THE USE OF OESTRADIOL BENZOATE AND PROGESTERONE TO SYNCHRONISE OESTRUS IN DAIRY CATTLE

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GENERAL ABSTRACT

Current oestrus synchronisation regimes for cattle are based on synchronising the end of the progestational phase of the oestrous cycle so that ovulation occurs simultaneously in treated animals. The end of the progestational phase can be synchronised through inducing premature luteolysis using prostaglandin $F_{2\alpha}$ and its analogues or by artificially extending dioestrus using exogenous progesterone treatment. The time taken for subsequent follicular maturation and ovulation tends to be inconsistent between animals, which contributes to the poor fertility obtained following fixed-time insemination after oestrus synchronisation treatments. The variable rate of follicular development occurring after a synchronous decline in plasma progesterone levels is a major limiting factor in achieving a degree of synchrony of oestrus and ovulation which would allow for fixed-time insemination.

Controlling the time of ovulation using exogenous oestrogen to induce a pre-ovulatory LH surge is a potential method by which the variability in timing of ovulation may be reduced. Alternatively, re-setting follicular wave patterns in different animals at the commencement of synchrony treatments using exogenous oestrogen, so that follicular wave emergence is synchronised, is another method by which the variability in timing of ovulation could be reduced.

A clinical trial was conducted involving 750 dairy heifers in 13 herds to determine the effects of 0.5 mg oestradiol benzoate administered intramuscularly 24 hours after removal of progesterone-containing intravaginal devices (CIDR-B) on the occurrence and timing of oestrus, synchronised pregnancy rate and synchronised conception rate in dairy heifers. Within each herd heifers were randomly allocated to one of two oestrus synchronisation treatments. All heifers received a CIDR-B progesterone-containing intravaginal device with an attached 10 mg oestradiol benzoate capsule for 12 days. Twenty-four hours after CIDR-B removal one group received an intramuscular injection of 0.5 mg oestradiol benzoate and the other group received an intramuscular injection of a placebo. Heifers were inseminated to detected oestrus 48 and 72 hours after device

removal. Administration of oestradiol benzoate 24 hours after removal of CIDR-B devices significantly increased the number of heifers exhibiting oestrus within the observation period (96.1 % vs 90.5%, $p<0.01$). It also altered the onset of oestrus so that significantly more heifers were in oestrus (86.6% vs 72.3%, $p<0.01$) and conceived (47.1 % vs 37.5%, $p<0.05$) by 48 hours after device removal. The overall synchronised conception rate and synchronised pregnancy rate were unaffected by treatment.

The effects of the same oestrus synchronisation treatment, on the time to oestrus, ovulation, and peak LH concentration were examined in dairy heifers. Treatment with oestradiol benzoate tended to reduce the time from device removal to LH peak in randomly cycling heifers (median time to LH peak 40.1 hr vs 63.9 hr, $p=0.07$), but treatment with oestradiol had no significant effect on the time to LH peak, standing oestrus or ovulation in heifers synchronised during late dioestrus.

The effects of oestradiol benzoate on the dominant follicle and corpus luteum of cows treated with progesterone (CIDR-B) at different stages of the oestrous cycle were investigated. Treatment with oestradiol benzoate on day 3 of the oestrous cycle caused atresia of the dominant follicle present at CIDR-B insertion and resulted in the early emergence of the subsequent follicular wave. Treatment with oestradiol benzoate on days 6, 9, 12 and 15 of the oestrous cycle had no effect on follicular characteristics or the emergence of the subsequent follicular wave. Treatment with oestradiol benzoate had no effect on the day of onset of regression of the corpus luteum regardless of the stage of the oestrous cycle at CIDR-B insertion.

The effectiveness of re-using CIDR-B devices to synchronise returns to oestrus in non-pregnant dairy heifers was examined. After an initial CIDR-B synchronisation programme in dairy heifers, the used CIDR-B devices were re-inserted 14 or 16 days after first insemination, for a period of 5 days. Re-insertion of used CIDR-B devices significantly increased the number of non-pregnant heifers detected in oestrus and inseminated by 48 hours after device removal (45.2% vs 27.3%, $p<0.05$, in herds where CIDR's were re-inserted on day 14; 48.8% vs 13.6%, $p<0.05$, in herds where CIDR's

were re-inserted on day 16). Re-insertion at 14 or 16 days after first insemination was equally effective in increasing visible returns to service. Conception rate was unaffected by CIDR-B treatment.

In conclusion, intramuscular administration of oestradiol benzoate 24 hours after the removal of CIDR-B progesterone-containing intravaginal devices increases the number of heifers exhibiting oestrus at an earlier time after device removal. The administration of oestradiol benzoate appears to reduce the variability in timing of LH peaks typically occurring in a herd of synchronised heifers due to different stages of follicular development being present at the time of CIDR-B removal. Treatment with oestradiol benzoate at the start of CIDR-B treatment appears to have no significant effect on synchronising follicular wave emergence in different animals other than those in early metoestrus. Administration of oestradiol benzoate after treatment with exogenous progesterone therefore appears to offer the most potential in controlling the time of oestrus and ovulation and allowing for fixed-time insemination.

