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RESEARCH NOTES

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

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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 PGF2*α* 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 numbersaffected 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

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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 Ovsynch56 (GnRH-7d-PGF2α-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≥ 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 PGF2α (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 PGF2α 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/AI 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 GnRH1 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 PGF2α injection, ovarian US was performed to determine ovulatory response to GnRH1.

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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≥ 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 PGF2*α* 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 (Table3).

Conception rate at 32 and 60 days after

Table 1. Effects of service numbers on ovulation to the first GnRH, Luteolytic response to $PGF_{2\alpha}$ injection and ovulation to the second GnRH.[a]

	Service number					
Item	AI1	AI2	AI3	AI4	AI≥ 5	P
Ovulation to first GnRH	89.5±0.12[a]	84.2±0.30[b]	80.0±0.19[b]	68.0±0.11[c]	64.0±0.42[c]	0.01
Luteolytic response to $PGF_{2\alpha}$ injection	94.2±0.33[a]	88.7±0.09[a]	86.6±0.27[a]	74.8±0.42[b]	70.8±0.15[b]	0.04
Ovulation to second GnRH	86.4±0.22[a]	84.8±0.14[a]	82.1±0.09[a]	70.9±0.61[b]	70.1±0.53[b]	0.03

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

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.[a]

	Service number					
Item	AI1	AI2	AI3	AI4	AI≥ 5	P
CR at 32 days after AI	30.6±0.02[a]	29.4±0.02[a]	27.4±0.02[a]	20.3±0.02[b]	19.2±0.03[b]	0.02
CR at 60 days after AI	22.4±0.03[a]	22.6±0.03[a]	20.4±0.04[a]	10.6±0.04[b]	10.2±0.04[b]	0.02
Pregnancy loss	8.2±0.62	6.8±0.54	7.0±0.84	9.7±1.25	9.0±0.93	0.61

[a] 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.[a]

	Response to the GnRH1			Response to the GnRH2		
Item	(+)	(-)	P	(+)	(-)	P
(LSM±SE)						
CR at 32 days after AI	42.1±0.02	34.6±0.01	0.03	56.6±0.01[a]	12.1±0.02[b]	0.01
CR at 60 days after AI	30.6±0.01	22.4±0.02	0.03	44.6±0.02[a]	22.4±0.01[b]	0.01

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

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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 numbersor 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 numbersaffected 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.

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چکیده

هدف از پژوهش حاضر بررسی تاثیر نوبت‌های تلقیح بر درصد گاوهایی که به همزمانی دوباره پاسخ میدهند و باروری گاو هلشتاین در فصل گرم بود. برای انجام این پژوهش ۷۵۰ رأس گاو هلشتاین با نوبت تلقیح مختلف و بر اساس آخرین تلقیح به طور تصادفی در بین پنج تیمار قرار گرفتند. گروه‌های آزمایشی عبارتند از: ۱- تلقیح اول، (۱۵۰ = n، AI1)، ۲- تلقیح دوم (۱۵۰ = n، AI2)، ۳- تلقیح سوم (۱۵۰ = n، AI3)، ۴- تلقیح چهارم (۱۵۰ = n، AI4) و ۵- تلقیح پنجم و بیشتر (۱۵۰ = n، AI5). اوسینک در روز ۲۸ پس از تلقیح بدون توجه به وضعیت آبستنی آغاز شد. از اولتراسونوگرافی برای معاینه وضعیت تخمدان‌ها در طی سه تزریق اوسینک و تست آبستنی در روز ۳۲ پس از تلقیح و تایید آبستنی در روز ۶۴ پس از تلقیح استفاده شد. نتایج نشان داد درصد گاوهایی که در پاسخ بهGnRH اول اوسینک تخمک‌ریزی کردند در اولین تلقیح نسبت به سایر تلقیحات بیشینه بود ($P = 0.01$). گاوهایی که به GnRH اول اوسینک پاسخ دادند به تزریق PGF2α پاسخ بیشتری دادند (۹۱/۸ درصد 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 GnRH اول اوسینک پاسخ دادند بیشتر از گروهی بود که به GnRH اول اوسینک پاسخ ندادند (۳۲/۱ درصد در مقایسه با ۲۴/۶ درصد). درصد گیرایی در روز ۳۲ و ۶۰ پس از تلقیح تا نوبت تلقیح سوم مشابه بود (۲۹/۱ درصد و ۲۱/۸ درصد به ترتیب در روز ۳۲ و ۶۰ پس از تلقیح) ولی بعد از تلقیح سوم کاهش یافت (۱۹/۷۵ درصد و ۱۰/۴ درصد به ترتیب در روز ۳۲ و ۶۰ پس از تلقیح، $P = 0.02$). به طور کلی نتایج پژوهش حاضر نشان داد نوبت تلقیح بر درصد گاوهایی که به همزمانی دوباره پاسخ می‌دهند تاثیر دارد. درصد گیرایی تا نوبت تلقیح سوم منطقی بود ولی بعد از تلقیح سوم درصد گاوهایی که به همزمانی دوباره پاسخ دادند کاهش یافت و درصد گیرایی به زیر ۲۰ درصد رسید.

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