

Estrous Synchronization Using PGF_{2α} with GnRH or Estradiol Benzoate to Improve Reproductive Performance of Lactating Dairy Cows

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ABSTRACT

Lactating dairy cows (n= 470) were used to evaluate the effect of two estrous synchronization protocols starting on d 10 of the estrous cycle on reproductive performance. OVS: Gonadotropin Releasing Hormone (GnRH)-Prostaglandin F_{2α} (PGF_{2α})-GnRH and timed Artificial Insemination (TAI) and EPG: Estradiol Benzoate (EB)-PGF_{2α} and GnRH and TAI. Pregnancy rates on d 45 and 90 were greater for the OVS (43.0%, $P = 0.004$ and 41.1%, $P = 0.002$) compared to the EPG group (30.2% and 27.4%) and were lower for multiparous cows (29.9%, $P = 0.008$ and 27.2%, $P = 0.004$) compared to primiparous cows (42.0% and 39.9 %), respectively. Pregnancy rates to first AI ($P = 0.002$), and overall AI were greater ($P = 0.001$) for the OVS (40.7% and 97.7%) compared to the EPG group (26.9% and 89.6%; respectively). Moreover, pregnancy rates of first, second, third and overall AI were lower ($P = 0.001$) for multiparous cows (26.6, 57.6, 77.7 and 90.2%, respectively) compared to primiparous cows (39.5, 71.3, 89.2 and 96%, respectively). Days open and the number of services per pregnancy were lower ($P = 0.014$; $P = 0.001$) for the OVS (81.7 d and 2.01) compared to the EPG group (88.5 d and 2.14) and lower ($P = 0.001$) for primiparous (80.6 d and 1.94) compared to multiparous cows (89.6 d and 2.22). Results indicate that a significant improvement in reproductive performance of timed AI cows following OVS than EPG program as measured by pregnancy rate, days open and number of services per pregnancy. In addition, primiparous cows had better reproductive performance than multiparous cows.

Keywords: Dairy Cows, Estrous-Synchronization, GnRH, Estradiol, TAI, Reproductive Performance.

1. INTRODUCTION

Failure to detect cows in estrus accurately is a major factor limiting reproductive performance of lactating dairy cows and this is the main reason for the increase in calving interval in dairy herds (Rounsaville et al., 1979). A calving interval of 12 to 13 months is generally considered economically optimal for dairy cows, but this is rarely achieved on commercial farms. Heat detection efficiency is usually less than 50% in most herds. Heat detection accuracy varies widely because up to 5% of all cows presented for artificial insemination (AI) have been reported as not being in standing estrus based on high milk progesterone levels (Reimers et al., 1985). Therefore, the efficient and accurate detection of estrus and the timing of AI remain current challenges in dairy

production. In synchronization of estrus and controlled ovulation schemes, clear guidelines must also be established to optimize insemination time with respect to ovulation. Research has been conducted to control the estrous cycle of the cow with the administration of hormones. Through research, a number of protocols for synchronization of estrus and ovulation have been developed; however, they differ in type of hormones, time of hormone administration, time of insemination, and cost.

Timed artificial insemination (TAI) protocols, such as Ovsynch, have been developed to decrease reliance on the detection of estrus in reproductive management programs (Pursely et al., 1995, 1997; Burke et al., 1996). Such a protocol consists of an injection of GnRH 7 days before and 48 h after PGF_{2α} before TAI which takes place 16 h after the second GnRH injection (Pursely et al., 1995, 1997). The optimum time to start the Ovsynch protocol is between days 5-12 of the estrous cycle which gives greater pregnancy rate than Ovsynch started at a random stage of the cycle (Vasconcelos et al., 1999).

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Estradiol Benzoate (EB) has been shown to induce the turnover of follicular wave, or emergence of a new follicular wave within 3-5 days in the majority of treated cows regardless of the stage of the follicular wave when treatment is started (Bo et al., 1995; Martinez et al., 2000). The mechanism responsible for estradiol-induced synchronization of follicular growth appears to involve the suppression of plasma FSH concentration followed by synchronous surge of FSH after atresia or removal of the dominant follicle.