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CHAPTER 1

LITERATURE REVIEW:
OESTRUS SYNCHRONISATION IN CATTLE

Introduction

New Zealand's predominantly pasture-based, seasonally calving dairy-farming system imposes restrictions on reproductive management of dairy herds. After entering the adult dairy herd, New Zealand cows must be bred annually during a restricted breeding period of approximately 12 weeks if they are to maintain the required 365 day calving interval. Cows not conceiving during this period are usually culled. Reproductive wastage, i.e. failure to conceive or conception that occurs unacceptably late in the breeding period, is one of the main reasons for the removal of cows from New Zealand dairy herds (Harris, 1989). The development of strategies such as oestrus synchronisation to aid in the reproductive management of New Zealand dairy herds is therefore vital in order to optimise reproductive performance.

Since it was discovered that daily injections of progesterone suppressed heat and prevented ovulation during the treatment period (Christian and Casida, 1948), intensive research into oestrus synchronisation in cattle has been conducted. During this time many regimes have been investigated but few have proven suitable to control the oestrous cycle.

Synchronising the oestrous cycle so that a large proportion of a group of cows or heifers is in oestrus at a similar time is used for several management-related reasons which are outlined below.

a) To enable the practical use of artificial insemination (AI)

Beef cattle and replacement dairy heifers are usually managed under extensive conditions. For herd owners to utilise AI in randomly cycling animals involves daily yarding for oestrus detection and insemination, a time-consuming and labour-intensive operation. Synchronising the oestrous cycle concentrates oestrus detection and AI into a narrow, predetermined time period. The ability to use AI in these animals has several advantages: a) it enables the selection of genetically superior sires thereby increasing the

rate of genetic gain within the herd, b) it allows the selection of "easy-calving" sires, thereby minimising losses due to dystocia, and c) it provides for increased selection pressure by utilising maiden heifers as a source of herd replacements. In New Zealand dairy herds maiden heifers are, on average, of higher genetic merit than older cows in the herd (Anon., 1994). In addition to the long-term advantages in terms of genetic improvement, short-term advantages of AI in heifers are: a) a concentrated calving pattern, b) an earlier mean calving date and c) a longer lactation length (Macmillan and Asher, 1990). An earlier, concentrated calving pattern is advantageous because first-calf cows have the longest calving to first-oestrus interval (Macmillan and Curnow, 1976) and the highest incidence of post-partum anoestrus (Fielden and Macmillan, 1973; Roche *et al*, 1992).

b) To facilitate reproductive management in seasonally calving dairy herds

In seasonally calving dairy herds in New Zealand, the breeding period is usually restricted to approximately 12 weeks, comprising of 4 to 8 weeks of artificial breeding (AB) (Macmillan and Asher, 1990) followed by a period of natural mating using herd bulls. Cows not conceiving during this 12 week period are usually culled. Those cows conceiving late in the breeding period are either culled or are induced to calve so that their calving coincides with the remainder of the cows in the herd. Induction of calving has several detrimental effects on the cow and calf. These include a higher incidence of calf mortality, retained foetal membranes and metabolic disease, and reduced production (Morton and Butler, 1995a; 1995b; 1995c). Synchronising the oestrous cycle of cows enables a greater proportion of the herd to be mated to AB early in the breeding period (Macmillan and Curnow, 1976). Armer *et al* (1993) applied a whole-herd synchronisation programme to three seasonal herds in New Zealand. This resulted in 87% to 95% of the cows being inseminated in the first four days of the breeding period. Proposed benefits of a subsequently concentrated calving pattern were that fewer cows would need to be induced to calve and that there would be a lower incidence of anoestrum due to the longer interval from calving to the planned start of mating (PSM).

Oestrus synchronisation has the additional advantage of increasing the number of breeding opportunities for each cow during the breeding period (Odde, 1990; Woolly and Thurston, 1993), hence reducing cow wastage due to culling.

c) To facilitate reproductive management in non-seasonally calving dairy herds

Various oestrus synchronisation programmes have been described for use in post-partum cows so that oestrus detection and insemination are conducted during only 7 out of each 21 days (Folman *et al*, 1984; Smith *et al*, 1986; Ferguson and Galligan, 1993). This has a favourable impact on reducing the percentage of non-pregnant cows in herds that have previously experienced poor reproductive performance due to inadequate oestrus detection efficiency (Ferguson and Galligan, 1993).

d) To increase the efficiency of oestrus detection

Inadequate oestrus detection is a major cause of poor reproductive performance in many dairy herds. In a study conducted in a Victorian dairy herd, Williamson *et al* (1972a) found that only 56% of cows in oestrus were identified by herdsmen and that this was reduced to 48% when observation of cows in the milking parlour was the sole method of oestrus detection. Stevenson and Britt (1977) reported similar results with approximately 50% of oestrus periods being missed. Lack of diligence in oestrus detection in New Zealand seasonally calving dairy herds is a major limitation to effective herd management once herd size exceeds 600 cows, or where contract labour is largely responsible for routine herd management (Armer *et al*, 1993). When cows are synchronised, increased oestrus activity can be predicted, thereby allowing farm staff to concentrate their efforts into observation of oestrus (Larson and Ball, 1992; Armer *et al*, 1993; Woolly and Thurston, 1993).

e) To facilitate embryo transfer (ET) programmes

Successful embryo transfer requires that recipients and donors are at the same

stage of the oestrous cycle when embryos are transferred. To achieve this in a large number of recipients, the oestrous cycle of the donor and recipients can be synchronised.

