Anim. Reprod., v.8, n.1/2, p.32-39, Jan./Jun. 2011

A comparison of fertility with a Cosynch protocol versus a modified Ovsynch protocol which included estradiol in lactating dairy cows during the summer season in Jordan

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Abstract

A total of 332 postpartum (pp) lactating Friesian cows allocated in two treatment groups at a commercial dairy farm were used to study the effect of a modified Ovsynch protocol supplemented with exogenous estradiol (E2) on fertility in lactating dairy cows during the summer season. All cows were injected with gonadotrophin releasing hormone (GnRH), followed 7 days later by an injection of prostaglandin (PGF2α). Then cows were randomly assigned to receive GnRH and TAI 72 h after PGF2α (CO-72; control group) or E2, GnRH and TAI at 48, 56 and 72 h, respectively after PGF2α (OV-56+E2). Estrus response for cows in the OV-56+E2 (59.8%) group was higher ($P < 0.05$) than for cows in the CO-72 (40.2%) group. Pregnancy per insemination (P/AI) at two diagnoses (days 30 to 35 and confirmed at days 45 to 50 after AI) were similar between the two treatment groups, but were higher ($P < 0.05$) for cows that showed estrus (59.8 and 41.2%, respectively) than for cows that did not show estrus (45.5 and 7.9%, respectively). Moreover, P/AI at the two diagnoses were higher ($P < 0.05$) for cows with a body condition score (BCS) >2.5 (60.1 and 36.4%, respectively) compared to those with BCS $\leq$2.5 (44.3 and 16.5%, respectively). Pregnancy losses were higher ($P < 0.05$) for cows that did not show estrus (82.6%) or with BCS $\leq$2.5 (62.8%) than for cows that showed estrus (31.0%) or with a BCS >2.5 (39.5%). No differences were observed in P/AI or pregnancy losses between primiparous and multiparous cows. Results indicate that the OV-56+E2 synchronization protocol resulted in a higher expression of estrus than the CO-72 without improving fertility as measured by P/AI and pregnancy losses. However, cows with high BCS had higher estrus expression, pregnancy rates, and lower pregnancy losses than cows with low BCS during the summer season.

Keywords: Cosynch, dairy cows, estradiol, fertility, Ovsynch.

Introduction

High ambient temperature and relative humidity adversely affect various reproductive processes in dairy cattle and cause high economic losses for dairy producers. The problem is aggravated when associated with high milk production because the rise in metabolic heat production leads to further increase in body temperature (Wolfenson *et al*., 2000). Worldwide, heat stress is a major cause of low fertility with pregnancies per artificial insemination (P/AI) decreasing from 40 to 60% in winter to 10 to 20% in the summer in tropical and subtropical areas (Wolfenson *et al*., 2000; De Rensis and Scaramuzzi, 2003). In Jordan, during the months of June through October (High temperature), P/AI to first service are reduced to about 20%. Conversely, during the months of October through January (cold temperature), P/AI are increased to about 39% (Alnimer *et al*., 2009). Numerous factors have been shown to decrease fertility in lactating dairy cows during heat stress. Heat stress has been reported to alter follicular development and reduces steroid hormone production (Wolfenson *et al*., 1997). These changes in follicular steroid concentration could disrupt oocyte growth, decrease expression of estrous behavior (Nebel *et al*., 1997), and increase 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).