Previous studies demonstrated that pregnancy rates were not different between Ovsynch protocol compared to either protocols of using estradiol injection 7 d before and 24 h after PGF_{2α} (Alnimer, 2005a) or using GnRH injection 7 d before and estradiol 24 h after PGF_{2α} and insemination at detected estrus (Alnimer, 2005b). Therefore, the aim of this study was to evaluate reproductive performance in lactating dairy cows through comparing Ovsynch protocol (OVS) to a program involving Estradiol Benzoate as a substitute for the first GnRH injection, followed 7 d by PGF_{2α} and TAI with GnRH injection 48 h later (EPG) in order to reduce labor time, stress and labor cost.

2. MATERIALS AND METHODS

This study was conducted on a commercial dairy farm at Alkhalidia area in the northern part of Jordan at 32° 33'N, 35° 51' E during the period between December, 2005 to April 2006. Lactating dairy cows were housed in free-stall barns provided with shade and were milked three times daily at 8-h intervals. The rolling average milk yield for the herd was around 9000 kg per lactation (305 d). Cows were fed according to NRC recommendations (NRC, 2001) a total mixed ration (TMR) of 40% forage (corn silage and alfalfa hay) and 60% concentrate (corn, barley, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins) containing 1.80 Mcal of NE_l/kg and 18.8% CP (DM bases). Cows had *ad libitum* access to fresh water.

A total of 600 lactating Friesian dairy cows were subjected to estrus detection between 25-30 d postpartum (pp) by visual observation and ALPROP^{TMP} system. The program included an activity meter (Delaval International AB, Tumba, Sweden) fitted to the neck of every cow to detect and record the activities exhibited by the cow when she approaches to heat and transmits data every hour to a

computer. If estrus was not observed till d 30, cows were rectally palpated to determine ovarian activity. All cows previously detected in estrus before d 30 and cows with a corpus luteum on rectal palpation received an i.m. injection of 25 mg PGF_{2α} (Lutalyse, Pharmacia & Upjohn S.A. Puurs, Belgium) on d 30.

Only cows showing estrus before or around d 34 pp (554 cows; primiparous, n= 332, and multiparous, n= 222) were used in this study. On d 10 after the observed estrus (d 45 ± 3 pp), cows were assigned randomly into two treatments (OVS: GnRH-PGF_{2α}-GnRH-TAI, n = 285) and (EPG: EB-PGF_{2α}-GnRH-TAI, n = 269). Cows in the OVS (control) were i.m. injected with 10 µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH), followed 7 d later by an i.m. injection of 25 mg PGF_{2α}, then another injection of 10 µg GnRH 48-h later and artificially inseminated from 16 to 20 h after the second GnRH injection, which is considered as standard procedure to improve reproductive performance in many dairy farms in Jordan. Cows in the EPG group were i.m. injected with 2 mg estradiol benzoate (EB) dissolved in oil (Intervet International B.V. Boxmeer, Holland), followed 7 d later by an i.m. injection of 25 mg PGF_{2α}, then injected with 10 µg GnRH and TAI 48-h later. Because this study was performed on a private farm, cows remained 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 54.4 ± 0.2 and 53.9 ± 0.3 d pp for cows in OVS and EPG, respectively.

Pregnancy diagnosis was conducted 30 d after TAI using transrectal ultrasonography (scanner 100 Vet, equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands). Pregnancy was determined by visualization of an embryonic heartbeat and surrounded by a fluid-filled cavity representing the allantoic cavity in the uterine lumen as described by Pierson and Ginther (1984). On d 45 and 90, pregnancy status was determined by rectal palpation. Cows, which were diagnosed pregnant on d 30 and later diagnosed non-pregnant on d 45 were considered as having experienced late embryonic losses, while cows that were diagnosed pregnant on d 45 and later diagnosed non-pregnant on d 90 were considered as having experienced fetal losses. Cows in both groups, which were not diagnosed pregnant on d 30, 45, or 90 received a new injection of 25 mg PGF_{2α} and inseminated after estrus

detection with GnRH injection within 30 minutes until the fourth time. Pregnancy rate was defined as the proportion of all treated cows that were pregnant on d 30, 45 and 90 post insemination and overall pregnancy rate (up to fourth AI). Days open were defined as the number of days from calving to conception for pregnant cows until the fourth insemination.

