Comparison of Two Estrous Synchronization Protocols on Reproductive Performance of Dairy Cows

Omer S. Darras¹ and Mufeed A. Alnimer²

ABSTRACT

Lactating dairy cows (n=53) were used to evaluate the effect of two estrous synchronization protocols starting between d 10-12 of the estrous cycle on reproductive performance. Cows in the Ovsynch (Control) treatment (n = 24) received an i.m. injection of 10µg Gonadotropin Releasing Hormone (GnRH) agonist (Buserelin, Receptal®), followed 7 d later by an i.m. injection of 25 mg Prostaglandin F2α (PGF2α) and 48 h later by a second i.m. injection of 10µg GnRH. The protocol was concluded with the timed artificial insemination (TAI) 16-20 h after the second GnRH. Cows in the Short-ES estrous Synchronization treatment (Short-ES; n = 29) received an i.m. injection of 25 mg PGF2α and an i.m. injection of 1 mg estradiol benzoate (EB) dissolved in oil and 10µGnRH 48 and 60 h after PGF2α, respectively. These cows were inseminated artificially at detected estrus. Blood samples were collected from all cows at days 0 (day of AI), 7, 14, 21, 22, and 23 after AI to determine P₄ concentrations. Pregnancy rates from first AI were based on plasma P₄ concentrations on d 22±1 post AI, and confirmed by rectal palpation between days 45 and 50 post AI. Results showed that more (P <0.01) cows in the Short-ES group (86.2%) exhibited estrus than in the Ovsynch group (41.7%). Pregnancy rates to first AI were greater (P < 0.05) in the Short-ES group (37.4%) than in the Ovsynch (12.5%) group. In contrast, pregnancy losses were lower (P < 0.05) in the Short-ES group (38.9%) than in the Ovsynch (76.9%) group. Pregnancy rates to second, third, and overall pregnancy rates were similar between both groups and between primiparous and multiparous cows. Days open for pregnant cows tended to be higher (P < 0.09) in the Ovsynch group than in the Short-ES group. Plasma P₄ concentrations were similar between pregnant and nonpregnant cows on days 0 and 7 after AI, however, were greater (P < 0.01) for pregnant than nonpregnant cows on days 14, 21, 22, and 23 after AI. Results indicate that Short-ES protocol improves reproductive performance by increasing the number of cows exhibiting estrus and pregnancy rates to first AI compared with Ovsynch protocol.

Keywords: Dairy cows, Short synchronization, PGF₂α, GnRH, Estradiol, Reproductive Performance.

INTRODUCTION

Increasing demand for milk production has led to a decline in the reproductive performance of dairy cows because of a prolonged calving interval (Butler, 2000). Several factors such as longer time period from parturition to first estrus, poor estrus expression or detection, improper timing of artificial insemination (AI), and reduction in conception rates at first AI have contributed to a longer calving interval, and in doing so, have compromised profitability in dairy farming (Sturman et al., 2000). The situation is further complicated by the fact that high yielding early postpartum dairy cows do not often develop regular...
ovarian activity (Opsomer et al., 2000).

Synchronization of estrus implies the manipulation of the estrous cycle or induction of estrus to bring a large percentage of a group of cows into estrus at a predetermined time (Odde, 1990). For this purpose, various synchronization programs allowing timed artificial insemination (TAI) have been developed for cows. Synchronization of ovulation (Ovsynch) was the first protocol developed that synchronizes ovulation within a period of 8 hours (Pursley et al., 1995 & 1997). This protocol involves two injections of gonadotrophin releasing hormone (GnRH) 7 d before and 48 h after a prostaglandin F$_2\alpha$ (PGF$_2\alpha$) injection with timed AI after 16-20 h of the second GnRH. This protocol has been shown to improve pregnancy rates in several studies (Moreira et al., 2001; Lubbage and Alnimer, 2003; El-Zarkouny et al., 2004). In Jordan, pregnancy rates at first AI after Ovsynch application range from about 20 to 40% (Lubbadeh and Alnimer, 2003; Alnimer, 2005a & b). However, this protocol requires several days to sort cows and perform the treatment, and it is difficult to reduce the interval between the start of treatment and AI to less than 9 days (Lopes et al., 2000).