Accurate estrus detection is a major factor in maintaining high reproductive efficiency. Development of timed artificial insemination (TAI) programs eliminated the dependence on estrus detection. Synchronization of ovulation (Ovsynch) was the first well-developed protocol (Pursley *et al*., 1995, 1997). The Ovsynch protocol was modified to Cosynch with TAI at the time of the second GnRH injection. Alnimer *et al*. (2009) found that cows treated with Cosynch 72 (GnRH and TAI 72 h after PGF2α) had higher P/AI than those treated with Cosynch 48 (GnRH and TAI 48 h after PGF2α). We have suggested that TAI with ovulation synchronization protocols should be tailored according to the season and parity (Alnimer *et al*., 2009). Currently, several large dairy farms have adapted CO-72 as part of their standard reproductive management of postpartum cows. Despite the applications of different Ovsynch protocols, low percentages of cows show estrus and fertility is still low,

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Received: July 27, 2010
Accepted: July 26, 2011

particularly in the summer, because of a relatively lower estradiol (E2) concentration around TAI (Lopez *et al*., 2004; Souza *et al*., 2007). Sellars *et al*. (2006) and Brusveen *et al*. (2008) added an E2 treatment at the time of second GnRH injection in the Ovsynch protocol and found higher rates of estrus expression with no significant differences in P/AI when compared to the Ovsynch protocol without E2. However, when E2 was injected 8 h before the second GnRH injection, Souza *et al*. (2007) reported that P/AI in E2-treated cows that exhibited estrus (49%) tended to be higher than those that exhibited estrus in the control group (40%). Furthermore, cows that exhibited estrus within 24 h of TAI had higher concentrations of estradiol and pregnancy rates compared with cows that did not exhibit estrus (Ahmadzadeh *et al*., 2003; Perry *et al*., 2007; Perry and Perry, 2008). DeJarnette *et al*. (2001) reported that conception rates were higher ($P < 0.05$) in cows detected in estrus (46%; 23/50) compared with cows not detected in estrus (25%; 24/95) after application of the modified Ovsynch program.

Heat stress causes a decrease in estradiol synthesis. In Jordan during the summer season, 70% of the cows were observed in estrus within 24 h after estradiol benzoate injection (Alnimer, 2005). Therefore, the aim of the current study was to compare a modified Ovsynch protocol that included the addition of E2 with a commonly used CO-72 protocol in affecting estrus expression and to determine whether increased estrus expression would increase P/AI in lactating dairy cows during the summer season.

Materials and Methods

This study was conducted on a commercial dairy farm in the Alkhaldia region in northeast Jordan at 32^o33^o N, 35^o51^o E from May to October 2009. Lactating Friesian cows were housed in free-stall barns, provided with shade and were milked three times daily at 8 h intervals. The rolling herd average milk production was approximately 8500 kg for a whole lactation cycle. Environmental data for overall mean maximum and mode temperatures (33.4 ± 0.3 and 34^oC), minimum temperature (16.3 ± 0.2^oC), and relative humidity (51.5 ± 0.9%) during the experimental period (May to October 2009) were obtained from the Official National Station in the Alkhaldia region (Fig. 1). Cows were fed 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 1.8 Mcal net energy of lactation (NE_L)/kg, 19% crude protein (dry matter basis), and adjusted according to National Research Council (2001) recommendations. Cows were given free access to fresh water.