Analyses of data were performed using General Linear Models Procedure of SAS (2000). Statistical model for reproductive responses included effects of treatments (OVS and EPG), parity (primiparous and multiparous) and their interactions. Estrus-detection rate before the last GnRH injection and for cows that returned to estrus within 7 d after TAI in the two treatment groups was tested by chi-square test. For all cows, days from calving to voluntary waiting period (VWP), days from calving to first AI, second, third, and fourth AI, services per conception, days open for pregnant cows were also included. Least squares means for significant effects were compared at $P < 0.05$ using *t*-test. Pregnancy rates on d 30, 45, and 90, cumulated pregnancy rates to first, second, third, and up to fourth AI (overall), late embryonic losses between d 30 and 45, fetal losses between 45 and 90 and overall losses between d 30 to 90 for both groups were compared using Chi-square test.

3. RESULTS AND DISCUSSION

The objective of this study was to evaluate reproductive performance in lactating dairy cows by using Ovsynch protocol or a program involving EB as a substitute for the first GnRH injection, followed 7 d by PGF_{2α} and TAI with GnRH injection 48 h later (EPG) so as to reduce labor cost, time and stress. Synchronization with OVS or EPG was initiated on d 10 of the estrous cycle. Three and half percent (10/285) of the cows in the OVS treatment and 17.5% (47/269) of the cows in the EPG treatment exhibited estrus and were inseminated prior to the last hormonal injection. Only 20% (2/10) cows in OVS and 42.6% (20/47) cows in EPG treatment were pregnant ($P > 0.05$) at d 45 and were excluded from the analysis because these cows did not complete the hormonal protocols. Similar observations were reported by Alnimer (2005a) who found 16.7% of the cows receiving estradiol benzoate on d 10 of the estrous cycle exhibited estrus before the last hormonal injection in the EPE protocol (EB + PGF_{2α} + EB and AI at detected estrus). It has been demonstrated that EB injection during

the luteal phase of the estrous cycle generally promotes a synchronous timing of emergence of a new wave. The intervals to new wave emergence and estrus are still variable (Kim et al., 2005). This variability could be due to a number of factors such as breed type, age, lactation status and dose of estradiol (Bo et al., 1995; Burke et al., 2003; Kim et al., 2005). Moreover, EB may also be used to induce estrus with or without concurrent ovulation. In addition, Vasconcelos et al. (1999) found that 6% of the cows ovulated before the second GnRH injection in OVS protocol and they speculated this occurrence. On the other hand, 9.5% (27/285) and 21.2% (57/269) of the cows in the OVS and EPG groups which returned to estrus after TAI protocols and were reinseminated at detected estrus for the same purpose (maximize pregnancy rate). These results are in agreement with the results reported by others (Pursley et al., 1997; Vasconcelos et al. 1999; Alnimer, 2005b). Therefore, the number of cows that enrolled in the analysis was 258 and 212 for OVS and EPG groups, respectively.