Estradiol-17β (E$_2$) was included in estrus synchronization programs as an alternative to the second GnRH or as an additional hormonal treatment in the Ovsynch protocol. Most studies (Alnimer 2005a; Souza et al., 2007, Hillegass et al., 2008; Brusveen et al, 2009) found no effect of using of the E$_2$ in the Ovsynch protocol on pregnancy rates. Previous studies demonstrated that administration of a luteolytic dose of PGF$_2\alpha$ followed by human Chorionic Gonadotrophin (hCG) plus estradiol synchronized estrus in all cows in the luteal phase produces a short synchronization program (Lopez - Gatius, 2000).

A short method for estrus synchronization in dairy cows to save time by decreasing the interval to AI was developed by Cirit et al. (2008). It involves injection of PGF$_2\alpha$ and then injection of E$_2$ after 48 h then GnRH injection after 12 h followed by TAI within 16-20 h after GnRH. This method needs a functional corpus luteum (CL) to succeed. They reported that estrus detection rate, ovulation synchronization rate and pregnancy rates for treated cows were 55, 94.1 and 35%, respectively. In addition, the same hormonal treatments (PGF$_2\alpha$, Estradiol Cypionate (ECP), and GnRH) were applied to another group of cows but at fixed interval between them (24 h). The protocol resulted in 59.4% estrus expression, 59.1% ovulation synchronization rate, and 31.3% pregnancy rates. However, Cirit et al. (2008) did not compare the short method of synchronization with the Ovsynch protocol. Therefore, the objective of this study was to test the hypothesis that using Short-ES estrus synchronization method in the middle of estrous cycle with AI after estrus in dairy cows would improve reproductive performance compared to the Ovsynch protocol.

MATERIALS and METHODS

Cows, Housing, and Management

This experiment was conducted between February to September 2009 on a commercial dairy farm (Jordan Union for Agricultural Zoological Investment) housing approximately 1600 Friesian lactating dairy cows located in the Al-Hallabat area of the northern of Jordan.

Cows were housed in free-stall barns provided with shades and were milked three times daily at 8-h intervals. The rolling herd average milk production was approximately 9000 kg per lactation cycle. Cows were fed a total mixed ration (TMR) of 42% forage (corn silage, alfalfa hay, and straw) and 58% concentrate (corn, barley, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins). The ration contained 1.7 Mcal net energy for 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. Weather data during the experimental period were obtained from the Official National weather Station in Alkhaldia area (Figure 1). Data included mean maximum (29.2 ± 2 °C), minimum temperature (12.8 ± 0.15 °C) and relative humidity (55.2%).

![Figure 1. Trend of maximum, minimum temperature, and relative humidity during the months of the experimental period.](image)

**Experimental Design**

A total 65 lactating Friesian dairy cows in completely randomized design (CRD) were subjected to an estrus induction program and observed for signs of behavioral estrus twice daily between day 25 to 32 postpartum. If estrus was not observed by day 32, cows were palpated by rectum to determine ovarian activity. Only cows with corpora lutea on rectal palpation were immediately injected (i.m.) with 25 mg PGF2α (Lutalyse; Pharmacia & Upjohn S.A., Puurs, Belgium) on day 32. Ten cows exhibited estrus between 28 and 32 day while twelve cows were excluded from the study because they either did not respond to PGF2α injection on day 32 or due to different reasons such as diseases and culling. Therefore, only fifty-three cows (8 primiparous and 45 multiparous) were used in the study.

