A comparative study of the effect of 2 hormonal treatment protocols on the reproductive performance of previously anoestrous dairy cows

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ABSTRACT

The objective of this study was to compare the reproductive performance of anoestrous dairy cows treated just prior to the mating start date (MSD) with a Controlled Internal Drug Releasing Device (CIDR) which was placed intravaginaly for either 6 or 8 days, and a combination of oestradiol benzoate. Lactating dairy cows (n = 926) that had been diagnosed with anovulatory anoestrus were divided into two sub-groups. A hormonal treatment protocol that involved the use of a CIDR device containing 1.9 g of progesterone was inserted into the vagina of each cow and left intravaginally for either 6 (6-day group, n = 441) or 8 days (8-day group, n = 485). Every cow in the trial was inseminated after being detected in oestrus from Day –2 onwards (where Day 0, was a herd's MSD), using an appropriate detection aid according to the herds' preference. Cows that had been seen in oestrus and were inseminated by Day 2 were selected for re-synchrony. The standardised re-synchrony involved the re-insertion of a previously used CIDR device into the vagina of each cow on Day 14, together with an injection of 1.0 mg oestradiol benzoate i.m. This CIDR device was removed on Day 22 and each of these cows injected with 1.0 mg oestradiol benzoate i.m. on Day 23. Each re-synchronised cow that was detected in oestrus was re-inseminated. Treatment with an 8-day CIDR increased the proportion of cows submitted for insemination within the first 3 days of the MSD, compared with the 6-day group (83.7 % *vs* 71.2 %, respectively, P < 0.001), as well as the proportion of cows conceiving within the first 3 days of MSD (36.2 % *vs* 27.7 %, P = 0.02), but reduced both the interval from MSD to the first service (4.5 ± 0.5 *vs* 6.8 ± 0.7, P = 0.01), and the interval from MSD to conception (28.1 ± 1.5 *vs* 34.0 ± 1.8, P = 0.009). A greater percentage of the cows in the 6-day group that were not pregnant to the first insemination were submitted for a second insemination by Day 28 compared with the 8-day group (81.1 % *vs* 68.3 %, P <0.001). Conception rates for cows submitted for this second insemination by Day 28 of the MSD were also higher in the 6-day than in the 8-day group (48.4 % *vs* 33.9 %, P = 0.009). The percentage of cows pregnant at the end of a herd's AI period of 6 weeks did not differ (57.1 % *vs* 54.8 % for 8-day and 6-day groups, respectively, P = 0.42); neither did the proportion of cows pregnant at the end of the a herd's combined AI and natural mating period of 21 weeks (81.4 % *vs* 79.2 %, for 8-day and 6-day groups respectively P = 0.36). Treatment of anovulatory anoestrous dairy cows with a combination of an 8-day CIDR and oestradiol benzoate before the MSD improved their reproductive performance by increasing the portion of cows submitted for insemination within the first 3-days of the MSD by reducing the interval from MSD to first service and by increasing the conception rate to the first insemination to collectively reduce the average interval from MSD to conception.

Key words: anovulatory anoestrus, dairy cow, re-synchrony.

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INTRODUCTION

The prevalence of anovulatory anoestrus in pasture fed dairy herds in the Macalister Irrigation district of Australia is a major factor affecting reproductive performance of seasonally bred Holstein cows. Recent trials in this area have shown that the prevalence of anovulatory anoestrus when mating start date (MSD) for herds in this district is about 15 %[25] and 23 %[11,18]. These findings are similar to studies performed in New Zealand, with a similar production system, where the prevalence of anovulatory anoestrus ranged from 9 % to 30 %[29] and could be as high as 52 %[35]. Factors that influence the prevalence of anovulatory anoestrus in these pasture-fed cows include the body condition score at calving, nutrition, milk production, breed, stocking rate, age and herd[12–14,22,27,28,29,35,36].

