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Effects of administration of gonadotropin-releasing hormone at artificial insemination on conception rates in dairy cows

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ABSTRACT

A controlled trial investigating the effect on conception of administration of 250 μg of gonadotropin-releasing hormone (GnRH) at artificial insemination (AI) in dairy cows in seasonal or split calving herds was conducted. Time of detection of estrus, body condition, extent of estrous expression, treatment, breed, age and milk production from the most recent herd test of the current lactation was recorded. Cows were tested for pregnancy with fetal aging between 35 and 135 days after AI. Sixteen herds provided 2344 spring-calved cows and 3007 inseminations. Logistic regression adjusting for clustering at herd level was used to examine the effect of treatment for first (2344) and second (579) inseminations separately. For first AI, treatment significantly improved conception rate in cows with milk protein concentrations of 3.75% or greater and for cows with milk protein concentrations between 3.00% and 3.50% and less than 40 days calved; increased conception rate from 41.2% to 53.4%. Treatment reduced conception rates in cows with milk protein concentrations of 2.75% or less. Treating only cows identified as responding positively to treatment (11% of all study cows) was estimated to increase first service conception rate in herds from 48.1% to 49.4%. There was no significant effect of treatment on conception to second AI, nor any significant interactions. These findings indicate that GnRH at AI should be limited to the sub-group cows most likely to respond. The positive effect of GnRH at AI may be mediated through improved oocyte maturation and/or improved luteal function, rather than by reducing AI-to-ovulation intervals.

1. Introduction

Conception rates in dairy cows have decreased over the last three decades associated with physiological and endocrine changes (Butler, 2000; Wiltbank et al., 2006), and with increases in genetic merit, altered management practices and increased milk yields. The uncoupling of the somatotropic axis from the gonadotropic axis in high-producing dairy cows, especially in early lactation, allows the cow to partition nutrients and mobilize body tissue for milk production; this partitioning, however, can be to the detriment of reproduction (Chagas et al., 2007; Lucy, 2001). In addition, hypothalamo-pituitary-gonadal axis functions may be directly suppressed in the postpartum period by negative energy balance (Butler, 2000) and endogenous opioids (Williams, 1990). Importantly, high-producing cows can metabolize sex-steroids more rapidly in comparison to cows operating at a lesser metabolic rate (Wiltbank et al., 2006). All of these influences can result in reduced gonadotropin release during the pre-ovulatory

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period, providing a mechanism for reduced estrous expression, delayed ovarian follicle maturation, delayed oocyte maturation, delayed ovulation relative to onset of estrus and compromised corpus luteum function.

GnRH analogs have been administered around the time of insemination to enhance oocyte maturation and induce ovulation (Mee et al., 1990; Thatcher et al., 1993), but effects on conception rates are inconsistent. A meta-analysis conducted about two decades ago of 40 trials reported in 27 papers, GnRH or analogs at the time of insemination on average increased conception rate. Across all studies, pooled treatment resulted in a 12.5% increase in probability of pregnancy and this increased to 22.5% for repeat-breeder cows (cows with three or more AIs). Responses to GnRH were greater with larger doses (>250 μg rather than <125 μg) (Morgan and Lean, 1993). The authors recommended further research to better characterize the population of cows likely to respond favorably to GnRH administration at AI. If the subset of cows most likely to respond positively to GnRH administration could be identified at AI, treatment could be restricted to these cows, potentially increasing the cost-benefit ratio of GnRH treatment.

The current study evaluated the effect of administration of GnRH at AI on conception rate in commercial dairy herds, and hypothesized interactions between treatment and potential risk factors for uncoupling of the somatotropic and gonadotropic axes.

