Jordan Journal of Agricultural Sciences, Volume **7**, No.**4** 2011

Effect of Different Hormonal Treatments for Synchronizing Estrus on Fertility of Lactating Dairy Cows

Ahmad I. Shamoun and Mufeed A. Alnimer✉*

ABSTRACT

This research was designed to study the effectiveness of applying three Timed Artificial Insemination (TAI) protocols on reproductive performance of Holstein Friesian lactating dairy cows. A group of 173 cows received an injection of GnRH on day 44 postpartum (pp) and $PGF_{2\alpha}$ 7 d later, and then assigned into three treatments: (1) CO-72 (n = 65), received GnRH injection and TAI on day 54 pp; (2) MOVS (n = 42), received GnRH injection at 56 h after $PGF_{2\alpha}$ followed by TAI 16 h later; (3) OVS-CO (n = 66), received two GnRH injections on day 54 and 61 followed by $PGF_{2\alpha}$ injection on day 68 before another GnRH injection with TAI on day 71 pp. Pregnancy rates to first AI for cows in both MOVS and OVS-CO treatments tended to be greater ($P < 0.1$) than those for cows in the CO-72 treatment while the overall pregnancy rates for cows in the MOVS treatment tended to be greater ($P < 0.1$) than those for cows in the other two treatments. Pregnancy losses for cows in the OVS-CO treatment were lower ($P < 0.05$) than those for cows in the CO-72 treatment. Number of services per conception for cows in the MOVS treatment were lower ($P < 0.05$) than those for cows in the CO-72 treatment. Cows calved through November, December had greater (P < 0.05) pregnancy rates to first AI, and lower ($P < 0.05$) number of services per conception than those cows calved through September and October. Cows in the CO-72 treatment had higher ($P < 0.05$) average daily milk yield during the first 5 months pp than for cows in both MOVS and OVS-CO treatments. In contrast, parity had no effect on any of reproductive performance measurements. In conclusion, cows in the MOVS treatment and those calved during November and December had better pregnancy rates than those cows in both CO-72 and OVS-CO treatments and those calved during September and October, respectively.

Keywords: Dairy Cows, Estrus Synchronization, Fertility, Timed Artificial Insemination.

INTRODUCTION

Reproductive performance is a major factor affecting profitability of dairy herds. Several factors such as efficiency of estrus detection, days open and calving interval were found to affect the reproductive performance (Bagnato and Oltenacu, 1994). The lack of an efficient and accurate method for estrus detection severely limits the reproductive performance of lactating dairy cows (Portaluppi and Stevenson, 2005; Rabiee et al., 2005). Therefore, many programs of estrus synchronization based on combination of different hormones were developed to control the estrous cycle length and follicular dynamics and to provide a fixed-time for artificial insemination (TAI) without the need for estrus detection.

Ovsynch was the first protocol developed for

*✉ Department of Animal Production, University of Jordan, Amman, Jordan, email: amufeed@ju.edu.jo
Received on 11/5/2010 and Accepted for Publication on 10/5/2011.

synchronization of ovulation. It involves injection of gonadotrophin releasing hormone (GnRH) 7 days (d) before and 48 hours (h) after prostaglandin $F_{2α}$ ($PGF_{2α}$) before TAI which takes place 16-24 h after the second GnRH injection (Pursley et al., 1995; 1997a & b). The optimum time to start Ovsynch protocol is between days 5 and 12 of the estrous cycle, which gives higher pregnancy rates than when Ovsynch started at random stage of the cycle (Vasconcelos et al., 1999; Moreira et al., 2001). The Ovsynch protocol improved pregnancy rates in cycling cows (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004), but it had no effect on anovular cows (Moreira et al., 2001). Gümen et al. (2003) found that fertility was reduced due to the increased percentages of short cycles in anovular cows following Ovsynch. There were a substantial percentage of cows that were anovular (20-30%) at the time of the first GnRH of Ovsynch protocol (Moreira et al., 2001; Lopez et al., 2005). The Ovsynch protocol was modified to Cosynch by changing the TAI to be with the second GnRH injection. This protocol required cows for handling only 3 times compared to 4 times in the Ovsynch, which meant less labor and cost (Geary and Whittier, 1998). Portaluppi and Stevenson (2005) found that cows treated with Cosynch 72 (GnRH and TAI 72 h after $PGF_{2α}$) had more pregnancies than that treated with Cosynch 48 (GnRH and TAI 48 h after $PGF_{2α}$) and Ovsynch (TAI after GnRH dose by 24 h). Recent study in Jordan by Alnimer et al. (2009) reported greater pregnancy rates for cows treated with Cosynch 72 and Ovsynch (GnRH 48 h and TAI 16 h after $PGF_{2α}$) than that treated with Cosynch 48. Souza et al. (2008) developed a new program involves using Double Ovsynch (GnRH, $PGF_{2α}$ 7 d later, and GnRH 3 d later, followed by Ovsynch-TAI protocol 7 d later) to induce cyclicity in anovular cows. They found that fertility following Double Ovsynch was improved compared to Ovsynch. Therefore, the objective of this study was to evaluate the reproductive performance of dairy cows by comparing Cosynch-72h (as control) to Modified Ovsynch and Ovsynch-Cosynch protocols.

MATERIALS and METHODS

Cows, Housing, and Management

This study was conducted from September 2008 to April 2009 at a commercial private dairy farm (Hammoudeh Farm) housing an approximately 1500 Holstein Friesian lactating dairy cows located in Alkhaldia area of the northern part of Jordan at 32°33' N, 35°51' E. Cows were housed in a free-stall barns provided with shades and were milked three times daily at 8 h intervals with an average milk yield of around 8000 kg per lactation (305 d). 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 1.75 Mcal net energy of lactation (NE_L)/kg, 17% crude protein (CP) (dry matter (DM) basis) and changed according to National Research Council (NRC) recommendations (2001). Cows had free access to fresh water. Environmental data for mean maximum temperature (31.4 ºC and 20 ºC), minimum temperature (14.9 ºC and 4.9 ºC), and relative humidity (59.5% and 58.7%) during calving months (September to October, and November to December; respectively) were obtained from the Official National Station at Dulail area.

Experimental Design

A total of 193 lactating Holstein Friesian dairy cows received an i.m. injection of 25 mg of $PGF_{2α}$ (Lutalyse; Pharmacia & Upjohn S.A., Puurs, Belgium) at day 30 pp. Twenty cows were excluded from the study due to different reasons such as diseases and culling while 173 cows (49 primiparous and 124 multiparous) were used in the study.