If left untreated, anovulatory anoestrous cows may have a 21-day submission rate of less than 55 %[15,20]. They are a major factor contributing to low submission rates often seen in dairy herds in Victoria[18]. Delayed submission and reduced conception rates may affect the calving pattern in the next season, since conception and submission rates were found to affect the calving pattern[23]. Furthermore, calving dates in seasonally calving herds have been identified as one of the major factors affecting the reproductive performance in many seasonally calving herds[10]. It is important to break the cycle of anovulatory anoestrous cows by concentrating inseminations and conceptions into the 3 or 4 weeks of the MSD to reduce the likelihood of late calved cows being culled and to optimise calving management to achieve reproductive performance goals.

Although recent studies have shown that resumption of cyclic activity was positively correlated with the negative energy balance nadir[27] the use of energy dense diets such as calcium salts of fatty acids[19] and total dietary fats[3] to advance the nadir and reduce the extent of negative energy in high yielding dairy cows to reduce the postpartum anoestrus duration has not been as successful as hormonal therapy[30].

Just prior to the time when this trial was done , the recommended treatment regime for treating anovulatory anoestrus involved inserting a controlled internal drug releasing device containing 1.9 g progesterone (CIDR device) for 6 days (6-day CIDR) with an injection of 1 mg oestradiol benzoate 24–48 hours after CIDR device withdrawal[34,43]. When using this treatment regime, submission rates of 84 to 88 % were achieved within 7 days of removing the CIDR devices but conception rates were 25 % lower than that of the untreated cycling herdmates (36–45 % for anovulatory anoestrus *vs* 62–70 % for cycling herdmates)[34,43]. Recent studies have shown that only 6 % of cows will display behavioural oestrus without ovulation after treatment with a CIDR device for 6 days and administration of oestra-

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diol benzoate 24 hours after removal of the CIDR[34].

One option to improve precision of oestrus and submission rates could be to inject oestradiol benzoate at CIDR insertion. This would cause synchronised emergence of a new wave of ovarian follicles within 4 days of treatment[4,5]. However, if oestradiol benzoate were administered at the start of a 6-day period of treatment with CIDR devices, pre-ovulatory follicles would be expected to emerge only 2 days prior to when CIDR devices were removed. This could lead to ovulation of immature follicles that may have poor fertility. It has recently been shown that the young immature dominant follicle in lactating anoestrous cows, which had emerged 1 day before CIDR withdrawal could be induced to ovulate but subsequent luteal development was compromised[8]. It would be expected that if oestradiol benzoate were to be used at CIDR insertion to ensure the synchronous emergence of a follicle, the duration of progesterone treatment would have to be increased.

The objectives of this study was to compared the reproductive performance of anovulatory anoestrous cows treated with a CIDR device for 6 days with a single injection of oestradiol benzoate with CIDR treatment for 8 days with oestradiol benzoate administered twice. It was hypothesised that anovulatory anoestrous cows treated with an 8-day CIDR device and oestradiol benzoate would have higher 3-day submission rates, and higher conception rates to the 1st service compared with anovulatory anoestrous cows treated with a 6-day CIDR.

MATERIALS AND METHODS

A clinical trial was conducted during the 1999/2000 breeding seasons. A total of 926 cows in 14 herds were enrolled in the trial. These herds were located within a 50 km radius of Maffra, Australia (latitude –37.96972, longitude 146.97667). The herds were selected on the basis that they were managed at an acceptable level that allowed meaningful results to be obtained in relation to individual animal ID, accurate records, competent oestrous detection, acceptable feeding management and willingness to comply with the trial protocol. Herd owners carried out the monitoring of herds to identify non-cycling and cycling cows. This was done using tail-paint as previously described[24] from at least 3 weeks after calving. Cows not seen or recorded in oestrus at least 10 days before a herd's MSD were selected after a morning milking to be presented for a veterinary examination.