Ten to twelve days after the observed estrus (d 46 ± 3), cows were randomly assigned into two treatments (Figure 2). Cows in the Ovsynch (Control) treatment (n = 24) received an i.m. injection of 10µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH, Wiesbaden, Germany), followed 7 d later by an i.m. injection of 25 mg PGF2α and 48 h later by a second i.m. injection of 10µg GnRH. The protocol was concluded with the timed artificial insemination (TAI) 16-20 h after the second GnRH. Cows in the Short-ES estrous Synchronization treatment (Short-ES; n = 29) received an i.m. injection of 25 mg PGF2α and an i.m. injection of 1 mg estradiol benzoate (EB) dissolved in oil (Intervet International B.V. Boxmeer, Holland) and 10µg GnRH
48 and 60 h after PGF$_{2\alpha}$, respectively. Later cows were inseminated artificially at detected estrus according to the AM-PM rule (12 h after standing heat). Cows in both treatments were subjected to an estrous detection program by visual observation. One experienced AI technician using proven fertility semen (ABS Global, Inc., Deforest, Wisconsin, USA) performed AI throughout the experiment. All cows were diagnosed for pregnancy on days 21, 22 and 23 (22±1 d), (first examination) after either TAI or AI based on P$_4$ concentrations. Progesterone concentrations equal to or exceeding 1 ng/ml were considered as an indication for a functional CL. Pregnancy was confirmed by rectal palpation between days 45 and 50 (second examination) after AI. Pregnancy losses were calculated as cows that were diagnosed pregnant at the first examination and diagnosed non-pregnant at the second examination. Cows in both treatments, which were diagnosed not pregnant on day 22±1 or between 45-50 d received a new i.m. injection of 25 mg PGF$_{2\alpha}$ and inseminated after estrus detection with an i.m. injection of GnRH within 30 minutes until the fourth AI. 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. Days from calving to first, first to second, second to third, and third to fourth AI were calculated. Days open were defined as the number of days from calving to conception for pregnant cows until the fourth AI while number of AI were calculated as number of AI for pregnant cows until the fourth AI.

**Figure 2. A schematic diagram of the hormonal treatments of lactating dairy cows in the study.**

**Blood Sampling and Radioimmunoassay (RIA)**

Blood samples were collected via the coccygeal venipuncture from all cows into heparinized tubes on days 0 (day of AI), 7, 14, 21, 22, and 23 after AI to determine P$_4$ concentrations. Plasma was harvested by centrifugation within 30 minutes at 3000 rpm for 15 minutes. Plasma samples were stored at -20 °C until assayed for P$_4$ concentrations. Plasma P$_4$ concentration was determined by RIA (Immunotech a Beckman Coulter Company, Marseille, France) employing highly specific polyclonal antibodies in a radioimmunoassay 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 overall effects of factors. The effect of treatment (Ovsynch and Short-ES) on estrus detection rate after hormonal treatment and pregnancy rate based on P₄ concentration at days 22+1 and by rectal palpation between 45-50 d post AI and pregnancy losses between the two diagnoses were tested by Chi-square test using the FREQ procedure of SAS. Using General Linear Model (GLM) procedure of SAS, least square analysis of variance was applied on reproductive responses including effects of treatment (Ovsynch and Short-ES), parity (primiparous and multiparous), 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, days from calving to first AI, first to second AI, and second to third AI, days open, and number of services per conception. Least square means for significant effects were compared at P < 0.05 using t-test Analysis of variance was performed to determine effects maximum temperature (from calving until 10 weeks) to first service and between treatments and on pregnancy rates. In addition, the effect of plasma P₄ concentration and milk production for the first 20 weeks after parturition on treatments was estimated. All these responses were estimated using the FREQ procedure of SAS.

**RESULTS AND DISCUSSION**

According to our knowledge, this study is the first conducted to evaluate the effect of two estrous synchronization protocols during postpartum on improving reproductive performance in dairy cows by using Ovsynch protocol or a Short-ES involving PGF₂α, E₂ and GnRH then insemination at estrus. The average daily milk production at the time of starting experiment did not differ (P > 0.05) between cows in the Ovsynch (36.9 ± 1.3 kg) and Short-ES (38.9 ± 1.4 kg) treatments. Likewise, mean lactation numbers for cows in the Ovsynch (3.0 ± 0.26) and Short-ES (2.6 ± 0.22) treatments were not significantly different.

