

1-1-2010

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EL-ZARKOUNY, SAMIR Z. (2010) "Conception rates for standing estrus and fixed-time insemination in dairy heifers synchronized with GnRH and PGF₂ α ," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 34: No. 3, Article 4. <https://doi.org/10.3906/vet-0808-22>
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Conception rates for standing estrus and fixed-time insemination in dairy heifers synchronized with GnRH and PGF_{2α}

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Received: 13.08.2008

Abstract: The objective of this study was to evaluate the effectiveness of GnRH/PGF-based protocols on reproductive outcome in Holstein heifers. Heifers were randomly assigned to the control group (n = 14), or treatment groups (n = 50) treated with GnRH (day -7) and PGF_{2α} (day 0). Heifers that exhibited estrus were inseminated (Select Synch, n = 34) and heifers that did not exhibit estrus up to 48 h after PGF_{2α} injection received a second GnRH injection and were fixed-time inseminated (Ovsynch, n = 16). Conception rates were 40%, 55.9%, and 31.3%, and overall pregnancy rates (P < 0.05) were 64.3%, 97%, and 87.5% in the control, Select Synch, and Ovsynch groups, respectively. Ovsynch heifers had 1.8 ± 0.5 and 3.2 ± 0.6 ng mL⁻¹ of serum P4, versus 3.3 ± 0.5 and 3.8 ± 0.6 ng mL⁻¹ in the Select Synch heifers at the time of the first GnRH and PGF_{2α} injection, respectively. Conception rates were 55.6% and 58.3% when P4 concentrations were high, and were 50% and 25% when P4 concentrations were low at the time of the first GnRH and PGF_{2α} injection, respectively. Dairy heifers synchronized with GnRH and PGF_{2α} should be inseminated when estrus is observed, and heifers that do not exhibit estrus up to 48 h after PGF_{2α} injection should be given a second GnRH injection and fixed-time inseminated 16-20 h later.

Key words: Heifers, conception rates, progesterone, Ovsynch, Select Synch

Introduction

Replacement heifers are the future of the dairy farm (1). Reproductive management of dairy heifers has always been a challenge. In recent years a combination of GnRH and PGF_{2α} has been used to increase reproductive efficiency in lactating dairy cows and heifers (2). The ovulation synchronization (Ovsynch) protocol was the first protocol designed to synchronize ovulation in dairy cows and allow for TAI with acceptable pregnancy rates (3); however, heifers did not respond favorably and pregnancy rates were 20%-40% lower than in heifers allowed to exhibit estrus before insemination (4,5). Peckelhoff et al. (6)

and Tenhagen et al. (1) assessed the reproductive outcomes of estrus synchronization in dairy heifers using GnRH and PGF_{2α} 7 days apart, followed by AI upon observation of estrus. Some studies have assessed the effectiveness of synchronizing ovulation with GnRH and PGF_{2α}, followed by TAI in dairy heifers, but results were inconsistent due to a low number of heifers (7) or an unknown synchronization response (5). Decreased pregnancy rates in nulliparous heifers on the Ovsynch protocol could be due to the number of follicular waves (5) or to inconsistent follicular wave emergence (8). Ovulation of the ovulatory follicle occurred within 24-48 h after

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the second GnRH injection in dairy cows in the Ovsynch protocol (1,9-11). A higher rate of ovulation (47.1%) was detected in dairy heifers 18-24 h after the second GnRH injection in the Ovsynch protocol, as compared to 10% in multiparous cows (12). As such, late application of TAI and/or aged oocytes could be the cause for decreased pregnancy rates in dairy heifers on the Ovsynch protocol (12).

The objective of the present study was to evaluate the effectiveness of the estrus synchronization protocol that allows for AI based on the observation of estrus in heifers treated with GnRH before PGF_{2α}-induced luteolysis and estrus (GnRH + PGF_{2α} [Select Synch]), and the Ovsynch protocol plus TAI.

Materials and methods

The study was conducted on a private dairy farm (Alalameia for Animal Production and Land Reclamation) located 90 km on the desert road from Alexandria to Cairo. All heifers were born on that dairy farm to Holstein cows inseminated with frozen semen imported from the USA.

Heifer management

The heifers were housed in open barns in groups of 10, based on age and body weight. All heifers were raised on heifer feed, consisting of silage and concentrate. Heifers were randomly assigned to

treatment protocols based on their age and body weight at the start of treatment. The inclusion criteria were minimum age of 13 months and body weight of 350 kg. Heifers that did not meet the health criteria, and minimum age and body weight were not included in the study.

