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Effect of a deslorelin implant in a timed artificial insemination protocol on follicle development, luteal function and reproductive performance of lactating dairy cows[☆]

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Abstract

This study examined the influence of a GnRH agonist containing either 450 or 750 µg of deslorelin in an implant form or a gonadorelin injection (control) to induce ovulation in the Ovsynch protocol on pregnancy rates (PR), embryonic loss, and ovarian function in 593 lactating Holstein cows. Cows were given two injections of PGF_{2α} 14 days apart, followed 14 days later by the Ovsynch protocol, and were timed artificially inseminated (TAI) at 68 ± 3 days postpartum. Blood samples for determination of plasma progesterone concentrations were collected at 24 and 10 days prior to and 11 days after TAI. Pregnancy was diagnosed on Day 27 and reconfirmed on Day 41 after TAI. Non-pregnant, not re-inseminated cows at Day 27 had their ovaries examined by ultrasonography, and the number and size of follicles and presence of luteal tissue were determined. Simultaneously, these cows were re-synchronized with the Ovsynch protocol. Pregnancy during the re-synchronization period was determined between 35 and 41 days after insemination. On Day 27, PR were higher for control (39.0%) and deslorelin 450 µg (DESLORELIN 450) implant (41.3%) than for those receiving the deslorelin 750 µg (DESLORELIN 750) implant (27.5%; $P < 0.05$). Pregnancy losses tended to decrease for DESLORELIN 450 compared with control (5.0% versus 12.7%; $P < 0.13$). Plasma progesterone concentrations did not differ significantly among treatments. Deslorelin suppressed ovarian activity and decreased PR during the re-synchronization period compared with control. The percentage of non-pregnant animals that were re-inseminated by Day 27 was less for deslorelin compared with control. In conclusion, incorporation of an implant of the GnRH agonist

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deslorelin to induce ovulation in the Ovsynch protocol has the potential to reduce pregnancy losses, but the response was dependent upon implant concentration. Evaluation of lower doses to minimize the negative effects on subsequent fertility is warranted.

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1. Introduction

Reproductive efficiency is a major component of economic success in dairy herds. Pharmacological control of the estrous cycle involves synchronization of follicular development, control of corpus luteum (CL) regression, and synchronization of ovulation to improve conception and pregnancy rates (PR), enabling development of a successful timed artificial insemination (TAI) protocol with adequate PR [1]. Systematic breeding programs that involve TAI have become an integral part of reproductive management in high-producing dairy herds [2]. However, TAI protocols have not increased conception rates or altered pregnancy losses in lactating dairy cows [3].

Recently, manipulation of the estrous cycle prior to initiation of the TAI protocol resulted in increased PR in lactating cyclic cows [4]. Optimizing fertility in TAI protocols should result in higher PR and greater adoption of such protocols to improve reproductive performance in dairy herds.

Embryo mortality in lactating dairy cows between 27 and 45 days after TAI has been reported to be as high as 21% [4]. Losses of pregnancy in lactating dairy cows have been similar when insemination is performed at detected estrus or after the Ovsynch/TAI protocol [3]. Furthermore, conception rate in cows inseminated at detected estrus is higher than that in cows inseminated following TAI [3]. Therefore, although TAI protocols such as the Ovsynch have improved service rates, they have not increased conception rates or improved embryo survival in cattle.

During the late stages of the estrous cycle, there is an upregulation of estradiol receptors, oxytocin mRNA, and prostaglandin F synthase mRNA in cyclic, non-lactating dairy cows [5]. This is thought to be coordinated partially by ovarian function, because it coincides with development of the second wave dominant follicle and is associated with a high estrogenic environment. Thus, follicle development during the period of maternal recognition of pregnancy may contribute to the development of luteolytic signals that compromise embryo survival. In fact, high levels of estradiol during late diestrus may decrease fertility [6]. Therefore, attenuation of follicle development during this period might reduce early pregnancy losses.

The GnRH agonist deslorelin-induced ovulation in dairy cows when used as the last GnRH injection in the Ovsynch protocol [7] or on Day 5 of the estrus cycle [8]. It induces a greater secretion of LH when compared with Buserelin [8], which might improve CL differentiation, resulting in higher mid-luteal phase progesterone concentrations when compared with a Buserelin injection [7]. Cows with higher plasma progesterone have more developed embryos that secrete more interferon- τ [9]. Moreover, chronic GnRH stimulation (due to deslorelin) on the gonadotrophs down-regulates GnRH receptors and creates a

period of GnRH insensitivity [10]. This transient state of insensitivity to GnRH decreases gonadotropin support for follicle growth and maturation. Collectively, use of a low dose, biodegradable deslorelin implant results in normal or improved luteal function [7], associated with a transient suppression in follicle development [7,8,10,11], that might increase fertility.

We hypothesized that the use of an implant of a GnRH agonist deslorelin in a TAI protocol would synchronize ovulation, delay follicular growth, and improve CL differentiation and development (as indicated by increased progesterone concentrations during diestrus). These effects were expected to improve embryo survival in lactating dairy cows.

The objectives of the present experiment were to evaluate follicular development, luteal activity, PR, and embryo survival in lactating dairy cows given a biodegradable implant containing 450 µg (DESLORELIN 450) or 750 µg (DESLORELIN 750) deslorelin versus 100 µg gonadorelin to synchronize ovulation in the Presynch/Ovsynch TAI protocol [4] during the first postpartum AI.