All cows received an i.m. injection of 10µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH, Wiesbaden, Germany) 14 d after $PGF_{2\alpha}$, followed 7 d later by another injection of 25 mg $PGF_{2\alpha}$ and then assigned randomly into three treatments of TAI protocols without consideration for milk production as shown in Figure 1. Cows in the control treatment (Co-72; n = 65) received a 10µg GnRH and TAI 72 h after $PGF_{2\alpha}$. In the Modified Ovsynch treatment (MOVS; n = 42), cows were injected with a 10µg GnRH 56 h after $PGF_{2\alpha}$ and TAI 16 h later. Cows in the Ovsynch – Cosynch treatment (OVS-CO; n = 66) were injected with a 10µg GnRH 3 and 10 d after $PGF_{2\alpha}$, then received another injection of 25 mg $PGF_{2\alpha}$ after 7 d of the final GnRH before termination of the protocol by a dose of 10µg GnRH and TAI 72 h after $PGF_{2\alpha}$. Cows were subjected to an estrus detection program by visual observation and ALPRO™ system with an activity meter (Delaval International AB, Tumba, Sweden). An experienced AI technician performed insemination with semen obtained from commercial proven fertility (ABS Global, Inc., Deforest, Wisconsin, USA).

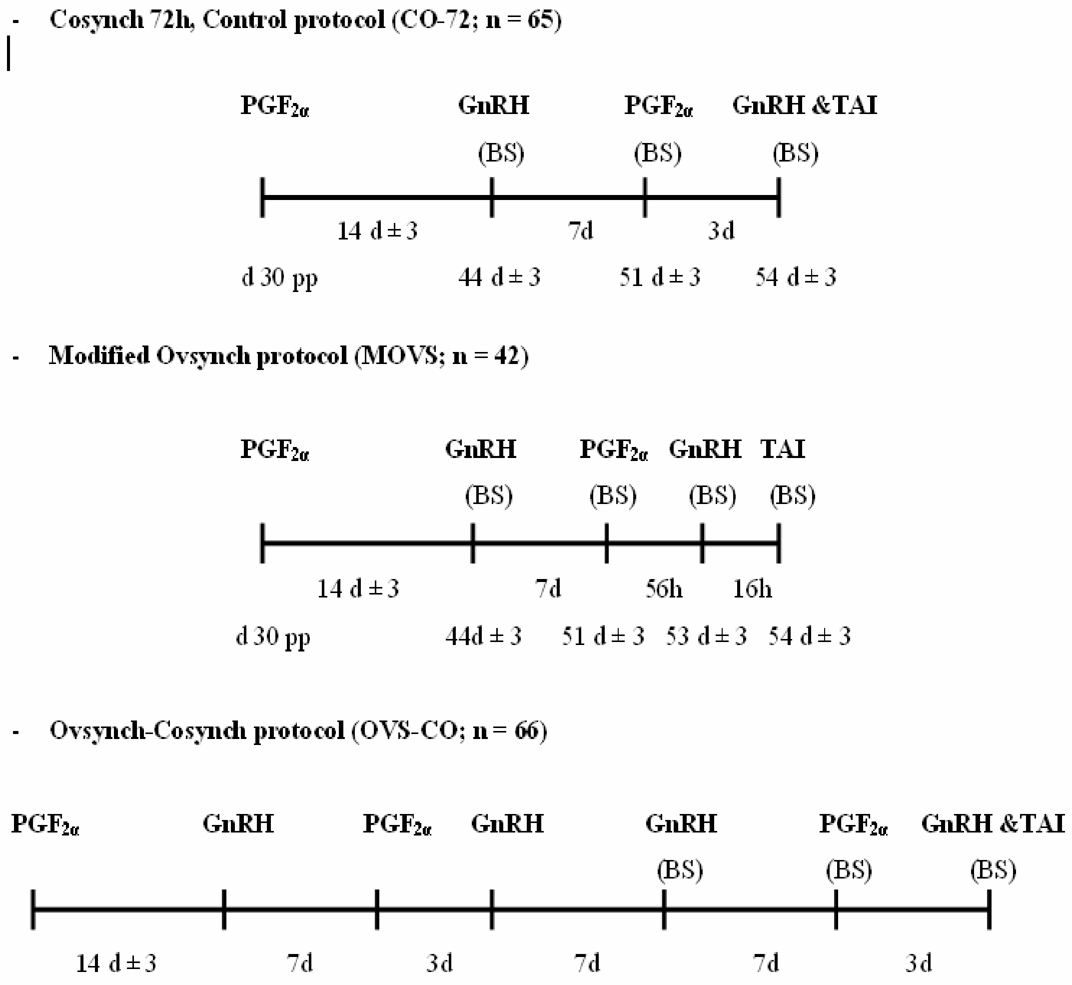

Figure1. Schematic diagram of the hormonal treatments, and blood samples (BS) of the lactating cows in the study. 3

At day 30 of either TAI or AI, cows were diagnosed for pregnancy by using an ultrasound (scanner 100 Vet; Pie Medical, Maastricht, The Netherlands) using a 7.5 - MHz probe. Pregnancy was determined by visualization of an embryonic vesicle with heartbeat as described by Pierson and Ginther (1984). Pregnancy status was confirmed by rectal palpation between days 45 and 50 after insemination. Pregnancy losses were calculated as the difference between cows pregnant at the first examination and cows non-pregnant at the second examination. Cows in the three treatments, which were diagnosed not pregnant on day 30 or between 45-50 d received a new injection of 25 mg $PGF_{2\alpha}$ and inseminated after estrus detection with GnRH within 30 minutes until the fourth insemination. Pregnancy rates were calculated for the two examinations and for the next three inseminations, while the overall pregnancy rates were defined as the percentage of cows that become pregnant after the fourth AI. Number of services per conception was calculated as the number of services for pregnant cows until the fourth insemination.

Blood Sampling and Radioimmunoassay (RIA)

Blood samples were collected via the coccygeal venipuncture from twenty cows of the CO-72 and MOVS treatments and twenty-two in the OVS-CO treatment into heparinized tubes just before hormonal treatment with first GnRH (third in the OVS-CO treatment), $PGF_{2\alpha}$ and at TAI (Figure 1). Plasma was harvested by centrifugation within 30 minutes at 3000 rpm for 15 minutes. Plasma samples were stored at - 20 ºC until assayed for P_4. Concentrations of plasma P_4 were determined by RIA (Immunotech a Beckman Coulter Company, Marseille, France) employing highly specific polyclonal antibodies in a RIA for the quantitative determination of P_4 in plasma. The standards used for plasma were 0, 0.11, 0.5, 1.75, 9.6, and 60 ng/ml. The inter- and intra-assay coefficients of variation (CV %) were 9.0% and 5.8%, respectively.

