**Effect of Two Estrous Synchronization Programs on Reproductive Performance of Dairy Cows under Summer Condition in Jordan**

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**ABSTRACT**

Two treatment groups of postpartum Friesian cows (n = 276) were synchronized for estrus starting on d 10 of the estrous cycle. GPG: GnRH-PGF2α-GnRH and timed AI, GPE: GnRH-PGF2α-EB and AI at detected estrus.

Pregnancy rates for first AI at d 28 (46.7 ± 4.4 and 48.4 ± 4.9%), at d 45 post-AI (34.7 ± 4.2 and 34.1 ± 4.7%) and the cumulative (87.3 ± 2.3 and 86.5 ± 3.3%) for GPG and GPE cows, respectively, did not differ between the two treatments. Whereas pregnancy rates to the first AI for primiparous and multiparous cows (38.8 ± 4.8 and 24.1 ± 4.3%, respectively) were significantly different (P < 0.02). Meanwhile, the cumulative pregnancy rates (87.6 ± 2.4 and 85.5 ± 3.8% for primiparous and multiparous, respectively) were not different. No differences were observed in days open for pregnant cows between GPG and GPE cows (85.2 ± 3.4 and 87.2 ± 3.8 d, respectively) and between primiparous and multiparous cows (84.9 ± 2.7 and 87.4 ± 4.3 d, respectively).

Results indicate no additional improvement in reproductive performance of cows inseminated at detected estrus following GnRH-PGF2α-EB program compared to cows inseminated at determined time following GnRH-PGF2α-GnRH program as measured by pregnancy rate and days open under summer condition.

**KEYWORDS:** dairy cows, estrous-synchronization, TAI, Estradiol, reproductive performance.

**INTRODUCTION**

Ambient temperature is an important determinant of reproductive performance in dairy cows (Wolfenson et al., 2000). In tropical and subtropical areas, reproductive efficiency is depressed in female cattle reared under hot and humid environments (Thatcher and Collier, 1986). In Jordan, during the summer months of June through October, pregnancy rates to first service are reduced; days from calving to first service and conception are increased (Alnimer and Lubbadeh, 2003). Several factors affecting pregnancy rate postpartum: among them are cyclicity, energy balance, heat stress, parity, milk production, diet, and diseases (Hansen and Arechiga, 1999; Cartmill et al., 2001a; Lucy, 2001; Moreira et al., 2001; Santos et al., 2004). Heat stress is associated with a decreased expression of estrous behavior (Nebel et al., 1997) and increase in the percentage of undetected estrus (Thatcher and Collier, 1986). The consequence of these changes is a reduction in the number of inseminations and an increase in the proportion of inseminations that do not result in pregnancy (Hansen, 1997). Heat stress has been reported to alter follicular development by reducing steroid hormone production (Wolfenson et al., 1997) and these changes in follicular steroid concentration could disrupt oocyte growth. In addition, heat stress reduces growth of the dominant follicle (Badinga et al., 1993) and causes incomplete dominance so that it increased growth of subordinate follicles (Wolfenson et al., 1995). Thus, the duration of dominance of the preovulatory follicle is increased in summer and the duration of dominance is negatively correlated with fertility (Mihm et al., 1994). Therefore, estrous synchronization programs should aim to synchronize follicular wave development as well as onset of estrus and ovulation. The Timed Artificial Insemination protocol (TAI) is an effective planned breeding program, developed in lactating dairy cows, that allows for AI without estrus detection (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997).
Conception rates after this program vary in literature and numerous factors influencing the risk of conception have been identified such as body condition score (Moreira et al., 2000a), stage of estrous cycle at the initiation of the protocol (Vasconcelos et al., 1999; Tenhagen et al., 2001), parity (Stevenson et al., 1999; Tenhagen et al., 2001), and heat stress (De La Sota et al., 1998; Cartmill et al., 2001b).

In cattle, estradiol normally promotes the preovulatory LH surge by stimulating the number of GnRH receptors in the anterior pituitary while concentrations of progesterone are basal (Hansel and Convey, 1983). Heatsynch is a newly developed synchronization protocol that uses ECP, an esterified form of estradiol-17β, in place of the second GnRH injection of the TAI protocol. It has been demonstrated that 1 or 0.5 mg of ECP induces an LH surge in lactating dairy cows (Pancarci et al., 2002; Stevenson et al., 2002) when given 24 h after a luteolytic dose of PGF2α. The aim of this study was to evaluate the effect of two estrous synchronization programs after postpartum for improving reproductive performance in dairy cows by using TAI protocol or a program involving GnRH, PGF2α, and Estradiol Benzoate (EB) then inseminated at detected estrus under summer condition of Jordan.