Desirable features of oestrus synchronisation programmes

The desirable features of an oestrus synchronisation programme were outlined by Larson and Ball (1992), namely:

a) high response rates to treatments initiated at any stage of the cycle,
b) tight synchrony in time of oestrus and time of ovulation,
c) normal fertility at the regulated ovulation, and
d) normal return to oestrus and fertility at repeated services.

In addition, the economic benefits from increasing the percentage of cows that conceive during the optimum period and from reducing the labour required for routine observations must be greater than the costs of the synchronisation programme.

The ultimate objective of any oestrus synchronisation programme is to achieve sufficient synchrony to allow insemination of treated cattle at a fixed-time without recourse to oestrus detection, while still achieving pregnancy rates at least equal to those with natural mating (Roche, 1976c; Macmillan and Asher, 1990).

Methods of oestrus synchronisation

Procedures to synchronise oestrus and ovulation in cycling cattle are based on synchronising the end of the progestational phase of the oestrous cycle (Wright and Malmo, 1992). Progesterone exerts a negative feedback effect on the oestrous cycle by decreasing pulsatile luteinising hormone (LH) release from the anterior pituitary gland (Rajamahendran *et al*, 1979; Stumpf *et al*, 1993). In the presence of progesterone, follicles fail to ovulate and behavioural oestrus is suppressed (Sirois and Fortune, 1990). When progesterone concentrations decline, the inhibitory effects on the pituitary are

removed, the pulse frequency and amplitude of LH secretion increases and the sequence of neuro-endocrine events leading to ovulation and oestrus occurs (Hafs *et al*, 1975; Fogwell *et al*, 1978).

Plasma progesterone levels can be controlled in two ways:

a) by prematurely removing the corpus luteum (CL) and therefore the source of endogenous progesterone; and
b) by administering an exogenous source of progesterone for a time which allows natural luteal regression, and termination of the progestational phase occurring when progesterone treatment ceases.

These methods have been used alone, in combination with each other and with various other compounds such as oestrogens, gonadotrophin releasing hormone (GnRH) and pregnant mare serum gonadotrophin (PMSG) to synchronise the oestrous cycle of cattle.

Oestrus synchronisation by premature removal of the corpus luteum

Manual enucleation

Manual enucleation of the corpus luteum via rectal palpation is the oldest method for controlling the oestrous cycle of cattle (Oxender *et al*, 1974; Seguin, 1979). Removing the corpus luteum in this manner eliminates the production of progesterone and the cow is in oestrus 3 to 5 days later. Haemorrhage at the time of enucleation and subsequent ovarian adhesions which can cause infertility, have caused some investigators to deem this an undesirable procedure for synchronising oestrus in cows (Oxender *et al*, 1974; Larson and Ball, 1992).

Luteolysis

During the normal bovine oestrous cycle natural luteal regression occurs in late

dioestrus with an associated decrease in blood progesterone levels. The naturally occurring luteolysin in cattle is $PGF_{2\alpha}$, synthesised from arachidonic acid within the endometrium and released into the uterine veins (Boothe, 1984). The counter-current exchange mechanism formed between the ovarian artery and utero-ovarian vein transports $PGF_{2\alpha}$ to the corpus luteum where it causes luteolysis (Inskeep, 1973; Garverick and Smith, 1993). The mechanisms involved in luteolysis are not known but probably involve immune-mediated cellular destruction (Pate, 1994).

Following the discovery in 1969 that $PGF_{2\alpha}$ was luteolytic in laboratory animals, research began into the effects of $PGF_{2\alpha}$ in large domestic animals (sheep, cattle and horses) (Louis *et al*, 1975; Walpole, 1975). At the same time, biochemical synthesis of several analogues such as cloprostenol, dinoprost, fenprostalene and prostianol was achieved. These analogues have luteolytic activity equal to, or greater than $PGF_{2\alpha}$, but with lower toxicity (Walpole, 1975). $PGF_{2\alpha}$ and its analogues are potent luteolytics in cattle provided treatment is initiated after day 4 or 5 of the oestrous cycle and before spontaneous luteal regression on day 16 or 17 (Rowson *et al*, 1972; Tervit *et al*, 1973; Cooper, 1974; Cooper and Rowson, 1975). More reliable luteolysis occurs between days 7 and 16 (Macmillan, 1983). Changes in plasma concentrations of progesterone (Louis *et al*, 1975; Seguin, 1979; Kazmer *et al*, 1981; Macmillan, 1983), luteinising hormone (Cooper and Rowson, 1975; Louis *et al*, 1975; Refsal and Seguin, 1980) and oestradiol 17-β (Cooper and Rowson, 1975; Louis *et al*, 1975) following luteolysis induced by $PGF_{2\alpha}$ and its analogues closely resemble those occurring around a spontaneous oestrus. The subsequent response of the ovary with follicular development and ovulation is morphologically similar to that following natural luteolysis (Cooper, 1974; Scaramuzzi *et al*, 1980; Quirk *et al*, 1986).

Route of administration

Various routes of administering prostaglandins to cattle are effective in causing luteolysis. Administration into the uterine horn ipsilateral to the ovary bearing the corpus luteum is effective in causing luteal regression between days 5 and 16 (Rowson *et al*,

1972). Intravenous administration is equally effective (Stellflug *et al*, 1975). The interval to oestrus following intravaginal administration has been shown to be longer than for the intrauterine route (Oxender *et al*, 1974). Intramuscular and subcutaneous administration are both effective in causing luteolysis provided treatment occurs between day 5 and 16 (Tervit *et al*, 1973; Cooper, 1974; Oxender *et al*, 1974). For practical purposes the intramuscular or subcutaneous routes are used routinely.