2. Materials and methods

2.1. Cattle management

A controlled trial was undertaken in spring 2012 in 16 seasonal or split calving dairy herds in the Macalister Irrigation Area, East Gippsland, Victoria, Australia. Seasonal and split calving systems predominate in south-eastern Australia. Seasonal systems use a restricted breeding period characterized by set dates for the start and cessation of matings for all cows in the herd. Inseminations and natural services are withheld from all cows until the start of mating (the mating start date). From mating start date onwards all estrus cows are inseminated regardless of their time since calving. Thus, each cow's calving to mating start date interval is her voluntary waiting period. Accordingly, voluntary waiting periods vary between cows within herds. The mating period typically begins in most herds with a period in which only AI is used, followed by a period when bulls are run with the milking herd. All cows detected in estrus during the AI period are inseminated. To ensure sufficient AI-sired heifer replacement calves are born each year approximately half of the cows must become pregnant to AI. This requires an AI period of between 4 and 8 weeks in most herds. Split-calving systems have more than one calving and mating periods (typically two calving periods, one each in spring and autumn). Cows calving in each group are managed as described above for seasonal calving herds, except that cows failing to conceive during the first mating period after their calving may be inseminated at observed estrus or mated by bulls during the next breeding period.

Herds were selected by convenience sampling from those that were seasonal (spring) calving or split calving with a spring calving group, had a numerical cow identification system, used computerized herd records, and, for the spring mating period, planned to use professional AI technicians with all inseminations each day performed at one time immediately after either the morning or afternoon milking, to undertake milk recording, and to use early pregnancy diagnosis with fetal aging, and the farm manager was willing to monitor the herd at least twice daily for cows in estrus and record time of first detection of estrus.

To be eligible, cows were required to have clear and unique numerical identification, to have calved either during the spring calving period in 2012 or, in split calving herds, during the previous autumn calving period in 2012 but not have conceived during the autumn mating period, and have no recorded reproductive disease or treatment in the 60 days preceding mating start date in spring 2012. Cows treated with either progesterone and/or GnRH between calving and first AI were ineligible for enrolment. All eligible cows within study herds were enrolled in the study.

Inseminations at estrus events where the cow received AI on consecutive days were ineligible for enrolment. Estrus periods induced using prostaglandin alone were eligible. All other inseminations of study cows within each herd's AI period were eligible and enrolled for the study.

The unit of allocation was the individual cow. Each cow was allocated into one of two groups based on whether the last digit of their identification number was an odd or even number. One of these groups was then randomly selected to receive treatment, the remaining group was the control group; cows with odd identification numbers were assigned to the treatment group. Assignment to treatment was at the cow level to ensure that any cow failing to conceive to first AI and subsequently returning to estrus would receive the same treatment for all subsequent AIs.

From each herd's mating start date, farm staff recorded the time of first detection of estrus for each insemination. Cows first detected in estrus at or immediately before the milking preceding insemination were categorized as "early AI". Cows first detected in estrus at or before the preceding milking were categorized as "late AI". The extent of estrous expression was recorded at the time of AI using a 1–3 scale (1 – weak, 2 – normal, 3 – strong) based on the farm staff's assessment of the cow's estrous behavior. Four experienced professional technicians performed all artificial inseminations across the 16 herds.

Cows in the treatment group received 250 μg gonadorelin (Ovurelin; Bayer Australia Ltd. (Animal Health); Pymble NSW) by deep intramuscular injection administered at the time of AI. Cows in the control group were not treated; no placebo treatment was used. Treatment status of all cows at each insemination was individually recorded. The AI technician classified the body condition of each cow as either below average for the district, or at or above average for the district. Herd, cow, and AI technician identities, and date and time of the insemination visit were also recorded. Cow breed, date of birth, most recent calving date, AI number for each cow for the study mating period, and milk production data (24-h milk volume and fat and protein yields for the cow's current lactation at the most recent milk recording preceding each AI (mean = 23.8 days and SD = 14.5 days before AI) were obtained electronically from herd management software.

In all herds, either an experienced veterinarian or technician undertook pregnancy diagnosis with fetal aging using trans-rectal ultrasonography. Pregnancy diagnosis was conducted at multiple visits to each herd, timed to ensure that all cows were tested for pregnancy at least once between 35 and 135 days after each study AI. For cows diagnosed pregnant, conception date was estimated based on physical examination findings and the cow's AI dates. Bull services were not recorded. Pregnancy diagnosis data were entered into herd management software.

