

The reproductive performance of dairy cows with anovulatory anoestrus that were injected with either gonadotrophin-releasing hormone or oestradiol benzoate as part of a re-treatment process after insemination

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ABSTRACT

This experiment compared the reproductive performance of synchronised anoestrous dairy cows that were treated initially with a combination of progesterone and oestradiol benzoate and then with either gonadotrophin-releasing hormone (GnRH) or oestradiol benzoate to resynchronise returns to service. It was hypothesised that injecting anoestrous dairy cows with GnRH 12–15 days after insemination and coinciding with the time of insertion of a controlled intravaginal progesterone-releasing (CIDR) device would increase conception rates to the preceding 1st insemination compared with oestradiol benzoate treated cows; both GnRH and oestradiol benzoate would resynchronising the returns to service of those cows that did not conceive to the preceding insemination. Groups of cows in 11 herds were presented for a veterinary examination after they had not been seen in oestrus postpartum. Those cows diagnosed with anovulatory anoestrus ($n = 1112$) by manual rectal palpation and/or ultrasonography were enrolled in the trial. Each enrolled cow was injected with 2 mg oestradiol benzoate i.m. on Day -10, (where Day 0 was the 1st day of the planned insemination) concurrently with vaginal insertion of a CIDR device. The device inserted was withdrawn on Day -2 and then each cow injected i.m. with 1 mg of oestradiol benzoate on Day -1 unless it was in oestrus. Observation for oestrus preceded each insemination. Every cow that had been inseminated on Days -1,0,1 or 2 was presented for treatment for resynchrony on Day 14 ($n = 891$). They were divided into 2 groups; those with an even number were each injected i.m. with 250 μg of a GnRH agonist (Treatment group $n = 477$); each of the cows with an odd number injected i.m. with 1mg of oestradiol benzoate (control group, $n = 414$). Each GnRH or oestradiol benzoate injection preceded reinsertion of a CIDR device previously inserted from Days -10 to -2. It was withdrawn on Day 22, 24 hours before injecting 1 mg oestradiol benzoate. Cows observed in oestrus were submitted for a 2nd insemination. Every enrolled cow still present in the herd was pregnancy tested by palpation of uterine contents *per rectum* about 6 weeks later and again at the end of a herd's seasonal breeding programme. The alternative use of GnRH instead of oestradiol benzoate did not affect the percentage of cows conceiving within 3 days of the mating start date (MSD) (35.6 % vs 35.3 %, $P = 0.90$), resubmission rates for a 2nd insemination among cows not pregnant to the 1st insemination (81.6 % vs 83.5 %, $P = 0.41$), 6-week pregnancy rate (59.3 % vs 60.6 %, $P = 0.65$), 21-week pregnancy rate (86.6 vs 85.0, $P = 0.36$), mean interval from MSD to conception (32.5 ± 1.8 days vs 29.9 ± 1.8 days, $P = 0.26$) or conception rate of cows reinseminated by Day 28 (43.3 % vs 38.8 %, $P = 0.39$). When GnRH was compared with oestradiol benzoate, it did not increase conception rates to the 1st service; it was as effective as oestradiol benzoate in synchronising returns to service in previously treated anoestrous cows that did not conceive to the 1st service. Its use affected neither conception rates to the preceding 1st inseminations nor to the following 2nd inseminations.

Key words: anovulatory anoestrus, controlled internal drug-releasing device, GnRH, oestradiol benzoate, resynchrony.

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the basis of availability of pasture, and/or seasonal fluctuations in milk prices. Fertility is one of the most critical determinants of pasture-based seasonal production systems⁸. Breeding must commence on a management set date, known as the mating start date (MSD); the MSD is the reference point for most reproductive indices used in seasonally calving herds. In order to maintain the target inter-calving interval of 365 days optimum reproduction efficiency of cows must be maintained so as to achieve at least 87 % submission rates within the 1st 3 weeks of the MSD¹⁶. This can be achieved through controlled breeding programmes as well as timely identification and treatment of reproductive disorders such as anovulatory anoestrus. Delayed conception affects calving patterns¹⁶ and cows conceiving late are more likely to be induced to calve prematurely²⁸.

