Some effects of using progesterone and oestradiol benzoate to stimulate oestrus and ovulation in dairy cows with anovulatory anoestrus

K.L. MacMILLAN, V.K. TAUFA, A.M. DAY[1] & S. McDOUGALL

Dairying Research Corporation, Private Bag 3123, Hamilton, New Zealand.

ABSTRACT

Anovulatory anoestrus (AA) is a major form of infertility in pasture-fed dairy cows in New Zealand and is most frequently treated by injecting equine chorionic gonadotrophin (eCG) after pre-treatment with progesterone (P_4). The effectiveness of this treatment varies between herds. Injecting oestradiol benzoate (ODB) from 24 h to 48 h following P_4 and ODB priming can also induce oestrus and ovulation in AA cows. Two field trials compared responses in 572 cows with AA treated with P_4 alone, P_4 + eCG and P_4 + ODB. The P_4 was administered per vaginum for 5 or 7 days using a CIDR device.

An injection of 1 mg ODB at 48 h after device removal in Trial 1 increased the insemination submission rate during the following 4 days from 55% among cows treated with P_4 or P_4 + eCG to 75%, and reduced the incidence of non-responding cows from 21% to 3%. In Trial 2, the comparable results to ODB treatments were 86% inseminated and 3% not responding.

The increased submission rates were not due to the ODB producing oestrus without ovulation. Consequently, 42% of anoestrous cows treated with ODB conceived during the first week of AB, 76% conceived after 6 weeks of AB and only 5.5% were finally not pregnant. Comparable figures for herdmates treated with P_4 but not injected with ODB were 29%, 66% and 12% respectively.

Injecting a low dose of ODB from 24 to 48 h after P_4 pre-treatment can be used to successfully treat AA in dairy cows.

Keywords: dairy cows; anovulatory anoestrus; progesterone; oestradiol; infertility.

INTRODUCTION

Anovulatory anoestrus (AA) is a major form of temporary infertility among dairy cows in New Zealand. Initial attempts at treatment were ineffective because they did not include a period of priming with progesterone (P_4) (Fielden et al., 1973). More recent studies showed that at least 7 days of treatment with P_4 administered per vaginum combined with an intramuscular injection of equine chorionic gonadotrophin (eCG; 400 IU previously called PMSG), could stimulate oestrus and ovulation within the following 2 weeks in about 75% of anoestrous cows (Macmillan & Day, 1987; Macmillan & Peterson, 1993). This overt response rate varied among herds from 40% to 100% for undefined reasons and affected confidence in this treatment.

When studying the effects of priming with P_4 on the intensity of behavioural responses to oestradiol benzoate (ODB) injected into anoestrous animals, we observed that most cows showed oestrus and ovulated followed by a luteal phase of normal duration (McDougall et al., 1992). A second study showed that the combination of P_4 priming and an ODB injection (0.6 mg) administered within 3 weeks postpartum caused 80% of the anoestrous cows to show oestrus and 86% to ovulate (McDougall & Macmillan, 1993). Combining the administration of these two steroid hormones could potentially reduce the variation in responses to treating AA. This potential was evaluated in two field trials by comparing responses to P_4 alone with either P_4 + eCG or P_4 + ODB at either of 2 dose rates.

MATERIALS AND METHODS

Animals

In Trial 1, cows which had not been detected in oestrus in each of 8 herds by the seventh day before the start of a herd's seasonal artificial breeding (AB) programme were presented for a veterinary examination in October or November, 1992. Those diagnosed as AA because there was no evidence of a post-partum ovulation were divided into 3 groups randomised within herd on age, breed, calving date and current body condition score. A similar selection procedure was followed in Trial 2 in 1993 in the same 8 herds.

Initial Treatment

In both trials an EAZI-breed CIDR B™ containing 1.9 g of P_4, (InterAg, Hamilton, NZ) including a CIDIROL capsule containing 10 mg ODB (Douglas Pharmaceuticals, Auckland, NZ) was inserted into the vagina of each animal with AA following diagnosis. In Trial 1, the 3 treatments were:

(a) CIDR treatment for 7 days;
(b) As for (a) with 400 IU eCG as Pregnecol (Pastoral Consultants, Otane, NZ) injected at CIDR device removal; and,
(c) CIDR treatment for 5 days with 1.0 mg ODB (in 2 ml oil) injected 48 h after device removal if the animal was not recently or currently in oestrus.