Statistical Analysis

Statistical analysis was performed using the SAS (2000) to test the effects of factors. The effect of treatment (CO-72, MOVS, and OVS-CO) on estrus detection rate (before, at, and after expected breeding period (EBP)) and pregnancy rates to first AI were tested by Chi-square test using the FREQ procedure of SAS. Then data of cows inseminated before EBP were removed from subsequent analysis.

Using General Linear Model (GLM) procedure of SAS, least square analysis of variance was applied on reproductive responses included effects of treatment (CO-72, MOVS, and OVS-CO), parity (primiparous and multiparous), calving months (September and October, November and December), and their two-way interactions. Responses included percentage of pregnancy rates at two diagnoses, the accumulated pregnancy rates up to the fourth AI, pregnancy losses between the two examinations, and number of services per conception. Least square means for significant effects were compared at $P < 0.05$ using *t*-test. In addition, the distribution of cows within P_4 categories at first GnRH (third in the OVS-CO treatment), $PGF_{2\alpha}$ injections, and at TAI ($P_4 \leq 1$ and $P_4 > 1$) and the effect of the average milk yield for the first 5 months on treatments were estimated. All these responses were estimated using the FREQ procedure of SAS.

RESULTS AND DISCUSSION

This study was designed to evaluate the efficiency of using three TAI protocols on reproductive performance of lactating dairy cows at a commercial dairy farm. Pregnancy rates and services per conception were significantly affected by treatment and calving month while parity did not. In addition, pregnancy losses were affected by treatment while calving month and parity had no effect. On the other hand, the average daily milk yield in the first five months after calving differed significantly ($P < 0.05$) among the three treatments while the distribution of cows between the two categories of P_4 concentrations at GnRH, $PGF_{2\alpha}$ injections, and at TAI did not differ among the treatments.

Treatment Effect on Estrus

Distribution of cows in the three treatments according to EBP is shown in Table 1. Around 16.9% (11/65) of cows in the CO-72 treatment, 11.9% (5/42) of cows in the MOVS treatment and 21.2% (14/66) of cows in the OVS-CO treatment exhibited estrus and were inseminated before the last hormonal injection, then they were excluded from the study analysis. According to Vasconcelos et al. (1999), those cows showed estrus and inseminated before completion of hormonal protocol failed to ovulate after the first GnRH injection because they were in the second half of the estrous cycle when they received the first GnRH injection. Therefore, the corpus luteum regressed early and the cows naturally came into estrus.

Table1. Distribution of cows in the three treatments according to the expected breeding period (EBP)

Estrus Detection Rate[2]	Treatment[1]		
	CO-72 (n = 65)	**MOVS (n = 42)**	**OVS-CO (n = 66)**
	(n) %	(n) %	(n) %
Before EBP [3]	(11) 16.9	(5) 11.9	(14) 21.2
At EBP [4]	(52) 80.0	(35) 83.3	(48) 72.7
After EBP [5]	(2) 3.1	(2) 4.8	(4) 6.1

[1]CO-72 (GnRH+$PGF_{2\alpha}$+GnRH & TAI 72 h after $PGF_{2\alpha}$); MOVS (GnRH+ $PGF_{2\alpha}$+GnRH+TAI 16 h after second GnRH); OVS-CO (GnRH+ $PGF_{2\alpha}$+GnRH+GnRH+ $PGF_{2\alpha}$+GnRH & TAI 72 h after $PGF_{2\alpha}$).

[2]By visual observation and ALPRO™ system.

[3]Cows exhibited estrus and were inseminated before the last GnRH injection in the three treatments.

[4]Cows were inseminated at the EBP in the three treatments.

[5]Cows exhibited estrus within 7 days after the end of the protocol and were re-inseminated in the three treatments.

The majority of cows were inseminated at EBP [CO-72, 80.0% (52); MOVS, 83.3% (35); and OVS-CO, 72.7% (48)]. Most previous studies reported around 10% (6% to 10.3%) or less of cows observed in estrus before the final GnRH injection of the TAI protocol (Mialot et al., 1999; Stevenson et al., 1999; Vasconcelos et al., 1999; Alnimer et al., 2009). However, DeJarnette et al. (2001) reported that 20% of cows showed estrus 48 h after $PGF_{2\alpha}$ injection of the TAI protocol. It seems likely that cows in both CO-72 and MOVS treatments in this study had acceptable percentages of estrus before TAI (16.9% and 11.9%) and synchronization rate (80% and 83.3%) to the second GnRH injection. Several studies reported around 85% synchronization rate in different TAI protocols (Mialot et al., 1999; Vasconcelos et al., 1999; Cartmill et al., 2001a; El-Zarkouny et al., 2004; Alnimer et al., 2009). In this study, the protocols were initiated on day 12 of the estrous cycle (around midcycle), which was the optimal period to start TAI protocols that aimed to enhance synchronization rate and consequently pregnancy rates (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004). In contrast, the high numerical percentages of estrus before TAI (21.2%) and the lower synchronization rate (72.7%) for cows in the OVS-CO treatment might be due to using extra hormonal injections. This permitted more cows to exhibit estrus before the last GnRH. Recently; Souza et al. (2008) excluded 28% of cows in the Double Ovsynch treatment because these cows failed to complete the protocol or were culled before the end of the study. On the other hand, a low percentage of cows showed estrus within a week after TAI and were re-inseminated [3.1 (2), 4.8 (2), and 6.1% (4) for CO-72, MOVS, and OVS-CO; respectively]. These results agree with previous studies reported 4% to 8.7% cows returning to estrus and were re-inseminated after the pre-determined TAI (Mialot et al., 1999; Alnimer, 2005; Alnimer et al., 2009).

