protocol, cows that ovulated in response to first GnRH had greater response to PGF2α (92.7 vs. 77.1%). Mean (±SEM) diameter (mm) of ovulatory follicle at TAI was larger in DO (16.1 ± 0.3) than PGO (15.6 ± 0.21) and PO (15.2 ± 0.12). Cows subjected to DO had greater P/AI at 32 days and at 60 days after TAI (26.6 and 24.4%) compared with those in PGO (21.4 and 20.0%) and PO (17.2 and 15.9%). However, TAI protocol had no significant effect on the incidence of pregnancy loss (6.1%, 6.6%, and 7.4% for DO, GO, and PO, respectively). In summary, cows in the DO protocol had a greater ovulation rate to the first GnRH and a greater synchronization rate, larger ovulatory follicles and greater P/AI. Of the 3 protocols used, DO yield the best reproductive performance in heat-stressed, lactating dairy cows.

1. Introduction

There are several GnRH-based protocols that increase insemination risk for dairy cattle, as they facilitate timed artificial insemination (TAI) without the necessity to detect estrus [1]. The Ovsynch protocol, commonly used to synchronize ovulation for TAI in dairy cattle, consists of 2 GnRH treatments given 9 days apart, with PGF2α given 7 days after the first GnRH [2]. During the past few years, the Ovsynch protocol has been modified to further improve pregnancy per artificial insemination (P/AI). A presynchronization strategy that involves 2 injections of PGF2α, each given 14 days apart, increased P/AI when Ovsynch was initiated 11 [3] or 12 days [4,5] after the last PGF2α of the Presynch protocol (ie, Presynch-Ovsynch protocol [PO]). However, acyclic cows are unlikely to benefit from presynchronization with PGF2α, because they do not have a CL. Hence, alternate presynchronization protocols that

* Corresponding author. Tel.: +98 1133822741; fax: +98 1133822565.
E-mail address: Dirandeh@gmail.com (E. Dirandeh).

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integrate GnRH into the Presynch have been proposed to optimize follicular development in acyclic cows [6]. A presynchronization protocol that combines a single GnRH treatment with PGF2α (ie, presynch-GnRH-Ovsynch [PGO]) decreased the percentage of acyclic cows over the PO [3]. Most recently, a new presynchronization protocol that included PGF2α and GnRH (double Ovsynch; DO) improved P/AI compared with the PO protocol in primiparous cows (65.2 vs. 45.2%) [7]. In addition, Akbarabadi et al. [8] reported that DO compared with PO increased the percentage of cows with a CL at initial GnRH and improved most aspects of synchronization during an Ovsynch protocol.

It is well known that lactating dairy cows inseminated during heat stress have impaired ovarian follicular dynamics and decreased fertility [9,10]. These negative effects on follicular steroidogenesis and oocyte quality were still manifest during early autumn [11,12], with fertility not restored until early winter [13] when temperatures had moderated. Recently, Ayres et al. [14] reported that service per conception tended to increase during a warm season (1.95 ± 0.14) compared with a cold season (1.62 ± 0.17) in dairy cows subjected to a PO protocol. Therefore, there is a need to identify new approaches to improve fertility in lactating dairy cows exposed to heat stress. One of those approaches could be elimination of compromised follicles with hormonal treatments that induce ovulation, synchronize emergence of a new follicular wave, and allow TAI [12,15].

We hypothesized that cows with their estrous cycle presynchronized with GnRH and PGF2α (ie, DO and PGO) have greater cyclicity and P/AI than cows with their estrous cycle presynchronized with only PGF2α (ie, PO). The objectives of this study were to compare ovarian response, P/AI, and pregnancy loss of multiparous Holstein cows subjected to 3 TAI protocols (DO, PGO, or PO) during heat stress.

2. Materials and methods

2.1. Animals and management

This experiment was conducted at a commercial dairy farm between July and November 2013 (temperature humidity index = 78–84). A total of 1374 multiparous lactating Holstein cows (yielding 29.4 ± 0.3 kg of milk/d) were enrolled. Cows were housed in free-stall barns with fans and bedded with sand. Cows received a total mixed ration formulated for lactating dairy cows producing 40 kg of 3.5% fat milk according to Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, National Research Council [16] guidelines and had free access to water. Diets were fed twice daily (07:00 and 16:00) for *ad libitum* intake (10% of refusals on as fed basis). Main ingredients were silage (corn and alfalfa), grain (barley or corn), hay (alfalfa or grass), and mineral supplements. All cows participating in this experiment were milked thrice daily at approximately 8-hour intervals and monitored daily for signs of diseases. If any health issues occurred, animals were moved to hospital pens, and appropriate treatments were performed (following standard treatment protocols) until their total recovery.

2.2. TAI protocols

Weekly, a cohort of cows at 34 ± 5.1 days in milk was assigned randomly to receive 1 of 3 TAI protocols: DO (n = 486), PGO (n = 453), or PO (n = 435). The DO cows received GnRH (100 μg of gonadorelin acetate, im; Parnell Technologies PTY. Ltd., Alexandria, Australia) followed by PGF2α (500 μg of cloprostenol, im; Parnell Technologies) 7 days later and GnRH 3 days after PGF2α, then began the Ovsynch protocol 7 days later. The PGO cows received 2 injections of PGF2α 14 days apart, followed by GnRH 2 days after last PGF2α, then began the Ovsynch 12 days later. The PO cows received 2 injections of PGF2α 14 days apart, and then began the Ovsynch protocol 12 days later. All cows received the same Ovsynch protocol (so called Ovsynch56) [17] that consists of GnRH followed by PGF2α 7 days later and a second GnRH treatment administered 56 hours after PGF2α. Cows were timed-inseminated 16 hours after second GnRH treatment. Estrus detection was performed by visual observation (3 times daily for at least 30 minutes each) from PGF2α to the second GnRH treatment of Ovsynch56. The following symptoms were used to characterize estrus: vaginal mucous discharge, bellowing, increased nervousness and activity, walking the fence line, swelling and reddening of the vulva, mounting other cows, or observed in standing estrus. Cows that showed signs of estrus were inseminated approximately 12 hours after onset of estrus and were considered to have an early AI.

Two professional AI technicians performed all inseminations, with semen from 3 commercially available sires equally balanced among the 3 experimental groups. Treatment protocols and activities during this study are shown (Fig. 1).

2.3. Ultrasonographic examinations

Ovarian examinations were performed by transrectal ultrasonography (BCF equipped with a 6–8 MHz linear transducer; Ultrasound Australas, Victoria, Australia) during the Ovsynch56 (at first GnRH injection, at PGF2α injection, at TAI, and 7 days after TAI) in all cows.

Cycling status and proportion of cows ovulating to initial GnRH treatment were determined as previously described [18]. Ultrasonography at TAI was used to determine diameter of the ovulatory follicle and along with ultrasonography 7 days after TAI were used to determine ovulation to second GnRH. Ovulation was confirmed by the presence of a CL 7 days after TAI in the same ovary, which had the largest follicle at TAI.

Cows with early ovulation included those with no dominant follicle ≥10 mm at TAI and those that were inseminated before TAI. Response to PGF2α was considered attained when the functional CL regressed. Synchronization rate was defined as the number of cows responding to PGF2α and ovulating after second GnRH over the total number of cows [19].

Pregnancy diagnosis was performed by ultrasonography at 32 days after AI. Pregnancy was characterized by the presence of fluid, an embryo, and a heartbeat. Cows diagnosed pregnant at 32 days were re-examined at 60 ± 5 days after AI to confirm pregnancy. Pregnancy loss