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### The effect of early postpartum intervention on the reproductive performance of anovulatory anestrus New Zealand dairy cows

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#### Introduction

Due to the seasonal nature of New Zealand's dairy industry all cows within a herd commence breeding on the same calendar date regardless of their individual calving date (planned start of mating; PSM). This enables a concentrated calving pattern and the associated time of greatest energy demand to be aligned with the period of predicted peak pasture growth. Cows which have not resumed cyclical activity postpartum (pp) by PSM are termed anovulatory anoestrus (AA) and are a significant cause of reproductive wastage as a direct result of a decreased number of cows inseminated and reduced conception, 6 week-in-calf and pregnancy rates.<sup>1, 2</sup> In New Zealand dairy herds the proportion of AA cows ranges from 15 to 27%.<sup>1, 3-5</sup>

Assessment of all cows failing to display signs of estrus by PSM is carried out in the majority of well-managed herds via transrectal palpation of the ovaries. Identification of AA cows allows initiation of an appropriate treatment protocol aimed at inducing estrus and ovulation, thereby improving the overall reproductive efficiency of the herd.<sup>1, 5, 6</sup> The current "industry standard" is to wait at least 35 d pp to examine and treat AA cows.<sup>6</sup> This protocol therefore dictates that all cows in the herd that calve in the 5 weeks leading up to PSM are not eligible for AA treatment and breeding at PSM.

Another management technique aimed at concentrating the calving season is the induction of calving in cows with a calving date late in the season using long-acting corticosteroids. This has previously been commonplace in the seasonal New Zealand dairy industry, however the procedure was proscribed in New Zealand in 2014 on animal welfare grounds, resulting in an increased number of cows calving later in the season and an associated broadening of the calving period. Late calving cows are a significant source of economic loss as they are less likely to conceive by the end of mating, are more likely to conceive later in the mating period, produce less milk in the subsequent season and are less likely to remain in the herd for more than two consecutive seasons.<sup>1</sup> Therefore recent focus has been on additional tools to advance potential conception dates within herds, particularly focusing on late-calving cows.

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One potential method of advancing conception dates within a herd is to reduce the wait period pp before AA cows are eligible for treatment. Therefore the objective of the current study was to determine the reproductive performance of AA cows treated with intravaginal P4, eCG and GnRH as early as 14 to 35 d pp. Additionally, clinical and historical parameters were assessed pre-treatment to determine their influence on reproductive performance. A prospective cohort study was performed involving 9,630 cows in 24 herds. We hypothesized that early intervention and treatment of AA cows would improve their reproductive performance by increasing the number of cows available for breeding at PSM. Additionally, the examination of measurable clinical and historical parameters may be useful to predict the success of treatment, enabling immediate on-farm recommendations to be made.

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## Materials and Methods

**Animals:** 534 cows from 24 seasonally-calving, predominantly pasture-based dairy herds that were 14 to 35 d pp and not showing visible signs of estrus were examined at PSM. All cows presented had no history of dystocia or pp disease during the current season. Cows diagnosed with uterine pathology or the presence of a CL were excluded from the trial. 507 cows were confirmed via ultrasound examination as being truly AA. Breed representation was; Friesian ( $n = 149$ ), Jersey ( $n = 93$ ) and Friesian-Jersey cross ( $n = 265$ ). A pilot study was performed during the first study season. Due to all herds involved being commercial operations and the unknown potential outcomes of treatment or non-treatment, all cows in the pilot study were allocated into the treatment group (treated;  $n=231$ ). After attaining results of the pilot study, cows during the subsequent and second season were randomly assigned to the treatment group (treated;  $n=142$ ) or left untreated (control;  $n=134$ ).

**Clinical examination:** On initial examination, each cow was body condition scored on a scale of 1 (emaciated) to 10 (obese) and examined for the presence of vaginal exudate as an indication of endometritis using a Metrichheck™ device as previously described.<sup>7</sup>

**Ultrasound examination:** A transrectal ultrasound examination of the uterus and ovaries was performed. Combined uterine horn diameter was determined by the addition of the largest, cross-sectional diameter of each horn 4 cm cranial to the bifurcation of the uterine horns. The entire uterus was visualized to assess for the presence of intra- uterine fluid. Visualization of each ovary enabled calculation of ovarian diameter by averaging two perpendicular measurements of cross-sectional diameter.