Cows that were selected for the initial treatment had calved at least 21 days before that herd's MSD. Each one was evaluated by rectal palpation as having undergone complete uterine involution. Only those cows considered to be not suffering from lameness or from uterine/ovarian pathology were included. Cows that had experienced conditions such as dystocia, retained placenta or endometritis, but which, in the opinion of the attending veterinarian, had fully recovered from such conditions and with the uterus completely involuted, were included in the study. The diagnosis of anovulatory anoestrus was as described by Malmo *et al.* (2000)[25]. Specifically, the reproductive tract of each cow was examined by rectal palpation.

Measurements that were used to evaluate uterine involution were the position of the uterus relative to the pelvis, uterine tone and uterine and ovarian size. An ultrasound with a 7.5 MHz a transrectal transducer (Aloka SSD-500®, Aloka, Tokyo, Japan) was used to examine structures on the surface of the ovaries of some cows when, in the opinion of the attending veterinarian, findings from manual rectal palpation were inconclusive. From these examinations, an anovulatory anoestrous cow was classified as one with a normal uterus that had undergone complete involution, but had small ovaries, no corpus luteum and sometimes a follicle on one or both ovaries.

Animal treatments

Cows were divided into 2 groups, based on odd and even freeze brand or ear tag numbers and allocated to the 6-day and 8-day group, respectively. Each cow in the 8-day group was treated with a CIDR device, impregnated with 1.9 g progesterone (CIDR-B®, Genetics Australia, Bacchus Marsh, Victoria) on Day –10 (where Day 0, the MSD, occurred 48 hours after the day of CIDR device removal) and was injected intramuscularly (i.m.). On the same day (Day –10) each cow in the 8-day group was injected i.m. with 2 mg oestradiol benzoate (CIDIROL®, Genetics Australia, Bacchus Marsh, VIC). On Day –8, a CIDR device was inserted into the vagina of each cow in the 6-day group but they were not injected with oestradiol benzoate.

Every CIDR device was withdrawn by gently pulling the protruding nylon filament from the vagina of each cow enrolled in the trial on Day –2. Every cow enrolled in the trail was injected i.m. with 1 mg oestradiol benzoate on Day –1. In cases where the nylon filament was not protruding from the vulva, a vaginal examination was undertaken to ensure that the CIDR device was not retained within the vagina. Cows were monitored for oestrus using tail paint as previously described[24] and presented for insemination after being detected in oestrus using the herd's preferred AI technique.

Selection for re-synchrony

Those treated animals that received a 1st insemination on Days –1, 0, 1 or 2 (*i.e.* 24, 48 or 72 hours after the administration of oestradiol benzoate), and which did not have a 2nd insemination during the following 12 days, were enrolled for re-synchrony on Day 14. Only those cows that were inseminated by Days –1,0,1 and 2 were selected for resynchronisation because it was desired for the purpose of the trail that they be in the middle of dioestrus (12–15 days after insemination) at the time of CIDR device reinsertion. Each cow in the 6-day and 8-day groups received the same treatment. Specifically, a previously used device was re-inserted into the vagina of each cow enrolled for re-synchrony on Day 14 and each cow injected i.m. with 1 mg oestradiol benzoate. Each CIDR device was withdrawn on Day 22. They were each injected i.m. with 1 mg oestradiol benzoate on Day 23. Second inseminations were preceded by observed symptoms of behavioural oestrus.

Pregnancy testing

Each cow was pregnancy tested by manual rectal palpation 6 to 9 weeks after the conclusion of a herd's AI programme. These cows were pregnancy tested a 2nd time along with the rest of the cows in the herd about 6 weeks after the joining programme had been completed (*i.e.* after bulls had been removed for at least 6 weeks).