Treatment Effects on Pregnancy Rates, Pregnancy Losses, and Services per Conception

Pregnancy rates and pregnancy losses for cows completed the hormonal protocols based on treatment are illustrated in Table 2. Pregnancy rates on day 30 were greater ($P < 0.05$) for cows in both CO-72 (68.5%) and MOVS (64.9%) treatments than those for cows in the OVS-CO (59.6%) treatment. Brusveen et al. (2008) found greater pregnancy rates at day 30 for cows in the MOVS treatment than those in the CO-72 treatment while Alnimer et al. (2009) reported opposite result. Pregnancy rates to first AI tended to be greater ($P < 0.1$) for cows in both MOVS (40.5%) and OVS-CO (40.4%) treatments than those for cows in the CO-72 (33.3%) treatment. In addition, pregnancy rates to second and third AI were greater ($P < 0.05$) for cows in the MOVS (83.8% and 89.2%) treatment than those for cows in both CO-72 (55.6% and 75.9%) and OVS-CO (63.5% and 73.1%) treatments. Collectively, overall pregnancy rates for cows in the MOVS (94.6%) treatment tended to be greater ($P < 0.1$) than those for cows in both CO-72 (77.8%) and OVS-CO (78.9%) treatments. In a previous study, Brusveen et al. (2008) reported a greater pregnancy rates for cows in the MOVS treatment over cows in the CO-72 treatment. In the current study, the difference between the two protocols (MOVS and CO-72) was only at the time of the second GnRH injection, which was earlier in the MOVS than in the CO-72. According to Pursley et al. (1995), ovulation occurs between 24 and 32 h after the second GnRH injection of the Ovsynch protocol (GnRH- $PGF_{2\alpha}$-GnRH-TAI 16 h after the second GnRH injection). Therefore, the time from AI to ovulation for cows in the MOVS treatment seemed shorter (between 8 and 16 h) than those for cows in the CO-72 treatment (between 24 and 32 h). In addition to that, around 6 h are needed for sperm capacitation to acquire fertilizing ability (Ball and Peters, 2004), which means that insemination should occurs at least 6 h before ovulation with maximum 18 h to obtain greater conception rates (Ball and Peters, 2004). Moreover, Pursley et al. (1998) reported greater pregnancy rates when insemination occurred between 8 and 16 h before ovulation with the greatest pregnancy rates at 16 h after the second GnRH injection. In contrast, Portaluppi and Stevenson (2005) recorded greater pregnancy rates to first AI for cows in the CO-72 treatment than for cows in the Ovsynch treatment. Portaluppi and Stevenson (2005) explained that cows in the CO-72 treatment were inseminated after the spontaneous LH surge and ovulation while those of the Ovsynch were inseminated at the same time expected for ovulation (72 h). Recently, Alnimer et al. (2009) reported similar pregnancy rates to first AI between cows in the CO-72 and Ovsynch treatments while overall pregnancy rates were greater for cows in the CO-72 treatment. On the other hand, similar pregnancy rates to first AI for cows in both MOVS and OVS-CO treatments disagreed with the findings of Souza et al. (2008) who reported greater pregnancy rates to first AI for cows treated with Double Ovsynch than those for cows treated with Ovsynch alone. Souza et al. (2008) found that improvement in pregnancy rates following the Double Ovsynch treatment was due to the primiparous cows. In the current study, the low number of primiparous cows might be responsible for the similarity in pregnancy rates to first AI between cows in the MOVS and OVS-CO treatments.

Table2. Pregnancy rates and pregnancy losses for cows completed the hormonal protocols based on treatment.

Parameter	**Treatment[1]**		
	CO-72 (n = 54)	**MOVS (n = 37)**	**OVS-CO (n = 52)**
Pregnancy Rates	(n) %	(n) %	(n) %
Day 30	(37) 68.5[a]	(24) 64.9[a]	(31) 59.6[b]
First AI[2]	(18) 33.3[d]	(15) 40.5[c]	(21) 40.4[c]
First and Second	(30) 55.6[b]	(31) 83.8[a]	(33) 63.5[b]
First, Second, and Third	(41) 75.9[b]	(33) 89.2[a]	(38) 73.1[b]
Overall[3]	(42) 77.8[d]	(35) 94.6[c]	(41) 78.9[d]
Pregnancy Losses[4]	(19) 51.4[b]	(9) 37.5[ab]	(10) 32.3[a]

[1]CO-72 (GnRH+$PGF_{2\alpha}$+GnRH & TAI 72 h after $PGF_{2\alpha}$); MOVS (GnRH+ $PGF_{2\alpha}$+GnRH+TAI 16 h after second GnRH); OVS-CO (GnRH+ $PGF_{2\alpha}$+GnRH+GnRH+ $PGF_{2\alpha}$+GnRH & TAI 72 h after $PGF_{2\alpha}$).

[2]Cows that were pregnant 45 to 50 d after AI.

[3]Up to fourth AI.

[4]Percentage of cows diagnosed pregnant at day 30 after AI that were diagnosed non-pregnant between day 45 and 50 after AI.

[a, b]Percentages among treatments with different superscripts differ ($P < 0.05$).

[c, d]Percentages among treatments with different superscripts tend to differ ($P < 0.1$).

Pregnancy losses were lower ($P < 0.05$) for cows in the OVS-CO (32.3%) treatment than those for cows in the CO-72 (51.4%) treatment while similar for cows in the MOVS (37.5%) treatment. Similar results were also found in lactating dairy cows treated with either CO-72 or Ovsynch treatments (Alnimer et al., 2009). It seems that anovular cows were responsible for increasing pregnancy losses of cows in the CO-72 treatment. Previous studies reported greater pregnancy losses in anovular cows than in cyclic cows (Cartmill et al., 2001a

& b; Gümen et al., 2003; Cerri et al., 2004). Reducing the percentage of anovulatory cows prior to first AI was expected to minimize pregnancy losses in dairy cows because anovulation poses a risk to establishment and maintenance of pregnancy (Santos et al., 2004). According to Souza et al. (2008), Double Ovsynch protocol might be effective in treating anovular cows through inducing cyclicity in those cows. In the present study, using the first Ovsynch protocol after one $PGF_{2\alpha}$ injection at day 30 pp without AI might synchronize tightly the stage of the estrous cycle at initiation of the Cosynch protocol with TAI for cows in the OVS-CO treatment. Moreover, the prolonged interval to first AI, which mean occurrence of more estruses before TAI (Friggens and Labouriau, 2010), may contribute in improving pregnancy rates for pp lactating dairy cows in the OVS-CO treatment.

In the present study, it was proposed that anovular cows were treated at the first AI in the OVS-CO treatment and at later services in both CO-72 and MOVS treatments. This may have contributed to the similar overall pregnancy rates for cows in the CO-72 and OVS-CO treatments while the MOVS treatment continued in producing greater accumulated pregnancy rates because of its superiority since the first AI.