Dose rate

The recommended intramuscular dose for $PGF_{2\alpha}$ in cattle is 25 mg although doses of between 12.5 mg and 60 mg have shown no differences in terms of oestrus response, degree of synchrony of oestrus, time to oestrus and time to ovulation (Roche, 1974c; Stellflug *et al*, 1975; Donaldson *et al*, 1982). Lower doses are effective in causing luteolysis if given by the intra-uterine route (Rowson *et al*, 1972). Investigation into the effects of varying the dose of cloprostenol showed that 0.5 mg consistently induced luteolysis (Cooper, 1974), but lower doses lead to fewer cows in oestrus and a higher proportion of cows showing silent heats (Macmillan, 1978).

Stage of cycle at administration and effects on synchrony and time to oestrus

The stage of the oestrous cycle at prostaglandin treatment has a major effect on the oestrous response. During the first 4 to 7 days of the cycle, the developing corpus luteum is insensitive to the luteolytic action of prostaglandin (Rowson *et al*, 1972; Tervit *et al*, 1973; Cooper, 1974; Cooper and Rowson, 1975; Macmillan, 1983). After day 16 or 17 the corpus luteum regresses spontaneously and therefore exogenous prostaglandin has no effect in hastening oestrus and ovulation (Cooper, 1974). The oestrus response following prostaglandin induced luteal regression follows a consistent pattern. The majority of responding animals exhibit oestrus approximately 3 days after treatment, generally ranging between 1 and 5 days (Cooper, 1974; Macmillan, 1978; Dailey *et al*, 1983; Macmillan and Henderson, 1984; Tanabe and Hann, 1984; Watts and Fuquay, 1985; Kastelic *et al*, 1990).

There is a progressive increase in the number of cows in oestrus after a single prostaglandin injection between days 5 and 17 of the oestrous cycle (Watts and Fuquay, 1983; Macmillan and Henderson, 1984; Tanabe and Hann, 1984; Watts and Fuquay, 1985). Cows and heifers injected in early dioestrus have a shorter interval to oestrus than do those injected in late dioestrus (Refsal and Seguin, 1980; King *et al*, 1982; Tanabe and Hann, 1984; Watts and Fuquay, 1985). Less variation in the interval to oestrus is achieved when treatment is initiated in early dioestrus compared to late dioestrus (Stevenson *et al*, 1984; Tanabe and Hann, 1984).

The post-injection interval to oestrus in cattle treated with prostaglandin is related to the stage of development of the dominant follicle present at the time of treatment because of the time required for such a follicle to complete its development (Scaramuzzi *et al*, 1980; Kastelic *et al*, 1990). Cows with follicles near their maximum diameter at the time of prostaglandin treatment have a shorter time to ovulation (Kastelic *et al*, 1990).

The stage-of-cycle effect is notable when two injections of prostaglandin are given 10 to 12 days apart. The onset of oestrus is sooner and the degree of synchrony greater following the second injection than is observed after the first (Cooper, 1974). At the time of the second injection the stage of cycle is less variable than at the first injection (Johnson, 1978).

The number of cows which exhibit oestrus after treatment is significantly correlated with the progesterone concentration at the time of prostaglandin injection (Watts and Fuquay, 1985). It is therefore likely that the functional maturity of the corpus luteum affects prostaglandin induced luteolysis.

Conception rates

Conception rate following prostaglandin induced oestrus is consistently equal to (Rowson *et al*, 1972; Cooper, 1974; Roche, 1974c; Burfening *et al*, 1978; Young and

Henderson, 1981; Tanabe and Hann, 1984) or greater than (Macmillan and Day, 1982; Seguin *et al*, 1983; Macmillan and Henderson, 1984) that achieved in control animals. It has been proposed that the enhanced conception rate seen in some trials is due to a more rapid decline in plasma progesterone concentrations following prostaglandin treatment (Macmillan and Henderson, 1984). The stage-of-cycle has also been demonstrated to influence the conception rate following prostaglandin induced oestrus. Lower conception rates are seen when luteolysis is induced in early dioestrus compared to late dioestrus (Watts and Fuquay, 1983; Watts and Fuquay, 1985).

Responses in lactating cows vs maiden heifers

Variation exists between lactating cows and maiden heifers in terms of the interval from prostaglandin injection to oestrus and the degree of response to treatment. In comparative studies, heifers consistently show a shorter post-injection interval to oestrus than cows (King *et al*, 1982; Seguin *et al*, 1983; Stevenson *et al*, 1984), however the reason has not been identified. A higher proportion of maiden heifers respond to treatment with prostaglandin when compared to lactating cows (Macmillan, 1978; King *et al*, 1982; Seguin *et al*, 1983). This difference is mainly due to a higher incidence of anoestrum in post-partum cows (Macmillan, 1978; Stevensen *et al*, 1987; Kiracofe, 1988), but was still evident when injection followed palpation of a corpus luteum (Seguin *et al*, 1983).