**Effect of Treatment and Parity on Estrus Detection Rate**

Synchronization in Ovsynch and Short-ES cows was initiated between 10 to 12 d of the estrous cycle. No cows in Ovsynch and Short-ES treatments exhibited estrus prior the last hormonal injection. Most previous studies reported around 10% (6% to 10.3%) or less cows observed in estrus before the final GnRH injection of the Ovsynch protocol (Stevenson et al., 1999; Vasconcelos et al., 1999; Alnimer et al., 2009). However, Cirit et al. (2008) did not show in their study how many cows exhibited estrus before the end of Short-ES estrus synchronization protocol. An effect (P < 0.01) of treatment but not parity on estrus detection rate was observed. On day 0 (day of AI), all cows in the Ovsynch treatment were inseminated and only 41.7% (10/24) of cows exhibited estrus within 24 h following the second GnRH injection in the Ovsynch group. In contrast, 15, 7 and 3 cows in the Short-ES treatment exhibited estrus, which is around 86.2% (25/29) and inseminated within 24, 48 and 72 h, respectively after GnRH injection. Among those cows in both groups observed in estrus, 20 % (2/10) and 40 % (10/25) of cows exhibited estrus within 24 h following the second GnRH injection in the Ovsynch group. In contrast, 15, 7 and 3 cows in the Short-ES treatment exhibited estrus, which is around 86.2% (25/29) and inseminated within 24, 48 and 72 h, respectively after GnRH injection. Among those cows in both groups observed in estrus, 20 % (2/10) and 40 % (10/25) of cows in the Ovsynch and Short-ES treatments, respectively, become pregnant after the first AI. Souza et al. (2007) found that 44.4% of the cows in the Ovsynch protocol expressed estrus 48 h after PGF₂α injection. On the other hand, Cirit et al. (2008) reported that the rate of estrus in cows with an active CL was higher (72.7%) than those cows without an active CL (30.0%) at the start of a short synchronization.
program. In the present study, similar estrus detection rate was observed between primiparous and multiparous cows as previously cited by Souza et al. (2007). In contrast, Hillegass et al. (2008) found that primiparous cows were more likely to be detected in estrus than multiparous cows.

**Effect of Treatment and Parity on Pregnancy Rates and Pregnancy Losses**

Pregnancy rates and pregnancy losses for cows based on treatment and parity are illustrated in Table 1. A greater pregnancy rate was observed by $P_4$ concentrations on d 22±1 post AI than that based on rectal palpation (d 45). These results are in agreement with those of Ababneh et al. (2007), who found that pregnancy rates at 19 to 22 d post AI in lactating dairy cows range from 50 – 60%. The sustained increase in $P_4$ levels on day 22±1 post AI might represent established pregnancies that were lost before pregnancy diagnosis on day 45 post AI.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment 1</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovsynch</td>
<td>Short-ES</td>
</tr>
<tr>
<td></td>
<td>(n = 24)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td>Pregnancy Rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Based on Plasma $P_4$ at day 22±1</td>
<td>(13) 54.2</td>
<td>(18) 62.1</td>
</tr>
<tr>
<td>First AI</td>
<td>(3) 12.5c</td>
<td>(11) 37.9d</td>
</tr>
<tr>
<td>First and Second</td>
<td>(14) 58.3</td>
<td>(14) 48.3</td>
</tr>
<tr>
<td>First, Second, and Third</td>
<td>(17) 70.8</td>
<td>(15) 51.7</td>
</tr>
<tr>
<td>Overall</td>
<td>(19) 79.2</td>
<td>(25) 86.2</td>
</tr>
<tr>
<td>Pregnancy Losses</td>
<td>(10) 76.9c</td>
<td>(7) 38.9d</td>
</tr>
</tbody>
</table>

1 Ovsynch (GnRH + PGF$_{2\alpha}$ + GnRH + TAI 16 to 20 h after second GnRH); Short-ES (PGF$_{2\alpha}$ + EB + GnRH + AI at detected estrus).