Oestrus and ovulation can be induced successfully in anovulatory anoestrous cows using a combination of a controlled intravaginal progesterone releasing (CIDR) for 6 to 8 days and oestradiol benzoate^{23,33}. Variations to this treatment protocol include the injection of oestradiol benzoate 24 hours after CIDR withdrawal³⁴, commencing treatment before MSD^{9,17}, or inseminating after a 2nd induced oestrus³². Although resynchronising returns to service did not improve resubmission rates in cycling heifers¹⁰, contrasting results have shown the benefits in cycling¹⁵ and anovulatory anoestrous cows⁶. Increasing the duration of progesterone treatment from 6 to 8 days will reduce the interval from MSD to 1st service and the mean interval from MSD to conception³⁹. However, the number of cows that had conceived by the end of the artificial insemination (AI) period still remained 10 % lower than in spontaneously cycling herd mates.

The prevalence of anovulatory anoestrous in Australian dairy herds, factors predisposing to its occurrence, as well as its effect on overall dairy herd fertility have recently been described³⁹. Anovulatory anoestrous cows have poor conception rates as well as prolonged inter-oestrous intervals than cycling

INTRODUCTION

Seasonal calving dairy production system is a system where breeding and calving are concentrated within a limited period of the year. This system is widely practiced in Australia and New Zealand. The choice of the calving system may be selected on

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herdmates for reasons that have not been identified. Early embryo mortality at Day 25 post-AI in dairy cows that had spontaneously resumed normal ovarian activity has been reported as high as 30%¹⁴. Ultrasonographic examination has shown that 9% of treated anovulatory anoestrous cows with a prolonged return to oestrus were not pregnant by 13 weeks after insemination²⁹. Serial measurement of progesterone concentrations of treated anovulatory anoestrous cows showed lower concentrations compared with contemporary cycling cows³⁵. Post-insemination rise in progesterone will positively influence the development of the trophoblast, and consequently the secretion of INF- τ , an interleukin important for the maintenance of pregnancy in domestic ruminants²⁵. It is possible that the progesterone concentration of anovulatory anoestrous cows might be compromised and that increased early embryonic losses may occur more frequently. However, a large-scale field study involving the supplementation of progesterone post-insemination failed to increase conception or pregnancy rates in previously anoestrous cattle³⁴. Post-insemination forms of a supplementary treatment should be aimed at decreasing embryo losses and to increase conception rates to the 1st oestrus. This form of treatment must also ensure that those cows not pregnant to 1st insemination do return for a 2nd insemination within 28 days.

Previous reports showed that injecting a synthetic GnRH agonist (buserelin) 11 to 14 days after 1st insemination sometimes improved conception rates^{5,20,40}, but not always^{13,37}. The endocrine changes following treatment with buserelin delayed leuteolysis and extended the period for the maternal recognition of pregnancy²⁴. Treating normal cycling cows with 250 μ g of gonadorelin, another synthetic GnRH agonist, at the time of CIDR device insertion caused follicular turnover, with synchronous emergence of a new wave of ovarian follicle development within 2 days^{36,38}. GnRH injected 11–14 days after insemination could have the potential to increase 1st round conception rates as well as synchronising returns to oestrus of those cows not conceiving to 1st insemination following treatment for anovulatory anoestrus.

It was hypothesised that injecting previously treated anovulatory anoestrous cows with a GnRH agonist, as opposed to oestradiol benzoate, at the time of reinsertion of a CIDR device from 12 to 15 days after 1st insemination would increase conception rates to that insemination, as well as synchronising returns

to service of those cows that had not conceived to the 1st insemination, thereby increasing the 2nd round submission rate. The combined effect would be that the mean interval from MSD to conception would be reduced in those cows treated with GnRH at the time of CIDR device reinsertion.

MATERIALS AND METHODS

Experimental site and selection of animals

The clinical trial was conducted in the Macalister Irrigation District, Victoria, Australia during the 2000/01 breeding season. A total of 1112 anovulatory anoestrous cows in 11 herds were initially enrolled in the trial. These herds were located within a 50 km radius around Maffra (latitude -37.96972, longitude 146.97667).