2. Materials and methods

2.1. Animals, housing, and feeding

Early lactation Holstein cows (293 primiparous and 300 multiparous) on a commercial dairy farm in the Central Valley of California were assigned to one of two treatments in a randomized, complete block design. The average lactation (1.97 ± 0.05), postpartum interval at the beginning of the study (29.5 ± 0.12 days), and BCS (3.3 ± 0.01) did not differ among treatments ($P > 0.50$). The study period lasted for the first two postpartum AI. During the entire study, all cows received the same total mixed ration to meet the nutrient requirements for lactating Holstein cows weighing 650 kg and producing 40 kg of 3.5% fat-corrected milk [12].

The experiment was conducted from March to June of 2001 and from August to December of 2001. Cows were housed in two free-stall barns, and primiparous and multiparous cows were grouped separately during the entire study. Cows were milked twice daily and milk yields were recorded for individual cows once monthly during the official California dairy herd improvement association test. Body condition of all cows was scored [13] at enrollment in the study (30 DIM), at insemination (68 DIM), and at the first ultrasound examination (95 DIM). Changes in BCS from enrollment in the study to AI and to pregnancy diagnosis were calculated. Also, BCS at AI was categorized as low ($BCS \leq 2.75$), medium ($BCS = 3.0-3.25$), and high ($BCS \geq 3.5$).

2.2. Treatments, ovarian ultrasonography, and pregnancy diagnosis

Cows were subjected to a pre-synchronization treatment with i.m. injections of PGF_{2α} (25 mg dinoprost tromethamine; Lutalyse[®]; Pharmacia Animal Health, Kalamazoo, MI, USA) at 30 ± 3 and 44 ± 3 days postpartum [4]. Fourteen days after the second PGF_{2α}, (58 ± 3 DIM), all cows received an i.m. injection of 100 µg of GnRH (gonadorelin diacetate tetrahydrate; Cystorelin[®]; Merial, Athens, GA, USA). On Day 7.5 after the

GnRH injection, cows received a third injection of PGF_{2α}. Forty-eight hours after the last PGF_{2α} injection, cows received either 100 µg of GnRH (i.m.), or a 450 or 750 µg biodegradable deslorelin implant (Peptech Animal Health, North Ryde, Australia) subcutaneously in the neck area. Timed AI was done in all cows 16–18 h later; the same technician inseminated all cows throughout the study. After the initial TAI, cows were observed for signs of estrus once daily in the morning by tail chalking [14] using paintsticks (All-weather Paintstick; LA-CO Industries, Chicago, IL, USA). Cows detected in estrus were inseminated the same morning.

Pregnancy was diagnosed by ultrasonography 27 days after AI. Observation of embryonic fluid, appearance of the embryo, and embryonic heartbeat were used as determinants of pregnancy. A real-time ultrasound scanner (Aloka SSD 500 V; Aloka Co. Ltd., Tokyo, Japan) equipped with a 7.5 MHz rectal probe was used. Cows diagnosed pregnant on Day 27 had their pregnancies reconfirmed by palpation per rectum of an embryonic vesicle 14 days later.

Ovaries of non-pregnant cows that had not been re-inseminated were examined on Day 27 to determine the presence of CL and follicles, and the number of follicles in the different class sizes: Class I ≤ 5.0 mm; Class II = 6–9 mm; and Class III = 10–19 mm; Class IV ≥ 20 mm. Non-pregnant cows 27 days after AI that had not been re-inseminated were re-synchronized with the Ovsynch protocol [1]. Ultrasound examination of the ovaries was performed again, 7 days later, at the moment of the PGF_{2α} injection during the re-synchronization protocol, to determine the presence of CL and the size of the largest follicle. Cows re-synchronized were submitted to TAI 16–18 h after the last GnRH injection of the Ovsynch protocol. Pregnancy during the re-synchronization period was determined by palpation per rectum of an embryonic vesicle at 38 ± 3 days after the second postpartum AI (Fig. 1).

The interval between the first and second postpartum AI for those cows re-inseminated prior to pregnancy diagnosis on Day 27 after the initial TAI was evaluated. The proportion of cows diagnosed as non-pregnant on Day 27 that were re-inseminated prior to pregnancy diagnosis in each treatment group also was evaluated.

2.3. Blood sample collection and analyses

At –24, –10, and 11 days relative to the day of the first postpartum AI, blood samples (10 ml) were collected from all cows by puncture of the coccygeal vein or artery into evacuated tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were placed immediately on ice and centrifuged at 1500 × g for 15 min at 10 °C for separation of plasma. Plasma was frozen at –25 °C and later analyzed for progesterone using a single antibody RIA procedure [15]. The assay sensitivity was 0.10 ng/ml, and the intra- and inter-assay CVs were 9.8 and 7.9%, respectively.

Progesterone concentrations in plasma on Days –24 and –10 relative to AI were used to determine whether a cow was cycling or anovulatory/anestrous. Cows with plasma progesterone concentration ≥ 1.5 ng/ml in any of the two samples were considered cycling. Cows with progesterone concentrations < 1.5 ng/ml in both plasma samples prior to the TAI protocol were considered as anovulatory. Plasma progesterone concentrations on Day 11 after AI were used to determine whether the treatment affected luteal function in lactating

