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

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Abstract

The objective of the current study was to determine the reproductive performance of anovulatory anestrus (AA) cows treated with a routine protocol at 14 to 35 d postpartum (pp). A prospective cohort study was performed over two seasons involving 9,630 cows in 24 herds. Cows not showing signs of estrus and without a corpus luteum (CL) identified on transrectal ultrasound were classified as AA (n=507). In the first season all cows were treated with an intravaginal progesterone device (Cue Mate 1.56 g w/w progesterone [P4]). The device was removed six days later and equine chorionic gonadotropin (eCG; 400 IU) administered IM. Two days later, gonadotropin releasing hormone (GnRH; 100 ug) was administered IM and fixed-time AI (FTAI) performed 16 to 20 h later. In the first season, all cows determined to be AA were allocated to the treatment group. In the second season, cows were randomly assigned to the treatment group or untreated control group. Multivariable logistic regression analysis was used to determine the effect of treatment, cow age, parity, days pp, uterine horn diameter, ovarian diameter, ovarian follicle diameter, Metricheck™ score and body condition score on reproductive performance. Treatment of AA cows was associated with significant reductions in both the interval from presentation date to conception date and the interval from individual calving date to conception date by an average of 7 d. Additionally, pregnancy rate to FTAI was improved in treated cows when compared to control cows (25% (95CI: 21-29%) vs. 14% (95CI: 9-19%) respectively; P = 0.01).

Keywords: Bovine, anestrus, intravaginal progesterone, uterine involution

Introduction

The pasture-based dairy industry of New Zealand relies on a concentrated seasonal calving pattern to align peak pasture growth with the time of greatest energy demand. This system requires all cows within a herd, regardless of their individual calving date, to commence breeding on the same calendar date (planned start of mating; PSM). Cows which have not resumed cyclical activity pp by this date are termed AA. Anovulatory anestrus cows are a significant cause of reproductive wastage as a direct result of a decreased number of cows inseminated and reduced conception and pregnancy rates.[1,2] In the majority of well-managed herds, all cows failing to display signs of estrus by PSM are commonly assessed by a veterinarian via transrectal palpation. Cows without a CL are classified as being AA. The prevalence of AA cows within a herd is influenced by calving date, breed, nutrition and peri-partum disease incidence and is documented to range from 15 to 27% in New Zealand dairy herds.[1,3-5]

Uterine involution and the resumption of normal ovarian cyclicity is reported to occur on average within 35 d pp in pasture-fed New Zealand dairy cows.[6] The process of involution involves uterine contractions, cellular shrinkage and necrosis, sloughing of the caruncles and regeneration of the endometrium.[7,8] Transrectal ultrasonography of the uterus enables an accurate measurement of uterine diameter and the associated degree of involution, as well as visualization of the presence of any free intrauterine fluid.[8] Given the accepted average time for uterine involution to occur and the associated return to cycling and accompanying fertility, the current "industry standard" is to wait at least 28 to 35 d pp to examine and treat AA cows.[6] This protocol therefore dictates that all cows in the herd that calve in the five weeks leading up to PSM are not eligible for AA treatment.

Pharmacologic programs for AA cows are initiated with the aim of inducing estrus and ovulation in these animals to improve the overall reproductive efficiency of the herd.[1,5,6] Standard AA treatment protocols initiated at least 28 d pp involve continuous P4 exposure via a controlled intravaginal releasing device for a period of six to eight days to suppress luteinizing hormone (LH)

and follicle stimulating hormone (FSH) pulses and prime the hypothalamus for an LH surge. At the initiation of P4 treatment, administration of GnRH is commonly performed to synchronize follicular waves.[9] Following removal of the intravaginal P4 device, a second dose of GnRH is administered 48 h later to induce ovulation.[9] Often the administration of eCG at the time of device removal is performed as it has been shown to increase pregnancy rates and decrease median days to conception in AA cows.[10,11]

The routine induction of calving in cows with a calving date late in the season using long-acting corticosteroids is a management technique that has previously been commonplace in the seasonal New Zealand dairy industry.[12] Premature induction of calving enables artificial tightening of the calving spread by advancing the calving date of cows otherwise due to calve late in the season. This 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, and are less likely to remain in the herd for more than two consecutive seasons.[1] In seasonally calving herds, late calving cows produce less milk in the subsequent season because all cows are 'dried off' on the same calendar date, regardless of individual calving dates. Therefore, late calving cows have reduced lactation length and fewer days in milk (DIM), reducing the income from that season. Therefore recent focus has been on additional tools to bring forward potential conception dates within herds, particularly focusing on late-calving cows.