Pregnancy rates and pregnancy losses between d 30 to 45 and d 45 to 90 between groups and parity are described in Tables (1 and 2). No differences were detected in pregnancy rates on d 30 between groups, parity or the interaction between group and parity. A treatment group, parity, but not interaction between group and parity effects was detected in the pregnancy rates on d 45 and 90 and from first TAI and overall AI. Pregnancy rates on d 45 and 90 were greater ($P = 0.004$; $P = 0.002$) for cows in the OVS (43.0 and 41.1%) compared to cows in the EPG (30.2 and 27.4%). In addition, pregnancy rates from first TAI and overall AI were greater ($P = 0.002$; $P = 0.001$) for cows in the OVS group (40.7 and 97.7 %) compared to cows in the EPG group (26.9 and 89.6 %). Furthermore, no treatment effect ($P > 0.05$) was detected on pregnancy rates to second and third AI (Table 2). No studies in the literature discussed reproductive performance between cows in the OVS and EPG protocols. Yet, pregnancy rates in this trial are in agreement with our previous studies at the same farm on d 28 in dairy cows (Alnimer, 2005a and b). In addition, Fricke et al. (1998) found that pregnancy rates at 28 to 32 d post AI in lactating dairy cows ranged between 40 to 47%. In the present study, pregnancy rate was greater in OVS group than in EPG group on d 45 post AI. In contrast, similar pregnancy rates were found in the previous studies (Alnimer, 2005a and b). Moreover, pregnancy rates on d 45 and 90 were similar between

Ovsynch protocol and Heatsynch protocol (estradiol replaces the second GnRH injection in the Ovsynch protocol) or between parities (Pancarci et al., 2002; Bartolome et al., 2002). On the other hand, Cerri et al. (2004) demonstrated that pregnancy rates on d 45 after AI were greater for cows receiving a Heatsynch management program compared to a program involving injections of GnRH and PGF_{2α} given 7 d apart and AI following estrus detection (i.e., 43% > 35%). Pregnancy rates from first, second, third and overall AI were similar between TAI and Heatsynch, GPE protocols or cows inseminated at detected estrus after one or two PGF_{2α} injections (Bartolome et al., 2002; Pancarci et al., 2002; Alnimer, 2005b). Previous studies indicated that pregnancy rates following Ovsynch program varied between 22 to 42% (Stevenson et al., 1996; Pursley et al., 1997; Fricke et al., 1998; Stevenson et al., 1999). In the present study, pregnancy rate (40.7%) for the OVS group was within the reported range.

Pregnancy rates were lower ($P = 0.008$; $P = 0.004$) for multiparous cows (29.9 and 27.2%) compared to primiparous cows (42.0 and 39.9 %) on d 45 and on d 90, respectively. Moreover, pregnancy rates to first, second, third, and up to fourth AI were greater ($P = 0.005$) for primiparous cows (39.5, 71.3, 89.2 and 96%, respectively) compared to multiparous cows (26.6, 57.6, 77.7 and 90.2%, respectively). These findings are consistent with the results of same (Tenhagen et al., 2001 and 2004; Portaluppi and Stevenson, 2005; Alnimer and Lubbadah, 2008) but not all previous studies (Jobst et al., 2000; Navanukraw et al., 2004; Alnimer, 2005a). Huszenicza et al. (1987) reported that primiparous cows had fewer reproductive problems compared to older cows. There are contradictory reports about differences in pregnancy rates between primiparous and multiparous cows. These differences may be explained by the use of different breeds, drugs, nutritional status, body condition score and time of initiation of the protocol (Grohn et al., 2000).

A treatment group, parity, but not interaction between group and parity effects was detected in pregnancy losses between d 30 and 45 and overall losses between 30 to 90 d (Table 1). Pregnancy losses between d 30 to 45 and overall losses between d 30 to 90 were lower ($P = 0.001$) for cows in the OVS group (5.8 and 7.8%) and primiparous cows (6.3 and 8.4%) compared to cows in the EPG group (15.6 and 18.4%) and multiparous cows (16.3 and 19.0%). No differences were detected in