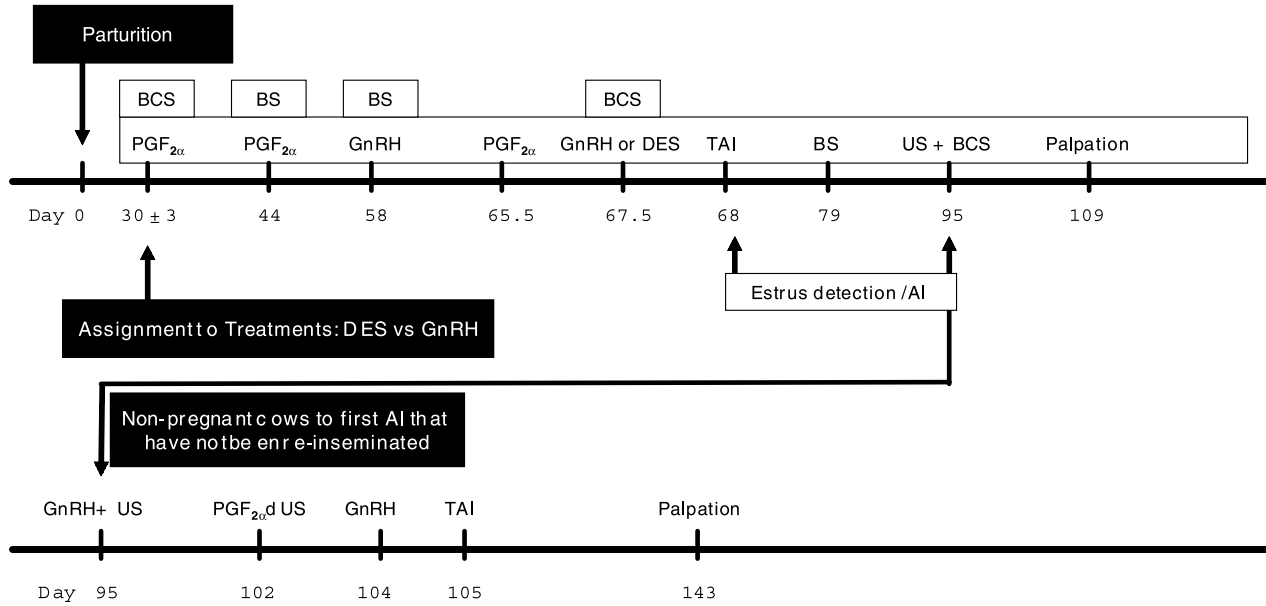


Fig. 1. Diagram of activities during the study. BCS, body condition score; BS, blood sample; DES, deslorelin implant; GnRH, gonadorelin; PGF_{2α}, prostaglandin F_{2α}; TAI, timed artificial insemination; US, ultrasound.

dairy cows. Induction of cyclicity after TAI was determined by the proportion of cows that were anovulatory prior to TAI and subsequently had a plasma progesterone concentration >1.5 ng/ml on Day 11 after TAI.

2.4. Experimental design and statistical analyses

The experimental design was a randomized-complete block design [16]. Cows were blocked according to parity, BCS at 30 days postpartum, and previous lactation 305 days mature equivalent milk yield (multiparous), and randomly assigned to one of the two treatments. Cows in the deslorelin treatment were randomly assigned to receive either a 450 or a 750 µg implant. Plasma progesterone concentration on Day 11 after TAI was analyzed by the GLM procedure of SAS [17], with the effects of treatment, parity, cyclicity, body condition score at AI, and higher-order interactions. Follicular populations and interval between the first and second postpartum AI were analyzed by the GLM procedure of SAS [17], with the effects of treatment, parity, cyclicity, body condition score at the first AI, plasma progesterone concentration on Day 11, and higher-order interactions. Orthogonal contrasts were performed to determine the effect of type of GnRH (control versus DESLORELIN 450 and DESLORELIN 750) and the effect of dose of deslorelin (DESLORELIN 450 versus DESLORELIN 750) on the outcome variables evaluated.

Binomially distributed data were analyzed by logistic regression [18], using the logistic procedure of the SAS program [17], with a model that included the effects of treatment, parity, cyclicity, body condition score at AI, BCS change from 30 DIM to AI, plasma progesterone concentration on Day 11, and higher-order interactions. Contrasts were performed to determine the effect of type of GnRH (control versus DESLORELIN 450 and DESLORELIN 750) and the effect of dose of deslorelin (DESLORELIN 450 versus DESLORELIN 750) on the outcome variables evaluated as described previously.

Regression analyses were performed to determine the best-fitted line between BCS at AI and the frequency of anovulatory/anestrous cows prior to the first AI. Linear, quadratic and cubic effects were tested. Cows were grouped based on their BCS at AI, which ranged from 2.50 to 4.00, and within each BCS group the frequency of anovulatory/anestrous was calculated. The number of cows within each BCS group ranged from 21 to 172. Body condition score was then plotted against the frequency of anovulatory/anestrous cows using the regression procedure of MINITAB [19] to determine the best-fitted line.

Treatment differences with $P \leq 0.05$ were considered significant, while tendencies were considered when $P > 0.05$ and $P \leq 0.15$.

3. Results

Plasma progesterone concentration prior to TAI was not determined in two of the 593 cows. Of the remaining 591 cows, 75 were not cycling (12.7%) by 58 DIM, and a greater proportion of primiparous cows were anovulatory compared to multiparous cows (18.2 versus 7.4; $P < 0.001$). Body condition score at AI ($P < 0.0001$), but not changes in BCS from Day 30 postpartum to AI ($P = 0.16$) affected the proportion of cyclic cows in the first

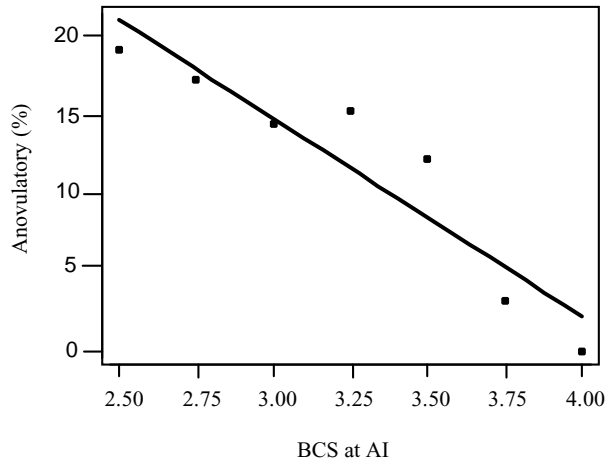


Fig. 2. Relationship between body condition score (BCS) at first postpartum artificial insemination (AI) and frequency of anovulatory dairy cows. Frequency of anovulatory cows = $52.2393 - 12.5 \times \text{BCS at AI}$ ($P < 0.002$).

