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Effects of progesterone inclusion in a gonadotropin–prostaglandin–gonadotropin programme on follicular dynamics and ovulation synchronisation of pasture-based dairy cows with anoestrous

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ABSTRACT

The aim of this study was to evaluate how the addition of a progesterone device to a gonadotropin–prostaglandin–gonadotropin (GPG) programme in dairy cows with postpartum anovulatory anoestrus affected ovarian follicular dynamics and the synchronisation of ovulation. Twenty-two dairy cows were randomly assigned to either GPG (Day 0: 100 μg GnRH, Day 7: 500 μg $PGF_{2\alpha}$, Day 9: 100 μg GnRH, Day 0–7: Day 10) or GPG with a progesterone device from Day 0 to 7 (GPG + P4). Ovarian follicular dynamics and ovulation synchronisation were studied using transrectal ultrasonography. Compared to the GPG + P4, GPG alone resulted in a relatively larger mean dominant follicle size and a higher mean peripheral oestradiol concentration (74.9 pg * day vs. 60.6 pg * day; P = 0.002); however, there was much greater variation in follicle diameter in the group treated with GPG (7.8–22 mm vs. 10.8–17.5 mm in GPG + P4) and this may, at least partly explain why only 7/11 cows in this group ovulated within 48 h of the Day 9 GnRH injection compared to 10/10 of the cows in the GPG + P4 group. These results suggest that differences in follicular dynamics between GPG and GPG + P4 programmes are not a key driver of the difference in conception rate, but further studies are required to better assess the role of ovulation synchronisation.

1. Introduction

Anovulatory anoestrus is a significant problem in the New Zealand dairy industry, as cows which are not observed in oestrus prior to the start of the breeding season (non-cyclers) have significantly lower conception and pregnancy rates than cows which are seen in oestrus (Xu and Burton, 2000; McDougall, 2001). Typically, approximately 20% of New Zealand dairy cows are classified as non-cyclers; with anovulatory anoestrus being the underlying problem rather than a failure of oestrus detection (Rhodes et al., 2003). This has a major impact on the profitability of dairy farming in New Zealand, principally through delayed conception and failure to conceive by the end of the breeding season (McDougall, 2010).

Hormonal intervention to induce ovulation by stimulating maturation of ovarian follicles is the main treatment option. In 2007, oestradiol treatments for dairy cows were prohibited in New Zealand following a policy change in Europe (European Union, 2003), leading to the loss of the most commonly used treatment programme (McDougall, 2010). Treatments were revised, with GnRH being substituted for oestradiol, whilst retaining progesterone as the basis of the programme (McDougall, 2010). These changes led to a new recommended treatment protocol for anoestrous dairy cows: a GPG/fixed-time artificial insemination (FTAI) + Progesterone programme (GPG + P4: two treatments of GnRH 9 days apart, a progesterone releasing intravaginal device from Day 0 to Day 7, $PGF_{2\alpha}$ injection on Day 7, followed by FTAI 16–20 h after the second GnRH injection) (Laven, 2008).

Progesterone has been included in GPG programmes, typically between the first GnRH and the $PGF_{2\alpha}$ treatments, as a means of improving the effectiveness of synchronisation protocols. Such improvements are largely based upon the risk that a GPG programme (i.e. one that lacks exogenous progesterone) can lead to a short luteal phase after ovulation of the induced follicle (Sheffel et al., 1982; Gümen et al., 2003), leading to lower conception rates than in cycling cows (Moreira et al., 2001). Indeed, several reports have shown that conception rates can increase by 10 to 20% by combining progesterone with a GPG programme in dairy (El-Zarkouny et al., 2004; Stevenson et al., 2008; McDougall, 2010) and beef (Lamb et al., 2001) cows. However, the exact mechanism of action of the progesterone device in such programmes has yet to be elucidated. The improved conception rate may be due in part to the premature oestrus that occurs in 5–12% of cows between the first GnRH and the $PGF_{2\alpha}$ injections (Roy and Twagiramungu, 1999; DeJarnette et al., 2001); the positive effects of progesterone on the endometrium (Clemente et al., 2009) and conception rate (Fonseca et al., 1983; Folman et al., 1990); or to a lower incidence of short luteal phases after synchronisation.

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Most studies comparing the efficacy of GPG and GPG + P4 programmes have been based on assessing conception and pregnancy rates rather than evaluating their effects on ovarian follicle and luteal development. This is particularly so for cattle with postpartum anovulatory anoestrus. It is important to understand the role of progesterone in a GPG programme, as whilst it increases conception rate it also markedly increases the cost of the programme. Identifying how progesterone improves conception rate could provide valuable information on how to improve the response of anoestrous cows to GPG-based programmes without the expense of an intravaginal progesterone-releasing device

Therefore, the aim of this study was to evaluate how the addition of a progesterone device to a GPG programme in dairy cows with postpartum anovulatory anoestrus affected ovarian follicular dynamics and the synchronisation of ovulation.

2. Materials and methods

All animal use was approved by Massey University Animal Ethics Committee, Palmerston North.

This study was conducted at Massey University's No. 4 Dairy Farm, a spring-calving pasture-based 460-cow dairy farm in Palmerston North (latitude 40°2′S, longitude 175°4′E), New Zealand, during spring (September to November) 2010.