2.2. Data management and statistical analyses

Insemination docket data were manually entered into an Access database (Microsoft Corporation, 2010) and merged with data exported from the herd management software program. Data were exported into R 3.0.1 (R Core Team, 2012) and also into Stata (StataCorp, 2011) for statistical analysis. The AIs were classified as resulting in conception if the cow's conception date was the same as the date of AI; all other AIs were classified as not resulting in conception. Each cow's breed was categorized as either Holstein-Friesian (75% or more Holstein-Friesian parentage) or other. Cows were classified as 'carryover' cows (cows that did not calve in spring 2012 but had calved previously) if they had calved more than 200 days before their first AI. Age at calving was calculated in years as the number of months of age divided by 12 rounded to the nearest whole number. Milk fat and protein concentrations were calculated on a weight/volume basis as, respectively, fat and protein yield divided by milk volume, and expressed as g/100 mL or 'percentages'.

The *a priori* hypotheses were that GnRH treatment would increase conception rates amongst cows at a greater risk of uncoupling of the somatotropic and gonadotropic axes. Factors influencing this risk include: breed (Berry et al., 2003), age at calving (Royal et al., 2002), calving to AI interval (Royal et al., 2002), body condition score (Butler and Smith, 1989), extent of estrous expression (Dransfield et al., 1998) and milk production (Buckley et al., 2003) in early lactation cows.

Effects of treatment on the binary outcome of conception to AI were analyzed using generalized linear mixed logistic models, with herd fitted as a random effect to control for clustering of the outcome within herds. Separate analyses were performed for first and second study insemination. Analyses were restricted to inseminations of cows calved during the spring calving period in 2012; inseminations of cows during the previous autumn calving period in 2012 were excluded.

The effect of treatment across all cows was assessed by fitting treatment in univariable models. Hypothesized interactions between treatment and potential interacting factors were examined using separate models for each risk factor. Continuous variables were assessed for linear relationships with the logit of conceived to AI by categorizing into equally spaced groups and assessing plots of logits *versus* median value for the risk factor for each group and by assessing plots of predicted logits after fitting fractional polynomials. Variables that did not display linearity in the logit were converted into categorical variables. For each potential risk factor, the main effects of both treatment and the factor were fitted along with the interaction term(s). Likelihood ratio test *P*-values for the interaction term (or for risk factors with more than 2 categories, the joint likelihood ratio test *P*-value for all interaction terms) were used. The 95% confidence intervals and Wald *P*-values were reported for the estimated effects of treatment.

For first AIs significant two-way interactions were detected between treatment and each of two potential risk factors (one categorical data and one continuous data). Main effects of treatment, these factors and both two-way interactions were simultaneously fitted as the final model. The three-way interaction was tested using the likelihood ratio test. This was not significant and the three-way interaction was not retained in the model.

Predicted conception rates for treated and control cows at each level of the categorical variable and from example values across the range of the continuous variable were determined from the final logistic model equation. Confidence intervals for predicted conception rates were calculated using the logistic model variances for each of the intercept, and main effect and interaction term coefficients and covariances between these.

3. Results

For the sixteen herds, maximum milking herd size during the study varied from 134 to 598 cows (mean 291). All herds grazed pasture during the study period, with grain-based supplements fed during milking. Approximately 44% of cows were Holstein-Friesian, 8% were Jersey and 48% crossbred. Herd mean 300-day milk yield for study lactations varied from 4243 to 7294 l per cow (mean 5910 l), 182 to 321 kg of fat per cow (mean 250 kg) and 143 to 245 kg of protein per cow (mean 198 kg). Mating start dates for the spring calving groups varied between herds from 01 October 2012 to 12 November 2012. AI period durations for the spring calving groups varied between herds from 28 to 62 days with a mean of 40 days. Thus, all inseminations were performed from October to December 2012. Estrous detection was performed at least twice daily using visual inspection of cows by the herd manager and key staff in all herds. Estrous mount detectors and tail paint were used in most herds. The herd manager recorded the time of first detection of estrus relative to the time of AI for each cow in estrus.

A total of 3508 cows (123–354 per herd) received eligible inseminations across the study period. There were 333 carryover cows and 831 other cows without an early pregnancy test. Removal of these cows left 2344 spring-calved cows (22–295 per herd) with early pregnancy test data yielding a total of 3007 study inseminations (29–344 per herd). A total of 1135 treated and 1209 control spring 2012 calved cows provided a first AI with 271 treated and 308 of these also providing a second AI during the study period.

Table 1
Distribution of first AIs by treatment status potential interacting factors.