A 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. Clinical examination included an assessment of body condition score (BCS), endometritis using the Metricheck™ (Simcro Ltd, Hamilton, New Zealand) tool and transrectal ultrasound to identify uterine diameter, ovarian diameter and ovarian structures present. Historical parameters analyzed included cow age, parity, herd, breed and season. A prospective cohort study was performed involving 9,630 cows in 24 herds. We hypothesized that the 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.

Materials and methods

Animals

All 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 presented for veterinary examination at PSM (n = 534, mean age: 5.1 years). Pre-mating estrus detection was carried out with the aid of tail paint in all herds prior to presentation.[13] All cows presented had no history of dystocia or pp disease during the current season. All herds were located in the Waikato region of New Zealand and were examined during the 2014 and 2015 spring breeding seasons. Cows diagnosed with uterine pathology or the presence of a CL were excluded from the trial. Of the 534 cows examined meeting the initial criteria, 507 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)[14] and examined for the presence of vaginal exudate as an indication of endometritis using a

Metricheck™ device as previously described.[15] Briefly, the intravaginal device consists of a steel rod attached to a concave silicon hemisphere. Advancement of the hemisphere to the cranial extent of the vaginal fornix followed by caudal retraction enables visual assessment of any material gathered in the silicon surface. Material obtained by the Metricheck™ device was scored as follows: 0 = no discharge, 1 = clear mucus, 2 = flecks of purulent material within otherwise clear mucus, 3 = mucopurulent but <50% purulent material, 4 = mucopurulent with >50% purulent material and 5 = mucopurulent with >50% purulent material and an odor.[15] In each season, all examinations were performed by the same veterinarian (2014 = JH, 2015 = GJ).

Ultrasound examination

A transrectal ultrasound (Easi-Scan; BCF Ultrasound Australasia; Auckland, New Zealand; broadband straight linear rectal probe; 5MHz) 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.[8] Pressure with the ultrasound transducer was minimized in order to obtain a circular cross section. The entire uterus was visualized to assess for the presence of intra- uterine fluid. If present, fluid was measured at its largest cross-sectional diameter to the nearest mm. Visualization of each ovary enabled calculation of ovarian diameter by averaging two perpendicular measurements of cross-sectional diameter. Ovarian activity was described as follows: MSF = multiple small follicles ($\leq$ 5 mm), MMF = multiple medium follicles (> 5 mm), CL = corpus luteum, or the diameter of the largest follicle/s if there were $\leq$ 2 large follicles present.

Treatment protocol

An intravaginal controlled release progesterone device (Cue Mate 1.56 g w/w P4, Bioniche Animal Health [A/Asia] Pty. Ltd., Armidale, NSW, Australia) was inserted per vagina into all treatment group animals on day 0. On Day 6, the intravaginal device was removed and cows administered 400 IU eCG IM (Pregnecol, Bayer New Zealand Ltd., Auckland, New Zealand). On day 8, 100 ug GnRH (Ovurelin, Bayer New Zealand Ltd., Auckland, New Zealand) was administered IM. Fixed-time artificial insemination 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 four to six weeks following FTAI. Thereafter, herd sires were used for natural service for three to five weeks. All cows were examined for pregnancy by transrectal sector ultrasonography at 42 d and 100 d after FTAI. Fetal age assessment of trunk diameter at both 42 d and 100 was performed to enable accurate calculation of conception dates.[16]

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 (Infovet, Zoetis New Zealand Ltd, Auckland, New Zealand).