58 DIM. A linear relationship between BCS at first postpartum AI and the incidence of anovulatory cows was established; anovulatory cows decreased 3.13% for every 0.25 unit increase in BCS between 2.50 and 4.00 (Fig. 2).

Frequency of cycling cows prior to TAI was 88.2, 87.9, and 85.0% for cows treated with GnRH, DESLORELIN 450, and DESLORELIN 750, respectively ($P = 0.90$). Subsequent to TAI, the proportion of cows with plasma progesterone higher than 1.5 ng/ml on Day 11 was 94.0%, and 92.9% of the anovulatory cows resumed cyclicity. Resumption of cyclicity in anovulatory cows was unaffected by treatment, BCS, or parity.

Pregnancy rates on Day 27 after TAI did not differ between control cows and cows treated with DESLORELIN 450 (Table 1). However, PR was lower for cows induced to

Table 1
Effect of a deslorelin implant in a timed AI protocol on fertility of lactating dairy cows

End point	Treatment ¹			P	
	Control	DESLORELIN 450	DESLORELIN 750	C1 ²	C2 ²
Pregnancy rate, Day 27 (%)	39.0 ^a	41.3 ^a	27.5 ^b	<0.19	<0.01
Pregnancy rate, Day 41 (%)	33.5 ^a	38.0 ^a	24.8 ^b	<0.49	<0.01
Pregnancy loss (%)	12.7 ^c	5.0 ^d	9.5 ^{cd}	<0.18	<0.36
Pregnancy rate second AI (%)	25.2 ^{ac}	16.7 ^d	9.6 ^b	<0.01	<0.18
Progesterone Day 11 (ng/ml ± S.E.M.)	7.08 (± 0.33)	7.01 (± 0.44)	7.75 (± 0.38)	<0.42	<0.16

Superscripts (a, b) within the same row differ ($P < 0.05$); superscripts (c, d) within the same row tend to differ ($P < 0.13$).

¹ GnRH: 100 µg of gonadorelin; DESLORELIN 450: biodegradable implant containing 450 µg of deslorelin; DESLORELIN 750: biodegradable implant containing 750 µg of deslorelin.

² C1: control vs. deslorelin; C2: DESLORELIN 450 vs. DESLORELIN 750.

ovulate with DESLORELIN 750 compared with DESLORELIN 450 ($P < 0.01$) and with controls ($P < 0.05$). On Day 41 after TAI, PR for control and deslorelin were similar (32.4%; $P = 0.49$), but cows treated with DESLORELIN 750 had lower PR than DESLORELIN 450 ($P < 0.01$) and controls ($P < 0.05$). Primiparous cows had higher PR than multiparous cows on Days 27 (43.0 versus 30.3; $P = 0.08$) and 41 (39.4% versus 25.5%; $P = 0.04$) after TAI. Interestingly, cyclicity prior to TAI had no effect on PR of dairy cows ($P > 0.50$). At Day 27 (36.6% versus 37.3%) and Day 41 (32.4% versus 32.9%), cycling and anovulatory cows had similar PR.

Although pregnancy loss between Days 27 and 41 after TAI almost doubled for control cows (12.7%) compared to cows receiving deslorelin (6.9%), incorporation of a deslorelin implant to induce ovulation in the Ovsynch protocol did not affect pregnancy loss ($P = 0.18$) during the respective period. However, pregnancy loss tended to decrease for cows receiving the DESLORELIN 450 compared to cows in the control group ($P = 0.13$).

Plasma progesterone concentrations during mid-cycle after TAI did not differ for control and deslorelin cows (7.08 ng/ml versus 7.44 ng/ml; $P < 0.34$). Although a similar proportion of cycling and anovulatory cows had plasma progesterone on Day 11 after TAI >1.5 ng/ml, anovulatory cows prior to TAI had higher concentrations of plasma progesterone than cycling cows (7.86 ng/ml versus 6.67 ng/ml; $P = 0.02$). In addition to cyclicity, BCS at TAI also affected mid-cycle concentrations of plasma progesterone. Cows in higher BCS also had higher plasma progesterone on Day 11, with a steady increase as BCS changed from low to medium to high (6.40 ng/ml versus 7.59 ng/ml versus 7.84 ng/ml; $P = 0.03$). Plasma progesterone concentrations on Day 11 after TAI affected PR of dairy cows on Day 27 ($P < 0.001$), and pregnant cows had higher plasma progesterone than cows diagnosed non-pregnant (8.68 ng/ml versus 6.51 ng/ml; $P < 0.001$).

During the re-synchronization period in cows diagnosed as non-pregnant to the first TAI, PR decreased in cows receiving deslorelin compared to controls (12.8% versus 25.2%; $P < 0.01$). Similarly, cows receiving the higher implant dose had lower re-synchronization PR than those in the control and DESLORELIN 450 combined (9.6% versus 22.3%; $P < 0.01$).

Body condition score at first postpartum AI tended to affect re-synchronization PR, with a gradual increase in PR as BCS changed from low to medium to high (11.5% versus 16.1% versus 25.0%; $P = 0.12$). Plasma progesterone on Day 11 after the TAI also tended to affect PR during the re-synchronized cycle and cows with plasma concentrations of progesterone equal to or greater than 1.5 ng/ml had higher PR than those with progesterone below 1.5 ng/ml (19.4% versus 10.7%; $P = 0.11$).