Variable	TreatedNo. (%)	ControlNo. (%)
Breed		
Holstein-Friesian	485 (42.8)	527 (43.5)
Other	603 (53.3)	638 (52.6)
Not recorded	47 (3.9)	44 (3.9)
Age at calving (years)		
2	269 (23.7)	284 (23.5)
3	185 (16.3)	193 (16.0)
≥4	643 (56.7)	692 (57.2)
Not recorded	38 (3.3)	40 (3.3)
Calving to first AI (days)		
1–39	29 (2.6)	38 (3.1)
40–59	118 (10.4)	126 (10.4)
60–79	286 (25.3)	312 (25.7)
80–99	418 (36.9)	397 (32.8)
100–119	140 (12.4)	159 (13.1)
≥120	140 (12.4)	166 (13.7)
Calving date not recorded	4 (0.4)	11 (0.9)
Body condition score at first AI		
Below average	436 (38.5)	468 (38.6)
Average or above	697 (61.6)	740 (61.1)
Not recorded	2 (0.2)	1 (0.1)
Strength of estrus expression at first AI		
Weak	181 (16.0)	178 (14.7)
Normal	600 (53.0)	656 (54.1)
Strong	353 (31.2)	375 (30.9)
Not recorded	1 (0.1)	0 (0.0)
AI timing relative to onset of estrus		
Early AI	641 (56.5)	644 (53.3)
Late AI	494 (43.5)	565 (46.7)
Milk volume[a] (l/cow/day)		
Mean (SD)	27.7 (7.30)	27.8 (7.49)
Number	1571	1440
Milk fat concentration[a] (%)		
Mean (SD)	4.01 (0.07)	4.02 (0.07)
Number	1571	1440
Milk protein concentration[a] (%)		
Mean (SD)	3.27 (0.03)	3.25 (0.03)
Number	1571	1440

[a] For the cow's current lactation at her most recent milk recording preceding first AI; milk production data were not recorded before first AI for 275 and 297 treated and control cows, respectively.

Distributions of first AIs by potential interacting factors for treated and control cows are presented in Table 1. For all of these variables, distributions were similar for treatment and control groups.

Treatment was not a significant predictor of odds of conception at first AI on univariable analysis (odds ratio: 1.0; 95% CI: 0.9–1.2; Wald *P*-value = 0.75). *P*-values for interactions between treatment and each of breed, age at calving, body condition score, extent of estrous expression, AI timing relative to onset of estrus, milk volume, and milk fat concentration were moderately high to high; likelihood ratio *P*-values for the interaction term (or terms jointly) were 0.85, 0.55, 0.96, 0.73, 0.69, 0.49 and 0.78, respectively. Wald *P*-values for the effects of treatment at all levels of these risk factors were greater than 0.05.

The *P*-values for interactions between treatment and each of milk protein concentration and calving to AI interval were both 0.02. This latter interaction was almost entirely due to effects of treatment differing between cows that were less than 40 days postpartum and cows 40 or more days postpartum at the time of AI. The odds ratio for the effect of treatment for first AI's that occurred at less than 40 days after calving was 3.4 (95% CI 1.1 to 11.0; Wald *P*-value = 0.04). Odds ratio point estimates for the effects of treatment for cows with calving to first AI intervals of more than 40 days ranged between 0.7 and 1.1.

Based on these results, two-way interactions between treatment and each of calving to first AI interval class and milk protein concentration were fitted along with the main effects. The likelihood ratio test *P*-value for the three-way interaction terms jointly was high (0.67) so these were not retained. The *P*-values for the two-way interactions between treatment and each of calving to first AI interval and milk protein concentration were, respectively, 0.20 and 0.02. Calving to AI interval was retained in the final model due to the large odds ratio for the effect of treatment for first AIs that occurred at less than 40 days after calving (3.4; 95% CI 1.1–10.9; Wald *P*-value = 0.04). Estimates from this model are presented in Table 2. Of the 1135 treated and 1209 first AIs for control cows, 854 and 908 were included in the final model respectively. The AIs from 281 treated and 301 control cows were excluded, as the cow's calving date and/or milk protein concentration were not recorded.