Statistical analysis

Multivariable logistic regression analysis was used to assess the effect of cow age, breed, BCS, Metricheck™ score, herd, days pp, season, combined uterine horn diameter, ovarian diameter, ovarian follicular diameter and treatment on each of 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 independent variables (including interactions) were initially included (full model) and then a backward, step-wise model building process was used where non-significant (Wald's test, P > 0.05) interactions were removed sequentially, followed by non-significant main effects. After each variable was dropped from the model, a likelihood ratio test was performed to compare the new, reduced model to the previous model in order to determine significance of the reduced model. Survival analysis was used to analyze the time-to-event outcomes of days to conception after the PSM and days to conception after GnRH treatment. All independent variables (including interactions) were entered into a Cox proportional

hazards regression model. A backward, step-wise model building process was then used where non-significant (Wald's test, $P > 0.05$) interactions were removed sequentially, followed by non-significant main effects. All analyses were performed using the software package R Version 3.1.3.[17]

Results

Effect of treatment on reproductive performance
        Treated cows had a significantly higher PR to FTAI when compared to control cows (25% (95CI: 21-29%) vs. 14% [95CI: 9-19%], respectively; $P = 0.01$, Table 1). There was no difference between the treatment and control groups in terms of 21 d ICR (39% [95CI: 34-45%] vs. 33% [95CI: 25-41%], respectively; $P = 0.2$, Table 1) or SPR (72% [95CI: 68-77%] vs. 73% [95CI: 65-80%], respectively; $P = 0.8$, Table 1).
        The mean interval from the administration of GnRH to conception date was significantly shorter in treated cows when compared to control cows (24 ± 20 d vs. 31 ± 22 d, respectively; $P = 0.007$, Table 2). Similarly, the interval from calving to conception was significantly reduced in treated cows (60 ± 21 d vs. 67 ± 23 d; $P = 0.005$, Table 2).

Effect of days postpartum on reproductive performance
        The number of days pp was categorized into three groups; ≤ 24 d, 25 to 29 d and ≥ 30 d. There was no difference in PR to FTAI (23% [95CI: 16-32%] vs. 21% [95CI: 15-27%] vs. 22% [95CI: 17-28%], respectively; $P = 0.8$, Table 3), 21 d ICR (42% [95CI: 33-52%]%) vs. 33% [95CI: 26-40%] vs. 39% [95CI: 33-46%], respectively; $P = 0.2$, Table 3) or SPR (69% [95CI: 60-78%] vs. 73% [95CI: 66-80%] vs. 73% [95CI: 67-79%], respectively; $P = 0.8$, Table 3) amongst the three groups.

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% [95CI: 10-29%] vs. 22.6% [95CI: 9-29%], respectively; $P = 0.8$), 21 d ICR (35% [95CI: 29-47%] vs. 43% [95CI: 32-54%], respectively; $P = 0.6$) or SPR (72% [95CI: 68-78%] vs. 73% [95CI: 69-78%], respectively; $P = 0.9$).

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 aimed to determine the reproductive performance of AA cows identified and treated between 14 and 35 d pp with intravaginal P4, eCG and GnRH. Assessment of clinical and historical parameters pre-treatment was also performed with the objective of determining any possible effect on reproductive performance to aid in predicting the success of treatment. This is the first study in New Zealand to assess reproductive performance of treated AA cows compared to non-treated controls in the very early pp period.
        A significant increase in pregnancy rate to FTAI was demonstrated in treated cows when compared to non-treated controls (25% vs 14%, respectively). This is most likely a direct result of P4 'priming' of the reproductive tract during the period of intravaginal device insertion, with increased P4 concentrations in the preceding luteal phase increasing the likelihood of conception.[5,18-20] However, the overall PR to FTAI of treated cows in this study was reduced compared to previous studies that demonstrated PR to FTAI following AA treatment to be 48.9%,[21] 47.1%,[22] 38.8%.[5] This is likely a result of those studies using a treatment protocol involving intravaginal P4 and estradiol benzoate (EB) which is no longer commercially available in New Zealand.[23] Alternatively, this result may have been influenced by the reduced pp wait period in the current study.
        Numerous studies have examined the impact of AA cows in both New Zealand and overseas.[1,5,21,22,24-28] A delay in the resumption of normal ovulatory estrus cycles has been associated with environmental factors including decreased BCS, peri-partum disease, reduced energy intake, negative energy balance and suckling.[1,29] The current study has demonstrated that the innovative

early treatment of AA cows can lead to conception occurring on average by 60 d pp, significantly less than non-treated controls. In a seasonal herd, this advancement of conception date has considerable production and economic benefits.