**Treatment protocol:** An intravaginal controlled release progesterone device (Cue Mate 1.56 g w/w P4, Vetoquinol Australia, Brisbane, Australia) was inserted per vagina into treatment group animals on day 0. On Day 6, the intravaginal device was removed and cows administered 400 IU eCG IM (Pregnecol, Vetoquinol Australia, Brisbane, Australia). On day 8, 100 ug GnRH (Ovurelin, Bayer New Zealand Ltd., Auckland, New Zealand) was administered IM. Fixed-time artificial insemination (FTAI) with frozen semen was performed 16 to 20 h following GnRH administration. All inseminations were performed by experienced artificial insemination (AI) technicians. Detection of estrus and AI continued for 4 to 6 weeks following FTAI. Thereafter, herd sires were used for natural service for 3 to 5 weeks. All cows were examined for pregnancy by transrectal sector ultrasonography at 42 d and 100 d after the initial FTAI. Foetal age assessment of trunk diameter at both 42 d and 100 was performed to enable accurate calculation of conception dates.

**Additional Data:** Additional data collected for each cow enrolled in the study included herd, breed, calving date and age at the time of treatment. Data were retrieved from veterinary practice records, the herd owner and the record-keeping database Infovet.

**Statistical Analysis:** Multivariable logistic regression analysis was used to assess the effect of cow age, breed, BCS, Metrichheck™ score, herd, days pp, season, combined uterine horn diameter, ovarian diameter, ovarian follicular diameter and treatment on the following reproductive performance parameters: pregnancy rate to FTAI (PR to FTAI; proportion of cows conceiving within 2 d of the initial FTAI/number of enrolled cows), 21 d in-calf rate (21 d ICR; proportion of cows conceiving within 21 d of FTAI/ number of enrolled cows), and end of season in-calf rate (SPR; proportion of cows pregnant at 100 d after FTAI/ number of enrolled cows). All analyses were performed using the software package R Version 3.1.3 <https://www.r-project.org>.

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## Results

**Effect of treatment on reproductive performance:** The mean interval from the administration of GnRH to conception date was significantly shorter in treated cows when compared to control cows (24 d vs. 31 d respectively). Similarly, the interval from calving to conception was significantly reduced in treated cows (60 d vs. 67 d). Treated cows had a significantly higher PR to FTAI when compared to control cows (25% vs. 14% respectively; Table 1). There was no difference between the treatment and control groups in terms of 21 d ICR (39% vs. 33% respectively) or SPR (72% vs. 73% respectively).

*Table 1: Reproductive performance of treated and control cows*

Group	n	PR to FTAI (%)	95% CI	21 d ICR (%)	95% CI	SPR (%)	95%CI
Treatment	373	25	(21-29)	39	(34-45)	72	(68-77)
Control	134	14	(9-19)	33	(25-41)	73	(65-80)
<i>P</i> -value		0.01		0.2		0.8	

**Effect of days postpartum on reproductive performance:** The number of days pp was categorised into three groups; ≤ 24 d, 25 to 29 d and ≥ 30 d. There was no difference in PR to FTAI (23% vs. 21% vs. 22% respectively; Table 2), 21 d ICR (42% vs. 33% vs. 39% respectively; Table 2) or SPR (69% vs. 73% vs. 73% respectively; Table 2).

*Table 2: Reproductive performance of days postpartum*

Days pp	n	PR to FTAI (%)	95%CI	21 d ICR (%)	95%CI	SPR (%)	95%CI
≤ 24	111	23	16-32	42	33-52	69	60-78
25-29	188	21	15-27	33	26-40	73	66-80
≥ 30	208	22	17-28	39	33-46	73	67-79
Total	507	22		38		73	
<i>P</i> -value		0.8		0.2		0.8	

**Effect of uterine horn diameter on reproductive performance:** Combined uterine horn diameter was categorized into < 4.5 cm and ≥ 4.5 cm. Overall the combined horn diameter did not affect PR to FTAI (21.6% vs. 22.6% respectively; P = 0.8), 21 d ICR (35% vs. 43% respectively; P = 0.6) or SPR (72% vs. 73% respectively; P = 0.9). However when analysing the 2014 season alone, cows with a uterine horn diameter of < 4.5 cm at the time of P4 device insertion had a higher PR to FTAI compared to cows with a uterine horn diameter of > 4.5 cm (31% vs. 21% respectively; P = 0.03)

**Effect of clinical parameters on reproductive performance:** When controlled for treatment and days pp, there was no significant effect of herd, cow age, breed, BCS, Metricheck™ score, ovarian diameter or ovarian follicular diameter on any of the reproductive performance outcomes.