Statistical analysis

Logistic regression[17] was used to analyse the binary outcome variables. The following main effects were included in the model; treatment (6 [reference] *vs* 8 days), farm (1 to 14 [reference]), condition score group, using the 1 to 8 scale, (less than 4.5, 4.5 [reference], greater than 4.5), calving to MSD interval (less than 51, 51–70 [reference] greater than 70) and age (2[reference], 3, greater than 3 years old). Adding the single interaction term to a model that had all the 5 effects and then determining the likelihood ratio statistic evaluated the statistical significance of an interaction involving treatment and any of the main effects. The odds ratios for treatment were adjusted for the 4 main effects described above. A similar procedure was used to evaluate the effects of treatment on 2 continuous variables, the MSD to 1st service

Table 1: **Time sequence of activities conducted throughout the trial.**

Day	Activity	
	6-day	8-day
–10	None	Insert CIDR Inject 2.0 mg oestradiol benzoate i.m
–8	Insert CIDR	None
–2	Withdraw CIDR	Withdraw CIDR
–1	Inject 1.0 mg ostradiol benzoate i.m.	Inject 1.0 mg ostradiol benzoate i.m
–1 to +2	Observe for oestrus Inseminate (AI)	Observe for oestrus Inseminate (AI)
14	Re-insert used CIDR Inject 1.0 mg oestradiol benzoate	Re-insert used CIDR Inject 1.0 mg oestradiol benzoate
22	Withdraw CIDR	Withdraw CIDR
23	Inject 1.0 mg ostradiol benzoate i.m	Inject 1.0 mg ostradiol benzoate i.m
23–28	Observe for oestrus (AI)	Observe for oestrus (AI)
28–42	Observe oestrus AI	Observe oestrus AI
42 (6 weeks from MSD – '6 week incalf rate')	1st pregnancy diagnosis (PD)	1st pregnancy diagnosis (PD)
42–147	Bull Mating	Bull mating
147 (21 weeks from MSD – '21-week incalf rate')	2nd PD	2nd PD

and MSD to conception intervals using multi-way analyses of variance.

The log-rank test and the Kaplan-Meier survival curve were used to evaluate the similarity of the 2 treatments for the cumulative proportion of cows becoming pregnant after the MSD. Effects were considered to be statistically significant when the *P*-value was less than 0.05. The statistical program NCSS[16] was used.

RESULTS

The prevalence of anoestrus for all herds in the Maffra District was 25.0 % (926/3710) and varied between herds from 10.5 % (34/325) to 43.0 % (173/402). These 926 cows had a calving to MSD interval of 60.9 ± 0.8 days, ages of 4.7 ± 0.1 and an average body condition score of 4.32 ± 0.02. None of these parameters differed between the 2 treatment groups, as well as between and within herds ($P >$ 0.31). A greater percentage of cows were inseminated within Days 3 and 21 of the MSD in the 8-day group than in the 6-day group ($P <$0.05, Table 2, Figure 1). Cows in the 8-day group had a shorter interval from MSD to the 1st insemination ($P <$ 0.001, Table 1). Similarly, conception rates to the respective inseminations and pregnancy rates were higher in the 8-day than 6-day group ($P<$ 0.05, Table 2,). There was a treatment by farm interaction for MSD to 1st service interval (P = 0.04), a treatment by farm interaction for 21-day pregnancy rates (P = 0.04) and treatment by farm interaction for 21-week pregnancy rates (P = 0.02).

A greater proportion of cows that did not conceive to the 1st service in the 6-day group were submitted for the 2nd insemination than in the 8-day group between Day 21 and Day 28 in the 6-day than 8-day group (Table 3, $P <$ 0.001). Conception rates among cows submitted for the 2nd insemination by Day 28 in the 6-day group were also higher (48.4 *vs* 33.9, respectively, P = 0.009, Table 3). The cumulative pregnancy rates by Day 28 were similar between the treatment groups at the conclusion of 2nd round inseminations (P = 0.61, Table 3, Figure 2).