Number of services per conception were lower ($P < 0.05$) for cows in the MOVS (1.5 ± 0.2) treatment than those for cows in the CO-72 (2.1 ± 0.17) treatment while it was similar for cows in the OVS-CO (1.9 ± 0.26) treatment. Because pregnancy rates to first AI were lower and pregnancy losses were higher for cows in the CO-72 treatment than those for cows in both MOVS and OVS-CO treatments; additional services were needed for cows in the CO-72 treatment to become pregnant.

Parity Effects on Pregnancy Rates and Pregnancy Losses

Pregnancy rates in all parameters and pregnancy losses did not differ between primiparous and multiparous cows. In addition, treatment x parity interaction did not affect pregnancy rates and pregnancy losses. Jobst et al. (2000), Navanukraw et al. (2004), and Alnimer (2005) reported similar pregnancy rates between primiparous and multiparous cows. In contrast, Tenhagen et al. (2004), Chebel et al. (2004), Brusveen et al. (2008), Souza et al. (2008), Alnimer et al. (2009), and Santos et al. (2009) found greater pregnancy rates and lower pregnancy losses for primiparous cows than multiparous cows. It is well documented that pregnancy rates are decreased with increased lactation number (Peters and Pursley, 2002; Grimard et al., 2006). In this study, primiparous cows had numerically greater pregnancy rates to first AI than multiparous cows (40.5% vs. 36.8%) but the small number of primiparous cows might be responsible for producing similar pregnancy rates between the two parities.

Calving Months Effects on Pregnancy Rates, Pregnancy Losses, and Services per Conception

The effect of calving months on pregnancy rates, pregnancy losses, and services per conception are presented in Table 3. Interaction of treatment x calving month was not significant in any of reproductive performance measurements. Cows delivered through November and December had greater ($P < 0.05$) pregnancy rates at day 30 (76.1%), to first (47.9%), and second AI (69.0%) than those cows delivered through September and October (52.8%, 27.8%, and 62.5%; respectively). On the other hand, calving months had no effect on pregnancy rates to third AI or overall pregnancy rates. Similar observations in calving months were found in pregnancy losses although it was numerically higher for cows delivered during September and October than that delivered during November and December. It seems that the elevated ambient temperature above the upper critical temperature (around

26 °C) was responsible for impaired pregnancy rates and pregnancy losses for cows calved during September and October. Alnimer et al. (2002; 2009) reported lower pregnancy rates and greater pregnancy losses for cows inseminated in summer than those for cows inseminated in winter. Negative effects of heat stress on reproductive efficiency of dairy cows are manifested through several routes; an alteration in follicular development and oocyte competence, steroidogenic capacity, P_4 production from CL, gonadotrophins secretion, and embryo development and quality (Badinga et al., 1994; Wolfenson et al., 1995; 2000; Roth et al., 2001a & b; Al-Katanani et al., 2002; De Rensis and Scaramuzzi, 2003).

Previous studies reported that heat stress had a delayed negative effect on pregnancy rates up to 50 d before AI (Roth et al., 2001a & b; Chebel et al., 2004). In addition, heat stress coinciding with high milk production may be the reasons of greater pregnancy losses for cows in the CO-72 treatment (51.4%). Cartmill et al. (2001b) reported greater pregnancy losses (42.7%) for high producing cows susceptible to high ambient temperature. Moreover, number of services per conception were lower ($P < 0.05$) for cows delivered through November and December (1.60 ± 0.15) than those for cows delivered through September and October (2.10 ± 0.19). Alnimer et al. (2002) found no differences in services per conception between cows inseminated in winter and summer months.

Table3. Pregnancy rates, pregnancy losses, and services per conception for cows completed the hormonal protocols based on calving months.

Parameter	Calving months	
	September and October (31.4 °C)[1] (n = 72)	**November and December (20.0 °C)[1] (n = 71)**
Pregnancy rates	(n) %	(n) %
Day 30	(38) 52.8^b	(54) 76.1^a
First AI[2]	(20) 27.8^b	(34) 47.9^a
First and Second	(45) 62.5^b	(49) 69.0^a
First, Second, and Third	(56) 77.8	(56) 78.9
Overall[3]	(61) 84.7	(57) 80.3
Pregnancy Losses[4]	(18) 47.4	(20) 37.0
Services per conception[5]	2.10 ± 0.19^b	1.60 ± 0.15^a

[1]Maximum temperature.

[2]Cows that were pregnant 45 to 50 d after AI.

[3]Up to fourth AI.

[4]Percentage of cows diagnosed pregnant at day 30 after AI that were diagnosed non-pregnant between day 45 and 50 after AI.

[5]For pregnant cows.

[a, b] Percentages between calving months with different superscripts differ ($P < 0.05$).

Milk Production Effects on Pregnancy Rates and Pregnancy Losses

A correlation between milk yield and pregnancy rates was not found, but cows in the CO-72 treatment had higher ($P < 0.05$) average daily milk yield (30.7 kg) during the first 150 days pp than for cows in both MOVS and OVS-CO (26.9 and 26.8 kg) treatments. This may explain the reduced pregnancy rates for cows in the CO-72 treatment. Previous studies reported a negative effect of high milk production on pregnancy rates of lactating dairy cows (Cerri et al., 2004; Grimard et al., 2006; Gabor et al., 2008). Cows are in negative energy balance (NEB) after calving and milk production gets preference over the other physiological processes including reproduction, so that the anovulatory anestrus period may be extended (Stevenson et al., 1997; Mwaanga and Janowski, 2000; Grimard et al., 2006). This means that more high-producing cows in the CO-72 treatment were anovular and contributed to the low pregnancy rates. On the other hand, cows in the OVS-CO treatment were inseminated late in the pp period (around day 70) compared to cows in the other two treatments (around day 56). Grimard et al. (2006) proposed that any additional delay to the first AI after calving especially for high producing cows would enhance the reproductive efficiency. The previous mentioned factors may contributed to the higher pregnancy rates to first AI and the lower pregnancy losses for cows in the OVS-CO treatment than those for cows in the CO-72 treatment.