Treatment protocols

Two injection regime

Given that exogenous prostaglandin treatment has the potential to induce luteolysis only between days 5 and 17 of the oestrous cycle, a single injection of prostaglandin given to a group of animals cycling at random will only be followed by oestrus and ovulation in a proportion of them. To overcome this problem, two injections of prostaglandin can be given 10 to 12 days apart (Cooper, 1974; Roche, 1974c). After the

first injection there are three types of responses. Animals treated between day 0 and 5 of the oestrous cycle will be unaffected by treatment and 10 to 12 days later will be in the prostaglandin-sensitive mid-luteal phase of the cycle. Animals treated between days 6 and 17 of the cycle will undergo luteolysis, show oestrus within 2 to 4 days, ovulate and begin a new oestrous cycle. Ten to 12 days after the first injection they will be in the mid-luteal phase of the cycle. Cows treated between days 18 and 21 of their cycle will be unaffected by treatment. They will complete natural luteal regression, followed by oestrus and ovulation. Ten to 12 days after treatment they will be in the sensitive luteal phase of the cycle. Hence, if a second injection of prostaglandin is given 10 to 12 days after the first, all animals should be sensitive to its luteolytic effect. The two injection protocol is now widely used to synchronise oestrus in cattle although the results of this treatment regime have been inconsistent (King and Robertson, 1974; Roche, 1976c; Macmillan *et al*, 1977; Odde, 1990; Larson and Ball, 1992; Wright and Malmo, 1992).

Single injection regimes

Several strategies using single injections of prostaglandin combined with intensive herd management are routinely used. One such strategy involves weekly palpation of cows after the planned start of mating and treatment with prostaglandin if a corpus luteum is present (Seguin *et al*, 1983; Malmo, 1991). Seguin *et al* (1983) found that this regime altered the oestrous pattern from a random distribution to one in which 88% of 1st inseminations and 82% of all inseminations were conducted on 4 days of the week.

Another prostaglandin-based reproductive management system involves daily heat detection and insemination for 5 to 6 days and then injecting all those cows that have not been inseminated on day 6 (Macmillan *et al*, 1977; Donaldson *et al*, 1982; Kiracofe, 1988; Malmo, 1991). A variation on this programme involves a second prostaglandin injection in some cattle. Initially, all cows are injected with prostaglandin and those exhibiting oestrus in the next 6 to 12 days are inseminated. A second injection can then be given at any time from 6 to 12 days after the first injection to cows that have not been inseminated (Kiracofe, 1988).

The identification of cycling cattle prior to programmed prostaglandin treatment has been used in seasonally calving dairy herds (Macmillan *et al*, 1977; Macmillan *et al*, 1978; Malmo, 1991). Cows on heat 6 to 11 days before the PSM are injected with prostaglandin on the first day of mating, whilst cows on heat 0 to 6 days prior to the PSM are injected 6 days after the PSM. Such a programme leads to a high proportion of cycling cows having their first service early in the breeding period.

Fixed-time insemination vs insemination to detected oestrus

Conception rates to fixed-time insemination depend on the stage of the cycle at treatment in prostaglandin synchronisation programmes. Improper timing of insemination relative to oestrus and ovulation limits conception rates (Stevenson *et al*, 1984). Generally conception rates are higher following fixed-time insemination after the second injection of a two injection regime, when compared to a single injection in randomly cycling animals. In maiden heifers, studies on response rates and the timing of ovulation following two injections of prostaglandin administered 11 days apart indicated that the best time for fixed-time insemination is between 72 and 96 hours after treatment (Cooper, 1974; Roche, 1977; Macmillan, 1983). Two inseminations between 72 and 96 hours after treatment have consistently resulted in conception rates equal to those following insemination to detected oestrus (Lauderdale *et al*, 1974; Burfening *et al*, 1978; Young and Henderson, 1981). Single insemination at 80 hours after the second prostaglandin injection has resulted in varying conception rates (Burfening *et al*, 1978; Young and Henderson, 1981; King *et al*, 1982; Stevenson *et al*, 1984).

Conception rates to fixed-time insemination of heifers between 72 and 96 hours after a single prostaglandin injection at random stages of dioestrus are variable and generally lower than those obtained following insemination to detected oestrus. This is due to the earlier onset of oestrus and ovulation in heifers treated early in dioestrus (King *et al*, 1982; Dailey *et al*, 1983; Stevenson *et al*, 1984) and therefore improper timing of insemination. Conception rates of heifers in late dioestrus bred by fixed-time insemination at 80 hours, are the same as those following breeding at a detected oestrus

(King *et al*, 1982; Stevenson *et al*, 1984).

The variation in the interval to oestrus following one or two injections of prostaglandin in lactating dairy cows is too great to recommend fixed-time insemination (Macmillan *et al*, 1977; Stevenson *et al*, 1987).

Reasons for failure of prostaglandin synchronisation programmes

Prostaglandins are approximately 90% effective in causing luteolysis in cows treated between days 5 and 17 of the oestrous cycle (King *et al*, 1982). Between 71% and 98% of cows show oestrus within 5 days of the second injection of a two injection system (Cooper, 1974; Macmillan *et al*, 1978; King *et al*, 1982; Seguin *et al*, 1983; Stevenson *et al*, 1984). Failure of prostaglandin synchronisation programmes in heifers has been attributed to a high proportion of anoestrous animals (pre-pubertal), pregnancies to previous (unrecorded) matings and abnormalities of the reproductive tract (Macmillan *et al*, 1978). A single or double injection protocol to synchronise oestrus can fail in lactating cows due to: a high incidence of anoestrous animals (Macmillan, 1983; Lucy *et al*, 1986; Stevensen *et al*, 1987); failure of luteal regression (Lucy *et al*, 1986; Stevensen *et al*, 1987; Kiracofe, 1988); ovulatory failure following the first of a two injection system with subsequent absence of corpus luteum development (Stevensen *et al*, 1987); luteolysis with failure of detection of oestrus (Kiracofe, 1988); and variation in the interval from treatment to oestrus (Macmillan, 1983).