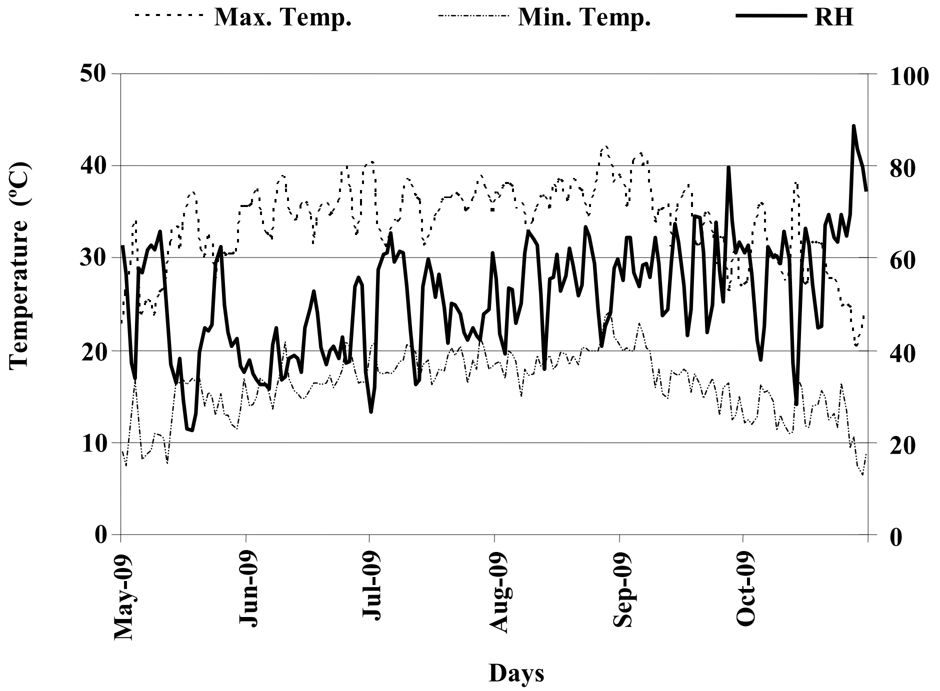

Figure 1. Trend of maximum and minimum daily temperature and relative humidity during the experimental period (May to October 2009).

Three hundred and sixty (primiparous, n = 120 and multiparous, n = 240) lactating Friesian dairy cows were subjected to an estrus detection program between days 25 and 30 postpartum (pp). The program included an ALPRO system with an activity meter (Delaval International AB, Tumba, Sweden) that transmits data every hour to the computer. In addition, standing estrus was confirmed by visual observation. If estrus was not observed by day 30, cows were rectally palpated to determine ovarian activity. All cows previously detected in estrus and cows with a corpus luteum on day 30 received an i.m. injection of 25 mg PGF2α (Lutalyse, Pharmacia & Upjohn S.A. Puurs, Belgium) at that time.

Only cows showing estrus before or around day 34 pp (332 cows) were used in this study. Twelve days after the observed estrus (day 46 ± 3 pp), cows were injected with 10 µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH, Wiesbaden, Germany), followed 7 days later by an i.m. injection of 25 mg PGF2α and were then randomly assigned into two treatment groups (Fig. 2). The control group was treated with GnRH and TAI 72 h after PGF2α (CO-72; n = 165), which is part of the standard postpartum reproductive management of cows on this farm. The treatment group received 1 mg estradiol benzoate (E2) dissolved in oil (Estradiol Benzoate ®, Intervet International B.V. Boxmeer, Holland) at 48 h and GnRH at 56 h after PGF2α and then TAI 16 h after the second GnRH injection i.e., 72 h after PGF2α (OV-56+E2; n = 167). Cows detected in estrus before the second GnRH injection were inseminated according to AM-PM rule to maximize pregnancies and were excluded from the experiment. Throughout the experiment, artificial insemination was performed by one experienced technician using commercially available proven fertility semen (ABS Global, Inc, USA). Frozen semen from seven sires was used at random across the two protocols to maintain similar distributions of sires across treatment groups. Days from calving to first TAI were 55.9 ± 0.6 and 56.8 ± 0.6 pp in the CO-72 and OV-56+E2 groups, respectively ($P > 0.05$). Cows were scored for body condition (BCS) on the day of TAI (1 = emaciated; 5 = obese; Edmonson *et al*., 1989).

