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
synchronization of ovulation. It involves injection of gonadotrophin releasing hormone (GnRH) 7 days (d) before and 48 hours (h) after prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\)) 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\(_2\alpha\)) had more pregnancies than that treated with Cosynch 48 (GnRH and TAI 48 h after PGF\(_2\alpha\)) 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\(_2\alpha\)) than that treated with Cosynch 48. Souza et al. (2008) developed a new program involves using Double Ovsynch (GnRH, PGF\(_2\alpha\) 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\(_{\text{L}}\))/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 °C and 20 °C), minimum temperature (14.9 °C and 4.9 °C), 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\(_2\alpha\) (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 (MOV; 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).

- **Cosynch 72h Control protocol (CO-72; n = 65)**

<table>
<thead>
<tr>
<th>PGF$_{2\alpha}$</th>
<th>GnRH</th>
<th>PGF$_{2\alpha}$</th>
<th>GnRH &amp; TAI</th>
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<tbody>
<tr>
<td>(BS)</td>
<td></td>
<td>(BS)</td>
<td></td>
</tr>
<tr>
<td>14 d ± 3</td>
<td>7d</td>
<td>3d</td>
<td></td>
</tr>
<tr>
<td>d 30 pp</td>
<td>44 d ± 3</td>
<td>51 d ± 3</td>
<td>54 d ± 3</td>
</tr>
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</table>

- **Modified Ovsynch protocol (MOV; n = 42)**

<table>
<thead>
<tr>
<th>PGF$_{2\alpha}$</th>
<th>GnRH</th>
<th>PGF$_{2\alpha}$</th>
<th>GnRH</th>
<th>TAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(BS)</td>
<td></td>
<td>(BS)</td>
<td>(BS)</td>
<td>(BS)</td>
</tr>
<tr>
<td>14 d ± 3</td>
<td>7d</td>
<td>56h</td>
<td>16h</td>
<td></td>
</tr>
<tr>
<td>d 30 pp</td>
<td>44 d ± 3</td>
<td>51 d ± 3</td>
<td>53 d ± 3</td>
<td>54 d ± 3</td>
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- **Ovsynch–Cosynch protocol (OVS-CO; n = 66)**

<table>
<thead>
<tr>
<th>PGF$_{2\alpha}$</th>
<th>GnRH</th>
<th>PGF$_{2\alpha}$</th>
<th>GnRH</th>
<th>PGF$_{2\alpha}$</th>
<th>GnRH &amp; TAI</th>
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<tr>
<td>(BS)</td>
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<td>(BS)</td>
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<tr>
<td>14 d ± 3</td>
<td>7d</td>
<td>3d</td>
<td>7d</td>
<td>7d</td>
<td>3d</td>
</tr>
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</table>

Figure 1. Schematic diagram of the hormonal treatments, and blood samples (BS) of the lactating cows in the study.
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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estrus Detection Rate</th>
<th>CO-72 (n = 65)</th>
<th>MOVS (n = 42)</th>
<th>OVS-CO (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before EBP</td>
<td>(11) 16.9%</td>
<td>(5) 11.9%</td>
<td>(14) 21.2%</td>
<td></td>
</tr>
<tr>
<td>At EBP</td>
<td>(52) 80.0%</td>
<td>(35) 83.3%</td>
<td>(48) 72.7%</td>
<td></td>
</tr>
<tr>
<td>After EBP</td>
<td>(2) 3.1%</td>
<td>(2) 4.8%</td>
<td>(4) 6.1%</td>
<td></td>
</tr>
</tbody>
</table>

1\(^{\text{CO-72 (GnRH+PGF}_{2\alpha}\text{+GnRH & TAI 72 h after PGF}_{2\alpha}\text{); MOVS (GnRH+ PGF}_{2\alpha}\text{+GnRH+TAI 16 h after second GnRH); OVS-CO (GnRH+ PGF}_{2\alpha}\text{+GnRH+PGF}_{2\alpha}\text{+GnRH & TAI 72 h after PGF}_{2\alpha}.}}}^{\text{1CO-72 (GnRH+PGF}_{2\alpha}\text{+GnRH & TAI 72 h after PGF}_{2\alpha}; MOVS (GnRH+ PGF}_{2\alpha}\text{+GnRH+TAI 16 h after second GnRH); OVS-CO (GnRH+ PGF}_{2\alpha}\text{+GnRH+PGF}_{2\alpha}\text{+GnRH & TAI 72 h after PGF}_{2\alpha}.}}$

2\(^{\text{By visual observation and ALPRO™ system.}}}^{\text{2By visual observation and ALPRO™ system.}}$

3\(^{\text{Cows exhibited estrus and were inseminated before the last GnRH injection in the three treatments.}}}^{\text{3Cows exhibited estrus and were inseminated before the last GnRH injection in the three treatments.}}$

4\(^{\text{Cows were inseminated at the EBP in the three treatments.}}}^{\text{4Cows were inseminated at the EBP in the three treatments.}}$

5\(^{\text{Cows exhibited estrus within 7 days after the end of the protocol and were re-inseminated in the three treatments.}}}^{\text{5Cows 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 (MOV and CO-72) was only at the time of the second GnRH injection, which was earlier in the MOV 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 occur 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.

Table 2. Pregnancy rates and pregnancy losses for cows completed the hormonal protocols based on treatment.

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

\(^1\)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+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.

\(^a\)Percentage among treatments with different superscripts differ (P < 0.05).

\(^b\)Percentage 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.
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 PGF₂α 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.

### Table 3. Pregnancy rates, pregnancy losses, and services per conception for cows completed the hormonal protocols based on calving months.

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

\(^1\)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 \(\text{PGF}_{2\alpha}\) 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 \((\leq 1.0 \text{ ng/ml})\) \(P_4\) 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 \(\text{PGF}_{2\alpha}\) 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 \(\text{PGF}_{2\alpha}\) injections, respectively, there were anestrous 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 \(\text{PGF}_{2\alpha}\) 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 \(\text{PGF}_{2\alpha}\) 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 \(\text{PGF}_{2\alpha}\) injections. On the other hand, the low proportion of cows that had low concentrations of \(P_4\) at both GnRH and \(\text{PGF}_{2\alpha}\) 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.

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