In Trial 2, the 5 treatments were:

(a) CIDR treatment for 5 days;
(b) CIDR treatment for 5 days with 0.75 mg ODB injected

[1] AgResearch, Ruakura Agricultural Research Centre, Private Bag 3123, Hamilton, New Zealand.

24 h after device removal;
(c) as for (b) but with 1.0 mg ODB injected at 24 h;
(d) as for (b) but with 0.75 mg ODB injected at 48 h; and,
(e) as for (b) but with 1.0 mg ODB injected at 48 h after device removal.

Some cows in every herd were allocated to Group A, but an individual herd only included 2 of the other 4 treatment groups so that only one visit had to be made to each herd when the 2 dose rates of ODB were used, or one dose rate was used at 24 and 48 h. This minimised the likelihood of errors. As in Trial 1, ODB was not injected if an animal was previously or currently in oestrus at the time of injection.

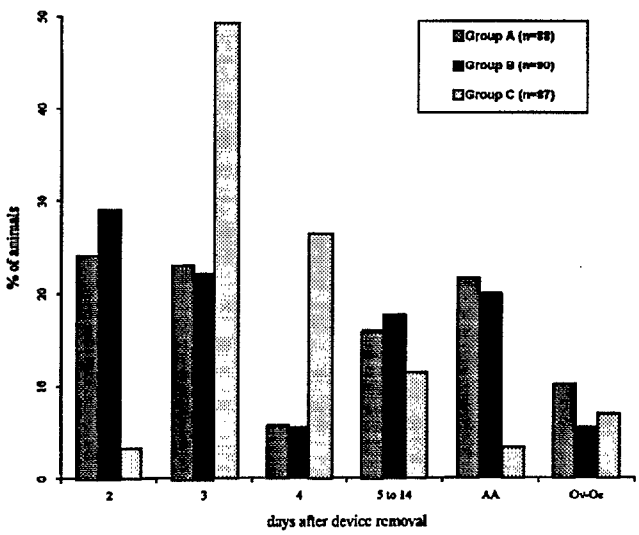
Device removal and associated injections occurred at morning milkings. Animals in both trials were observed for symptoms of oestrus with the rest of the herd and presented on a daily basis for insemination.

Responses and Re-examinations

Animals not detected in oestrus within 14 to 17 days of device removal were presented for a second examination. They were diagnosed as either having ovulated without oestrus, or having failed to respond to treatment and were still AA. The former were injected with a luteolytic dose of prostaglandin $F_{2\alpha}$ (PGF; Lutalyse, Upjohn NZ Ltd); the latter had a CIDR device (+ capsule) inserted for a second treatment, but it was always for 5 days with 1.0 mg ODB injected 24 h after device removal. This revisiting process was repeated with unmated animals not inseminated within 14 days after removal of a CIDR device for the second time.

A milk sample was taken from every cow in Trial 1 at the morning milking on the fourteenth day after device removal. This sample was refrigerated before being assayed for P_4 (Coat-a-Count; Los Angeles, USA). Those cows which had ovulated within 4 days of device removal should have reached mid-cycle by this time and had milk P_4 concentrations >1 ng/ml. Herdmates which had a behavioural oestrus but no ovulation would have produced milk containing <1 ng P_4/ ml.

FIGURE 1: Intervals to oestrus among cows diagnosed with anovulatory anoestrus (AA), or which ovulated without a detected oestrus (Ov-Oe) after treatment with a CIDR device (Groups A) as well as eCG (Group B) or ODB (Group C) (Trial 1).



Pregnancy Testing and Data Presentation

Animals inseminated within 14 days of removal of the original CIDR device were pregnancy tested by rectal palpation from 7 to 9 weeks after first insemination. All cows were pregnancy tested 6 weeks after the end of the herdís breeding programme to confirm their pregnancy status and conception dates.

RESULTS

The distribution of intervals from device removal to detected oestrus or re-examination are shown in Fig. 1 for the 265 cows included in Trial 1. The distributions of these intervals did not differ significantly for Treatments A and B with 71% of animals inseminated within 14 days, mostly on the second (27%) or third (22%) days after device removal. Another 21% failed to respond and remained AA. In contrast, 89% of cows administered Treatment C had been inseminated within 14 days ($p<0.01$), with 49% and 26% on the third and fourth days respectively. Only 3% remained AA ($p<0.01$; Fig. 1).