Cows in the 8-day group had a shorter interval from MSD to conception than

Table 2: **Comparison of submission and conception rates to the 1st insemination between the 2 treatment cows.**

Reproductive parameter	Treatment group		Adjusted odds ratio	*P*-value
	6-day	8-day	(95 % CI)	
Cumulative percentage of cows inseminated within 3 days of MSD	71.2 (314/441)	83.7 (406/485)	2.1 (1.5–2.9)	<0.001
Conception rates of cows inseminated within 3 days of MSD	27.7 (87/314)	36.2 (147/406)	1.5 (1.0–2.0)	0.02
Cumulative pregnancy rates within 3 days of MSD	19.7 (87/441)	30.3 (147/485)	1.7 (1.3–2.3)	<0.001
Cumulative percentage of cows inseminated within 21 days of MSD	86.8 (383/441)	91.3 (443/485)	1.6 (1.0–2.4)	0.04
Conception rates of cows inseminated within 21 days of MSD	28.5 (109/383)	36.8 (163/443)	1.4 (1.1–2.0	0.02
Cumulative pregnancy rates within 21 days of MSD	25.4 (112/441)	35.1 (170/485)	1.5 (1.2–2.1)	0.003
Interval from MSD to 1st service (days, mean ± SEM)	6.8 ± 0.7 (*n* = 441)	4.5 ± 0.5 (*n* = 485)	2.2 (0.5–3.9)	0.01

Table 3: **Comparison of the reproductive performance of cows in the 2 treatment groups at the 2nd insemination.**

Reproductive indices	Treatment group		Adjusted odds ratio	*P*-value
	6-day	8-day	(95 % CI)	
Cumulative number of cows re-inseminated by Day 28	81.1 (87/313)	68.3 (146/403)	0.5 (0.3–0.7)	<0.001
Cumulative number of Non pregnant cows not re-inseminated by Day 28	18.9 (87/441)	31.7 (146/485)	2.2 (1.4–3.5)	<0.001
Conception rates to cows re-inseminated by Day 28	48.4 (89/184)	33.9 (60/177)	0.6 (0.4–0.9)	0.009
Cumulative pregnancy rate by Day 28	50.8 (224/441)	49.9 (242/485)	0.9 (0.7–1.2)	0.61

those in the 6-day group ($P = 0.01$, Table 4). There were no differences in the pregnancy rates between the treatment groups at the end of AI breeding, or natural breeding (42 and 147 days, respectively, Table 4, Fig. 2). The log-rank test showed that there were no differences in the distribution of cows becoming pregnant after the MSD (Log-rank statistic = 0.14, df = 1, $P = 0.71$). The median time from the MSD to conception (the time that 50 % of cows had conceived) for the 6 and 8-day treatments were 27 days (95 % CI 25 to 35 days) and 29 days (95 % CI 25 to 37 days), respectively.

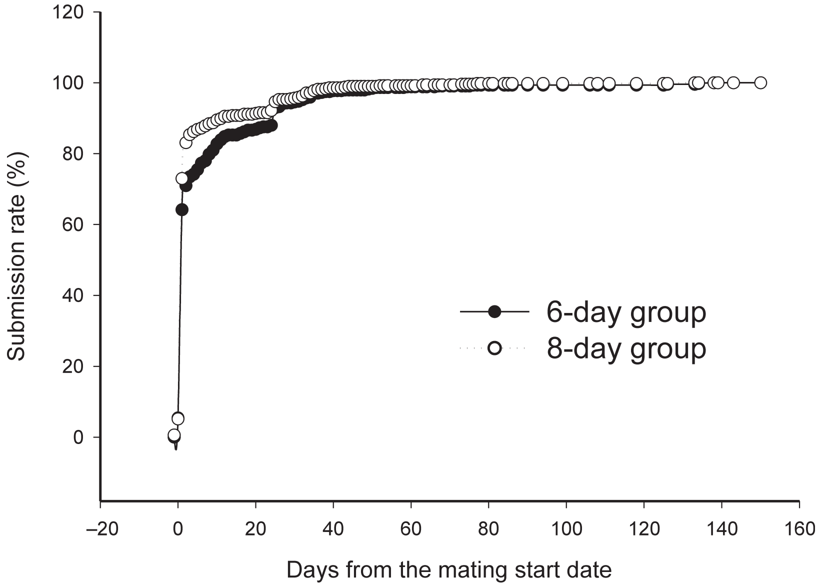

Fig. 1: **Submission patterns after treating anoestrous cows with a 6- or 8-day CIDR.**