Oestrus synchronisation by administration of exogenous progestagens

The main naturally occurring progestagen is the steroid hormone progesterone. Synthetic progestagens include 6-methyl-17-acetoxyprogesterone (MAP), dihydroxyprogesterone acetophenide (DHPA), 6-chloro-6-dehydro-17-acetoxyprogesterone (CAP), melengestrol acetate (MGA), norgestomet and cronolone. Only some of these compounds are suitable for routine use to synchronise oestrous.

Roche (1976c) outlined the desirable features of methods of administering progesterone to cattle to synchronise oestrus, ie. such a method must be easily applied and terminated; parallel endogenous levels and patterns of progesterone in blood; allow a precipitous drop in plasma and tissue levels of progesterone when the treatment is terminated; and give a predictable and precise onset of oestrus after the end of treatment. Progestagens have been administered to cattle via several routes with the most common being daily subcutaneous or intramuscular injections, subcutaneous implants, oral preparations, and intravaginal devices.

Daily injection

Daily injections of progesterone prevent oestrus and ovulation during the treatment period (Christian and Casida, 1948; Ulberg *et al*, 1951). Treatment periods of between 14 and 24 days result in conception rates to the synchronised oestrus well below that of control animals (Trimberger and Hansel, 1955; Wiltbank *et al*, 1965; Carrick and Shelton, 1967).

Subcutaneous implants

Silastic implants containing 4 g progesterone placed subcutaneously in the dewlap are capable of suppressing oestrus during the treatment period (Roche, 1974b). Fertility at the synchronised oestrus following a 10 or 20 day treatment period is significantly lower than in control animals.

Oral preparations

Several progestagens are effective in suppressing oestrus and ovulation when administered orally. Those that have been investigated include: 6-methyl-17- acetoxy-progesterone (MAP), dihydroxyprogesterone acetophenide (DHPA), 6-chloro-6-dehydro-17-acetoxyprogesterone (CAP) and melengestrol acetate (MGA). All of these compounds are capable of effectively synchronising the oestrous cycle when given for extended

periods. Unfortunately, fertility at the synchronised oestrus is markedly reduced (Hansel *et al*, 1961; Hansel *et al*, 1966; Wiltbank and Kasson, 1968; Zimbelman *et al*, 1970; Lamond *et al*, 1971; Patterson *et al*, 1989) and as such they are not routinely used alone for oestrus synchronisation (Odde, 1990).

Intravaginal devices

Pessaries

Intravaginal, progesterone-impregnated, polyurethane sponge pessaries were found to have an unsatisfactory retention rate (86.7%) when inserted for 20 days to synchronise oestrus (Sreenan and Mulvehill, 1975). When the treatment period was reduced to 9 days pessary retention was improved to 96% (Scanlon *et al*, 1972). Similar retention rates are reported for cronolone impregnated intravaginal sponges left in place for 18 to 21 days. Between 69.4% and 98.0% of treated animals retain the sponges (Carrick and Shelton, 1967; Wishart and Hoskin, 1968; Sreenan and Mulvehill, 1975). Between 53.5% and 91.8% of treated animals are in oestrus within 5 days of sponge removal. Pregnancy rate to the synchronised oestrus is between 23% to 46.6%. The decreased fertility following cronolone treatment makes the method unacceptable for oestrus synchronisation.

Progesterone releasing intravaginal devices (PRIDs)

Silastic coils consisting of a core of stainless steel covered with 3 mm of silastic rubber and impregnated with progesterone were developed by Roche (1976a) to deliver progesterone intravaginally. The retention rate is higher in heifers (96%) than in lactating dairy cows (92%) (Roche, 1976b). Oestrus synchronisation response is correlated to the length of the treatment period, a 14 day period resulting in a tighter degree of synchrony than a 9 or 12 day period (Roche, 1978). Fertility is inversely proportional to the length of the treatment period, higher conception rates are obtained when treatment is less than 12 days (Roche and Ireland, 1981).

Altering the concentration of progesterone in PRIDs from 4% to 20% did not result in increased serum concentrations of progesterone and PRIDs were found to be ineffective in maintaining luteal phase concentrations of progesterone (4 to 7 ng/ml) in serum for longer than 7 days (Roche and Ireland, 1981).

Depending on the length of the treatment period some cows treated in the early stages of the oestrous cycle will have a corpus luteum persisting after cessation of treatment, which leads to a prolonged interval from PRID removal to oestrus (Cumming *et al*, 1982; Sprott *et al*, 1984; Munro and Moore, 1985a).

Variation in conception rate and oestrus response has meant that PRIDs alone are not suitable for routine oestrous synchronisation in cattle.