Progesterone Concentrations

The proportion of cows with high (> 1 ng/ml) P_4 concentrations at GnRH injection were 75, 90, and 95.5% for cows in the CO-72, MOVS, and OVS-CO treatments, respectively. Similar trend was found at $PGF_{2\alpha}$ injection in the three treatments (85, 90, and 90.9% for cows in the CO-72, MOVS, and OVS-CO treatments, respectively). In addition, the proportion of cows with low (≤ 1.0 ng/ml) P_4 concentrations at the TAI were 95, 90, and 86.4% for cows in the CO-72, MOVS, and OVS-CO treatments, respectively. However, the differences among treatments at each P_4 class were not significant. Increased proportion of cows with high concentrations of P_4 at GnRH and $PGF_{2\alpha}$ injections before TAI produced more synchronization rates and consequently pregnancy rates to first AI (Moreira et al., 2001; Lubbadeh and Alnimer, 2003; El-Zarkouny et al., 2004). In the present study, anovular cows might be responsible for increasing proportion of cows had low concentrations of P_4 at GnRH injection in the CO-72 treatment. Moreira et al. (2000; 2001) suggested that among cows had low and high P_4 concentrations at GnRH and $PGF_{2\alpha}$ injections, respectively, there were anestrus cows that responded to the initial GnRH injection but it produced lower pregnancy rates than those had high concentrations of P_4 at both GnRH and $PGF_{2\alpha}$ injections due to increased probability of short estrous cycles after insemination. Recently, Souza et al. (2008) reported a significant decrease in the proportion of cows that had low concentrations of P_4 at GnRH injection from 33.3 % for Ovsynch cows to 9.4% for Double Ovsynch cows while it did not differ at $PGF_{2\alpha}$ injection. In the present study, it seems likely that using Ovsynch protocol before Cosynch induced cyclicity in anovular cows and decreased the proportion of cows that had low concentrations of P_4 at both GnRH and $PGF_{2\alpha}$ injections. On the other hand, the low proportion of cows that had low concentrations of P_4 at both GnRH and $PGF_{2\alpha}$ injections in the MOVS treatment might be an indication of the presence of few anovular cows in this treatment.

CONCLUSION

Pregnancy rates to first AI tended to be greater for cows in both MOVS and OVS-CO treatments than for cows in the CO-72 treatment while overall pregnancy rates for cows in the MOVS treatment tended to be greater than for cows in both CO-72 and OVS-CO treatments. Moreover, pregnancy losses were lower for cows in the OVS-CO treatment than for cows in the CO-72 treatment. On the other hand, cows calved during November and December had greater pregnancy rates to first AI than for cows calved during September and October while pregnancy losses were numerically greater for cows delivered during September and October than that delivered during November and December. In contrast, primiparous and multiparous cows had similar pregnancy rates and pregnancy losses. Further study with more number of animals and with consideration for milk production is needed to investigate the responsiveness of anovular cows to the OVS-CO protocol without $PGF_{2\alpha}$ injection.

ACKNOLEDGMENTS

The Deanship of Scientific Research at the University of Jordan is gratefully acknowledged for funding this project. The authors express their appreciation to Hammoudeh farm for providing animals and facilities, to Mohammad Abu Eid and all workers in the farm for their assistance.

REFERENCES

Al-Katanani, Y. M. Paula-Lopes, F. F. and Hansen, P. J. 2002. Effect of Season and Exposure to Heat Stress on Oocyte Competence in Holstein Cows. ***Journal of Dairy Science***, 85, 390-396.

Alnimer, M. A. 2005. Effect of Two Estrous Synchronization Programs on Reproductive Performance of Dairy Cows Under Summer Conditions in Jordan. ***Dirasat, Agricultural Sciences***, 32:248-257.

Alnimer, M. A., M. J. Tabbaa, M. M. Ababneh and W. F. Lubbadeh. 2009. Applying Variations of the Ovsynch Protocol at the Middle of the Estrus Cycle on Reproductive Performance of Lactating Dairy Cows During Summer and Winter. ***Theriogenology***, 72:731-740.

Alnimer, M., G. De Rosa, F. Grasso, F. Napolitano and A. Bordi. 2002. Effect of Climate on the Response to Three Oestrous Synchronization Techniques in Lactating Dairy Cows. ***Animal Reproduction Science***, 71:157-168.

Badinga, L. Thatcher, W. W. Wilcox, C. J. Morris, G. Entwistle, K. and Wolfenson, D. 1994. Effect of Season on Follicular Dynamics and Plasma Concentrations of Estradiol-$_{17\beta}$, Progesterone and Luteinizing Hormone in Lactating Holstein Cows. ***Theriogenology***, 42, 1263-1274.

Bagnato, A. and P. A. Oltenacu. 1994. Phenotypic Evaluation of Fertility Traits and Their Association with Milk Production of Italian Friesian Cattle. ***Journal of Dairy Science***, 77:874-882.

Ball, P. J. H. and A. R. Peters. 2004. ***Reproduction in Cattle***, (3rd ed.), Oxford: Blackwell Publishing.

Brusveen, D. J., A. P. Cunha, C. D. Silva, P. M. Cunha, R. A. Sterry, E. P. B. Silva, J. N. Guenther and M. C. Wiltbank. 2008. Altering the Time of the Second Gonadotropin-Releasing Hormone Injection and Artificial Insemination (AI) During Ovsynch Affects Pregnancies per AI in Lactating Dairy Cows. ***Journal of Dairy Science***, 91:1044-1052.

Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, G. C. Lamb and J. S. Stevenson. 2001a. Stage of Cycle, Incidence, and Timing of Ovulation, and Pregnancy Rates in Dairy Cattle After Three Timed Breeding Protocols. ***Journal of Dairy Science***, 84:1051-1059.

Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, T. G. Rozell, J. F. Smith and J. S. Stevenson. 2001b. An

Alternative AI Breeding Protocol for Dairy Cows Exposed to Elevated Ambient Temperatures Before or After Calving or Both. ***Journal of Dairy Science***, 84:799-806.

Cerri, R. L. A., J. E. P. Santos, S. O. Jushem, K. N. Galvao and R. C. Chebel. 2004. Timed Artificial Insemination with Estradiol Cypionate or Iinsemination at Estrus in High-Producing Dairy Cows. ***Journal of Dairy Science***, 87:3704-3715.

Chebel, R. C., J. E. P. Santos, J. P. Reynolds, R. L. A. Cerri, S. O. Jushem and M. Overton. 2004. Factors Affecting Conception Rate after Artificial Insemination and Pregnancy Loss in Lactating Dairy Cows. ***Animal Reproduction Science***, 84:239-255.

DeJarnette, J. M., R. R. Salverson and C. E. Marshall. 2001. Incidence of Premature Estrus in Lactating Dairy Cows and Conception Rates to Standing Estrus or Fixed-Time Inseminations after Synchronization Using GnRH and $PGF_{2\alpha}$. ***Animal Reproduction Science***, 67:27-35.