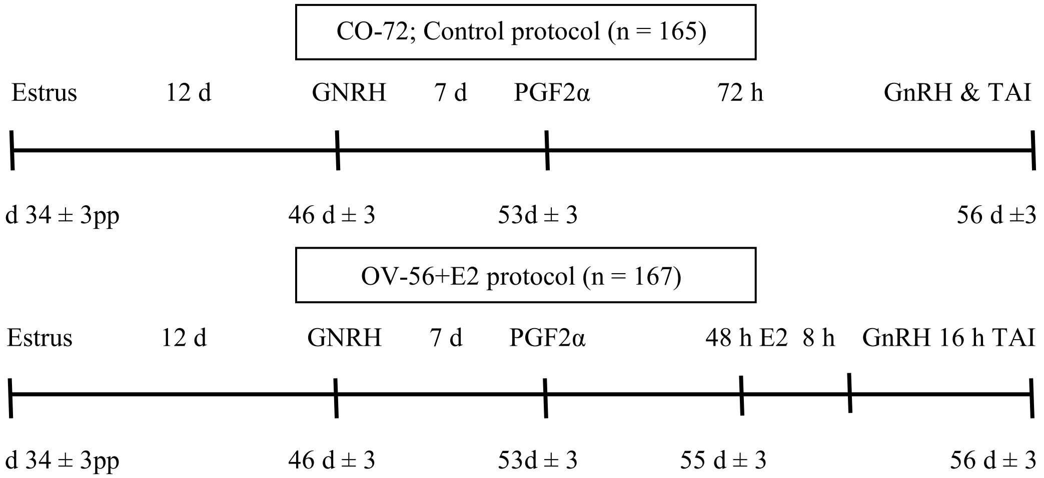

Figure 2. Schematic diagram of treatment protocols used in the experiment. Treatments: CO-72: (GnRH+PGF2α+GnRH+TAI 72 h after PGF2α), OV-56+E2: (GnRH+PGF2α+E2+GnRH+ TAI 16 h after the second GnRH injection).

All cows were examined for pregnancy by ultrasound (scanner 100 Vet; Pie Medical, Maastricht, The Netherlands) using 7.5 MHz probe 30 to 35 days after AI as described previously (Alnimer *et al*., 2009), and again at 45 to 50 days after AI. P/AI was defined as the proportion of all treated cows that were pregnant at 30 to 35 days and at 45 to 50 days post-AI. Pregnancy losses were calculated as cows that were diagnosed pregnant at first examination and diagnosed nonpregnant at the second exam.

Statistical analyses were performed using SAS (2000) to test the overall effects of factors. The effect of treatment (CO-72 and OV-56+E2) on estrus detection rate of cows and P/AI at days 30 to 35 and 40 to 45 post-AI, and pregnancy losses between the two examinations were tested by Chi-square test and Fisher exact test using the FREQ procedure of SAS. Cows inseminated before TAI were removed from subsequent analysis. Using General Linear Model (GLM) procedure of SAS, least square analysis of variance was applied to fertility responses including effects of treatment (CO-72 and OV-56+E2), parity (primiparous and multiparous) and their two-way interactions. Responses included percentage of P/AI at two diagnoses, pregnancy losses, and days from calving to first TAI. For the purpose of analyses, the effect of BCS on estrus detection rate, P/AI and pregnancy losses was estimated. Cows were classified according to BCS as low if BCS ≤ 2.5 and high if BCS > 2.5. The effects of average milk production for the first 4 months on treatment effects were measured. All responses were estimated using the FREQ procedure of SAS. Least square means for significant effects were compared at $P < 0.05$ using *t*-test.

Results

Synchronization of cows was initiated around day 12 of the estrous cycle. Mean lactation number did not differ ($P > 0.45$) between CO-72 and OV-56+E2 groups and were 1.65 ± 0.13 and 1.53 ± 0.1, respectively. Similarly, milk production between days 30 to 60 pp was 30.2 ± 0.75 kg/day and the average in the first 120 days pp was similar ($P > 0.05$) for cows in the CO-72 and OV-56+E2 groups (28.4 ± 0.31 and 28.9 ± 0.26 kg/day, respectively) and did not affect P/AI. Mean BCS were also similar ($P > 0.05$) between CO-72 and OV-56+E2 groups and were 2.68 ± 0.02 and 2.69 ± 0.02, respectively.