Treatment protocols

The experimental design is shown in Figure 1. The control group (n = 14) did not receive any treatment. Heifers in the control group were observed for the signs of behavioral estrus twice daily throughout the study period and were artificially inseminated (AI) according to the AM/PM rule. Estrus was synchronized in the remainder of heifers (n = 50) with gonadotropin releasing hormone (GnRH) and prostaglandin F_{2α} (PGF_{2α}). Synchronized heifers were injected with 20 µg of GnRH (GnRH 1) (buserelin acetate [Receptal®]) on day 0 and with 0.150 mg of PGF_{2α} (cloprostenol [Estrumate®]) on day 7. All heifers were observed for signs of behavioral estrus from 24 h before PGF_{2α} injection to 48 h after PGF_{2α} injection. Heifers that exhibited estrus were inseminated 8-16 h later (Select Synch; n = 34) and received no further injections. Heifers that did not exhibit estrus within 48 h of PGF_{2α} injection received a second injection of GnRH (GnRH 2) (Ovsynch, n = 14) and were fixed-time inseminated 16-20 h after GnRH injection.

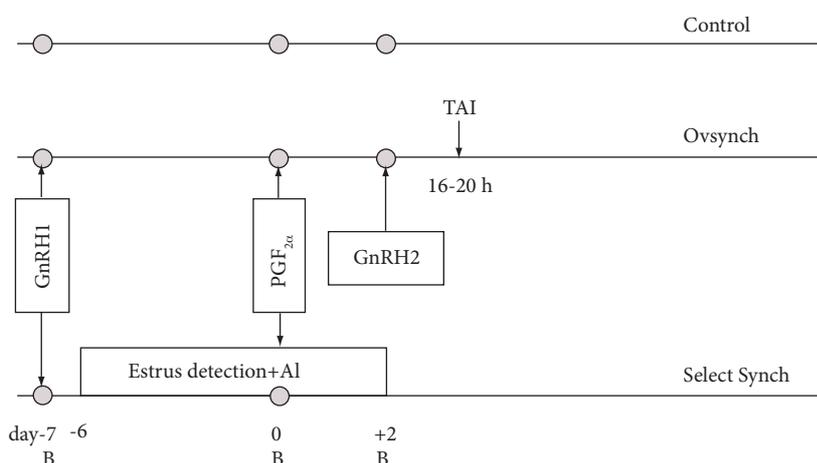


Figure 1. Experimental design for dairy heifers treated with control, Ovsynch, and Select Synch protocols. Blood (B) samples were collected from all animals on days -7, 0, and +2.

Blood collection and enzyme immunoassays

Blood samples were collected via tail vein puncture before hormonal treatments on day -7 (first GnRH injection), day 0 (PGF_{2α} injection), and day +2 (second GnRH injection) in the Select Synch and Ovsynch heifers, and on the corresponding days in the control heifers. Blood samples were centrifuged at 3000 rpm for 20 min and serum was harvested and stored at -20 °C for later analysis of progesterone. Progesterone (P4) was measured in serum samples using enzyme immunoassay kits. Intra- and interassay coefficients of variation were 4.4% and 4.3% for P4, respectively. First service conception rates were determined 35.0 ± 5 days after AI via ultrasound scanning of uterine contents using a Concept MLV ultrasound system equipped with a transrectal 5.0-7.5-MHz transducer. The number of services per conception was determined for the control, Ovsynch, and Select Synch heifers.

Statistical analysis

The rates of AI submission based on visual detection of estrus, conception (number of pregnant heifers after synchronized insemination divided by the number of heifers inseminated), overall pregnancy (number of pregnant heifers at the end of the AI period), and number of services per conception to first AI were calculated. The time interval from PGF_{2α} injection (day 0) to first standing of estrus was calculated. All the preceding variables were analyzed using the GLM procedure in SAS (13). Categories of P4 (high and low serum P4), time of injections (GnRH 1, PGF_{2α}, and GnRH 2), and treatments were used as the main sources of variation in conception rates to first AI in a separate model (SAS).

Results

Data for first service conception rates (%), number of services per conception, and overall pregnancy rates (%) are presented in Table 1. Heifers treated with the Ovsynch protocol had a 31.3% first service conception rate, as compared to 40.0% and 55.9% in the control and Select Synch heifers, respectively. There were no significant differences in the number of services per conception between the control, Ovsynch, and Select Synch heifers (2.3 ± 0.3, 1.8 ± 0.3, and 1.7 ± 0.2, respectively). Overall pregnancy rates in the Select Synch (97.0%) and Ovsynch (87.5%) heifers were higher ($P < 0.05$) than in the controls (64.3%).