In this study, treated cows demonstrated a 7 d reduction in the interval from calving and time of treatment to conception when compared to non-treated controls. The most significant advantage of earlier conception in seasonal herds is an extra 7 DIM per cow, increasing the income from that season. In seasonally calving dairy herds, all cows are dried off on the same date, regardless of their calving date. With the current milk price of $6/kg milk solids, seven days of extra production provides an additional $84 per cow per lactation assuming production of 2kgMS/day. In addition, 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 a potential alternative management tool to advance conception dates and bring about the consolidation of the calving pattern.[1] Furthermore, 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.[1]

The current study demonstrated no significant effect of days pp at the time of first presentation on the reproductive performance of both control and treated cows. This is very positive, indicating a significant economic benefit can be gained from treating AA cows sooner pp than the current industry standard of 35 d. This advantage is in agreement with a previous study in which no significant difference was noted in mean calving to conception interval when comparing the reproductive performance of cows treated 14 to 21 d pp with those 22 to 42 d pp and those greater than 42 d.[30] Whilst this aforementioned study also demonstrated a significant reduction in season pregnancy rate and submission rate in cows less than 21 d pp when compared to those cows greater than 21 d pp, control animals were not included in the trial and therefore conclusions are better drawn from the current study which did include control animals.

One of the objectives of the current study was to analyze the effect of specific clinical and historical parameters measured at the time of treatment on reproductive performance. This would allow for practical recommendations to be made on-farm at the time of treatment based on clinical findings to aid in predicting the success of treatment. In our study there was no effect of BCS at the time of presentation on the outcome variables. Whilst this is in agreement with a previous study,[24] an improvement in reproductive performance in cows with higher body condition scores has also been reported.[21] The narrow range of condition scores in the current study may account for the lack of effect of BCS in our study.

Whilst there was no significant effect of uterine diameter on reproductive performance when results from both seasons were combined, results from the 2014 season had indicated that cows with a combined uterine horn diameter of <4.5cm had significantly improved PR to FTAI than cows >4.5cm. Previous studies have identified that increased uterine volume and delayed involution can be associated with decreased fertility in multiparous, lactating dairy cattle, specifically a reduction in PR to FTAI.[21]

It is possible in the current study that the discrepancy in uterine diameter results between seasons may have been an operator effect, 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 further standardising the methodology of measurements taken transrectally. However, the 2014 results alone suggest that if consistent measurements are taken it may be possible for the measurement of uterine diameter to become a valuable predictive tool in the success of early AA treatment. Additional studies are necessary to further analyze the predictability of uterine horn diameter on reproductive performance.

The current study did not demonstrate an association between Metricheck™ score and reproductive performance. This is in contrast to previous studies that found a significant reduction in conception, pregnancy and non-return rates in Metricheck™ positive animals.[15,31,32] However, a previous New Zealand study described the reduced specificity of this diagnostic tool for the detection

of endometritis, with only 37% of cows Metricheck™ positive (scores 2 and greater) showing evidence of endometrial inflammation on cytology.[33] 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 Metricheck™ score and reproductive performance.[31-34]

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 provides economic benefits by increasing the subsequent lactation length and DIM per treated cow. Additionally, the resulting synchronization of ovarian cyclicity of late calving cows with the rest of the herd at PSM is likely to have significant positive economic and production impacts in a seasonally calving industry during subsequent seasons.

Acknowledgements

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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)
P-value		0.01		0.2		0.8	

Table 2: Effect on conception date of treated and control cows

Group	n	Mean GnRH to Conception interval (days)	Mean calving to conception interval (days)
Treatment	373	24 ± 20	60± 21
Control	134	31± 22	67± 23
P-value		0.007	0.005

Table 3: Reproductive performance of days postpartum

Days postpartum	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	
P-value		0.8		0.2		0.8	