## Discussion

The current study is the first in New Zealand to assess reproductive performance of treated AA cows compared to non-treated controls in the very early pp period. The study results were very positive, with a significant increase in pregnancy rate to FTAI demonstrated in treated cows when compared to non-treated controls. Additionally, the early treatment of AA cows can lead to conception occurring on average by 60 d pp, significantly less (an average of 7 d) than non-treated controls. In a seasonal herd, this advancement of conception date has considerable production and economic benefits. The most transparent advantage of earlier conception in seasonal herds is an extra 7 d of milk production per cow, increasing the income from the season per cow. More importantly, the cumulative positive effects of earlier conception continue into future seasons. Firstly, it brings the calving date for the following season forward, aligning the current season's late calving cows with the rest of the herd. With the banning of pharmaceutical calving induction in 2014, this presents an alternative management tool to advance conception dates and consolidate the calving pattern. Secondly, an earlier calving date next season lengthens the exposure of individuals to the following breeding season, increasing the likelihood of early conception and reducing the risk of not being pregnant at the end of the season. This effect is cumulative, with each season bringing forward the potential calving date and subsequent conception dates.

Another positive finding from the current study was the absence of effect of days pp at the time of first presentation on the reproductive performance of both control and treated cows. This indicates a significant economic benefit could be gained from treating AA cows sooner pp than the current industry standard of 35 d, regardless of time pp in the assessed time period (21-35 d pp).

An objective of the current study was to analyze the effect of specific clinical and historical parameters measured at the time of treatment on reproductive performance. From a clinician's standpoint this would allow for guided recommendations to be made on-farm at the time of treatment. In our study there was no effect of year (season) on outcome variables, therefore results from both seasons were combined for analysis. There was also no effect of BCS at the time of presentation on the outcome variables.

The current study did not demonstrate an association between Metrichick™ score and reproductive performance. This is in contrast to previous studies that found a significant reduction in conception, pregnancy and non-return rates in Metrichick™ 'positive' animals.<sup>7-9</sup> However, one previous New Zealand study described the reduced specificity of this diagnostic tool for the detection of endometritis, with only 37% of cows Metrichick™ 'positive' showing evidence of endometrial inflammation on cytology.<sup>10</sup> The short interval from parturition to diagnosis in the current study may further increase over-diagnosis of endometritis using this technique, which may explain the lack of correlation between Metrichick™ score and reproductive performance.

Whilst there was no significant effect of uterine diameter on reproductive performance when results from both seasons were combined, results from the 2014 season alone revealed that cows with a combined uterine horn diameter of <4.5cm had significantly improved PR to FTAI than cows >4.5cm. This suggests a relationship between a greater degree of uterine involution, and thus repair, at the time of FTAI and resultant enhanced chance of embryo viability. Delayed uterine involution is most commonly associated with intrauterine infection and/or inflammation, however it can also be secondary to dystocia, parity, RFM and metabolic or nutritional disorders.<sup>11, 12</sup> Increased uterine volume and delayed involution has been associated with decreased fertility in multiparous, lactating dairy cattle, specifically a reduction in PR to FTAI.<sup>11</sup> The discrepancy in uterine diameter results between seasons may be a direct result of operator difference within this study, with two different operators each performing measurements for a single season. Whilst this may indicate that ultrasound examination is a less useful aid in a practical setting, we have only examined the use of two individuals. These potential discrepancies may have been overcome by increasing the number of operators involved or by further standardising the methodology of measurements taken transrectally. However, the 2014 results alone suggest that if accurate and consistent measurements are taken there may be scope for the measurement of uterine diameter to become a valuable predictive tool in the success of early AA treatment.

### Conclusion

The present study demonstrated a significant improvement in reproductive performance when AA cows were treated as early as 14 d pp. The potential advancement of conception date by 7 d gains economic benefits by increasing the lactation length and DIM per treated cow by 7 d. Additionally, the resulting synchronization of ovarian cyclicity of late calving cows with the rest of the herd at PSM continues to have a significant positive economic and production impacts in a seasonally calving industry during subsequent seasons. Furthermore, accurate uterine diameter measurement at the time of treatment may be effective in predicting treatment success, however further studies are required to confirm this.

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