Odds ratios for effects of treatment, and associated 95% confidence intervals and *P*-values at various combinations of milk protein concentration at the most recent milk recording preceding the AI and calving to first AI categories are presented in Table 3. The model estimated conception rates and associated 95% confidence intervals for the same combinations of milk protein concentration at the most recent milk recording preceding the AI and calving to first AI categories are presented in Fig. 1.

Treatment had a positive effect on first service conception rates in cows with milk protein concentrations at or above 3.75% at a recent milk recording, and in cows with milk protein concentrations between 3.00% and 3.50% provided their calving to first AI interval was less than 40 days. Treatment effect was minimal in cows with milk protein concentrations between 3.00% and 3.50% and calved 40 days or more at first AI. Treatment appeared to have a negative effect on first service conception rate in cows with recent milk protein percentages equal to or less than 2.75%.

Treatment was not significant for second AI after calving on univariable analysis (odds ratio: 0.73; 95% CI: 0.46–1.16; Wald *P* = 0.64). *P*-values for interactions between treatment and each of breed, age at calving, calving to AI interval, body condition score, strength of estrous expression, AI timing relative to onset of estrus, milk volume, milk fat concentration and milk protein concentration were moderately high to high. Likelihood ratio test *P*-values for the interaction terms (or terms jointly) were 0.89, 0.77, 0.65, 0.22, 0.17, 0.98, 0.94 and 0.54, respectively.

4. Discussion

GnRH administration at the time of AI is increasingly being adopted within seasonal calving dairy herds in Australia, and is generally administered without considering milk production or the physiological status of the cow. Administration of GnRH to every cow at the time of AI adds substantially to herd costs, and would not improve farm profit if it does not increase conception rates at least modestly. Profitability would be reduced if the overall effect of