DISCUSSION

Treatment of anovulatory anoestrous cows before the MSD with a combination of an 8-day CIDR device combined with 2.0 mg of oestradiol benzoate at the time of CIDR device insertion followed by 1.0 mg oestradiol benzoate 24 hours after CIDR device removal significantly improved the reproductive performance of the anovulatory anoestrous cows in this study during the 1st 2 inseminations of the MSD. The interval from the MSD to 1st service was reduced from 6.8 days in the 6-day group to 4.5 days in the 8-day group. Similarly, the submission rates to the 1st insemination were significantly higher in the 8-day than in the 6-day group (Table 2). These results differ from those of McDougal (2001)[26] who found that the submission rates to the 1st insemination of anovulatory anoestrous cows treated with a CIDR for 6 or 8 days did not differ. Xu and Burton (1997)[43] reported that 85 % of anovulatory anoestrous cows were inseminated within 6 days of the MSD. In that protocol, a 5-day CIDR with a 10 mg capsule was used[43], and therefore direct comparison with this trial may not apply. In a trial similar to the current one, 60.3 % of cows treated with a CIDR device for 6-days were inseminated within 6 days of the MSD[34]. In a 7-day treatment protocol, the injection of either 1 mg or 0.5 mg oestradiol benzoate 48 hours after CIDR withdrawal reduced the proportion of cows that spontaneously showed behavioural signs of oestrus, but significantly increased the proportion of cows that were induced to ovulate and showed behavioural signs of oestrus[42]. This is in agreement with the current study.

The reasons why anovulatory anoestrous cows treated with CIDR device for 8 days had a shorter duration to onset of oestrus may be due to the developmental stage of the ovulatory follicle at the time of CIDR device withdrawal. Progesterone alone can cause follicular turnover in anoes-

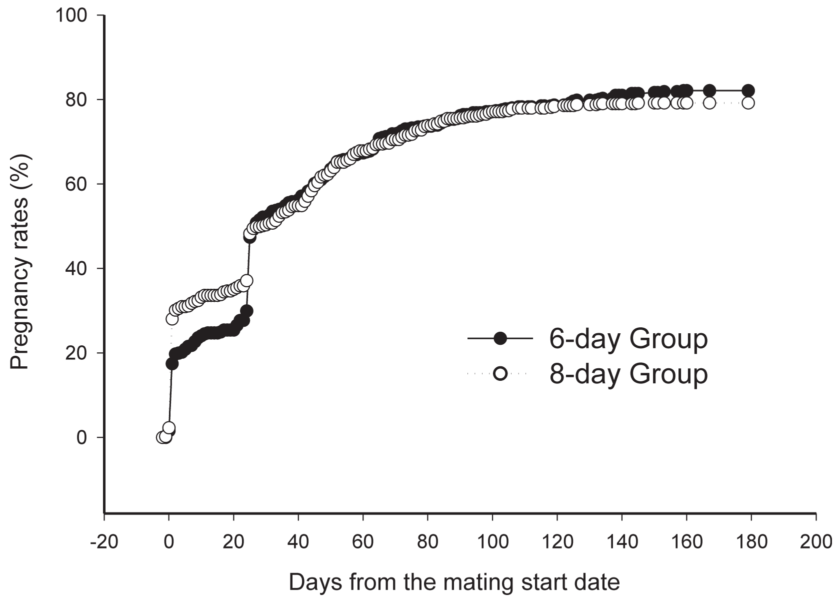

Fig. 2: **Comparison of cumulative pregnancy rates of 6- and 8-day treatment groups from the start of the mating start date.**

Table 4: **Overall reproductive performance of anoestrous cows enrolled in the trial.**