In cows diagnosed as non-pregnant to the first TAI and not re-inseminated by Day 27, follicular population was altered by treatment (Table 2). Deslorelin reduced the population of medium and large follicles and increased the number of follicles <5 mm in diameter. The proportion of cows with a follicle ≥ 10 mm on Day 27 after the initial TAI was higher for control cows than for cows induced to ovulate with deslorelin ($P < 0.001$). Similarly, the proportion of non-pregnant and not re-inseminated cows with a CL on Day 27 was lower for deslorelin than controls ($P < 0.001$). The inhibitory effect of deslorelin on the proportion of non-pregnant cows with a large follicle or a CL was more

Table 2

Effect of a deslorelin implant in a timed AI on ovarian activity of dairy cows diagnosed non-pregnant on Day 27 after AI

End point ¹	Treatment ²			P	
	GnRH	DESLORELIN 450	DESLORELIN 750	C1 ³	C2 ³
Number of follicles (mean ± S.E.M.)					
<5 mm	8.13 ^a (± 0.77)	11.08 ^b (± 1.06)	10.62 ^b (± 0.74)	<0.001	<0.68
5–9 mm	2.10 ^a (± 0.18)	0.89 ^b (± 0.24)	0.94 ^b (± 0.17)	<0.001	<0.83
10–19 mm	1.27 ^a (± 0.12)	0.58 ^b (± 0.16)	0.58 ^b (± 0.11)	<0.001	<0.97
≥20 mm	0.21 ^d (± 0.07)	0.15 (± 0.09)	0.07 ^e (± 0.06)	<0.12	<0.10
Follicle ≥ 10 mm (%)	83.1 ^a	60.2 ^{bf}	45.9 ^{bg}	<0.001	<0.09
Corpus luteum (%)	72.9 ^a	60.2 ^c	49.5 ^{bf}	<0.001	<0.10
Ovarian activity (%)	92.2 ^a	88.0 ^c	73.4 ^{bd}	<0.002	<0.03
Re-inseminated <27 days (%)	50.6 ^a	27.3 ^b	11.6 ^c	<0.001	<0.001
Re-insemination interval (days)	22.2 ^a (± 0.9)	26.8 ^b (± 1.2)	25.9 ^b (± 1.5)	<0.001	<0.60

Superscripts (a–c) within the same row differ ($P < 0.01$); superscripts (d, e) within the same row differ ($P < 0.05$); superscripts (f, g) within the same row tend to differ ($P < 0.15$).

¹ Ovarian activity: presence of a CL or a follicle greater than 10 mm; re-insemination interval: interval between the first and second AI for those cows re-inseminated prior to pregnancy diagnosis on Day 27 after the initial AI.

² GnRH: 100 µg of gonadorelin; DESLORELIN 450: biodegradable implant containing 450 µg of deslorelin; DESLORELIN 750: biodegradable implant containing 750 µg of deslorelin.

³ C1: control vs. deslorelin; C2: DESLORELIN 450 vs. DESLORELIN 750.

pronounced for DESLORELIN 750 than for DESLORELIN 450. The proportion of cows with ovarian activity, as indicated by the presence of a follicle ≥ 10 mm or by the presence of a CL, in cows treated with deslorelin was dose-dependent. Fewer cows treated with DESLORELIN 750 than those treated with DESLORELIN 450 had ovarian activity on Day 27 ($P < 0.05$).

A greater proportion of non-pregnant cows were re-inseminated prior to Day 27 in the control group compared to cows induced to ovulate with deslorelin (50.6% versus 18.5%; $P < 0.001$), with a more exacerbated effect of the higher dose of deslorelin ($P < 0.01$). Furthermore, re-insemination interval in non-pregnant cows prior to Day 27 after the initial TAI was longer for cows induced to ovulate with deslorelin than with gonadorelin ($P < 0.001$).

On Day 7 of the re-synchronization period (Table 3), at the moment of the PGF_{2α} injection, a lower proportion of deslorelin cows had a follicle with a diameter ≥ 10 mm compared to control cows (72.3% versus 88.6%; $P < 0.01$); the negative effect of deslorelin on the presence of a large follicle tended to be more pronounced in cows initially treated with the higher dose deslorelin ($P = 0.07$). Similarly, deslorelin also reduced the proportion of cows with a CL 7 days after the GnRH treatment, as well as ovarian activity, which was the proportion of cows on Day 7 of the re-synchronization with either a CL or a follicle > 10 mm. The diameter of the largest follicle when PGF_{2α} was given tended to be smaller for cows treated with deslorelin compared to controls, but no difference was observed for the different deslorelin doses.

Table 3

Effect of a deslorelin implant in a timed AI protocol on ovarian activity 7 days after re-synchronization with GnRH on Day 27 after the initial timed insemination

End point ¹	Treatment ²			P	
	GnRH	DESLORELIN 450	DESLORELIN 750	C1 ³	C2 ³
Follicle ≥ 10 mm (%)	88.6 ^a	83.3 ^c	66.3 ^{bf}	<0.01	<0.07
Corpus luteum (%)	75.7 ^{ac}	55.1 ^d	60.9 ^b	<0.01	<0.86
Ovarian activity (%)	96.0 ^c	91.8	84.8 ^d	<0.03	<0.26
Response to GnRH (%)	76.9	23.5	39.6	<0.001	<0.34
Size of largest follicle (mm \pm S.E.M.)	13.5 ^c (\pm 1.1)	12.2 (\pm 1.4)	11.7 ^f (\pm 0.9)	<0.15	<0.73

Superscripts (a, b) within the same row differ ($P < 0.01$); superscripts (c, d) within the same row differ ($P < 0.05$); superscripts (e, f) within the same row tend to differ ($P < 0.15$).