Monitoring of herds for oestrus commenced at least 3 weeks after parturition so as to identify those cows not displaying overt behavioural signs of oestrus, using a tail paint technique which has previously been described^{19,44}. Cows that had calved at least 3 weeks (21 days) before MSD were potentially eligible to be enrolled in the trial. The removal of tailpaint indicated that mounting had occurred, and positively identified cows that had been in oestrus. At the end of the monitoring period, cows that had retained their original application of tail paint were drafted out at the end of a morning milking, and presented for veterinary examination about 10 days before the herd's MSD.

The reproductive tract of each presented cow was examined by rectal palpation of the reproductive tract and/or transrectal ultrasonography using the procedures previously described by Malmo *et al.* (2000)²³. The anovulatory anoestrous cows were identified as those which had not been detected in oestrus and not ovulated since calving, yet had undergone an apparently normal reproductive recovery (involution). The criteria routinely used at the Maffra Veterinary Clinic²³, required anovulatory anoestrous cows to have a normally involuting or involuted uterus, and smooth-surfaced ovaries (which were often small in volume), sometimes with at least 1 well-developed follicle on an ovary, but without a Corpus luteum (CL). Cows that had ovulated, as determined by palpation of a CL or markedly increase uterine tone, but had not shown behavioural signs of oestrus (behavioural anoestrous, or silent ovulation), were not included in the trial. Any cow with incomplete uterine involution, or with any other form of uterine pathology, was excluded together with any

cow that had calved within 21 days before examination body condition scores on a 1 to 8 scale were recorded for each animal at the time of the initial treatment.

Animal treatments

Each anovulatory anoestrous cow was treated for anoestrus following its enrolment with the currently recommended protocol⁷ from around 10 days before the herd's MSD. A CIDR device (CIDR[®] Cattle insert, Genetics Australia, Bacchus Marsh, Victoria, Australia) was inserted into the vagina of each anovulatory anoestrous cow, using the manufacturer's guidelines⁷ in conjunction with an i.m. injection of the 4 ml of CIDROL[®] (Genetics Australia, Bacchus Marsh, Victoria, Australia) containing 2 mg of oestradiol benzoate. The device was removed 8 days later and 2 ml of CIDROL[®] (1 mg of oestradiol benzoate) injected i.m. 24 hours later. Visual detection of behavioural signs of oestrus aided by tail paint preceded insemination in accordance with the normal mating practice in that herd.

Re-treatment of every anovulatory anoestrous cow inseminated during the 4 days following device removal (Day -1 to Day 2 about MSD) involved reinserting devices originally withdrawn on Day -2. They were washed, disinfected and dried before being stored. These re-treated cows in each participating herd were divided into 2 groups based on permanent identification numbers being odd or even. On Day 14 CIDR devices were reinserted *per vaginam* into every cow ($n = 891$). On the same day, each even-numbered cow ($n = 477$) was injected i.m. with 2.5 ml of Fertagyl[®] (Intervet Australia, Castle Hill, New South Wales, Australia) containing 250 μ g gonadorelin while each odd-numbered cow ($n = 414$) was injected with 2 ml CIDROL[®] containing 1mg of oestradiol benzoate. CIDR devices were withdrawn after 8 days (Day 22) and 2 ml CIDROL[®] injected into each of those cows 24 hours after CIDR removal (Day 23). Re-insemination occurred following the detection of standing oestrus.

Pregnancy testing

Veterinarians from the Maffra Veterinary Centre tested every enrolled cow for pregnancy 12–15 weeks after MSD. Any cow not confirmed pregnant at the initial testing was re-examined at a 2nd visit 27–29 weeks from MSD.

Statistical analysis

Logistic regression¹² was used to analyse the binary outcome variables. The following main effects were included in all models: treatment (oestradiol benzoate

[reference] vs GnRH); farm, condition score (<5, 5[reference], >5); calving to MSD (<51, 51–70[reference], >70 days); and age (2[reference], 3, >3 year old). The odds ratio for treatment was adjusted for the 5 main effects described above. First-order interactions were tested between treatment and each of the other main effects. A similar procedure was used to evaluate the effect of treatment on the continuous variable MSD to conception using multi-way analysis 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 MSD. Cows becoming pregnant on Day -1 and 0 relative to MSD were given a MSD-conception of 0.1 days for the purposes of the log-rank test and Kaplan-Meier survival curve. Effects were considered to be statistically significant when the *P*-value was <0.05. The statistical program NCSS¹¹ was used.