2.1. Animals

Nine days before the planned start of the breeding season, all lactating cows which had been calved for >30 days, had not had an assisted calving, had not been diagnosed with endometritis, and had not been recorded in oestrus were examined. Oestrus detection had been undertaken by farm staff using a combination of tail paint and observation from 40 days prior to the planned start of mating. The reproductive tracts of all the selected animals (n = 22) were examined by transrectal ultrasonography and manual palpation per rectum to confirm the absence of utero-ovarian pathology as well as the presence or absence of a corpus luteum (CL). Cows with a CL in either of the ovaries, any abnormalities of the puerperium, or any evidence of uterine infections or adhesions or other pathology of the ovaries or uterus were excluded from the study. Body condition score (BCS, scale 1 to 10: Macdonald and Roche, 2004) of all the animals was also recorded at the time of enrolment.

2.2. Synchronisation protocols

Using a random allocation sheet (created using MS Excel, 2007), the cows were allocated to one of the two treatment groups, based on their order through the race on Day 0 (Fig. 1). Treatments were: (1) Day 0: 100 μg i/m GnRH (Ovurelin, Bomac Laboratories Ltd, Auckland, New Zealand) and placement of a progesterone-releasing intravaginal device (1.56 g progesterone: Cue-Mate, Bomac Laboratories Ltd); Day 7: 500 μg cloprostenol i/m (Ovuprost, Bomac Laboratories Ltd) and removal of the progesterone device; Day 9: a second dose of GnRH. Fixed-time AI (FTAI) was performed on Day 10 (16–20 h after the second GnRH treatment) (GPG + P4; n = 11); (2) As for Group 1, with the exclusion of the progesterone-releasing intravaginal device (GPG; n = 11).

2.3. Ultrasonography

Ovarian structures of all the cows were monitored and studied using a real time B-mode ultrasound scanner (DP-6600 Vet, Mindray, Szechuan, China), equipped with a variable linear transducer set at 7.5 MHz. Ultrasonography was performed on Days 0 to 9. On each occasion, digital ultrasound images of both ovaries were recorded and a corresponding ovarian map was also drawn manually on the recording sheet to locate and identify the structures on the ovary, particularly the presence or absence of a CL.

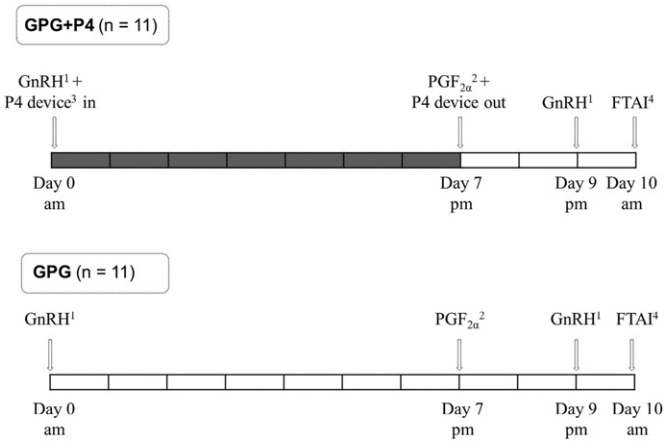

Fig. 1. Synchronisation protocol (grey shading indicates progesterone releasing intravaginal device). [1] 100 μg GnRH, i/m. [2] 500 μg $PGF_{2\alpha}$ i/m. [3] Progesterone releasing intravaginal device containing 1.56 g of progesterone. [4] Fixed-time artificial insemination.

The impact of treatment and time on follicular dynamics was evaluated by measuring the size of the largest dominant follicle using ImageJ (v1.46d, National Institutes of Health, USA). The diameter of the dominant follicle was estimated by taking the average of two measurements: (i) the size at the widest point and (ii) the size at right angles to the first measurement.

Between Days 0 and 7, the response of the dominant follicle to treatment (persistence, ovulation or atresia) was studied. Ovulation was defined as the disappearance of a dominant follicle followed by the development of a CL (or accessory CL) and atresia was defined as the disappearance of a dominant follicle followed with no CL development. Persistence was defined as no disappearance of the dominant follicle.

The time to the emergence of a new follicular wave was identified as the day, within 7 days of the first treatment with GnRH, that the dominant follicle was retrospectively identified to have had a diameter of ≥4 mm. If the dominant follicle was not detected until it reached 6 or 7 mm, the previous day was taken as the first day (Ginther et al., 1989). The growth rate of the pre-ovulatory follicle was established from the diameter reached on Day 9, minus the diameter on the day of its detection divided by the number of the days.

Timing of ovulation after Day 7 was determined by ultrasound examination of the ovaries every 12 h from the morning of Day 9 until the afternoon of Day 11 or ovulation, whichever was sooner. Ovulation was defined as the disappearance of a previously identified dominant follicle of ≥9 mm diameter.

2.4. Blood samples and hormone assays

Blood samples (10 mL) were collected via coccygeal venipuncture into heparinised vacutainers (BD New Jersey, USA) on Days 0, 1, 2, 3, 7, 8, 9, 12, 16 and 22 for determination of plasma oestradiol and progesterone concentrations. Plasma was separated within 2 h of collection by centrifuging at 1500 *g* at 4 °C for 20 min w. The plasma was then stored at −20 °C until final assay.

2.4.1. Progesterone assay

Plasma progesterone concentrations were measured in duplicate 10 μL aliquots by radioimmunoassay, using the ImmuChem Double Antibody Progesterone ^{125}I RIA kit for in vitro diagnostic use (MP Biomedicals, USA). The sensitivity of the assay was 0.14 ng progesterone/mL. The intra-assay coefficients of variation at 80, 50 and 20% binding on the standard curve were 16.1%, 8.4% and 9.9% respectively; the inter-assay coefficients of variation were 19.1%, 14.4% and 15.7% for low, medium and high solutions, respectively.