pregnancy losses between d 45 to 90 for cows between groups, parity or interaction between group and parity (Table 1). The reasons for differences in pregnancy losses between the two groups were not known. The overall incidence of pregnancy loss between d 30 to 45 for both groups was around 11%. Several studies on lactating dairy cows have indicated that pregnancy losses between 27 to 45 d after AI ranged from 8 to 21% (Humblot, 2001; Chebel et al., 2003; Santos et al., 2004; Alnimer, 2005a and b; Alnimer and Lubbadah, 2008). In some cases, pregnancy losses have been reported to be greater than 21% (Moreira et al., 2000; Cartmill et al., 2001). Cow parity was positively linked to pregnancy loss in this study. The incidence of pregnancy loss was greater in multiparous cows than primiparous cows, which is similar to the results of previous studies (Santos et al., 2004; Lee and Kim, 2007; Alnimer and Lubbadah, 2008; Santos et al., 2009). Similarly, Humblot (2001) showed that frequency of embryonic mortality increase with parity (1st to 3rd parity), although others have reported no effect of parity on pregnancy loss (Cerri et al., 2004; Chebel et al., 2004; Alnimer, 2005a; Moore et al., 2005). These discrepancies may reflect differences in geography, study population, case definitions and procedures among studies. In addition, some factors were associated with pregnancy losses like concentration of progesterone at week 5 of gestation, twin ovulation and body condition (Starbuck et al., 2004).

A treatment group effect was detected for the interval from calving to second AI (Table 2). Interval from calving to second AI was shorter ($P = 0.02$) for cows in the OVS group (82.5 d) compared to cows in the EPG group (86.2 d). No treatment effect was detected for interval from calving to first, third or up to fourth AI. In addition, no effect was detected between primiparous and multiparous cows on the interval from calving to AI (Table 2). Similar observations were reported for interval from calving to first, third and fourth AI (Alnimer, 2005b). Moreover, intervals from calving to inseminations in the present study are shorter than those reported in a previous study by Alnimer et al. (2002) as they did not use new strategy for estrus detection. In practice, most nonpregnant cows are in estrus 20 – 23 d after first insemination (Chenault et al., 2003). The variability in return to estrus may be explained by the normal variation in estrus length and early embryonic death (causing partial extension of the estrous cycle as in the EPG group in the second AI). In the current study,

pregnancy diagnosis by ultrasound was done on d 30 then the nonpregnant cows were supposed to be on d 5 – 10 of subsequent estrous cycle; a period when a PGF_{2α} responsive corpus luteum and a dominant follicle are present on the ovary. Therefore, cows could be diagnosed for pregnancy via ultrasound on d 27 – 29 then the nonpregnant cows could be timed AI on d 30 or 31 (Chebel et al., 2003).

A treatment group and parity effects were detected on days open and the number of services per pregnancy (Table 2). Days open and number of services per pregnancy were lower ($P = 0.014$; $P = 0.001$) for cows in the OVS group (81.7 d and 2.01) compared to those for cows in the EPG group (88.5 d and 2.14). Moreover, days open and the number of services per pregnancy were lower ($P = 0.001$) for primiparous (80.6 d and 1.94) compared to those for multiparous cows (89.6 d and 2.22). In the present study, cows in both groups were inseminated at predetermined time, but pregnancy rate at first AI for cows in the OVS group was greater than that in the EPG group which caused reduction in the days open and number of services. The average days open for cows in EPG group (88.5 d) was similar to that reported in a previous study at the same farm (87.2 d)

for GPE group (Alnimer, 2005b), while it was greater than that reported in another study (84.7 d) in the EPE group (Alnimer, 2005a). In addition, the number of services per pregnancy (2.14) was greater than that reported in a previous study (2.08) for GPE cows (Alnimer, 2005b) and (1.78) for cows in the EPE group (Alnimer, 2005a).

4. CONCLUSIONS

Results indicate that reproductive performance of dairy cows can be improved through using the protocol: GnRH-PGF_{2α} and TAI 16-20 h after the second GnRH better than the use of the protocol: EB-PGF_{2α}-GnRH and TAI at the time of GnRH injection.