Animal ethics approval

The Animal Experimentation Ethics Committee of the University of Melbourne approved this trial (Experiment number 0095).

RESULTS

A total of 1285 cows was initially submitted for examination. The number initially treated as anovulatory anoestrous cows was 1259. However the number of cows suitable for analysis was reduced to 1112 after excluding cows that had been culled (35), which died after enrolment (3), which had doubtful or incomplete records (108) or had outlying parameters such as a calving to MSD interval of over 400 days (1).

There were no differences in the age at calving (5.03 ± 0.122 vs 5.05 ± 0.11 years for oestradiol benzoate and GnRH, respectively, *P* = 0.90), body condition score at initial examination, on a 1 to 8 scale (4.83 ± 0.02 vs 4.82 ± 0.02 for oestradiol benzoate and GnRH groups, respectively, *P* = 0.76), or interval from calving to MSD (62.4 ± 1.2 vs 62.0 ± 1.2 days for oestradiol and GnRH groups, respectively, *P* = 0.80) for the cows in the 2 treatment groups which were included in the final analysis.

There was a tendency for more cows to be inseminated within the 1st 3 days of MSD in the GnRH treated group (82.2 % vs 77.8 %, *P* = 0.08, Figs 1, 2). The conception rates to 1st insemination for the oestradiol benzoate and GnRH cows inseminated by Day 2 were 35.3 % (*n* = 146) and 35.6 % (*n* = 170), respectively (*P* = 0.90, Table 2). In total, 891 cows were suitable for resynchrony because they had

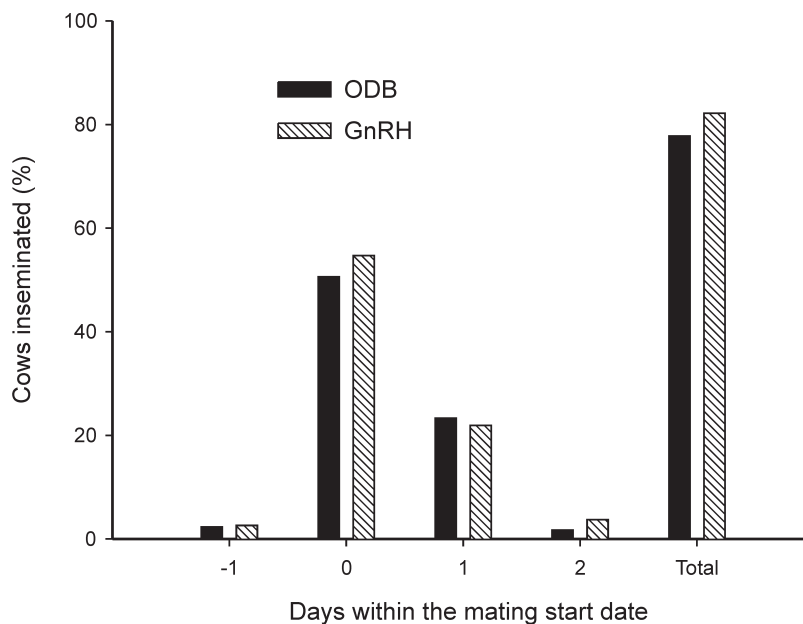


Fig. 1: Percentages of cows that were initially treated for anoestrus and were inseminated within the 1st 3 days of the breeding season, and subsequently allocated to the 2 treatment groups, the oestradiol benzoate (ODB) and GnRH groups.

been inseminated from Day -1 to Day 2 of a herd's MSD. They included 414 that were injected with oestradiol benzoate at device reinsertion and 477 injected with GnRH. The percentage of cows that did not conceive to the inseminations done from Day -1 to Day 2 and were submitted for a 2nd insemination between Days 22 to 28 after MSD did not differ between the 2 treatment groups (83.5 % vs 81.5 % of cows for oestradiol benzoate and GnRH groups, respectively, *P* = 0.41, Fig. 3, Table 1). Conception rates to the 2nd insemination made during this period were similar (43.4 % vs 38.8 %, for oestradiol benzoate and GnRH groups, respectively, *P* = 0.39).