Among cows used in the study (n = 332), 15% (25/165; CO-72) and 7.2% (12/167; OV-56+E2) exhibited estrus after PGF2α and before the scheduled last GnRH administration ($P = 0.08$). After insemination following the AM/PM rule, 28% (7/25; CO-72) and 66.7% (8/12; OV-56+E2) of those cows were pregnant ($P < 0.05$) at days 45 to 50 post-AI. These cows did not complete the hormonal treatment protocols (n = 37) and were excluded from further analysis. Therefore, 295 cows that completed the hormonal protocols were available for analysis. Estrus response, P/AI and pregnancy losses are presented in Table 1. Estrus expression rate for cows in the CO-72 group was lower ($P < 0.01$) than for cows in the OV-56+E2 group. P/AI at days 30 to 35 and 45 to 50 post-AI and pregnancy losses did not differ ($P > 0.1$) between groups (Table 1). However, there was a treatment by expression of estrus interaction ($P < 0.05$) effect on P/AI (Table 2). Among cows that expressed estrus, P/AI was similar between treatment groups. Regardless of treatment, cows that showed estrus had higher ($P < 0.02$) P/AI at days 30 to 35 and 45 to 50 post-AI (Table 2). Conversely, among cows that did not express estrus, cows in the OV-56+E2 group had lower ($P < 0.05$) P/AI at first and second pregnancy diagnoses than cows in the CO-72 group (Table 2). In addition, cows that did not express estrus in the OV-56+E2 group had lower ($P < 0.05$) P/AI than those cows that showed estrus in both groups (Table 2).

Pregnancy loss did not differ between CO-72 and OV-56+E2 groups (Table 1). Regardless of treatment, cows that showed estrus had lower ($P < 0.02$) pregnancy losses than cows in which estrus was not detected (Table 2). There was no interaction for treatment group by expression of estrus on pregnancy losses. However, in cows that failed to show estrus, pregnancy loss tended to be lower ($P < 0.1$) in the CO-72 than in the OV-56+E2 group (Table 2).

Reproductive performance of cows according to parity and BCS is presented in Table 1. No significant differences were found in P/AI or pregnancy losses between parities, or treatment x parity interactions. When data was classified according to high (>2.5) and low (±2.5) BCS regardless of treatments or parity, expression of estrus for cows that had high BCS was higher ($P < 0.01$) than for cows with low BCS. No interaction was observed between treatment or parity and BCS in expression of estrus or pregnancy rates. P/AI were higher ($P < 0.05$) for cows with high BCS at days 30 to 35 and 45 to 50 than in cows with low BCS. Pregnancy losses were lower ($P < 0.05$) for cows with high BCS compared to those with low BCS.

Table 1. Pregnancy rates and pregnancy losses for cows according to treatment, parity and BCS.

End point	Treatment[†]		Parity		BCS[‡]	
	CO-72 (n = 140)[#]	OV-56 + E2 (n = 155)[#]	Primiparous (n = 94)	Multiparous (n = 201)	Low (≤2.5) (n = 97)	High (>2.5) (n = 198)
Estrus n (%)	82 (58.5)[a]	116 (74.8)[b]	60 (63.8)	134 (66.7)	52 (53.6)[a]	142 (71.7)[b]
Pregnancy per AI	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
At day 30 to 35	80 (57.1)	82 (52.9)	51 (54.3)	111 (55.2)	43 (44.3)[A]	119 (60.1)[B]
At day 45 to 50	40 (28.6)	48 (31.0)	27 (28.7)	61 (30.4)	16 (16.5)[A]	72 (36.4)[B]
Pregnancy losses[§] (%)	40 (50.0)	34 (41.5)	24 (47.1)	50 (45.1)	27 (62.8)[A]	47 (39.5)[B]

[†] CO-72: (GnRH+PGF2α+GnRH+TAI 72 h after PGF2α), OV-56+E2: (GnRH+PGF2α+E2+GnRH+TAI 16 h after the second GnRH injection).