***Controlled internal drug releasing device* (CIDR)**

The CIDR device is produced by coating a premoulded annealed nylon spine weighing 10.35 g with 19 g of silicon-based elastomer containing 1.9 g (10% w/w) of progesterone (Macmillan *et al*, 1991). A cord or nylon filament is attached to the device to facilitate removal from the vagina. Retention rates averaged 99% in heifers with treatment insertion periods of 4 to 15 days, and 98% in dairy cows treated for periods of 4 to 7 days (Macmillan and Peterson, 1993). In the development of the CIDR device, design and shape changes were made to achieve a plasma progesterone concentration of at least 2 ng/ml for at least 12 days (Duirs *et al*, 1986; Macmillan and Peterson, 1993). Average plasma progesterone concentrations are influenced by the length of treatment, the type of animal (ovariectomised vs cycling), and the stage of the oestrous cycle when treatment is initiated or terminated (Macmillan *et al*, 1991).

Due to the persistence of corpora lutea at the cessation of treatment, CIDR treatment alone, as with PRID treatment, is not suitable for oestrus synchronisation. Increasing the treatment period from 7 to 14 to 21 days increased the degree of synchrony but decreased fertility to the synchronised oestrus (Macmillan and Asher,

1990).

Progestagen combinations

Progestagens used alone, regardless of the route of administration, are not suitable for routine oestrus synchronisation due to the associated low fertility (Trimberger and Hansel, 1955; Hansel *et al*, 1961; Wiltbank *et al*, 1965; Hansel *et al*, 1966; Carrick and Shelton, 1967; Wiltbank and Kasson, 1968; Wishart and Hoskin, 1968; Zimbelman *et al*, 1970; Lamond *et al*, 1971; Roche, 1974b; Sreenan, 1975; Sreenan and Mulvehill, 1975; Roche and Ireland, 1981; Patterson *et al*, 1989; Macmillan and Asher, 1990). In order to obtain the desired level of synchrony it is necessary to administer the exogenous progestagen for at least the length of the normal corpus luteum lifespan, usually 12 days. However, this duration of exposure to progestagens leads to the persistence of dominant follicles containing aged ova and an associated lower level of fertility (Hill *et al*, 1971; Lamond *et al*, 1971; Macmillan and Asher, 1990; Sirois and Fortune, 1990). As a result, progestagens have been combined with other hormones, namely oestrogens and prostaglandins, in an attempt to decrease the time needed for progestagen exposure without compromising fertility or the degree of synchrony of oestrus.

Progestagen/Oestrogen combinations

The inclusion of oestrogens (as either oestradiol benzoate or oestradiol valerate) at the start of synchronisation treatments using exogenous progestagens has been shown to increase the number of treated animals in oestrus and to shorten the interval from cessation of treatment to oestrus (Wiltbank and Kasson, 1968; Cumming *et al*, 1982; Sprott *et al*, 1984). In other studies, the addition of an oestrogen to CIDR (Macmillan *et al*, 1991; Macmillan and Peterson, 1993) or PRID (Roche, 1974a) treatments has not shown a beneficial effect on reducing the time to oestrus following device removal. The mechanism by which oestrogens increase the number of treated animals exhibiting oestrus at an earlier time after cessation of progestagen treatment is unknown. Oestrogens may have a luteolytic effect but the evidence supporting this is inconclusive (Loy *et al*, 1960; Wiltbank *et al*, 1961; Shelton and Casida, 1970; Lemon, 1975). Oestrogens have been

shown to re-set follicular waves by causing atresia of the dominant follicle present at the time of treatment (Bo *et al*, 1993; Bo *et al*, 1994; Adams, 1994; Bo *et al*, 1995). The increased number of treated animals in oestrus at an earlier time after cessation of progestagen treatment may be due to the induction of a synchronous stage of follicular development in treated animals.

Oestrogens are routinely included at the start of all of the commercially available programmes which utilise exogenous progestagens to synchronise oestrus because of the empirical evidence that they improve desired outcomes, although their mechanisms of action are unclear.

Treatment for 12 days with a PRID and 5 mg oestradiol benzoate and 200 mg of progesterone injected intramuscularly at device insertion is consistently effective in synchronising oestrus in cycling cattle with fertility equal to that of controls (Roche, 1976a; Roche, 1976c; Roche and Gosling, 1977; Cumming *et al*, 1982; Sprott *et al*, 1984).

A regime consisting of a subcutaneous ear implant containing 6 mg or 3 mg norgestomet and an injection of 3 mg norgestomet and 5 mg oestradiol valerate at the time of implant insertion is marketed under the "Syncro-Mate-B" (6 mg implant) or "CRESTAR" (3 mg implant) brand-names. The implant is removed after 9 days, and most cows are in oestrus 36 to 60 hours later (Wright and Malmo, 1992). This is an effective treatment regime which results in 77 to 100% of treated cows exhibiting oestrus within 5 days of implant removal. Fertility using this regime has varied, with reported first-service conception rates from 33 to 68% (Odde, 1990). Variability in conception rates using the Syncro-Mate-B regime is reportedly due to a high proportion of anoestrous cows prior to treatment; luteal dysfunction; poor body condition and delayed oestrus and ovulation (Mikeska and Williams, 1988). Fertility following Syncro-Mate-B treatment may be higher when treatment starts after day 11 of the oestrous cycle (Brink and Kiracofe, 1988). Infection at the site of implantation was found to hinder the removal of implants and could result in some implants not being found (Tregaskes *et al*, 1994).