2 Based on plasma $P_4$ concentration (> 1 ng/ml) on day 21, 22, and 23 post AI.

3 Cows that were pregnant 45 to 50 d after AI.

4 Up to fourth AI.

5 Percentage of cows diagnosed pregnant on day 22±1 after AI that were diagnosed non-pregnant between day 45 and 50 after AI.

a, b Percentages between treatments with different superscripts differ (P < 0.01).

c, d Percentages between treatments with different superscripts differ (P < 0.05).

Pregnancy rates based on progesterone concentrations at d 22±1 were similar for Ovsynch (54.2%) and Short-ES (62.1%) cows. In contrast, pregnancy rates to first AI were lower (P < 0.05) for cows in the Ovsynch (12.5%) than those for cows in the Short-ES (37.4%) treatments. Pregnancy rates to first AI for cows in the Short-ES group were in line with the study of Cirit, et al. (2008) who reported that pregnancy rates to first AI for cows in the Short-ES timed AI protocol starting at diestrus of the cycle were around 33.3%. Moreover, pregnancy rates were greater in the Ovsynch protocol supplemented with E$_2$ for cows that
showed estrus than those cows that did not show estrus (Souza et al., 2007; Brusveen et al., 2009). Unexpectedly, in the present study, pregnancy rates in the Ovsynch protocol were lower than previous studies (Alnimer 2005a&b; Alnimer et al., 2009), but the reason for that may be due to a tendency in P4 concentrations elevation in the Ovsynvh protocol at day 0 or due to some cows, which were in heat before day 0 and did not show estrus. In the current study, pregnancy rates to second, third AI, and overall pregnancy rates were not different for Ovsynch and Short-ES cows (Table 1). Similar results were found in lactating dairy cows between Ovsynch and protocols involving GnRH, PGF2α and E2 (Alnimer, 2005a) or Ovsynch supplemented with E2 (Brusveen et al., 2009).

Differences (P < 0.01) were observed in pregnancy rates based on P4 concentrations on day 22±1 between primiparous (100%) and multiparous (51.1%) cows. Balendran et al. (2008) reported that pregnancy rates based on P4 concentrations from d 0 to d 22 post AI among first parity cows were higher than third and fourth parity cows. In the current study, although a tendency of increase in pregnancy rates to first (50.0 vs. 22.2%), second (75.0 vs. 48.9 %), third (75 vs. 57.8%), and overall (100 vs. 80%) was found, there were no statistical differences between primiparous and multiparous cows, respectively. Navanukraw et al. (2004); and Alnimer (2005a) reported similar pregnancy rates between primiparous and multiparous cows. In contrast, several studies reported by (Chebel et al., 2004; Souza, et al., 2007; Brusveen 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 decrease with increasing in lactation number (Grimard et al., 2006; Balendran et al., 2008).

Effect of Maximum Temperature on Pregnancy Rates and Pregnancy losses

The weekly maximum temperature for the first 8 weeks after calving (during first AI) did not differ between both treatments while at the second AI it was greater (P < 0.01) for cows in the Short-ES (34.25 ± 0.62 °C) treatment than cows in the Ovsynch (29.43 ± 0.69 °C) treatment. At the third AI, the maximum temperature did not differ between both treatments while at the fourth AI it was greater (P < 0.01) for cows in the Ovsynch (37.05 ± 0.62 °C) treatment than for those in the Short-ES (34.3 ± 0.56 °C) treatment. The differences in pregnancy rates at first AI between two groups may be due to the treatment effects. On the other
hand, pregnancy rates at the second AI in the Ovsynch group were numerically higher than in the Short-ES group (table 1); this may be due to a higher maximum temperature at the time when cows of the Short-ES group were inseminated. In contrast, pregnancy rates at fourth AI in the Short-ES group were numerically higher than in the Ovsynch group due to the same reason. Al-Katanani et al. (2002) reported that heat stress before and after breeding, and on the day of breeding was associated with low pregnancy. In addition, heat stress had a delayed negative effect on pregnancy rates up to 50 d before AI (Roth et al., 2001; Chebel et al., 2004). Previous studies reported lower pregnancy rates and greater pregnancy losses for cows inseminated in summer than those for cows inseminated in winter (Alnimer et al., 2009).

**Effect of Treatment and Parity on Reproductive Performance Parameters**

Reproductive performance parameters for cows based on treatment and parity are illustrated in Table 2. Treatment differences (P < 0.01) but not parity were detected for the interval from calving to first AI for cows in the Ovsynch (58.3 ± 1.2 d) and Short-ES (47.3 ± 1.1 d) groups. The intervals from first to second, second to third and third to fourth AI were not different for Ovsynch and Short-ES cows. Similar observations in the same parameter were found between primiparous and multiparous cows, with the mean intervals between estruses approximately 28 ± 12 d, where all cows were observed for estrus twice daily by visual observation. Days open for pregnant cows tended to be higher (P < 0.09) for cows in Ovsynch (111.7 d) group than for cows in Short-ES (74.7 d) group, while no differences were observed between primiparous (91.9 d) and multiparous (94.5 d) cows. The number of services per conception was similar Ovsynch and Short-ES cows (2.6 ± 0.4 vs. 1.8 ± 0.4; respectively, table 2) and between primiparous and multiparous cows (2.1 ± 0.5 vs. 2.4 ± 0.2; respectively). Because pregnancy rates to first AI were lower for cows in the Ovsynch treatment than for cows in Short-ES treatment, additional inseminations were needed for cows in the Ovsynch treatment to become pregnant, while cows in the Short-ES treatment were inseminated shorter than other treatment by about 11 d due to differences in duration of both treatments. In practice, the return to estrus is variable with most cows returning to estrus 20 to 24 d after insemination (Chenault et al., 2003). The variability in return to estrus may be explained by the normal variation in estrus length and early embryonic death or due to high environmental temperature from the second AI until the end of experiment. Heat stress is associated with a decreased expression of estrous behavior (Nebel, et al., 1997) and increase 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, 2007).

**Table 2. Reproductive performance for cows based on treatment and parity.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovsynch (n = 24)</td>
<td>Short-ES (n = 29)</td>
</tr>
<tr>
<td>Interval (d) from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calving to First AI</td>
<td>58.3 ± 1.2 a</td>
<td>47.3 ± 1.1 b</td>
</tr>
<tr>
<td>First to Second AI</td>
<td>42.7 ± 9.4</td>
<td>43.6 ± 14.8</td>
</tr>
<tr>
<td>Second to Third AI</td>
<td>19.0 ± 12.3</td>
<td>18.5 ± 13.1</td>
</tr>
</tbody>
</table>
Milk Production for Cows According to Treatment and Pregnancy Status

The milk production for cows of the study according to the treatment and pregnancy status are shown in Figures 3 and 4. Milk production in the first 8 weeks did not differ between cows in the Ovsynch and Short-ES treatments. Differences (P < 0.05) were observed at weeks 9 (31.3 ± 2.0 vs. 38.1 ± 1.7 kg), 10 (32.7 ± 1.9 vs. 37.9 ± 1.6 kg), and 11 (32.5 ± 1.9 vs. 36.0 ± 1.7 kg), for cows in the Ovsynch and Short-ES groups, respectively. This may explain why pregnancy rates at second and third AI were higher numerically in the Ovsynch group than in the Short-ES group. Similar milk production was observed after that up to 20 weeks. In addition, milk production did not differ between pregnant and non-pregnant cows in the study. Regardless of treatment, overall pregnancy rates were lower than previous studies (Lubbadeh and Alnimer, 2003; Alnimer, 2005b). This may be due to a higher milk production for cows in the present study as reported by Lucy (2001). In addition, cows are in NEB after calving and milk production gets priority over other physiological processes including reproduction, thus, anovulatory anestrus period may extended (Mwaanga and Janowski, 2000; Grimard et al., 2006). Moreover, the reproductive efficiency of the dairy cows has decreased in the past two decades with an increase in milk production, which is related to the prolonged NEB (Grohn and Rajala-Schultz, 2000; Washburn et al., 2002).

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Figure 4. Weekly milk production for pregnant and nonpregnant cows

Progesterone Concentrations for Cows According to Treatment and Pregnancy Status

Plasma progesterone (P4) concentrations at days 0, 7, 14, 21, 22, and 23 after AI for cows in both treatments are illustrated in Figure 5. Plasma P4 concentrations tended to be greater (P < 0.1) for cows in the Ovsynch (0.67 ng/ml) treatment than for those in the Short-ES (0.44 ng/ml) treatment on day 0. Likewise, on day 7 after AI, P4 concentration was greater (P < 0.01) in the Ovsynch (7.38 ng/ml) than in the Short-ES (3.37 ng/ml) treated cows. However, plasma P4 concentrations did not differ between both treatments at days 14, 21, 22, and 23 after AI. This means that the CL of some cows did not regress completely or due to the start of new development of the CL, Therefore, the low pregnancy rate in the Ovsynch group may be due to the greater of P4 concentrations on day of AI since this protocol utilizes TAI without heat detection except for cows observed in heat at that time. A higher P4 concentration in the Ovsynch treatment, was due to some cows, which, were in heat before day 0 and did not exhibited estrus.

The effect of pregnancy status on plasma P4 concentrations after first AI is illustrated in Figure 6. Plasma P4 concentrations did not differ between pregnant and nonpregnant cows at days 0 and 7 after AI but it was greater (P < 0.01) for pregnant (9.08, 7.12, 7.34 and 8.33 ng/ml) than for nonpregnant cows (3.75, 2.98, 2.98 and 3.35 ng/ml) at days 14, 21, 22, and 23 after AI, respectively. Similar findings were reported by Herzog et al. (2011). In a previous study, peripheral P4 concentrations were higher in pregnant than nonpregnant cows as early as day 10 (Hansel, 1981). In contrast, it was reported that P4 concentrations were not significantly different between pregnant and nonpregnant inseminated cows from 7 to 15 d after insemination (Echternkamp et al., 2009). These apparent differences may be attributed to varying numbers of animals per study, management factors, and P4 assays.

CONCLUSIONS

Short-ES protocol improves reproductive performance by increasing number of cows observed in estrus and pregnancy rates to first AI than Ovsynch protocol. In contrast, pregnancy losses were higher in the Ovsynch protocol than in the Short-ES protocol. Pregnancy rates to second, third, and overall were similar between both groups and between primiparous
and multiparous cows. Further studies under controlled ambient temperature with higher number of animals are needed to investigate the lack of pregnancies at first AI in the Ovsynch protocol.

![Figure 5. Plasma P₄ concentrations for cows in the Ovsynch and Short-ES treatments after AI.](image)

![Figure 6. Plasma P₄ concentrations for pregnant and nonpregnant cows after AI.](image)
REFERENCES


The performance of bull semen on breeding period evaluation using Ovsynch (GnRH-PGF2α) and Short-ES (PGF2α – E2-GnRH) treatments, compared with the time of first ovulation and postpartum drying off. The results showed that Short-ES treatment resulted in a higher percentage of conception rates than Ovsynch treatment. Additionally, the short-term estrus synchronization treatment reduced the number of days to first ovulation. The results of this study suggest that Ovsynch treatment can be used as an effective method for synchronizing estrus in bulls.