The 6- and 21-week pregnancy rates,

the mean interval from MSD to 1st service and the mean interval from MSD to conception were similar for the 2 groups of resynchronised cows (Table 2). The percentage of cows that were not pregnant at the end of the breeding season (empty rates) were also similar (14.3 % vs 12.6 for the oestradiol benzoate and GnRH groups, respectively, *P* = 0.33, Table 2).

The log-rank test showed no differences in the distribution of the cumulative proportion of cows becoming pregnant after MSD (log-rank statistic = 0.025, *df* = 1, *P* = 0.87). The median time to conception from MSD for the oestradiol benzoate and GnRH treatments was 24 days in both cases (95 % CI 24 to 25 days, 95 % CI 24 to

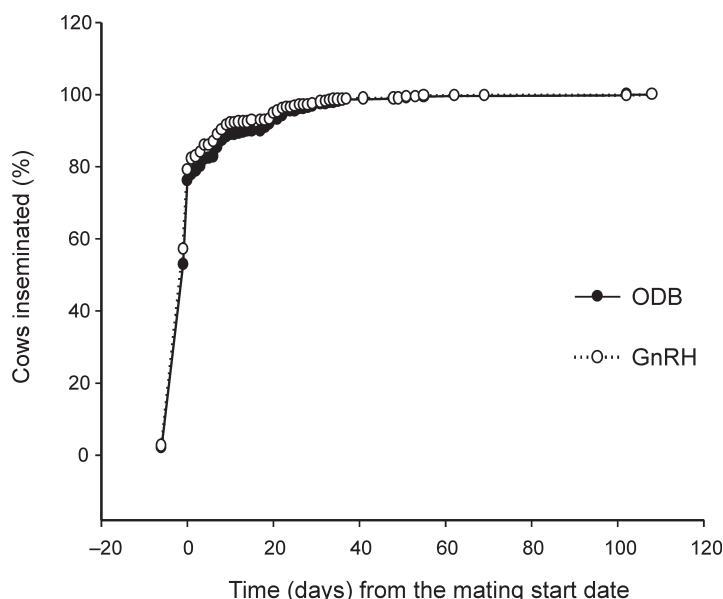


Fig. 2: Cumulative percentage of cows in the oestradiol benzoate (ODB) and GnRH groups inseminated for the 1st time after the initial anoestrous treatment.

Table 1: Conception rates of cows reinseminated by Days 24 and 28.

Reproductive index	Treatment group		Adjusted odds ratio (95 % CI)	P
	Oestradiol benzoate	GnRH		
Cumulative conception rates for cows reinseminated by Day 24 (%)	43.6 (65/149)	38.2 (63/165)	0.80 (0.50 – 1.28)	0.36
Cumulative conception rates for cows reinseminated by Day 28 (%)	43.3 (81/187)	38.8 (80/206)	0.83 (0.55–1.26)	0.39

Table 2: Comparison of overall reproductive performance between treatment groups.

Reproductive Index	Treatment group		Adjusted odds ratio (95 % CI)	P
	Oestradiol benzoate	GnRH		
Cumulative number cows conceiving within 3 days of MSD (%)	35.3 (146/414)	35.6 (170/477)	1.02 (0.77–1.35)	0.90
6-week pregnancy rates (%)	60.6 (251/414)	59.3 (283/477)	0.94 (0.71–1.24)	0.65
21-week pregnancy rates (%)	85.0 (352/414)	86.6 (413/477)	1.20 (0.81–1.76)	0.36
Mean interval from MSD to conception (mean ± SEM, days)	29.9 ± 1.8 (n = 355)	32.5 ± 1.8 (n = 417)	2.88 (–2.18 to 7.95)	0.26
Mean interval from MSD to 1st service (mean ± SEM, days)	4.2 ± 0.5 (n = 532)	3.5 ± 0.5 (n = 580)	–0.68 (–1.94 to 0.58)	0.29
Cumulative percentage of cows not resubmitted by Day 28	16.5 (37/224)	18.4 (47/255)	1.23 (0.75–2.03)	0.42
Empty rates at end breeding season (%)	14.3 (59/414)	12.6 (60/477)	0.82 (0.55–1.22)	0.33

28 days, for oestradiol benzoate and GnRH groups, respectively, Fig. 4).

DISCUSSION

The use of 250 µg of Fertagyl, as the GnRH agonist at the time of CIDR device reinsertion from 12 to 15 days after the 1st AI did not increase conception rates relative to those anovulatory anoestrous cows treated with oestradiol benzoate on device reinsertion. The rationale behind the use of GnRH in the present experiment was derived from the fact that GnRH had been shown to improve the embryo survival rates³¹ and its use with anovulatory anoestrous cows could be beneficial because of the low progesterone concentrations frequently seen after induction of oestrus and ovulation in these cows³³. Several factors may influence the response of cycling cows to treatment with GnRH. Peters *et al.* (2000)³¹ used meta-analyses to show that the variation in responses to treatment between different trials was associated with the trial environment, herd management and unidentified animal factors. Nonetheless, a saturated model showed a significant odds ratio for an increase in pregnancy rates when cycling cows were injected with GnRH 11–14 days after 1st insemination³¹.

The percentage of cows conceiving within 3 days of MSD was not altered by the subsequent use of GnRH instead of oestradiol benzoate. Some studies in New Zealand have reported a decline in conception rate to the preceding insemination when oestradiol benzoate was

injected 12 to 14 days after 1st insemination in cows treated for anoestrus, compared with untreated control cows^{26,27}. This could reflect the ability of oestradiol to enhance expression of oxytocin receptors by the endometrium⁴¹. In cycling non-pregnant cows, plasma

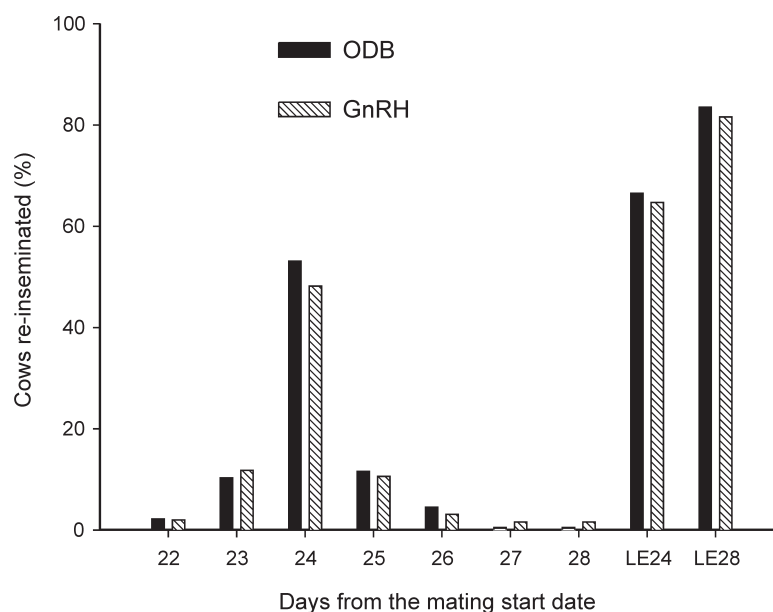


Fig. 3: Percentage of cows in the oestradiol benzoate (ODB) and GnRH groups that were not pregnant to inseminations performed between Day –1 and Day 2 and were re-inseminated between Days 22 and 28 as well as the cumulative percentage of cows reinseminated by Days 24 (LE24) or 28 (LE28).