Reproductive parameter	Treatment group		Adjusted odds ratio	*P*-value
	6-day	8-day	(95 % CI)	
MSD – conception interval (days, mean ± SEM)	34.0 ± 1.8 (n = 362)	28.1 ± 1.5 (n = 384)	6.0 (1.5–10.6)	0.009
Cumulative 42 days pregnancy rates (%)	57.1 (252/441)	54.8 (266/485)	0.9 (0.7–1.2)	0.42
Cumulative 147 days pregnancy rates (%) (21 week mating)	81.41 (359/441)	79.18 (384/485)	0.9 (0.6–1.2)	0.36
Empty rate (%)	17.91 (79/441)	20.82 (101/485)	1.2 (0.9–1.7)	0.22
Conception rate to the 1st insemination	32.0 (141/441)	38.1 (185/485)	1.3 (1.0–1.7)	0.07

trous cows[32]. This implies that a proportion of anoestrous cows treated with progesterone for 6 days will have an immature follicle at the time of CIDR device withdrawal. The maturity of the dominant follicle in suckling anoestrus cows was critical to its response to treatment with oestradiol benzoate and progesterone[8]. Only 4/9 of the treated cows showed ovulation with anoestrous cows with immature dominant follicle, compared with 9/9 of mature dominant follicle[8]. Similar conclusions on the maturity of the dominant follicle in cycling cows have been made[2]. Shorter durations of dominance (2–4 days) delayed the onset of oestrus by 7 hours[2].

This may explain the long interval to oestrus seen in cows treated with progesterone for 6 days.

The high submission rate seen in the 8-day group was associated with higher conception rate to that insemination, consequently reducing the mean interval to conception to 28.1 days instead of 34.0 days. This differs from the findings of McDougall (2001)[26] who found that the improved reproductive performance of cows treated with a CIDR device for 8 days was not due to the increased submission rate between Days 0 and 14, but due to only an increased conception rate to that of insemination. In agreement with this trial Eagles *et al.* (2001)[11] reported that cows treated with a CIDR device for 6 days as well as re-synchrony with a used CIDR device re-inserted for 6 days on Day 14, had a mean interval from the MSD to conception of 31 days. Nonetheless, the median intervals from the MSD to conception were similar between the 2 treatment groups in this trial. This was due to the higher submission and conception rates in the 6-day group following re-synchrony.

The proportion of cows not pregnant to the 1st service and re-inseminated between Days 21 and 28 were significantly higher in the 6-day group than in the 8-day group. The proportion of non-pregnant cows that were not re-inseminated was lower in the 6-day than in the 8-day group; these cows have been coloquially termed Phantom cows[31]. The percentages of non-pregnant cows that are not submitted for a 2nd insemination have also been reported in New Zealand dairy herds[26]. The conception rates of cows re-inseminated by Day 28 were also higher in the 6-day than in the 8-day group. The reason why there were more Phantom cows in the 8-day group is not clear. It may be related to the hypophyseal-pituitary negative feedback due to the combined effect of using oestradiol benzoate at the time of the initial insertion of the CIDR device in the 8-day group. A similar trial was performed in the subsequent breeding programme wher a CIDR device for 8 days at the initial treatment, combined with an injection of 2 mg oestradiol benzoate at the time of CIDR device insertion and 1 mg oestradiol benzoate 24 hours after device withdrawal; the re-synchrony treatment involved re-insertion of a CIDR device on Day 14 (Day 0 = MSD), and injection of 1 mg oestradiol benzoate at the time of CIDR device re-insertion. The CIDR device was withdrawn on Day 22, and a further i.m. injection of 1 mg oestradiol benzoate on Day 23[38]. In that trial the percentage of non-pregnant cows that were not submitted for a 2nd insemination was 16.5 %. The findings in another later trial that was conducted[38] compared with the 6-day results in this study, show that the injection of oestradiol benzoate at the time of insertion of a CIDR device did not have untoward results on the submission rates after re-synchrony. However, Rhodes *et al.* (1997)[33] treated anovulatory anoestrous cycling cows with sub-luteal plasma concentrations and found that persistence of dominant follicles could only be achieved with 12.5 % of treated cows, as opposed to 50 % of cycling cows. Such treatment of anoestrous cows with progesterone also lead to a decrease in the plasma concentrations of oestrodiol, and emergence of new follicular waves in 87.5 % of treated cows[33]. This ovarian response to low concentrations of progesterone differed from that seen in cycling cows where an increase in size of the dominant follicle and increase in production of oestrogen, as well as suppression of subordinate follicles occurred due to sub-luteal plasma concentrations of progesterone[37,39,40]. The strategic injection of 1 mg of oestradiol benzoate during the luteal phase[6], or at different stages of the oestrus cycle[1,4] has been demonstrated to be effective in synchronising follicular wave emergence in cycling cows, due to suppression of pituitary LH secretion[7]. These differences suggest that the hypothalamic-pituitary of anoestrous cows is highly sensitivity to the negative feedback effect of ovarian steroid feedback and that treating these cows with progesterone alone can cause follicular turnover. Combining oestradiol benzoate with progesterone may completely shut down the hypothalamic-pituitary axis, so that treated anoestrus cows revert to the stage of anoestrus after induction of ovulation. In fact, a new follicle emerged in only 55 % of anoestrous cows treated with 2 mg of oestradiol benzoate at the time of CIDR insertion[32]. Interestingly, in those anoestrous cows that had follicular turnover, treatment with oestradiol benzoate significantly delayed new follicular emergence, possibly due to a prolonged decrease in pituitary support for development of gonadotrophin-dependent follicles. Results of the trial by Rhodes *et al.*[32] may explain the lack of benefit seen in a trial where a 5-day CIDR protocol where oestradiol benzoate was injected at the time of CIDR device insertion[41], but support findings from the present study increasing the duration of progesterone treatment to 8 days in conjunction with the injection of 2 mg oestradiol benzoate at the time of CIDR device insertion may have allowed sufficient time for emergence of a new follicular wave. Whether the increase in submission rates was due to the increase in duration of treatment with progesterone alone, or due to the addition of oestradiol benzoate in an 8-day CIDR justifies further investigation.

The percentage of cows pregnant by Day 28, 6 or 21 weeks were not different between the 2 treatment groups. This is in agreement with conclusion of McDougall (2001)[26]. However, the 28-day pregnancy rates seen in this study were lower than that of McDougall (2001)[26]. This may be due to the high conception rates to inseminations done between Days 0–14 in the later study. This is not surprising since New Zealand dairy cows have higher fertility than those of Australian dairy cows (K L Macmillan, 2001, unpubl. data).

The percentage of cows not pregnant at the conclusion of the breeding period did not differ. This is in agreement with studies in New Zealand, where in one study the empty rates were 20 %[43]. Earlier work found that empty rates were around 7 % in treated anovulatory anoestrous cows[21]. In the current study cycling herdmates had a 10 % lower empty rates at Day 147 than treated anovulatory anoestrous cows over the same period. This is in agreement with Xu and Burton (1997)[43]. The reasons behind the low reproductive performance of anovulatory anoestrous cows after they had been treated are not clear. Recent studies have shown that previously anoestrous cows that did not conceive to the 1st insemination but expressed oestrus at the subsequent expected return to oestrus would be less likely to be in negative energy balance[27] Together, these results suggest that the major limitation to overall reproductive performance of these cows following resumption of ovarian cyclisity may be their negative energy balance.

In summary, the use of the combination of oestradiol benzoate and an 8-day CIDR improved the reproductive performance of anoestrous dairy cows by increasing 1st round submission rates as well as reducing the mean interval to 1st insemination by 2.3 days and the interval to conception by 5.9 days. Some non-pregnant cows that did not return for a 2nd insemination limited the success of this protocol. Consequently, the 6-week and 21-week-pregnancy rates as well as the empty rates were not different between cows treated with a CIDR device for 6 or 8 days. Future studies must investigate treatment regimes that will use the benefits of an 8-day CIDR, as well as increasing 1st round conception rates so as to reduce the proportion of treated cows that fail to conceive to a 1st insemination and also fail to respond to a re-synchrony treatment.

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