A milk sample taken during dioestrus was assayed from each of 173 cows included in Trial 1 and inseminated within the first 5 days after CIDR device removal. Progesterone concentrations exceeded 1 ng/ml in 95.4% of these samples. Their pregnancy rate to first insemination was 43.4% and this was not affected by treatment.

Only 48.1% of the 81 cows in Trial 2 which received P4 alone were detected in oestrus and inseminated during the 4 days following device removal. The comparable average among the 226 cows injected with ODB was 86.2% ($p<0.01$). This average was affected by dose (0.75 vs 1.0 mg = 83% vs 90%), and by the timing of the ODB injection (24 vs 48 h = 91% vs 82%; $p<0.05$) so that only 77.4% of cows injected with 0.75 mg ODB at 48 h after device removal were inseminated in the initial 4-day period compared to 93.7% of those administered 1.0 mg ODB at 24 h ($p<0.05$). Although fewer cows within the ODB groups were detected in oestrus from 5 to 14 days after device removal (19% vs 7%; $p<0.05$), more of the cows which were not injected with ODB remained AA (21% vs 3%; $p<0.01$) and had to be retreated. The average pregnancy rate to first inseminations made within 5 days of device removal was 45.3%. This was not affected by either ODB treatment or time of treatment.

In both trials, ODB treatment significantly altered conception patterns. For example, 42% of the cows injected with ODB in Trial 2 (n = 208) had conceived by the end of Week 1 (after device removal) compared to 29% of uninjected cows ($p<0.05$). This difference had diminished by the end of Week 3 (48% vs 45%), but had increased again by the end of Week 6 (76% vs 66%; $p<0.05$). The percentage of cows which finally failed to conceive was also reduced among cows administered ODB (5.5% vs 12%; $p<0.05$).

DISCUSSION

The routine treatment of cows with AA has become common in New Zealand's dairy herds. Using P_4 to prime the hypothalamic-pituitary system is a normal procedure in cases of suckling or seasonal anoestrum in ungulates. Although this

procedure worked satisfactorily in some dairy herds, results were unaccountably variable. The results described in this report show that the strategic use of a low dose of ODB can produce oestrus accompanied by ovulation in about 90% of treated animals.

Two specific areas of concern could be associated with this form of use of ODB. First, submission rates could be inflated by some cows displaying a behavioural oestrus in response to the ODB which was not accompanied by ovulation (false heat). Secondly, oestradiol has been implicated in producing low pregnancy rates in synchrony programmes. The results from Trials 1 and 2 showed that the incidence of false heats was not increased sufficiently to affect pregnancy rates to first insemination. Nonetheless, average pregnancy rates in Trials 1 and 2 (43.4% and 45.3%, respectively) were lower than those usually obtained in cycling cows (Macmillan & Peterson, 1993). In spite of the lower fertility at first insemination, arranging diagnosis and treatment so that most oestrous responses occurred during the first 3 or 4 days of a herd's AB programme should mean that about 75% of treated cows should conceive during an AB programme of 6 weeks if the treatment included the injection of ODB, and about 5% would have failed to conceive by the end of mating. The synchrony effect of the treatment should make the mean conception date earlier for treated anoestrous cows than for cycling herdmates which received no synchrony treatments. If current trials should demonstrate this effect, then the strategic use of a low dose of ODB following P_4 priming will substantially reduce the adverse effects of AA on conception patterns in many New Zealand dairy herds.

REFERENCES

Fielden, E.D.; Macmillan, K.L.; Watson, J.D. 1973. The anoestrous syndrome in New Zealand dairy cattle. 1. A preliminary investigation. *New Zealand Veterinary Journal* **21:** 77-81.

Macmillan, K.L.; Day, A.M. 1987. Treating the non-cycling cow. *Proceedings of the 39th Ruakura Farmers' Conference* pp. 65-68.

Macmillan, K.L.; Peterson, A.J. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. *Animal Reproduction Science* **33:** 1-25.

McDougall, S.; Burke, C.R.; Macmillan, K.L.; Williamson, N.B. 1992. The effect of pretreatment with progesterone on the oestrous response to oestradiol-17b benzoate in the post-partum dairy cow. *Proceedings of the New Zealand Society of Animal Production* **52:** 157-160.

McDougall, S.; Macmillan, K.L. 1993. Follicle waves, ovulation and oestrus in the postpartum cow. *Proceedings of the 10th Seminar for the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association* pp. 33-47.