In suckling beef cows, removing calves from their dams is recommended from the time of implant removal until breeding (Anon., 1995a), however, the response to calf removal is inconsistent (Odde, 1990; Kiser *et al*, 1980).

The CIDR intravaginal device successfully synchronises oestrus in cattle when used for a 12 day treatment period with 10 mg of oestradiol benzoate in an intravaginal gelatin capsule at device insertion. Onset of oestrus occurs between 48 and 96 hours after device removal with the majority of responding animals exhibiting oestrus at 48 and 72 hours after device removal (Duirs *et al*, 1986). Macmillan and Peterson (1993) found that 94% of heifers aged 12 to 14 months were in oestrus by 96 hours after device removal. The average pregnancy rate was 60%.

Progestagen/Prostaglandin combinations

Administration of prostaglandin at or before the time of removal of exogenous progestagen sources ensures that no residual source of endogenous progesterone remains after device removal. This allows the length of exposure to progestagens to be reduced and fertility maintained (Thimonier *et al*, 1975; Heersche *et al*, 1979; Smith *et al*, 1984; Macmillan and Peterson, 1993). Prostaglandin injection at the end of progestagen treatment results in a higher number of animals in oestrus (Thimonier *et al*, 1975; Heersche *et al,* 1979; Macmillan and Peterson, 1993). Progestagen must be given for at least 5 to 7 days before prostaglandin injection to ensure that prostaglandin administration does not occur during metoestrus (Heersche *et al*, 1979). Feeding MGA daily for 14 days then injecting $PGF_{2\alpha}$ 17 days after the last day of MGA feeding successfully synchronises oestrus in beef heifers (Mauck *et al*, 1994).

The injection of prostaglandin from 24 to 48 hours prior to progestagen device or implant removal, rather than at the time of removal, leads to a greater degree of oestrus synchrony (Thimonier *et al*, 1975; Heersche *et al*, 1979; Smith *et al*, 1984; Fogwell *et al*, 1986; Macmillan and Peterson, 1993), a shorter interval from device removal to luteinising hormone peak (Fogwell *et al*, 1986) and an increased number of

animals in oestrus after treatment. Half of the recommended dose of prostaglandin has been found to be effective in causing luteolysis in heifers treated with a CIDR (Macmillan and Peterson, 1993).

Progestagen/prostaglandin combinations are more effective than progestagen/oestrogen combinations in synchronising oestrus because of the unpredictable luteolytic action of oestrogens (Gyawu *et al*, 1991).

Progestagen/Oestrogen/Prostaglandin combinations

Development of treatment regimes using progestagen in combination with other treatments has lead to effective synchrony regimes incorporating oestrogen and prostaglandin. Studies in maiden dairy heifers treated with an intravaginal device (CIDR) have shown that oestrus synchrony is superior when heifers receive a 10 day treatment in association with 10 mg oestradiol benzoate as an intravaginal gelatin capsule at device insertion and then treatment with 250 μg cloprostenol or 12.5 mg $PGF_{2\alpha}$, 4 days before device removal (Duirs *et al*, 1986; Macmillan and Asher, 1990; Macmillan and Peterson, 1993). Such a regime achieved oestrus in 93% of heifers within 48 hours of device removal with normal fertility at the synchronised oestrus (Jellie, 1993; Macmillan, 1993). This degree of synchrony enables the recommendation of a single fixed-time insemination, 48 to 52 hours after device removal (Anon., 1995b). The level of fertility to fixed-time insemination using this regime has not been established under commercial conditions.

The labelled indication for CRESTAR (3 mg norgestomet ear implant plus an intramuscular injection of 3 mg norgestomet and 5 mg oestradiol valerate) to synchronise oestrus in lactating dairy cows includes an injection of prostaglandin 2 days before implant removal on day 10 (Anon., 1995a).

Other progestagen combinations

Other hormonal preparations, namely gonadotrophin-releasing hormone (GnRH) and pregnant mare serum gonadotrophin (PMSG), have been combined in progestagen-based synchrony treatments in an attempt to enhance the oestrus response. No beneficial effect was noted on pregnancy rate (Roche, 1976a) or time to oestrus (Mauer *et al*, 1975) in heifers when GnRH was administered 28 to 36 hours after the removal of PRIDs. When PMSG was administered 24 to 48 hours before removal of progestagen intravaginal pessaries, 83.3% of heifers were observed in oestrus between 24 to 72 hours after pessary removal, however the conception rate was unsatisfactory at 43.7% (Wishart and Hoskin, 1968).

Limitations of current oestrus synchronisation methods

As outlined previously the desirable features of a programme to synchronise oestrus in cattle should include a high response rate regardless of stage of cycle at which treatment is administered, tight synchrony in time of oestrus and ovulation, at least normal fertility at the synchronised ovulation and normal return to oestrus and fertility at subsequent services (Larson and Ball, 1992). The ability to perform a single insemination (appointment breeding) at a defined time after treatment would eliminate the need for oestrus detection (Macmillan and Asher, 1990; Larson and Ball, 1992). Low efficiency in oestrus detection is a major cause of poor reproductive performance in dairy herds (Williamson *et al*, 1972a; Roche, 1976c; Larson and Ball, 1992).

Current oestrus synchronisation regimes are effective in producing a synchronous decline in blood progesterone levels in different animals after treatment. