RESEARCH NOTES

Effect of Service Numbers on Resynchronization Responses in Lactating Dairy Cows during Warm Season

Z. Ansari Pirsaraei¹*, E. Dirandeh¹, and A. Rezaei Roodbari²

ABSTRACT

Our objective in this study was to consider the effect of service numbers on the percentage of lactating cows that responded to the resynchronization and fertility during warm season. Lactating dairy cows (n = 750) in five groups of 150 with different service numbers according to the last service (AI₁ = First service, AI₂ = 2nd, AI₃ = 3rd, AI₄ = 4th, AI ≥ 5) were assigned to receive Ovsynch56 starting on day 28 after fixed time AI (TAI). Ultrasonography was done during Ovsynch56 injections and also for confirmation of pregnancy in all cows at 32 and 60±4 days after fixed timed AI. Results showed proportion of cows that ovulated in response to the first GnRH injection of Ovsynch was greatest (P= 0.01) in the first service cows compared with other services. In addition, cows that ovulated in response to the first GnRH of Ovsynch had greater response to PGF₂α of Ovsynch (91.8 vs. 74.2%, respectively) and finally greater conception rate (CR) at 32 d after AI (32.1 vs. 24.6%, respectively) than those that did not ovulate. Conception rate at 32 and 60 days after AI was similar up to the third service (29.1 and 21.8% at days 32 and 60, respectively), but decreased after that (19.75 and 10.4% at days 32 and 60, respectively, P= 0.02). In conclusion, results of this study showed service numbers affected the proportion of cows that responded to resynchronization protocol. Conception rate was reasonable up to the third service, but, after the third breeding, proportion of cows that responded to resynchronization decreased and CR dropped below 20%.

Keywords: Conception rate, Ovsynch, Pregnancy loss, Ultrasonography.

INTRODUCTION

Excellent reproductive performance is essential for the success of any dairy operation. Regardless of location within the world, improving reproductive efficiency has a significant influence on profitability. Over the few last decades, reproduction efficiency has decreased whereas milk production per cow has increased (Lucy, 2001). Berger et al. (1981) showed that calving interval was increasing by one d/yr in Midwestern US herds. Washburn et al. (2002) reported large increases in Days Open (DO) in herds in the southeastern United States during the 1990s. After the initial postpartum AI, approximately 60% of lactating dairy cows do not conceive (Galvao et al., 2007). In addition, less than 60% of cows are re-inseminated before being presented for pregnancy diagnosis; thus, cows that do not become pregnant may have long interval between AI (Dewey et al., 2010). About 50% of standing heats are undetected during the postpartum period (Washburn et al., 2002).

Employing resynchronization of ovulation protocols is an effective tool for reducing the

¹Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, P. O. Box: 578, Islamic Republic of Iran.
*Corresponding author; e-mail: ansari2000@yahoo.com
²Department of Animal Science, University of Tehran, Karaj, Islamic Republic of Iran.
interval between inseminations for cows that are not pregnant (Dewey et al., 2010; Bruno et al., 2014; Lopes et al., 2013). Many studies evaluated different aspects of resynchronization strategies (Dewey et al., 2010; Giordano et al., 2012a; Chebel et al., 2013). On many farms, an Ovsynch protocol or modifications of Ovsynch are used to resynchronize ovulation for second and subsequent timed AI and are commonly referred to as resynch protocols (Fricke et al., 2003, Colazo et al., 2012). Although interval between AIs is decreased, conception rate of resynch protocols are less than those of the first service (Galvao et al., 2007).

Several studies have demonstrated that reproductive variables are significantly impaired during the warm period (Labe’rnia et al., 1998). Heat stress reduces the length and intensity of estrus and increases incidence of anestrous and silent ovulations (Hansen and Arechiga, 1999). The use of fixed Timed AI (TAI) to avoid the deleterious effects of reduced estrous detection has been well documented (De la Sota et al., 1998). Resynch protocols can be used to overcome silent heat problems of post AI non-pregnant cows during warm season.

All published researches have studied pregnancy performance of resynch protocols after postpartum first AI, while employing these protocols after the second or more services can shorten interval between calving to pregnancy. Till now, there is just one study (Lopes et al., 2013) on comparison of conception rate following resynch employed after the second, third, or more services. The objective of this study was to evaluate lactating dairy cows’ response to resynchronization employed at different service number.

**MATERIALS AND METHODS**

High-producing Holstein dairy cows (>30 kg day\(^{-1}\), n= 750) averaged 3.3±1.6 (mean±SEM) in parity and 3.4±0.6 (mean±SEM) in body condition score at calving. The diet was formulated to meet or exceed NRC requirements (NRC, 2001).

Treatments

Each week, cows at various days in milk DIM and having at least one previous AI were blocked by parity (primiparous vs. multiparous) and service numbers and randomly assigned to receive Ovsynch\(_{56}\) (GnRH-7d-PGF2\(_{a}\)-56h-GnRH-16hTAI) on day 28 after AI. According to the last service, five experimental groups, each of 150 cows, were as follows: (1) AI1; (2) AI2; (3) AI3; (4) AI4, and (5) AI\(\geq\) 5. During the experiment, all TAI were performed after synchronization of ovulation using an Ovsynch protocol (Bruno et al., 2014). All cows received an i.m. injection of GnRH (gonadorellin acetate, 100 mcg gonadorelin mL\(^{-1}\), i.m.; Parnell Technologies, PTY. LTD., Alexandria, Australia) 28 days after TAI regardless of their pregnancy status, and cows diagnosed not pregnant using transrectal ultrasonography 35 days after TAI received an i.m. injection of PGF2\(_{a}\) (cloprostenol, 250 mcg cloprostenol mL\(^{-1}\), i.m.; Parnell Technologies, PTY. LTD., Alexandria, Australia) and continued the protocol to receive the second injection of GnRH 56 h after PGF2\(_{a}\) and TAI 16 to 20 hours later. Ultrasonography

Pregnancy diagnosis 32 days after TAI and ovarian Ultrasonography (US) were performed using a portable scanner (Easi-Scan, BCF Technology Ltd., Livingston, UK) fitted with a 7.5-MHz linear-array transducer. For all cows, pregnancy diagnosis was performed 32 days after TAI regardless of their resynchronization treatment to compare P/TAI and pregnancy loss at similar time points between different service numbers. All cows diagnosed pregnant 32 days after TAI were rechecked using transrectal palpation 60 days after TAI.

Ultrasonography was performed at GnRH\(_1\) to determine the presence or absence of a CL and diameter of follicles present on the ovaries (Giordano et al., 2012b). Seven days later at the PGF2\(_{a}\) injection, ovarian US was performed to determine ovulatory response to GnRH\(_1\).
Ovulation was defined as the presence of a follicle at GnRH1 and presence of a new or an additional CL in the same location 7 d later at the second US examination. Ovulation to the second GnRH injection was confirmed by the disappearance of a large (> 10 mm) follicle that had been detected at the examination just before TAI (Dirandeh et al., 2015a).

**Statistical Analyses**

The experimental design was a complete randomized block design with parity as the blocking factor. Analyses of binary response data (CR, pregnancy loss, ovulatory response to GnRH, luteal response to PGF2α) were performed by logistic regression using the GLIMMIX procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). For analysis of pregnancy data, milk production was included as a covariate, and service sire and AI technician were considered for inclusion as random effects. Predicted probabilities of pregnancy and odds ratios were computed using the LOGISTIC procedure of SAS.

The final logistic regression model removed variables by a stepwise backward elimination based on the Wald statistic criterion when $P > 0.10$. Size of the ovulatory follicle was analyzed by ANOVA using the GLM procedure of SAS. Service number, parity, and their respective interactions were included in all models.

**RESULTS**

Proportion of cows that ovulated in response to the first GnRH injection of Ovsynch was greatest ($P= 0.01$) in the first service cows compared with other services. Overall, 89.5% of cows in the first service ovulated in response to G1 compared with 84.2, 80.0, 68.0 and 64.0% for AI2, AI3, AI4 and AI$\geq 5$, respectively (Table 1).

Luteolytic response to PGF2α of Ovsynch was greater in AI1, AI2, and AI3 compared with other services ($P< 0.05$ for each comparison). Synchronization rate in response to Ovsynch was increased ($P= 0.05$) in AI1, AI2, and AI3 compared with services more than three. Synchronization rate was not different between AI4 and more services. In addition, synchronization response to Ovsynch was improved ($P< 0.005$) in cows that ovulated in response to the first GnRH of Ovsynch compared with those that did not (86.8 vs. 64.4%, respectively). Ovulatory response to the second GnRH injection of Ovsynch56 was affected ($P= 0.03$) by treatment and was greater in the first three services (82.4%) compared with other services (70.5%, Table 1). In addition, cows that ovulated in response to the first GnRH of Ovsynch had greater response to PGF2α of Ovsynch (91.8 vs. 74.2%, respectively) and, finally, greater CR at 32 days after AI (42.1 vs. 34.6%, respectively) and 60 days after AI (30.6 vs. 22.4%, respectively) than those that did not ovulate (Table 3).

Conception rate at 32 and 60 days after...
TAI was similar up to the third service (29.1 and 21.8% at days 32 and 60, respectively), but decreased after that (19.75 and 10.4% at days 32 and 60, respectively; P= 0.02). Pregnancy loss from 32 to 60 d after TAI was not affected by service numbers (Table 2).

**DISCUSSION**

Ovulatory response to G1 was affected by service numbers (P= 0.02) and decreased after the third service. The present study results indicated that ovulation to the first GnRH of Ovsynch positively affected luteal and follicular responses to subsequent PGF2α and final GnRH of Ovsynch. Cows that ovulated in response to G1 had a greater luteal regression rate compared with cows that failed to ovulate. Ovulatory response to the first GnRH injection of Ovsynch is a critical determinant of the overall synchronization outcome to Ovsynch (Vasconcelos et al., 1999; Moreira et al., 2000). The Corpus Luteum (CL) in cows that ovulated after the first GnRH of Ovsynch, regardless of treatment, were more likely to undergo luteolysis in response to PGF2α of Ovsynch and had a greater synchronization rate compared with cows that did not respond to first GnRH. It is likely that the first injection of GnRH induced ovulation in cows bearing follicles ≥ 10 mm in diameter that resulted in the formation of a new CL and the recruitment of a new follicular wave approximately 36 to 60 hours later (Ryan et al., 1991; Pursley et al., 1995; Dirandeh et al., 2009). Moreover, if cows ovulated after the first GnRH, they were more likely to have a functional dominant follicle capable of ovulation at the final GnRH injection of Ovsynch (Vasconcelos et al., 1999). Most studies (Moreira et al., 2001; Galvao and Santos, 2010; Keskin et al., 2010; Dirandeh et al., 2014, 2015a, b) demonstrated that a higher response to GnRH1 increased the ovulatory response to GnRH2 and, therefore, the pregnancy rate. Pregnancy information indicated that concentrations of P4 at PGF2α of Ovsynch, and concentrations of E2 and

### Table 2. Effects of service numbers on conception rate (CR) at 32 and 60 days after AI and pregnancy loss from 32 to 60 days after AI.

<table>
<thead>
<tr>
<th>Item</th>
<th>AI1</th>
<th>AI2</th>
<th>AI3</th>
<th>AI4</th>
<th>AI5≥</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR at 32 days after AI</td>
<td>30.6±0.02</td>
<td>29.4±0.02</td>
<td>27.4±0.02</td>
<td>20.3±0.02</td>
<td>19.2±0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>CR at 60 days after AI</td>
<td>22.4±0.03</td>
<td>22.6±0.03</td>
<td>20.4±0.04</td>
<td>10.6±0.04</td>
<td>10.2±0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Pregnancy loss</td>
<td>8.2±0.62</td>
<td>6.8±0.54</td>
<td>7.0±0.84</td>
<td>9.7±1.25</td>
<td>9.0±0.93</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*p* Means in the same row with different superscript are significantly different (P< 0.05). Each value includes LSM±SE.

### Table 3. Conception rate (CR) at 32 and 60 days after AI according to response to the first GnRH (GnRH1) and second GnRH (GnRH2) treatments.

<table>
<thead>
<tr>
<th>Item</th>
<th>Response to the GnRH1</th>
<th>P</th>
<th>Response to the GnRH2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(LSM±SE)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>CR at 32 days after AI</td>
<td>42.1±0.02</td>
<td>34.6±0.01</td>
<td>0.03</td>
<td>56.6±0.01</td>
</tr>
<tr>
<td>CR at 60 days after AI</td>
<td>30.6±0.01</td>
<td>22.4±0.02</td>
<td>0.03</td>
<td>44.6±0.02</td>
</tr>
</tbody>
</table>

*p* Means in the same row with different superscript are significantly different (P< 0.05). Each value includes LSM±SE.
follicle size at final GnRH, were key indicators of fertility (Bello et al., 2006).

The result of current study showed cows that ovulated in response to the first GnRH of Ovsynch had greater CR at 32 and 60 days after TAI than those that did not ovulate. In agreement with our result, Chebel et al. (2006) demonstrated that cows that ovulated to the first GnRH injection of a timed AI protocol had P/AI approximately 10% greater than those that did not ovulate. Such a difference in P/AI was probably because cows that do not ovulate to the GnRH injection have a prolonged period of dominance of the ovulatory follicle and embryos of reduced quality (Cerri et al., 2009). Vasconcelos et al. (1999) and Moreira et al. (2000) demonstrated that ovulation in response to the first GnRH injection of timed AI protocols increased in cows and heifers that start such protocols between days 5 and 9 of the estrous cycle.

An effect (P= 0.02) of AI number was observed in the present experiment in which CR was 10% higher in AI1 than the AI2 groups and dropped to 9.4 and 13.6% for the fifth and sixth services, respectively. In agreement with current results, an effect of insemination number in other resynchronization experiments is reported (Sterry et al., 2006; Silva et al., 2009). Lopes et al. (2013) reported P/AI decreased by about 15 percentage points for cows receiving their third to fifth AI service. Several experiments support that strategies to presynchronize cows submitted to a resynchronization protocol can increase fertility (Silva et al., 2007; Dewey et al., 2010; Giordano et al., 2012a, b) suggesting that part of the problem with resynchronized inseminations is that cows initiate the protocol at a suboptimal stage of the estrous cycle. Nonetheless, the effect of AI number on P/AI also supports the notion that a subpopulation of lower fertility cows accumulates as AI number increases within a herd. No effect was observed of parity on P/AI to resynchronized services in the present experiment, and this result was in agreement with other experiments (Galvao et al., 2007; Silva et al., 2009).

The total pregnancy loss from 32 to 60 days after TAI was 7.1% and was not affected by service numbers. This observation agrees with studies reporting that treating cows of unknown pregnancy status with GnRH does not affect pregnancy loss (Fricke et al., 2003; Buttrey et al., 2010). Furthermore, it was not surprising that we failed to detect service number or parity differences in the rate of pregnancy loss because most studies comparing TAI programs after resynchronization have shown similar results (Silva et al., 2009; Thompson et al., 2010).

CONCLUSIONS

Results of this study showed that service numbers affected proportion of cows that responded to resynchronization protocol. Conception rate was reasonable up to the third service, but, after third breeding, proportion of cows responding to resynchronization decreased and CR dropped below 20%, therefore, performing resynchronization protocol may not be economical for cows conceived after the third breeding.

ACKNOWLEDGEMENTS

We are grateful to the Mahdasht Meat and Milk Complex Company for providing the necessary research facilities.

REFERENCES


تأثیر نوبت‌های تلقیح بر پاسخ به همزمانی دوباره در گاوهای شیری هلسنای طی فصل گرم

چکیده

هدف از یپوشه حاضر بررسی تاثیر نوبت‌های تلقیح بر درصد گاوهایی که به همزمانی دوباره پاسخ می‌دهند و باروری گاو هلسنای در فصل گرم بود. برای انجام این یپوشه ۷۵۰ رأس گاو هلسنای با نوبت تلقیح مختلف و بر اساس آزمون T لیکلیک به طور تصادفی در دو گروه گرفته شد. گروه‌های آزمایشی عبارتند از: ۱۰۰ نوبت دوم (P=0.01) (AI2)، ۲۰ نوبت دوم (P=0.02) (AI3)، تلقیح چهارم (P=0.05) (AI4) و ۵ تن نوبت پنجم و بیشتر (AI5).

اوسینک در روز ۲۸ پس از تلقیح بدون توجه به وضعیت آسیبی آغاز شد. از اولتراژئوگرافی برای معاینه وضعیت تخم‌دانان و در طی سه تزریق اوسینک و تست آسیبی در روز ۳۲ پس از تلقیح و تا پایان آسیبی در روز ۶۴ پس از تلقیح استخدام شد. نتایج نشان داد درصد گاوهایی که به پاسخ به اول اوسینک تخم‌دانان در اولین تلقیح نسبت به سابقه تلقیحات بیشتر بود (P=0.01).

گاوهایی که به اوسینک پاسخ دادند به ترتیب GnRH اول اوسینک پاسخ دادند به ترتیب GnRH 9/8 درصد PGF2α. در گروهی که پاسخ دادند در مقایسه با ۷۴/۲ درصد در گروهی که پاسخ ندادند، همچنین درصد گیرایی در روز ۳۲ پس از تلقیح در گروهی که به اوسینک پاسخ دادند بیشتر از گروهی GnRH به که به اوسینک پاسخ دادند (P=0.01) درصد ۲/۶ در مقایسه با ۲/۴ درصد گیرایی بود.

در روز ۲۳ و ۶۰ پس از تلقیح نتیجه تلقیح سیم مشابه بود (P=1/19 درصد و ۲۱/۸ درصد به ترتیب در روز ۳۲ و ۶۰ پس از تلقیح). ولی بعد از تلقیح سیم کاهش یافت (1/۵/۹ درصد و ۱۱/۴ درصد به ترتیب در روز ۳۲ و ۶۰ پس از تلقیح). P=0.02، به طور کلی نتایج یپوشه حاضر نشان داد نوبت تلقیح بر درصد گاوهایی که به همزمانی دوباره پاسخ می‌دهند تاثیر دارد. درصد گیرایی تا نوبت تلقیح سیم منطقی بود ولی بعد از تلقیح سیم درصد گاوهایی که به همزمانی دوباره پاسخ دادن کاهش یافت و درصد گیرایی، به زیر ۲۰ درصد رسید.