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Table 1. Percent pregnancy rates on days 30, 45, and 90 and pregnancy losses between days 30 to 45 and days 45 to 90 between groups and parity

Variable	Group ¹		P-value ²	Parity		P-value ³
	OVS (n=258)	EPG (n=212)		Primiparous (n=286)	Multiparous (n=184)	
Pregnancy rate (%)						
On day 30	48.8 (126) ⁴	45.8 (97)	0.505	48.3 (138)	46.2 (85)	0.663
On day 45	43.0 (111)	30.2 (64)	0.004	42.0 (120)	29.9 (55)	0.008
On day 90	41.1 (106)	27.4 (58)	0.002	39.9 (114)	27.2 (50)	0.004
Pregnancy losses (%)						
Days 30-45	5.8 (15)	15.6 (33)	0.001	6.3 (18)	16.3 (30)	0.001
Days 45-90	1.9 (5)	2.8 (6)	0.524	2.1 (6)	2.7 (5)	0.664
Days 30-90	7.8 (20)	18.4 (39)	0.001	8.4 (24)	19.0 (35)	0.001

¹Group: OVS = (GnRH-PGF_{2α}-GnRH and TAI). EPG = (Estradiol-PGF_{2α}-GnRH and TAI).

²Between groups

³Between parity

⁴Number of cows

Table 2. Characteristics of reproductive performance between groups and parity

Variable	Group ¹		P-value ²	Parity		P-value ³
	OVS (n=258)	EPG (n=212)		Primiparous (n=286)	Multiparous (n=184)	
Pregnancy rate (%)						
From first AI	40.7 (105) ⁴	26.9 (57)	0.002	39.5 (113)	26.6 (49)	0.004
First and second	69.0 (178)	62.3 (132)	0.125	71.3 (204)	57.6 (106)	0.002
First, second and third	87.2 (225)	81.6 (173)	0.093	89.2 (255)	77.7 (143)	0.001
Overall ⁵	97.7 (252)	89.6 (190)	0.001	96.5 (276)	90.2 (166)	0.005
Interval from calving to AI (d)						
First	54.6 ± 0.3 (258)	54.6 ± 0.3 (212)	0.576	54.5 ± 0.3 (286)	54.9 ± 0.4 (184)	0.315
Second	82.5 ± 1.1 (154)	86.2 ± 1.1 (155)	0.020	83.3 ± 1.0 (174)	85.5 ± 1.2 (135)	0.172
Third	107.4 ± 1.7 (80)	110.2 ± 1.7 (80)	0.247	107.9 ± 1.6 (82)	109.8 ± 1.7 (78)	0.412
Fourth	128.5 ± 2.1 (35)	129.7 ± 2.0 (39)	0.679	128.9 ± 2.2 (31)	129.2 ± 1.8 (43)	0.916
Days open	81.7 ± 1.8 (252)	88.5 ± 2.1 (190)	0.014	80.6 ± 1.7 (276)	89.6 ± 2.2 (166)	0.001
No. of AI per pregnancy	2.01 ± 0.1 (252)	2.14 ± 0.1 (190)	0.001	1.94 ± 0.1 (276)	2.22 ± 0.1 (166)	0.001

¹Group: OVS = (GnRH-PGF_{2α}-GnRH and TAI). EPG = (Estradiol-PGF_{2α}-GnRH and TAI).

²Between groups

³Between parity

⁴Number of cows

⁵From first till the fourth AI

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	(470)				
20-16	(OVS = GnRH-PGF _{2α} -GnRH)				
	(EPG = EB-PGF _{2α} -GnRH)		GnRH		
OVS			90	45	GnRH
	(%27.4	%30.2)	EPG	(P=0.002	%41.1
	(P=0.004	%27.2	P=0.008	%29.9)	P=0.004
	(P=0.002)				%43.0)
EPG	(%97.7	%40.7)	OVS		GnRH
					(P=0.001)
					(%89.6
	(%90.2	77.7	57.6	26.6)	%26.9)
					(P=0.001)
					(%96.0
	(2.01	81.7)	OVS		39.5)
	(P=0.001)		(P=0.001	P=0.014)	
			(2.14	88.5)	EPG
	(2.22	89.6)			(1.94
EPG					80.6)
			OVS		

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