Mean serum P4 concentration (ng mL⁻¹) at the time of the first GnRH, PGF_{2α}, and second GnRH injection in the controls, Ovsynch, and Select Synch heifers are presented in Table 2. At the time of the first GnRH injection, no differences were observed in mean serum P4 concentration between control, Ovsynch, and Select Synch heifers, but the first GnRH injection resulted in decreased ($P < 0.05$) serum P4 concentrations at the time of PGF_{2α} injection in the Ovsynch and Select Synch heifers (3.2 ± 0.6 and 3.8 ± 0.6 ng mL⁻¹, respectively), as compared to the controls (6.1 ± 1.0 ng mL⁻¹). Heifers treated with PGF_{2α} (Ovsynch and Select Synch) had lower ($P < 0.05$) serum P4 concentrations (1.7 ± 0.4 ng mL⁻¹) at the time of the second GnRH injection than the control heifers (5.7 ± 0.7 ng mL⁻¹).

At the time of the second GnRH injection 52.2% of the heifers had a high serum progesterone concentration, indicating incomplete luteal regression after PGF_{2α} injection. In the Ovsynch and Select

Table 1. Reproductive traits (Conception rate, CR; Number of services per conception, NSC) of dairy heifers treated with the Ovsynch and Select Synch protocols and controls.

Treatment	CR1 (%)	Overall (%)	NSC
Control	40.0 (n = 10)	64.3b (n = 14)	2.3 ± 0.3 (n = 10)
Ovsynch	31.3 (n = 16)	87.5a (n = 16)	1.8 ± 0.3 (n = 16)
Select Synch	55.9 (n = 34)	97.0a (n = 34)	1.7 ± 0.2 (n = 34)

CR1: Conception rate to first service

Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2. Average serum concentrations of progesterone (ng/mL⁻¹) and luteolysis (%) in dairy heifers receiving the Ovsynch and Select Synch protocols and controls.

	Control	Ovsynch	Select Synch
GnRH1	3.6 ± 0.8	1.8 ± 0.5	3.3 ± 0.5
PGF _{2α}	6.1 ± 1.0 ^a	3.2 ± 0.6 ^b	3.8 ± 0.6 ^b
GnRH2	5.7 ± 0.7 ^a	1.7 ± 0.4 ^b	1.7 ± 0.4 ^b
Luteolysis (%)	25.0	58.3	54.6

GnRH1: First GnRH injection
 GnRH2: Second GnRH injection
 Values within rows with different superscripts differ significantly (P < 0.05)

Synch protocols 58.33% and 54.55% of the heifers, respectively, had a decreased serum progesterone concentration at the time of the second GnRH injection. Only 25% of the control heifers had spontaneous luteal regression and decreased serum P4 concentrations at the time the Ovsynch and Select Synch heifers received a second GnRH injection.

Additional relationships between serum P4 and conception rates are summarized in Figure 2. The conception rate in heifers with luteal activity (P4 ≥ 1.0 ng mL⁻¹) before PGF_{2α} injection was 55.6% (45.5% and 71.4% in the Ovsynch and Select Synch heifers, respectively), whereas the conception rate in heifers that had low (<1.0 ng mL⁻¹) serum P4 concentrations was 50% (100% and 33.3% in the Ovsynch and Select Synch heifers, respectively). The conception rate in heifers with luteal activity (P4 > 1.0 ng mL⁻¹) at the

time of PGF_{2α} injection was 58.3% (57.1% and 60% in the Ovsynch and Select Synch heifers, respectively), whereas the conception rate in heifers with low (<1.0 ng mL⁻¹) serum P4 concentrations was 25% (33.3% and 0.0% in the Ovsynch and Select Synch heifers, respectively).

Among the treated heifers (n = 50), 34 (68%) were observed to be in estrus and were inseminated (Select Synch heifers), versus 16 heifers (32%) that were not observed to be in estrus up to 48 h after PGF_{2α} injection; these heifers received a second GnRH injection and were fixed-time inseminated (Ovsynch).

The proportional distribution of estrus behavior in the Select Synch heifers from the time of the first GnRH injection to 48 h after PGF_{2α} injection is shown in Figure 3. After PGF_{2α} injection 29.55% and 38.64% of the heifers treated with the first GnRH injection and PGF_{2α} exhibited estrus within 24 and 48 h,

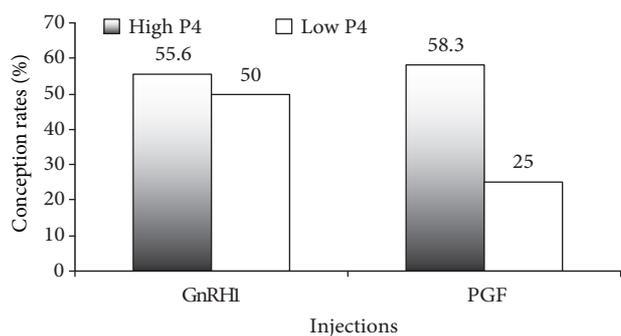


Figure 2. Conception rates in Holstein heifers when P4 concentration were high (≥ 1 ng/mL⁻¹) or low (< 1 ng/mL⁻¹) at the times of first GnRH and PGF_{2α} injections.