¹ Ovarian activity: presence of a CL or a follicle greater than 10 mm; response to GnRH: cows that had no CL on Day 27 when gonadorelin was given and had a CL on Day 34.

² GnRH: 100 μ g of gonadorelin; DESLORELIN 450: biodegradable implant containing 450 μ g of deslorelin; DESLORELIN 750: biodegradable implant containing 750 μ g of deslorelin.

³ C1: control vs. deslorelin; C2: DESLORELIN 450 vs. DESLORELIN 750.

4. Discussion

The present study was designed to determine the effects of replacing gonadorelin with a GnRH agonist implant (deslorelin) to induce ovulation in the Ovsynch/TAI protocol on PR, pregnancy loss, luteal function, and follicular development in lactating dairy cows.

The incidence of anovulatory cows during the first 58 DIM was similar between treatments and the higher incidence in primiparous than multiparous cows has been reported previously [2–4]. Incidence of anovulatory cows during the first 58 DIM was variable and was probably affected by herd, season of the year, level of milk production, nutrition, and BCS in the early postpartum period, among other factors. Clearly, a relationship between BCS at 68 DIM and frequency of anovulatory cows in the first 2 months postpartum has been established. Moreira et al. [4] and Santos et al. [3] also indicated that the frequency of anovulatory cows decreased as BCS increased prior to the first postpartum AI. Body condition score reflects the energy reserves of dairy cows [13]; cows in better energy status have earlier resumption of ovarian function and higher fertility.

Some GnRH agonist have increased binding affinity to GnRH receptors and a longer half-life [20]. The response of cattle to treatment with deslorelin involves an acute and chronic phase [10]. The acute phase lasts for several days, which increases the secretion of gonadotropins. When compared to buserelin, deslorelin induces a greater secretion of LH [8], which might improve CL formation, resulting in higher mid-luteal phase progesterone concentrations when compared with a Buserelin injection [7].

Different concentrations of deslorelin have been used successfully to induce ovulation in non-lactating dairy cows and dairy heifers when incorporated into the Ovsynch protocol [7,11]. Treatment with deslorelin at doses of 450–750 μ g increased plasma progesterone concentrations after Day 10 of the estrous cycle and suppressed follicle development for variable intervals [7,11]. Plasma progesterone concentrations during mid-diestrus were not

affected by treatment and there was no evidence for a deslorelin-induced decrease in luteal phase progesterone concentrations.

Pregnancy rates determined on Days 27 and 41 after TAI did not differ between cows in the control and DESLORELIN 450 groups, but it was lower for cows receiving DESLORELIN 750. It is not clear why the higher dose deslorelin suppressed fertility in lactating dairy cows. When Bartolome et al. [11] treated non-lactating dairy cows with a deslorelin implant containing either 750 or 1000 μg , all cows ovulated and formed a CL after treatment, and they had similar, or even higher, plasma concentrations of progesterone when compared to an injection of 100 μg of gonadorelin. Therefore, timing of ovulation and progesterone support are not expected to be the cause for the lower PR in cows treated with DESLORELIN 750. Higher concentrations of deslorelin are expected to prolong the chronic phase of the GnRH agonist effect on the gonadotrophs, which should result in a longer period of suppressed follicular development [10]. Perhaps more prolonged suppression of gonadotropin support from the pituitary when higher doses of deslorelin are used affects establishment of pregnancy, although no clear mechanism is known. Previously, non-lactating dairy cows were treated with either buserelin or deslorelin and more deslorelin cows became pregnant, although the number of animals was extremely limited for assessment of pregnancy data [7].

Pregnancy losses between 27 and 41 days after TAI were similar for control and deslorelin cows, but cows in the DESLORELIN 450 treatment tended to have lower pregnancy losses than cows treated with gonadorelin ($P = 0.13$). Plasma progesterone concentrations during mid-diestrus did not differ between DESLORELIN 450 and control cows, and progesterone concentrations on Day 11 did not affect pregnancy losses between Days 27 and 41 after TAI ($P = 0.28$). However, treatment with deslorelin attenuated follicular growth and the trend of reduced pregnancy losses for the DESLORELIN 450 supported the concept that follicular development during the period of early embryo development may adversely affect embryo survival.

During diestrus, the stimulation caused by the rise in progesterone re-establishes the endometrial lipid pool. In the absence of a viable embryo during the period of maternal recognition of pregnancy, the endometrium, under stimulation of estradiol and oxytocin, produces $\text{PGF}_{2\alpha}$, which is responsible for the lysis of the CL and termination of pregnancy or re-initiation of a new estrous cycle [5]. We speculate that suppressed follicular development as caused by deslorelin [11] is expected to result in a lower estrogenic environment during the early stages of pregnancy, which might reduce the ability of the endometrium to secrete $\text{PGF}_{2\alpha}$ and improve embryo survival.

When pregnant cows on Day 27 after TAI were either left as controls ($n = 90$) or treated with a deslorelin implant ($n = 89$), Bartolome et al. [21] observed that the number of CL and the concentration of progesterone on Day 45 was increased, at the same time that follicular population was reduced, but pregnancy losses between Days 27 and 45 and between Days 27 and 90 after insemination were not affected. Thus, late treatment with deslorelin did not alter pregnancy losses, but when cows were exposed to a low dose of deslorelin during the early stages of embryonic development and during the period when the CL is maintained for pregnancy, late pregnancy losses may have been attenuated as observed in the current study. Collectively, these studies suggest that there might be a period of sensitivity to attenuation of follicular growth on pregnancy losses.

The ability of deslorelin to suppress follicular growth in cows and heifers has been demonstrated previously [7,8,11]. When given during the early postpartum period, deslorelin attenuated resumption of cyclicity in lactating dairy cows [22,23]. Similarly, cows in different stages of the lactation cycle also have suppressed follicular development after treatment with deslorelin [11]. When non-lactating cows were treated with a biodegradable deslorelin implant containing either 750 or 1000 μg or with a gonadorelin product as the last injection of GnRH in the Ovsynch protocol, the first wave dominant follicle was smaller, emergence of the second follicular wave was not altered, and the largest follicle during the second follicular wave also was reduced in size during the first 16 days after treatment for the deslorelin cows compared to cows treated with gonadorelin [11]. The largest follicle during the second wave of follicular development did not grow to a diameter >7 mm in cows treated with deslorelin, compared to approximately 15 mm on Day 16 after treatment in cows that received gonadorelin.

The suppressive effect of deslorelin on follicle development was observed based on the lower proportion of non-pregnant cows on Day 27 after TAI that had a follicle with a diameter ≥ 10 mm, and by the reduced follicle population greater than 5 mm. The effect of deslorelin is dose-dependent [10]; a more pronounced effect on the proportion of non-pregnant cows with either a follicle greater than 10 mm or a CL on Day 27 was detected for DESLORELIN 750 than DESLORELIN 450. The suppressive effect of GnRH agonist on follicular development is related to the chronic phase of GnRH stimulation. Chronic exposure to GnRH, as observed when deslorelin implant is given, down-regulates LH and FSH beta subunits gene expression and reduces mRNA encoding the genes for LH transcription [24]. The down-regulation of GnRH receptors in the pituitary gonadotrophs creates a period of GnRH insensitivity [10]. This transient state of insensitivity to GnRH decreases the gonadotropin support for follicle growth and maturation.

Due to the suppressive effect of deslorelin on follicle development, re-synchronization of non-pregnant cows with a protocol that resumes follicular growth and induces ovulation at the moment of insemination is very important. We re-synchronized non-pregnant cows that had not been re-inseminated by Day 27 after the initial TAI with the Ovsynch protocol using only a gonadorelin product. A deslorelin implant reduced the response to GnRH on Day 27, based on detection of a new CL on Day 34 in cows that did not have a CL at gonadorelin injection. This effect is probably related to the uncoupling of GnRH receptors when the gonadotrophs are still insensitive to GnRH stimulation.

When lactating cows were treated with a deslorelin implant during the first week postpartum and challenged with GnRH on Day 14 after calving, the LH response to injection of GnRH was blocked [22], supporting the concept that deslorelin desensitizes the gonadotrophs. Furthermore, follicles need to reach a minimum size to express LH receptors in the granulosa cells [25] and to respond to an injection of LH [26]. The proportion of cows with a follicle >10 mm at the moment of GnRH injection on Day 27 after TAI was reduced for deslorelin compared to control, which would limit the ability of any LH surge to induce ovulation. Obviously, re-synchronization at Day 27 after TAI was not successful for those treated with deslorelin, based on the proportion of cows that responded to the first GnRH of the Ovsynch and the proportion of cows with a large follicle at the moment of the $\text{PGF}_{2\alpha}$ injection. All these factors should impair the ability of the last GnRH of the Ovsynch protocol to induce a synchronous ovulation of a healthy follicle.

The attenuation in follicular development and the lack of response to gonadorelin treatment during the re-synchronization period reduced PR to the second AI. Lack of ovulation and ovulation of a small incompetent follicle are possible reasons for the reduced fertility during the re-synchronization period. Vasconcelos et al. [27] demonstrated that reduction in the size of the ovulatory follicle compromised the ability of the resulting CL to sustain high concentrations of plasma progesterone and reduced PR. Although the means by which follicles did not reach adequate ovulatory size were different between the current study and the study reported by Vasconcelos et al. [27], perhaps part of the effect of deslorelin on reduced fertility during the second postpartum AI was due to a induced ovulation of a smaller ovulatory follicle that would compromise subsequent luteal function.

After cows underwent the Ovsynch protocol between 58 and 67 DIM, 94.0% of them had plasma progesterone concentrations ≥ 1.5 ng/ml. This indicates that a majority of the lactating cows in this study had progesterone concentrations compatible with the presence of a CL on Day 11 after TAI. Interestingly, on Day 27 after TAI, there were 34 cows treated with deslorelin that had luteal concentrations of progesterone (≥ 1.5 ng/ml) on Day 11 after TAI, but on Day 27 they were non-pregnant, had no CL, and only follicles < 9 mm in diameter. Although we did not ultrasound these cows throughout the postinsemination period, this response would indicate some cows underwent luteolysis in the absence of normal follicular development. Pritchard et al. [6] observed that high levels of estradiol during late diestrus reduce fertility. Large dominant follicles are more estrogenic than small subordinate follicles. It is suggested that the second wave dominant follicle in lactating cows that reach maximum size at approximately the same time the CL is maintained for pregnancy and raises circulating estradiol concentrations is partially responsible for stimulating the luteolytic cascade. However, luteolysis occurred even in the absence of large follicles during diestrus in cows treated with deslorelin [7,8]. Therefore, a lower estradiol threshold or an alternate pathway is responsible for triggering the luteolytic cascade in cows.

In conclusion, deslorelin induced the formation of a CL that resulted in normal mid-luteal phase plasma progesterone concentrations, decreased return to estrus in non-pregnant cows, reduced follicular population at Day 27 after treatment, and suppressed response to GnRH during re-synchronization of non-pregnant cows that had not been re-inseminated. Treatment with a DESLORELIN 450 resulted in similar PR to gonadorelin, but the higher-dose deslorelin impaired fertility. However, DESLORELIN 450 tended to reduce late embryonic losses compared to gonadorelin, which warrants further investigation. Nevertheless, additional studies should determine the ideal dose of deslorelin to minimize the carry over effect of the chronic stimulation by the GnRH agonist on suppressed fertility during the subsequent insemination.

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References

- [1] Pursley JR, Kosorok MR, Wiltbank MC. Reproductive management of lactating dairy cows using synchronization of ovulation. *J Dairy Sci* 1997;80:301–6.
- [2] Stevenson JS. Reproductive management of dairy cows in high milk-producing herds. *J Dairy Sci* 2001;84(E Suppl):E128–43.
- [3] Santos JEP, Juchem SO, Cerri RLA, Galvão KN, Chebel R, Thatcher WW, et al. Effect of bST and reproductive management on reproductive and lactational performance of Holstein cows. *J Dairy Sci* 2003;86, in press.
- [4] Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci* 2001;84:1646–59.
- [5] Thatcher WW, Guzeloglu A, Meikle A, Kamimura S, Bilby T, Kowalski AA, Badinga L, Pershing R, Bartolome J, Santos JEP. Regulation of embryo survival in cattle. *Reproduction* 2003;(Suppl. 61):253–66.
- [6] Pritchard JY, Schrick FN, Inskeep EK. Relationship of pregnancy rate to peripheral concentrations of progesterone and estradiol in beef cows. *Theriogenology* 1994;42:247–59.
- [7] Ambrose JD, Pires MFA, Moreira F, Diaz T, Binelli M, Thatcher WW. Influence of deslorelin (GnRH-agonist) implant on plasma progesterone, first wave dominant follicle and pregnancy in dairy cattle. *Theriogenology* 1998;50:1157–70.
- [8] Rajamahendran R, Ambrose JD, Schmitt EJ, Thatcher MJ, Thatcher WW. Effects of buserelein injection and deslorelin (GnRH-agonist) implants on plasma progesterone, LH, accessory CL formation, follicle and corpus luteum dynamics in Holstein cows. *Theriogenology* 1998;50:1141–55.
- [9] Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001;121:175–80.
- [10] D'Occhio MJ, Fordyce G, Whyte TR, Aspden WJ, Trigg TE. Reproductive responses of cattle to GnRH agonists. *Anim Reprod Sci* 2000;60/61:433–42.
- [11] Bartolome JA, Santos JEP, Pancarci SM, Melendez P, Arteché ACM, Hernandez O, et al. Induction of ovulation in nonlactating dairy cows and heifers using different doses of a deslorelin implant. *Theriogenology* 2004;61:407–19.
- [12] NRC. Nutrient requirements of dairy cattle. 7th rev. ed. Washington, DC: Natl Acad Sci USA; 2001.
- [13] Ferguson JD, Galligan DT, Thomsen N. Principal descriptors of body condition score in Holstein cows. *J Dairy Sci* 1994;77:2695–703.
- [14] Macmillan KL, Taufa VK, Barnes DR, Day AM, Henry R. Detecting estrus in synchronized heifers using tailpaint and aerosol raddle. *Theriogenology* 1988;30:1099–114.
- [15] Knickerbocker JJ, Thatcher WW, Bazer FW, Drost M, Barron DH, Fincher KB, et al. Proteins secreted by Day 16 to 18 conceptuses extend corpus luteum function in cows. *J Reprod Fertil* 1986;77:381–91.
- [16] Kuehl R. Complete block designs. In: *Statistical principles of research design and analysis*. Belmont, CA: Wadsworth Publishing Co.; 1994. p. 256–305.
- [17] SAS. SAS/STAT[®] User's guide (release 8.2). Cary, NC: SAS Inst. Inc.; 2001.
- [18] Allison, PD. Logistic regression using the SAS[®] system: theory and application. Cary, NC: SAS Institute Inc.; 1999.
- [19] MINITAB. Reference manual release 13.32. State College, PA: MINITAB Inc.; 2000.
- [20] Karten MJ, Rivier JE. Gonadotropin-releasing hormone analog design. Structure–function studies towards the development of agonists and antagonists: rationale perspectives. *Endocr Rev* 1986;7:44–66.
- [21] Bartolome JA, Kamimura S, Silvestre F, Arteché ACM, Bilby TR, Archbald LF, et al. The use of a deslorelin implant during the late embryonic period to enhance embryo survival. *J Dairy Sci* 2002;85(Suppl 1):301 [abstract].
- [22] Mattos R, Orlandi C, Williams J, Staples CR, Trigg T, Thatcher WW. Effect of an implant containing the GnRH agonist deslorelin on secretion of LH, ovarian activity and milk yield of postpartum dairy cows. *Theriogenology* 2001;56:371–86.
- [23] Padula AM, Macmillan KL. Reproductive responses of early postpartum dairy cattle to continuous treatment with a GnRH agonist (deslorelin) for 28 days to delay resumption of ovulation. *Anim Reprod Sci* 2002;70:23–36.

- [24] Anderson L. Intracellular mechanisms triggering gonadotrophin secretion. *Rev Reprod* 1996;1:193–202.
- [25] Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot F. Follicle selection in monovular species. *Biol Reprod* 2001;65:638–47.
- [26] Sartori R, Fricke PM, Ferreira JC, Ginther OJ, Wiltbank MC. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol Reprod* 2001;65:1403–9.
- [27] Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 2001;56:307–14.