De Rensis, F. and Scaramuzzi, R. J. .2003). Heat Stress and Seasonal Effects on Reproduction in the Dairy Cow-A Review. ***Theriogenology***, 60, 1139-1151.

El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley and J. S. Stevenson. 2004. Pregnancy in Dairy Cows after Synchronized Ovulation Regimens with or without Presynchronization and Progesterone. ***Journal of Dairy Science***, 87:1024-1037.

Friggens, N. C. and R. Labauriau. 2010. Probability of Pregnancy as Affected by Oestrus Number and Days to First Oestrus in Dairy Cows of Three Breeds and Parities. ***Animal Reproduction Science***, 118:155-162.

Gabor, G., F. Toth, L. Ozsvari, Zs. Abonyi-Toth and R. G. Sasser. 2008. Factors Influencing Pregnancy Rate and Late Embryonic Loss in Dairy Cattle. ***Reproduction of Domestic Animals***, 43:53-58.

Geary, T. W. and J. C. Whittier. 1998. Effects of a Timed Insemination following Synchronization of Ovulation using the Ovsynch or Cosynch Protocol in Beef Cows. ***The Professional Animal Scientist***, 14:217-220.

Grimard, B., S. Freret, A. Chevallier, A. Pinto, C. Ponsart and P. Humblot. 2006. Genetic and Environmental Factors Influencing First Service Conception Rate and Late Embryonic/Foetal Mortality in Low Fertility Dairy Herds. ***Animal Reproduction Science***, 91:31-44.

Gümen, A., J. N. Guenther and M. C. Wiltbank. 2003. Follicular Size and Response to Ovsynch Versus Detection of Estrus in Anovular and Ovular Lactating Dairy Cows. ***Journal of Dairy Science***, 86:3184-3194.

Jobst, S. M., R. L. Nebel, M. L. McGilliard and K. D. Pelzer. 2000. Evaluation of Reproductive Performance in Lactating Dairy Cows with Prostaglandin $F_{2\alpha}$, Gonadotropin-Releasing Hormone, and Timed Artificial Insemination. ***Journal of Dairy Science***, 83:2366-2372.

Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke and M. C. Wiltbank. 2005. Relationship Between Level of Milk Production and Multiple Ovulations in Lactating Dairy Cows. ***Journal of Dairy Science***, 88:2783-2793.

Lubbadeh, W. F. and M. A. Alnimer. 2003. Different Postpartum Hormonal Treatments for Improving Fertility in Dairy Cows. ***Dirasat, Agricultural Sciences***, 30:304-310.

Mialot, J. P., G. Laumonnier, C. Ponsart, H. Fauxpoint, E. Barassin, A. A. Ponter and F. Deletang. 1999. Postpartum Subestrus in Dairy Cows: Comparison of Treatment with Prostaglandin $F_{2\alpha}$ or GnRH + Prostaglandin $F_{2\alpha}$ + GnRH. ***Theriogenology***, 52:901-911.

Moreira, F., R. L. De La Sota, T. Diaz and W. W. Thatcher. 2000. Effect of Day of the Estrous Cycle at the Initiation of A Timed Artificial Insemination Protocol on Reproductive Responses in Dairy Heifers. ***Journal of Animal Science***, 78:1568-1576.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes and W. W. Thatcher. 2001. Effects of Presynchronization and Bovine Somatotropin on Pregnancy Rates to A Timed Artificial Insemination Protocol in Lactating Dairy Cows. ***Journal of Dairy Science***, 84:1646-1659.

Mwaanga, E. S. and T. Janowski. 2000. Anoestrus in Dairy Cows: Causes, Prevalence and Clinical Forms. ***Reproduction of Domestic Animals***, 35:193-200.

Navanukraw, C., D. A. Redmer, L. P. Reynolds, J. D.

Kirsch, A. T. Grazul-Bilska and P. M. Fricke. 2004. A Modified Presynchronization Protocol Improves Fertility to Timed Artificial Insemination in Lactating Dairy Cows. ***Journal of Dairy Science***, 87:1551-1557.

National Research Council. 2001. ***Nutrient Requirements of Dairy Cattle***, (7th ed.), Washington, D. C.: National Academy Press.

Peters, M. W. and J. R. Pursley. 2002. Fertility of Lactating Dairy Cows Treated with Ovsynch After Presynchronization Injections of $PGF_{2\alpha}$ and GnRH. ***Journal of Dairy Science***, 85:2403-2406.

Pierson, R. A. and O. J. Ginther. 1984. Ultrasonography for Detection of Pregnancy and Study of Embryonic Development in Heifers. ***Theriogenology***, 22:225-233.

Portaluppi, M. A. and J. S. Stevenson. 2005. Pregnancy Rates in Lactating Dairy Cows After Presynchronization of Estrous Cycles and Variations of the Ovsynch Protocol. ***Journal of Dairy Science***, 88:914-921.

Pursley, J. R., M. R. Kosorok and M. C. Wiltbank. 1997a. Reproductive Management of Lactating Dairy Cows Using Synchronization of Ovulation. ***Journal of Dairy Science***, 80:301-306.

Pursley, J. R., M. O. Mee and M. C. Wiltbank. 1995. Synchronization of Ovulation in Dairy Cows Using $PGF_{2\alpha}$ and GnRH. ***Theriogenology***, 44:915-923.

Pursley, J. R., R. W. Silcox and M. C. Wiltbank. 1998. Effect of Time of Artificial Insemination on Pregnancy Rates, Calving Rates, Pregnancy Loss, and Gender Ratio After Synchronization of Ovulation in Lactating Dairy Cows. ***Journal of Dairy Science***, 81:2139-2144.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick and L. L. Anderson. 1997b. Pregnancy Rates per Artificial Insemination for Cows and Heifers Inseminated at A Synchronized Ovulation or Synchronized Estrus. ***Journal of Dairy Science***, 80:295-300.

Rabiee, A. R., I. J. Lean and M. A. Stevenson. 2005. Efficacy of Ovsynch Program on Reproductive Performance in Dairy Cattle: A Meta-Analysis. ***Journal of Dairy Science***, 88:2754-2770.

Roth, Z., A. Arav, A. Bor, Y. Zeron, R. Braw-Tal and D. Wolfenson. 2001a. Improvement of Quality of Oocytes Collected in the Autumn by Enhanced Removal of Impaired Follicles from Previously Heat-Stressed Cows. ***Reproduction***, 122:737-744.