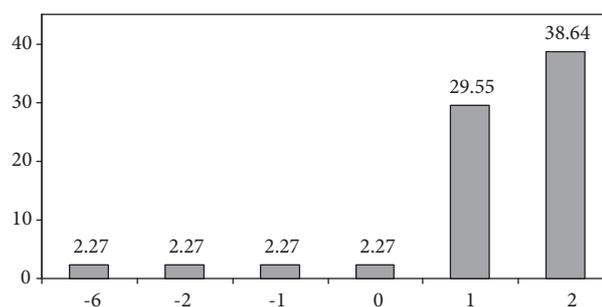


Figure 3. Percentage of distribution of estrus before and after PGF_{2α} injection for dairy heifers treated with GnRH and PGF_{2α} (Select Synch).

respectively. Four more heifers were observed to be in estrus on days -6, -2, -1, and 0 after PGF_{2α} injection (1 heifer each day).

Discussion

Pregnancy rates per AI were lower in heifers treated with Ovsynch (35.1%, n = 77) than in the Select Synch heifers (74.7%, n = 78) that were synchronized with PGF_{2α} (5). This might have been due to lower ovulatory response to the first GnRH injection and other differences in the follicular dynamics of the heifers. Some researchers (9,14) reported that the presence of CL or elevated progesterone concentrations at the start of the ovulation synchronization protocol could be a factor related to the success of such protocols.

In the present study heifers that exhibited estrus after PGF_{2α} injection (Select Synch) had elevated ($3.3 \pm 0.5 \text{ ng mL}^{-1}$) P4 concentrations at the time of the first GnRH injection, as compared to $1.8 \pm 0.5 \text{ ng mL}^{-1}$ in heifers that did not exhibit estrus (Ovsynch). It was shown that the success of the Ovsynch protocol is influenced by the stage of the estrus cycle when the first GnRH dose is administered (7,11,15). Pregnancy rates increased when dairy cows were pre-synchronized with 2 PGF_{2α} injections (7) or 1 PGF_{2α} injection (16) before the start of the Ovsynch protocol. Pre-synchronization of the estrous cycle results in cows that are in the proper stage of the estrous cycle (early or late diestrus) before the start of the Ovsynch protocol.

Overall conception rates in the present study are in accordance with previous reports of pregnancy rates that are higher in heifers with high P4 concentrations at the time of PGF_{2α} injection than in heifers with low serum P4 concentrations. Furthermore, the lower overall pregnancy rates we observed in heifers with low serum P4 concentrations at the time of PGF_{2α} injection are compatible with previous reports by Pursley et al. (5), who reported that the presence of a functional CL or high P4

concentration at the time of PGF_{2α} injection is crucial to the success of ovulation synchronization protocols.

Burke et al. (17) recorded higher conception rates in cows undergoing AI at detected estrus (Ovsynch without the second GnRH injection) than in cows fixed-time inseminated (Ovsynch). Timed AI following the Ovsynch protocol was strongly advocated by several investigators (17-19) as an effective tool for improving reproductive management in dairy cows, as it eliminates the need for estrus detection. DeJarnette et al. (20) reported that 20% of Ovsynch-treated cows exhibited premature estrus (48 h after PGF_{2α}) and concluded that detection of estrus during this period improved conception rates.

In the present study GnRH and PGF_{2α} injection caused more heifers (34/50) to exhibit estrus behavior, and as a result they were inseminated (Select Synch), and 16 of the 50 heifers did not exhibit estrus up to 48 h after PGF_{2α} injection and were injected with a second GnRH dose and were fixed-time inseminated (Ovsynch). Some researchers suggest that ovulation in cows occurs 24-48 h after the second GnRH injection in the Ovsynch protocol (1,9-11). Furthermore, the highest rates were obtained between 24 and 32 h (3,12). Demiral et al. (12) observed 47.1% of ovulation in dairy heifers 16-20 h after the second GnRH injection and concluded that late application of TAI in heifers treated with the Ovsynch protocol results in aging of oocytes and reduced pregnancy rates. Estrus detection after PGF_{2α} injection for 48 h is considered an acceptable practice to maximize pregnancy rates before the second GnRH injection with the Ovsynch protocol in dairy heifers (21).

Replacement dairy heifers synchronized with GnRH and PGF_{2α} should be monitored closely for signs of behavioral estrus and inseminated when estrus is observed, whereas heifers that do not exhibit estrus up to 48 h after PGF_{2α} injection should be given a second GnRH injection and fixed-time inseminated 16-20 h later.

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