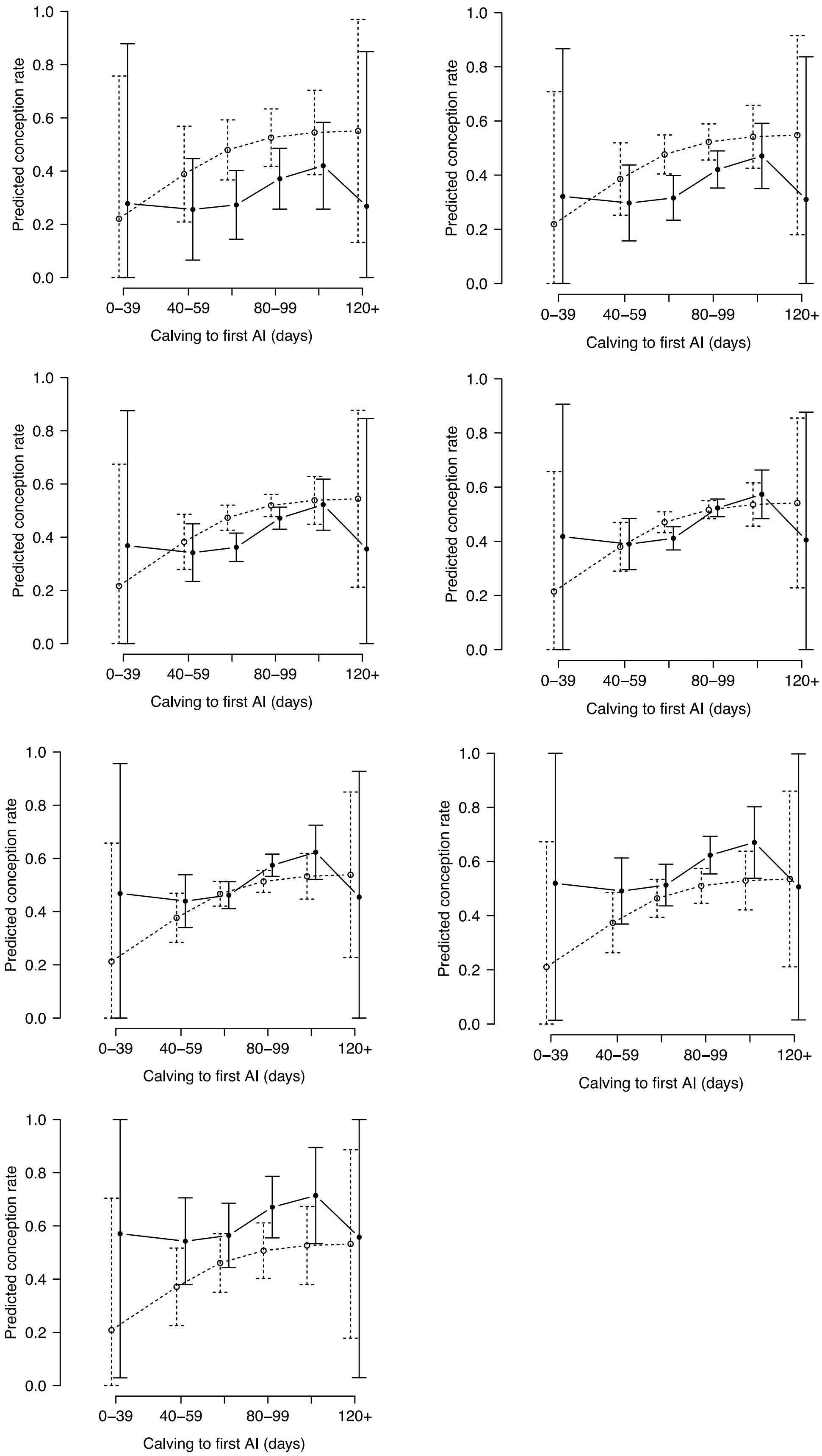

Fig. 1. Predicted first AI conception rates (proportions of cows conceiving to first AI) for treated (——▯— —) and control (—○—) cows at various milk protein percentages and calving to first AI interval categories.

Table 2
Final model for conception to first AI in dairy cows treated with GnRH at time of AI ('treated') or untreated ('control').

Term	Odds ratio	95%CI	*P*-value
Treatment status			
Control	Reference group		
Treated[a]	3.1	0.8–11.9	0.09
Calving to first AI interval			
1–39 days	Reference group		
40–59 days	2.2[b]	0.8–6.0	0.12
60–79 days	3.2	1.2–8.3	0.02
80–99 days	3.9	1.5–9.9	0.01
100–119 days	4.2	1.5–11.2	0.01
≥120 days	4.2	1.3–14.0	0.02
Treated * calving to first AI interaction			
Treated * 40–59 days	0.4[c]	0.1–1.6	0.20
Treated * 60–79 days	0.3	0.1–1.0	0.05
Treated * 80–99 days	0.3	0.1–1.3	0.11
Treated * 100–119 days	0.4	0.1–1.6	0.19
Treated * ≥120 days	0.2	0.0–1.2	0.08
Milk protein concentration	1.1[d]	0.6–2.1	0.65
Treated * milk protein concentration	2.8[e]	1.2–6.5	0.02

Milk protein concentration was centered at the overall mean value of 3.268%. Intercept estimate (estimated odds of conception for control cows at average milk protein concentration (3.268%) and calving to first AI of 1–39 days) was 0.3 (95% CI 0.1–0.7; $P = 0.01$).

[a] Estimated increase in odds of conception for treated cows relative to control cows at average milk protein concentration (3.268%) and calving to first AI of 1–39 days.

[b] Estimated increase in odds of conception in control cows for calving to first AI of 40–59 days compared to 1–39 days.

[c] The multiplicative change in the odds ratio for treatment relative to control on odds of conception for calving to AI intervals of 40–59 days relative to when the reference category of 1–39 days is used.

[d] Estimated increase in odds of conception for each 1% increase in milk protein concentration in control cows.

[e] The multiplicative change in effect of treatment (*i.e.* the change in the odds ratio for treatment relative to control) on odds of conception for each extra 1% increase in milk protein concentration.

GnRH in the herd is detrimental. Thus, the present study was designed to investigate whether GnRH administration at the time of AI improved conception rates, and to assess these effects in subsets of cows where administration at AI was potentially beneficial.

Findings from the present study do not support the administration of GnRH at AI to all cows. Based on the *a priori* hypotheses and findings from the present study, treatment at first AI is effective at increasing conception rate in cows with milk protein concentrations at or above 3.75% at a recent milk recording, and also in cows with milk protein concentrations between 3.00% and 3.50% provided their calving to first AI interval is less than 40 days. These findings do not support treatment of cows with recent milk protein concentrations between 3.00% and 3.50% when calving to first AI interval is 40 days or more. Treatment appears contra-indicated in cows with recent milk protein concentrations equal to or less than 2.75%.