Roth, Z., R. Meidan, A. Shaham-Albalancy, R. Braw-Tal and D. Wolfenson. 2001b. Delayed Effect of Heat Stress on Steroid Production in Medium-Sized and Preovulatory Bovine Follicles. ***Reproduction***, 121:745-751.

Santos, J. E. P., H. M. Rutigliano and M. F. SaFilho. 2009. Risk Factors for Resumption of Postpartum Estrous Cycles and Embryonic Survival in Lactating Dairy Cows. ***Animal Reproduction Science***, 110:207-221.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri and K. N. Galvao. 2004. The Effect of Embryonic Death Rates in Cattle on the Efficacy of Estrus Synchronization Programs. ***Animal Reproduction Science***, 82-83:513-535.

SAS. 2000. ***SAS Statistically User's Guide***. Version 8. SAS ins., Cary, NC, USA.

Souza, A. H., H. Ayres, R. M. Ferreira and M. C. Wiltbank. 2008. A New Presynchronization System (Double-Ovsynch) Increases Fertility at First Postpartum Timed AI in Lactating Dairy Cows. ***Theriogenology***, 70:208-215.

Stevenson, J. S., Y. Kobayashi and K. E. Thompson. 1999. Reproductive Performance of Dairy Cows in Various Programmed Breeding Systems Including Ovsynch and Combinations of Gonadotropin-Releasing Hormone and Prostaglandin $F_{2\alpha}$. ***Journal of Dairy Science***, 82:506-515.

Stevenson, J. S., G. C. Lamb, D. P. Hoffmann and J. E. Minton. 1997. Review: Interrelationships of Lactation and Postpartum Anovulation in Suckled and Milked Cows. ***Livestock Production Science***, 50:57-74.

Tenhagen, B. A., R. Surholt, M. Wittke, C. Vogel, M. Drillich and W. Heuwieser. 2004. Use of Ovsynch in Dairy Herds - Differences Between Primiparous and Multiparous Cows. ***Animal Reproduction Science***, 81:1-11.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley and M. C. Wiltbank. 1999. Synchronization Rate, Size of the Ovulatory Follicle, and Pregnancy Rate After Synchronization of Ovulation Beginning on Different days of the Estrous Cycle in Lactating Dairy Cows. ***Theriogenology***, 52:1067-1078.

Wolfenson, D. Roth, Z. and Meidan, R. 2000. Impaired Reproduction in Heat-Stressed Cattle: Basic and Applied Aspects. ***Animal Reproduction Science***, 60-61, 535-547.

Wolfenson, D. Thatcher, W. W. Badinga, L. Savio, J. D. Meidan, R. Lew, B. J. Braw-Tal, R. and Berman, A. 1995. Effect of Heat Stress on Follicular Development During the Estrous Cycle in Lactating Dairy Cattle. ***Biology of Reproduction***, 52, 1106-1113.

تأثير معاملات هرمونية مختلفة لتوحيد الشياع في خصوبة أبقار الحليب

*أحمد إبراهيم شمعون، ومفيد عوني النمر**✉

ملخـص

أجري هذا البحث لدراسة فعالية تطبيق ثلاثة برامج للتلقيح الإجباري (TAI) على الأداء التناسلي لأبقار الفريزيان الحلابة. حقنت جميع أبقار الدراسة و عددها 173 بقرة بهرموني المحفز للنخامية (GnRH) و البروستاجلاندين ($PGF_{2\alpha}$) في يومي 44 و51 بعد الولادة ومن ثم قسمت إلى ثلاث معاملات: (1) CO-72 (65 بقرة): حقنت بالهرمون المحفز للنخامية (GnRH) و لقحت إجباريا في اليوم 54 بعد الولادة؛ (2) MOVS (42 بقرة): حقنت بالهرمون المحفز للنخامية (GnRH) بعد 56 ساعة من حقن هرمون البروستاجلاندين ($PGF_{2\alpha}$) و من ثم لقحت إجباريا بعد 16 ساعة؛ (3) OVS-CO (66 بقرة): حقنت بالهرمون المحفز للنخامية (GnRH) في يومي 54 و 61 بعد الولادة ومن ثم بهرمون البروستاجلاندين ($PGF_{2\alpha}$)في اليوم 68 و بحقنة أخرى من الهرمون المحفز للنخامية (GnRH) ومن ثم التلقيح الإجباري في اليوم 71 بعد الولادة. أظهرت النتائج ميلا في نسب الحمل من التلقيحة الأولى ($P < 0.1$) لأبقار معاملتي MOVS و OVS-CO عنها لأبقار معاملة CO - 72 بينما مالت نسب الحمل التراكمية لأن تكون أفضـل ($P < 0.1$) لأبقار معاملة MOVS عنها لأبقار معاملتي CO-72 و OVS-CO. كانت نسب فقد الأجنة أقل معنويا ($P < 0.05$) لأبقار معاملة OVS-CO منها لأبقار معاملة CO-72 بينما كان عدد التلقيحات اللازمة لحدوث الحمل أقل ($P < 0.05$) لأبقار معاملة MOVS منها لأبقار معاملة CO-72. من ناحية أخرى، كانت نسب الحمل من التلقيحة الأولى أعلى معنويا ($P < 0.05$) وعدد التلقيحات اللازمة لحدوث الحمل أقل معـنـويا ($P < 0.05$) للأبقار التي ولدت خلال شهري تشرين الثاني وكانون الأول عنها للأبقار التي ولدت خلال شهري أيلول وتشرين الأول. في المقابل، كانت الأبقار الوالدة لمرة واحدة والأبقار متعددة الولادات متشابهة في جميع مقاييس الأداء التناسلي. دلت نتائج التجربة على أن هناك تحسنا في الأداء التناسلي لأبقار معاملة MOVS والأبقار التي ولدت خلال شهري تشرين الثاني وكانون الأول مقارنة مع تلك التي في معامـلتي CO-72 و OVS-CO والتي ولدت خلال شهري أيلول وتشرين الأول، على التوالي.

الكلمات الدالة: الأبقار الحلابة، توحيد الشياع، الخصوبة، التلقيح الاجباري.

*✉ قسم الانتاج الحيواني، الجامعة الأردنية. البريد الالكتروني amufeed@ju.edu.jo

تاريخ استلام البحث 2010/5/11 وتاريخ قبوله 2011/5/10.