[‡] Body condition score.

[#] Twenty five and 12 cows from CO-72 and OV-56 + E2 groups, respectively exhibited estrus and were inseminated before the last GnRH injection and were excluded from the analysis (included four cows in the CO-72 that exhibited estrus more than 48 h after PGF2α and before the second GnRH injection).

[§] Proportion of cows diagnosed pregnant at 30 to 35 days after TAI that were diagnosed nonpregnant at 45 to 50 days after AI.

[a,b] Percentages within major groups with different superscripts differ ($P < 0.01$). [A,B] Percentages within BCS groups with different superscripts differ ($P < 0.05$).

Table 2. Pregnancy rates and pregnancy losses according to estrus response for cows in CO-72 and OV-56+E2 groups.

End point	Estrus response[†]					
	Estrus[‡]			No estrus		
	CO-72 (n = 78)	OV-56 + E2 (n = 116)	Overall (n = 194)	CO-72 (n = 62)	OV-56 + E2 (n = 39)	Overall (n = 101)
Pregnancy per AI (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
At day 30 to 35	46 (59.0)^a	70 (60.3)^a	116 (59.8)^A	34 (54.8)^a	12 (30.8)^b	46 (45.5)^B
At day 45 to 50	32 (41.0)^a	48 (41.4)^a	80 (41.2)^A	8 (12.9)^c	0 (0.0)^d	8 (7.9)^B
Pregnancy losses[#] (%)	14 (30.4)	22 (31.4)	36 (31.0)^A	26 (76.5)^c	12 (100)^d	38 (82.6)^B

[†] Twenty five and 12 cows from CO-72 and OV-56+E2 groups that respectively exhibited estrus were inseminated before the last GnRH injection and were excluded from the analysis.

[‡] Determined by ALPRO system and visual observation.

[#] Cows diagnosed pregnant at 30 to 35 days after AI that were diagnosed not pregnant at 45 to 50 days after AI.

^{a,b}Percentages within a row with different superscripts differ ($P < 0.05$). ^{c,d}Percentages within a row in the No estrus groups with different superscripts differ ($P < 0.1$). ^{A,B}Percentages for overall differed between Estrus and No estrus groups ($P < 0.02$).

Discussion

The objective of this experiment was to study the effect of a modified Ovsynch protocol, which included E2, on fertility of lactating dairy cows compared to the standard CO-72 protocol used in Jordan under summer conditions. More cows in the CO-72 (15%) group were observed in estrus prior to the last GnRH injection than cows in the OV-56+E2 (7.2%) group. This result was expected because of the shorter interval between PGF2α injection and GnRH injection in the OV-56+E2 (56 h) than the CO-72 (72 h) and therefore less time for cows to develop a large follicle and express estrus. Alnimer *et al*. (2009) previously reported that 8 to 10% of cows showed estrus before the second GnRH in the TAI protocol, while DeJarnette *et al*. (2001) observed 20% of Ovsynch-treated cows displaying estrus within 48 h after PGF2α.

In the present study, more cows were observed in estrus (before and after GnRH injection) in the OV-56+E2 group than in the CO-72 group. These results are in agreement with those reported by Souza *et al*. (2007) and Brusveen *et al*. (2009) who found that the expression of estrus in cows in the E2-supplemented Ovsynch was higher than for cows in the Ovsynch without E2 supplementation. Other studies demonstrated higher estrus expression rates in dairy cows receiving ECP (an esterified long acting form of estradiol-17β) instead of the last GnRH injection in the Ovsynch protocol (Pancarci *et al*., 2002; Cerri *et al*., 2004; Kasimanickam *et al*., 2005). Hillegass *et al*. (2008) reported 49.6% of lactating dairy cows in estrus in a CO-72 protocol and a significant increase to 79.9% with an injection of ECP supplementation. According to Souza *et al*. (2005), treatment with 1 mg of E2 in the absence of follicles ≥5 mm will cause a dramatic increase in circulating E2 concentrations but not expression of estrus. Therefore, existence of an endogenous E2 source may be essential for estrus expression induced by this low dose of exogenous E2.

In the current study, approximately 30% of cows did not show estrus in response to E2 treatment (OV-56+E2 group), which is higher than that reported by Souza *et al*. (2007) who found that 20% of cows did not exhibit estrus. Moreover, Souza *et al*. (2007) used E-17β, which has a shorter half-life and reaches greater circulating levels compared to E-benzoate. This could be partially responsible for some differences in estrus expression seen in the current trial. Alternatively, in the present study, around 50% of cows in the CO-72 group did not show estrus. The current study was conducted during the summer season, and heat stress may have compromised ovarian follicle development and steroidogenic capacity (Wolfenson *et al*., 2000), with the consequences of a decrease in length and intensity of estrus (Younas *et al*., 1993). For example, in the summer motor activity and other manifestations of estrus such as mounting activity are reduced (Nebel *et al*., 1997) and the incidence of anestrus and silent ovulation are also increased (Gwazdauskas *et al*., 1981).

In the present study, cows that showed estrus in the OV-56+E2 group had higher P/AI than those that did not show estrus. Souza *et al*. (2007) also found higher P/AI for E2-supplemented cows that showed estrus in an Ovsynch protocol than control cows that did not show estrus. Regardless of treatment, cows that showed estrus had higher P/AI than those that did not show estrus. A positive effect of detected estrus on pregnancy was observed with TAI protocols when GnRH or ECP were used to induce ovulation (Pancarci *et al*., 2002; Cerri *et al*., 2004; Kasimanickam *et al*., 2005). It is noteworthy that the expression of estrus resulted in higher P/AI regardless of E2 injection. Furthermore, cows that exhibited estrus have been reported to have increased E2 concentrations at 48 h after luteolysis, immediately before GnRH induced ovulation. Conversely, similar P/AI at day 68 after AI were reported for cows that did and did not receive ECP in CO-72, suggesting that endogenous E2 is not critical for pregnancy in TAI protocols (Hilligass *et al*., 2008).

In the current study, the lower fertility and the failure to express estrus in the OV-56+E2 group may be related. In these cows, there may have been insufficient follicular E2 secretion even with exogenous supplementation to elicit estrus behavior, and the reduced fertility may have been associated with reduced follicular function. Lyimo *et al*. (2000) reported a correlation of 0.7 between estradiol concentration and estrus behavior. The reduced E2 concentration might be related to a defective follicle or altered follicular development and absence of a mature ovulatory follicle. Ovulation of immature follicles has been reported to lead to development of smaller and less functional corpus luteum (Burke *et al*., 2001). Therefore, cows that fail to express estrus, despite a high level of estrus detection, after E2-supplementation protocols should not be inseminated and resynchronized again. This conclusion needs to be tested in a large fertility study. Further work is necessary to determine if the dose of 1 mg E2/animal is the optimal dose or adjustments should be made according to the ovarian state, milk production, and BCS. In addition, the low fertility in this group may be attributed indirectly to low BCS, as 10 of these 12 cows were in the low BCS category (Table 2).

In the present study, P/AI at two diagnoses did not differ between the two treatment groups (Table 1). In cows that expressed estrus, P/AI at two diagnoses was also similar between the two treatment groups. Souza *et al*. (2007) reported that P/AI tended to be higher for cows supplemented with E2 than control cows that showed estrus. Estradiol cypionate treatment was successful in elevating serum E2 level when administered before or at the time of the second dose of GnRH in the Ovsynch protocol; however, P/AI were not affected by treatment (Souza *et al*., 2007; Hillegass *et al*., 2008; Brusveen *et al*., 2009).

The present study was performed during summer in the semi-arid environment of northeast Jordan. Therefore, P/AI in this study were lower than those reported by other researchers (Souza *et al*., 2007; Hillegass *et al*., 2008). Moreover, P/AI at first insemination have been reported to not differ between Ovsynch (without E2 supplement) and CO-72 treatments on the same farm during the summer season (Alnimer *et al*., 2009). High ambient temperatures have a number of detrimental effects on physiological processes that are important for establishment and maintenance of pregnancy after fertilization. These effects are well documented (Wolfenson *et al*., 2000; Cartmill *et al*., 2001; De Rensis and Scaramuzzi, 2003). In the current study, primiparous and multiparous cows had similar pregnancy rates. Navanukraw *et al*. (2004) and Alnimer (2005) reported similar P/AI between primiparous and multiparous cows in different TAI protocols. In contrast, several recent studies found higher P/AI for primiparous cows than multiparous cows (Brusveen *et al*., 2008; Alnimer *et al*., 2009; Santos *et al*., 2009).

Regardless of treatment or parity, P/AI was increased in cows with high BCS (Table 1). These results are in congruence with others (Hillegass *et al*., 2008; Santos *et al*., 2009). In general, studies conducted during the summer season have reported that cows with lower BCS (≤ 2.5) have reduced fertility after TAI protocols (Moreira *et al*., 2000). In contrast, Navanukraw *et al*. (2004) reported that BCS at TAI did not affect pregnancy rate in a TAI protocol.

Pregnancy losses were similar between OV-56+E2 and CO-72 groups for cows that showed estrus. Souza *et al*. (2007) previously cited similar results. However, pregnancy losses in the current study were higher than in previous studies on the same farm (embryonic mortality of around 23% from 28 to 45 days after AI in the two synchronization protocols; Alnimer, 2005). Recently, we have observed higher pregnancy losses during the summer (40%) compared to winter (8.5%) seasons and in multiparous cows than primiparous cows (Alnimer *et al*., 2009). The discrepancies in the results may be due to the differences in maximum temperatures during the experimental period for each study. Furthermore, others have reported 7 to 56% pregnancy loss in dairy cows and such losses were attributed to season (Cartmill *et al*., 2001) and a high incidence of embryonic mortality after day 27 (Vasconcelos *et al*., 1998).

Regardless of treatment, cows that showed estrus had lower ($P < 0.02$) pregnancy losses than cows in which estrus was not detected. This might be a delayed effect of the low estradiol concentrations during the periovulatory period. During the summer season, heat stress has adverse effects on the quality of ovarian follicles (Badinga *et al*., 1993; Wilson *et al*., 1998) causing a decrease in estradiol synthesis. The expression of estrus may simply reflect a decrease in estradiol synthesis, due to impaired follicular function, ovulation, and corpus luteum development, and endometrial gene expression during the luteal phase (Bridges, 2007). Maximal concentration of estradiol during proestrus was significantly correlated to total number of nuclei per embryo, and deficient preovulatory estradiol concentrations altered endometrial gene expression which was proposed to alter its function (Bridges, 2007). These changes might be the reason behind the increased rate of pregnancy loss. In cows that failed to show estrus, pregnancy loss tended to be lower ($P < 0.1$) in the CO-72 (76.5%) than the OV-56+E2 (100%) cows. There was no interaction for treatment group by expression of estrus on pregnancy losses. The low number of pregnancies in OV-56+E2 might be the reason behind the failure to detect such differences.

In conclusion, results indicate that the OV-56+E2 protocol resulted in a higher incidence of the expression of estrus than the CO-72 protocol, presumably because of E2 supplementation. However, fertility as measured by P/AI and pregnancy losses was not improved. Cows with high BCS had a higher rate of estrus expression, P/AI, and lower pregnancy losses than cows with low BCS.

Acknowledgments

The Deanship of Research University of Jordan is gratefully acknowledged for funding this project (1068/2007). The authors express their appreciation to Mohammad Eid and Hammodeh farm for facilities and assistance.

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