In the present study 2086 cows had the calving date recorded and data from at least one postpartum milk recording before first AI. The statistical model for first service conception predicted an average first service conception rate for these 2086 cows of 48.1% if no cows were treated and 48.0% if all cows were treated. However, if treatment was limited to the responsive subgroups in the present study, a total of 222 cows (11% of cows), comprising 162 cows (8%) with a milk protein concentration at the most recent milk recording preceding the AI at or above 3.75% and 60 cows (3%) with recent milk protein concentrations between 3.00% and 3.50% that were 40 days or less postpartum at first AI, would be treated at first AI. The increase in first AI conception rate due to treatment in these 222 cows was estimated from the model at 12.2% (increasing from 41.2% to 53.4%). Therefore treating these 222 cows only is estimated to increase the first service conception rate for the herd from 48.1% to 49.4%; a net increase of 1.3%.

It appears that administration of GnRH at first AI may be ineffective or mildly detrimental to conception for most combinations of calving to AI interval and milk protein concentration at the most recent milk recording preceding the AI. Of the cows in the present study, 89% (1864 of the 2086 cows with a calving date and at least one postpartum milk record before AI for the current lactation) were in this category. If 89% of the cows were not treated the average conception rate to first service was estimated at 48.9% from the model (increased from 47.3% if all were treated). Therefore, if all cows are treated, the potentially small negative impact of treatment on conception rate in the majority of cows would effectively counterbalance the relatively large improvement in conception rate in the small subset of cows identified in the present study as benefiting from treatment.

Effects of treatment on conception rates may be because GnRH induces a pre-ovulatory-like surge release of LH. Increased LH concentration enhances oocyte maturation, induces ovulation and may enhance corpus luteum function (Thatcher et al., 1993). Within approximately 2 h after GnRH administration, LH pulse frequency and amplitude increase (Thatcher et al., 1993). However it is unlikely that any beneficial effects of GnRH at AI are due to reduced intervals from AI to ovulation. Times from the pre-ovulatory surge release of LH to ovulation appear to be, on average, between 27 and 30 h (Senger, 2012). Therefore, if ovulation is induced by the exogenous GnRH, the interval from GnRH administration to ovulation will be approximately

Table 3
Odds ratios for effects of treatment on conception to first AI, and associated confidence intervals and *P*-values at various combinations of milk protein concentration at the most recent milk recording preceding the AI and calving to first AI interval categories from final model.

Milk protein conc. (%)	Calving to first AI interval (days)	Odds ratio for treatment[a]	95% CI	*P*-value
2.50	1–39	1.4	0.3–5.9	0.68
	40–59	0.5	0.2–1.2	0.14
	60–79	0.4	0.2–0.8	0.01
	80–99	0.5	0.3–1.0	0.04
	100–119	0.6	0.3–1.3	0.19
	≥120	0.3	0.1–1.2	0.08
2.75	1–39	1.7	0.4–6.9	0.46
	40–59	0.7	0.3–1.3	0.26
	60–79	0.5	0.3–0.8	0.01
	80–99	0.7	0.4–1.1	0.08
	100–119	0.8	0.4–1.4	0.38
	≥120	0.4	0.1–1.4	0.13
3.00	1–39	2.1	0.5–8.2	0.28
	40–59	0.8	0.5–1.5	0.57
	60–79	0.6	0.4–0.9	0.02
	80–99	0.8	0.6–1.2	0.28
	100–119	0.9	0.5–1.6	0.82
	≥120	0.5	0.1–1.6	0.22
3.25	1–39	2.6	0.7–9.9	0.16
	40–59	1.0	0.6–1.8	0.88
	60–79	0.8	0.6–1.1	0.17
	80–99	1.0	0.8–1.4	0.86
	100–119	1.2	0.7–2.0	0.58
	≥120	0.6	0.2–1.9	0.37
3.50	1–39	3.3	0.9–12.4	0.08
	40–59	1.3	0.7–2.3	0.37
	60–79	1.0	0.7–1.4	0.91
	80–99	1.3	0.9–1.8	0.17
	100–119	1.5	0.8–2.6	0.20
	≥120	0.7	0.2–2.4	0.59
3.75	1–39	4.1	1.0–15.8	0.04
	40–59	1.6	0.9–3.1	0.14
	60–79	1.2	0.7–2.0	0.43
	80–99	1.6	1.0–2.5	0.05
	100–119	1.8	0.9–3.5	0.08
	≥120	0.9	0.3–3.1	0.85
4.00	1–39	5.0	1.2–20.7	0.02
	40–59	2.0	1.0–4.2	0.07
	60–79	1.5	0.8–2.8	0.20
	80–99	2.0	1.1–3.7	0.03
	100–119	2.2	1.0–4.9	0.04
	≥120	1.1	0.3–4.0	0.88