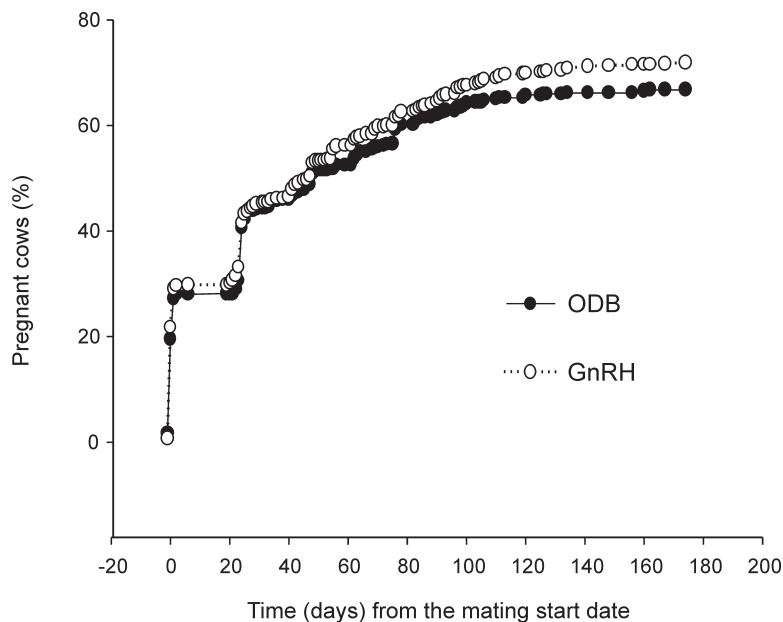


Fig. 4: Pregnancy rates of anoestrous cows in the oestradiol benzoate (ODB) and GnRH groups that were eligible for resynchrony treatment.

progesterone declined 24–48 hours after treatment with oestradiol benzoate³. However, treating pregnant cows which were previously either cycling⁴ or anoestrus⁶ with oestradiol benzoate 14 days from MSD did not reduce their conception rates. This is in agreement with other studies where oestradiol benzoate was used in this manner in cycling cows^{18,21}. Oestradiol benzoate does not reduce conception rates to the previous service because, firstly IFN- τ redirects metabolism of PGF 2α in favour of epoxy derivatives⁴³ and by downregulation of protein kinase c⁴²; and secondly IFN- τ together with progesterone, inhibit the expression of oestradiol and oxytocin receptors by the endometrium³⁰. These 2 mechanisms ensure that a viable foetus will be retained in the presence of briefly elevated plasma oestradiol before the spontaneous onset of luteolysis in non-pregnant animals.

The percentage of cows that did not conceive to the 1st insemination and were submitted for a 2nd insemination by Day 28 was similar for cows resynchronised with oestradiol benzoate or GnRH. Both oestradiol benzoate and GnRH cause turnover of follicular waves in cattle^{1,2,36}. The benefits of resynchrony in cycling^{4,15} and anoestrous cows^{6,22} using oestradiol benzoate and a CIDR device have been demonstrated. The response to resynchronisation exceeded those reported when a CIDR device had been inserted for 6 days (76 %)⁶. There is no information in the literature comparing the effects of reinsertion of a progesterone-releasing device for 6- or 8-day CIDR at resynchrony in cycling and anovulatory anoestrous cows. Eagles *et al.* (2001)⁶

reinserted a CIDR for 6 days. The resynchrony used in this study involved an reinsertion of a CIDR for 8 days. Further comparative studies need to be made to evaluate the effect of a 6- or 8-day CIDR at resynchrony.

In summary, treating anovulatory anoestrous dairy cows with GnRH instead of oestradiol benzoate from 12 to 15 days after 1st insemination did not increase conception rates among those cows previously inseminated within the 1st 3 days of the breeding programme. However, the injection of GnRH at the time of CIDR device reinsertion on Days 12–15 was as effective as oestradiol benzoate in synchronising the return to service of cows that had not conceived to the preceding 1st insemination. Cows that were submitted for a 2nd insemination after being injected with GnRH or oestradiol benzoate had similar conception rates to 2nd service. Consequently, the mean interval from MSD to conception was similar among cows treated with GnRH or oestradiol benzoate. However, 16.5 % and 18.4 % of anovulatory anoestrous cows that did not conceive to the 1st service were not successfully resynchronised using either GnRH or oestradiol benzoate. These cows were not resubmitted for a 2nd insemination within the expected 21–28 days. If any further success in the treatment of anovulatory anoestrous cows is to be achieved, the mechanisms that result in the quiescence of these cows needs to be investigated. The initial hypothesis that substitution of GnRH for oestradiol benzoate at the commencement of the resynchrony treatment would increase conception rates to the 1st service is rejected; the hypothesis that

both GnRH and oestradiol benzoate would resynchronising the returns to service of those cows that did not conceive to the preceding insemination is accepted.

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