**MATERIALS AND METHODS**

This study was conducted on a commercial dairy farm at Dulail area northeast of Jordan from May to October 2003. Lactating dairy cows were housed in free-stall barns provided with shade and were milked three times daily at approximately 8-h intervals. The average (± SEM) milk yield was 32.12 ± 0.69 kg per day between 40 to 60 day postpartum. Cows were fed according to NRC recommendations (NRC, 1989) a Total Mixed Ration (TMR) of 40% forage (corn silage and alfalfa hay) and 60% concentrate (whole cottonseed, barely, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins) containing 1.70 Mcal of NE3/kg and 17.8% CP (percentage of DM). Cows had ad libitum access to fresh water. The experiment was implemented on a weekly basis according to the predetermined day postpartum.

A total of 296 lactating Friesian dairy cows (primiparous, n = 200) and (multiparous n = 96) were subjected to estrus detection between 20-30 d postpartum (pp) by visual observation and ALPRO™ system (Delaval International AB, Tumba, Sweden), which was utilized to detect the onset of estrus and to record standing events associated with estrus. An activity meter was hung around the same neckband as the ALPRO transponder, to monitor the extra activity shown by a cow when she comes into heat and to transmit this data every hour to the computer. If estrus was not observed until d 30 pp, each cow received an i.m. injection of 25 mg PGF2α (Lutalyse, Phamacia and Upjohn S.A. Puurs, Belgium) then also observed for estrus the same as previous procedure (visual observations and ALPRO™ system).

On d 10 (mid estrous cycle) after heat, cows were assigned randomly into two treatments (GPG, n = 150) and (GPE, n = 126), 20 cows did not respond to PGF2α treatment on d 30 and were excluded from the study. Cows in the GPG (as control) were injected with 10 µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH), followed 7 d later by an i.m. injection of 25 mg PGF2α, then another injection of 10 µg GnRH 48-h later and time inseminated from 16 to 20 h after the second GnRH injection, and considered as standard procedure to improve reproductive performance in many dairy farms in Jordan. Cows in the GPE were injected with 10 µg GnRH, followed 7 d later by an i.m. injection of 25 mg PGF2α, then injected with 1mg Estradiol Benzoate (EB) dissolved in oil (Intervet International B.V. Boxmeer, Holland) 24-h later and inseminated at detected estrus. Because this study was performed in a private farm, cows continued under the estrus detection program during the experimental period and were inseminated at any detected estrus to maximize conception. The Voluntary Waiting Period (VWP) for breeding was set at 53.2 ± 0.4 and 50.5 ± 0.4 d pp for cows in GPG and GPE, respectively. Pregnancy was confirmed by a portable B-mode ultrasound scanner (scanner 100 Vet, equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands), at d 28 and reconfirmed by rectal palpation at 45 d post AI. Cows, which were diagnosed pregnant at d 28 and later diagnosed nonpregnant, were considered as having lost their pregnancy. Cows in the GPG and GPE, which returned to heat, were re-inseminated at detected estrus. Those cows, which were diagnosed not pregnant either on d 28 or 45, received another injection of 25 mg PGF2α and re-inseminated at detected estrus. For cows in GPE, estrus detection rate was defined as the proportion of cows that responded to synchronization within 96 h after EB injection. Pregnancy rate was defined as the
proportion of all treated cows that were pregnant at 28 d post AI. Days open were defined as the number of days from calving to conception for pregnant cows till the fourth insemination. Environmental temperatures and relative humidity were obtained from official national station at Dulail area. The Temperature Humidity Index (THI) was calculated following the formula of Kelly and Bond, reported by Ingraham and his associates (1979).

Logistic regression of SAS (SAS, 1998) was utilized to study the pregnancy rates using stepwise selection procedure to determine independent variables at predetermined significance levels (variable entered with $p \leq 0.30$ and stayed with $p \leq 0.20$ in the model) using the following model:

$$Y_{ijklmno} = \mu + t_i + p_j + (tp)_{ij} + (ijk)_{ijklmno} + \text{ol} + b_1 (m_{120}-m_{120Avg}) + b_2 (m_{pp}-m_{ppAvg}) + \text{ijklmno}$$

$Y_{ijklmno}$ represent pregnancy rate, pregnancy loss, estrus detection rate, days from calving to VWP, days from calving to first AI, Hours from last hormonal injection to first AI, days open for pregnant cows and number of insemination per pregnant cows, $\mu$: overall means of the measurements, $t_i$: is the effects of treatments (GPG and GPE), $p_j$: is the effects of parity (primiparous and multiparous), $w_k$: week entering experiment after pp, $o_l$: is the observed estrus of each cow, $b_1 (m_{120}-m_{120Avg})$: regression effect of 120-day total milk yield, $b_2 (m_{pp}-m_{ppAvg})$: regression effect of milk production between 40 to 60 d pp, $ijklmno$: is the overall error. Least squares mean were compared using t-test.

**RESULTS AND DISCUSSION**

The objective of this study was to evaluate the effect of two estrous synchronization programs after pp for improving reproductive performance in dairy cows by using TAI protocol (GPG) or a program involving GnRH, PGF$_{2\alpha}$, and EB then inseminated at detected estrus (GPE) under summer condition. Synchronization in GPG and GPE cows was initiated on d 10 of the estrous cycle. 6% (9/150) and 2.4% (3/126) of cows in GPG and GPE groups exhibited estrus and inseminated prior the last hormonal injection. Only 22.2% (2/9) and 0% (0/3) of cows in GPG and GPE were pregnant at d 45. Vasconcelos et al. (1999) reported that 6% of cows ovulated before the second GnRH injection in TAI protocol. These cows failed to ovulate to the first GnRH injection primarily they were in the second half of the estrous cycle when they received the first GnRH injection and exhibited estrus before the second GnRH injection because the corpus luteum regressed and the cows naturally came in heat (Vasconcelos et al., 1999).

A greater ($P < 0.001$) proportion of cows in GPG were inseminated within 24 h following the second GnRH injection compared with those of cows in GPE inseminated at detected estrus within 96 h following EB injection. Ninety four percent (141/150) of cows in GPG were inseminated at (19.6 ± 2.4 h) following the second GnRH injection, while 8.7% (13/150) of those cows showed estrus after that time and were re-inseminated to maximize pregnancy rate. These cows likely initiate growth of a new follicular wave in response to ovulation of dominant follicle; however, the follicle grows quickly and losses dominate during the nine-day interval between GnRH injections and failed to ovulate after the second GnRH injection (Vasconcelos et al., 1999). Therefore, only 85.3% of cows were synchronized, which agrees with the results of Pursley et al. (1995) and Vasconcelos et al. (1999). Recently, Cartmille et al. (2001a and b) reported that 83.8% of cows were cycling in TAI program, while 5 to 15% of cows were not cycling at the time of insemination.

Around 82% (103/126) of cows in GPE were inseminated at (44.7 ± 2.6 h) following EB injection. 68.3% (86/126) of cows in GPE group was exhibited estrus within 24 h after EB injection. These results are in line with those of Borman et al. (2002) who reported that treatment of lactating dairy cows with GnRH, 7 d later by PGF$_{2\alpha}$ and ECP after 24 h then AI at detected estrus, 63% of cows were observed in estrus. In another study, Stevenson et al. (2002) detected only 40% of cows in estrus when using similar synchronization method. Moreover, Pancarci et al. (2002) reported that estrus occurred at 29.0 ± 1.8 h with ovulation occurring at 55.4 ± 2.7 h after ECP injection of 66.6% of cows in Heatsynch protocol (ECP replaced the second GnRH injection in TAI protocol) on the day of TAI. It has been
reported that injected cows with 1 or 0.5 mg of ECP induced an LH surge in lactating dairy cows when given 24 h after a luteolytic dose of PGF$_{2\alpha}$ (Pancarci et al., 2002; Stevenson et al., 2002). Furthermore, cows with CL that regressed before the second hormonal injection in both groups were not pregnant.

In the present study, pregnancy rate has been tested during summer season (Figure 1) and a greater pregnancy rate was observed by ultrasound on d 28 post-AI than that based on rectal palpation (45 d). Pregnancy rates at d 28 were similar for GPG (46.7 ± 4.4%) and GPE (48.4 ± 4.9%) cows. There was no effect of parity or treatment × parity interaction on pregnancy rate for first AI. Also, milk yield between d 40 to 60 pp and the total yield at d 120 did not affect pregnancy rates. These results are in agreement with those of Fricke et al. (1998), who found that Pregnancy rates at 28 to 32 d post AI in lactating dairy cows range from 40 to 47%. Pregnancy rates to first, second, third, and fourth AI were not different for GPG and GPE cows (Table 1). A similar observation was found for cumulative pregnancy rate in GPG and GPE cows (87.3 ± 2.3 and 86.5 ± 3.3 %, respectively (Table 1). Similar results were found in lactating cows between TAI and Heatsynch protocols as reported by Bartolome et al. (2002; Pancarci et al. (2002); Stevenson et al. (2002). A previous study in the same farm during summer season (Lubbadeh and Alnimer, 2003) showed that pregnancy rate to first AI (30%) was higher and days open (104 d) were lower for cows following GPG program when the program was started at random stage of the estrous cycle compared to control group treated with PGF$_{2\alpha}$ and inseminated at detected estrus (17% and 121d for pregnancy rate and days open, respectively). In the present study, pregnancy rate from first AI of dairy cows was higher than that obtained in a previous study (Alnimer et al., 2001; Alnimer and Lubbadeh, 2003; Lubbadeh and Alnimer, 2003), since activity meter was not available to detect anestrus cows during summer. The use of ALPRO system improves estrus detection and thus fertility as has been seen in GPE group. Moreover, the effect of a synchronized estrus after pre-synchronization by PGF$_{2\alpha}$ on pregnancy rates for both treatment groups supports earlier studies indicating that day of estrous cycle and pre-synchronization influence efficiency of the TAI protocol (Vasconcelos et al., 1999; Moreira et al., 2000b, 2001).

Differences were observed in pregnancy rate to first (38.8 ± 4.8 vs. 24.1 ± 4.3%; P < 0.02), third (27.3 ± 5.4 vs 48.6 ± 8.1%; P < 0.05), and tended to be higher (P<0.1) to fourth (57.1 ± 6.7 vs. 33.3 ± 9.9%) AI between primiparous and multiparous, respectively, while cumulative pregnancy rate was similar for primiparous and multiparous cows (87.6 ± 2.4 and 85.5 ± 3.8%, respectively Table 1). Several authors reported higher conception rates from first insemination in primiparous than multiparous cows (Cartmill et al., 2001a; Tenhagen et al., 2004). Other studies did not demonstrate this effect (Jobst et al., 2000; Pancarci et al., 2002).

No differences were observed in pregnancy losses due to treatment effects, parity and treatment × parity interaction. Pregnancy losses from 28 to 45 d after insemination were 12.0 ± 2.9 and 14.3 ± 3.3% for cows in GPG and GPE groups, respectively. Previous studies reported 7 to 56% pregnancy loss in dairy cows and such losses were attributed to season (Cartmill et al., 2001b) and to the stage of pregnancy (Vasconcelos et al., 1998). Moreover, Daily et al. (2002) postulated that most loss of pregnancy occurs prior to d 45 of gestation. Depression of pregnancy rate during the warm or hot period of the year has been well demonstrated (Badinga et al., 1985). In addition, a TH1 value exceeding 72 indicates mild to extreme heat stress conditions for lactating dairy cows (Armstrong, 1994).

The intervals from calving to first, second, third, and fourth AI were not different for GPG and GPE cows (Table 2). Similar observations in the same parameter were found between primiparous and multiparous cows, while tended to be higher (P<0.06) in multiparous than primiparous cows (85.3 ± 1.7 vs. 81.2 ± 1.3 d, respectively), with the mean intervals between estruses was approximately 29 ± 3 d, since all cows were observed estrus by visual observations and ALPRO system. No differences were observed in days open for pregnant cows between GPG and GPE cows (85.2 ± 3.4 and 87.2 ± 3.8 d, respectively) and also between primiparous and multiparous cows (84.9 ± 2.7 vs 87.4 ± 4.3 d; respectively). The number of inseminations per pregnancy was similar (P > 0.05) for GPG and EPE (2.10 ± 0.10 vs. 2.08 ± 0.11, respectively (Table 2) cows. In practice, the return to estrus is variable with most cows returning to estrus 20 to 24 d after insemination (Chenault et al., 2003). In the present study, ultrasound pregnancy diagnosis is done at approximately d 28 then non-pregnant cows will be on d 6 - 10 of the subsequent estrous cycle; a period when PGF$_{2\alpha}$ responsive corpus luteum and a dominant follicle are present on the ovary.
Stevenson et al. (2003) demonstrated that cows could be diagnosed for pregnancy on d 27 – 29 and re-inseminated by timed AI using rapid re-synchronization either by PGF$_{2\alpha}$ alone or PGF$_{2\alpha}$ + GnRH. They found that days from first service to re-insemination after the non-pregnant diagnosis cows were around 31± 2 d. On the other hand, intervals from calving to inseminations in the present study are better than a previous study by Alnimer et al. (2001) since they did not use new strategy for detection estrus. Many studies have shown GPG protocol to be a highly effective and economic strategy for improving reproductive performance (number of AI for pregnant cows, days open etc.) in high-producing lactating dairy cows (Burke et al., 1996; Pursley et al., 1997; De La Sota et al., 1998), because no differences were observed in reproductive performance between the two groups. Therefore, early non-pregnancy diagnosis and detecting estrus, by ALPRO system, result in reducing intervals between inseminations and interval from calving to conception.

**CONCLUSIONS**

Results indicate that no significant improvement in reproductive performance of cows timed AI or AI at detected estrus following treatment with GnRH-PGF$_{2\alpha}$- GnRH (GPG) or GnRH-PGF$_{2\alpha}$-EB (GPE) as measured by the estrous response rate, pregnancy rate at induced estrous and reducing intervals between inseminations and days open under summer condition. Moreover, in GPE group, EB can substitute the second GnRH injection.

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Effect of Two Mufeed A. Alnim