[a] Odds ratio for treatment relative to control.

30 h. Semen deposited at the time GnRH is administered would generally not survive 30 h. *In utero* longevity of the frozen-thawed sperm reservoir varies depending on the quality of the frozen semen. Although some high quality semen is documented as lasting up to 32 h (Hockey and Norman, 2006), longevity is more typically between 12 and 24 h (Roelofs, 2005). Accordingly, it seems likely that any beneficial effects of GnRH at AI are due to other mechanisms than impacts on the timing of the surge release of LH. However, a logical further study would be to investigate the effects of administering GnRH 10–15 h prior to insemination (at or near the onset of estrus) to coordinate ovulation more closely with sperm reservoir longevity.

Despite differences between studies in treatment protocols, the benefit of GnRH administration at the time of AI to early postpartum cows identified in the present study is consistent with findings from a previous study (Archbald et al., 1990), where significant benefits associated with GnRH administration approximately 40 days postpartum were found. The reasons for reduced fertility in early postpartum cows have been described (Archbald et al., 1990) and include failure of resumption of ovarian folliculogenesis, resumption of folliculogenesis but failure of ovulation, and resumption of folliculogenesis, occurrence of ovulation, but premature regression of the corpus luteum. These aberrations in ovarian function may be affected by nutritional and metabolic demands from 20 days postpartum as the cow progresses toward peak lactation (Butler, 2000). Accordingly, the finding that GnRH improves conception rates in early postpartum cows but has much less or no effects in cows calved 40 days or more may be because GnRH improves oocyte maturation or enhances luteal function in early postpartum cows. Reasons for the beneficial effects of GnRH administration at the time of AI in cows with high milk protein concentrations are unclear. The rapid metabolism of sex steroids, particularly estrogens, in high-producing cows (Wiltbank et al., 2006) may lead to suppressed pre-ovulatory LH release and impaired luteal function. This process may be mitigated by the administration of GnRH at AI, leading to enhanced luteal function. However, it is unlikely that cows with greater milk protein concentrations have adversely greater rates of metabolism of sex steroids. Amongst Holstein-Friesian cows, those with greater milk protein concentrations have markedly superior reproductive performance to those with lesser concentrations (Morton, 2004). As GnRH administration is beneficial in cows with greater milk protein concentrations, this suggests that GnRH can be effective when reproductive physiological function is near to optimal.

In seasonal and split calving herds, cows that are less than 60 days postpartum at mating start date have reduced conception rates and are at greater risk of late conception or failure to conceive and involuntary culling than cows calved more than 60 days postpartum. Therefore the identification of a useful and effective treatment for this at-risk group of cows is of potentially great benefit for seasonal and split calving dairy herds where the maintenance of compact calving and mating periods is becoming increasingly more difficult due to delayed conceptions. The administration of selective treatment to a small subset of cows requires an effective, efficient and integrated herd management system to minimize errors. Farmers and their veterinarians will need to assess the potential benefits of treatment against the cost and practicalities of implementation on their farm.

5. Conclusions

Under conditions such as those in the herds used in the present study, GnRH at first AI increases conception rates in cows with milk protein concentrations at or above 3.75% at a recent milk recording, and also in cows with milk protein concentrations between 3.00% and 3.50% provided the calving to first AI interval is less than 40 days. Treatment of other cows results in small or no increases in conception rates. Conception rates may be reduced by the administration of GnRH at AI in cows with milk protein concentrations equal to or less than 2.75%. These findings indicate that GnRH at AI should be used only in the sub-group cows most likely to respond, those with high milk protein concentrations or intermediate milk protein concentrations and less than 40 days postpartum.

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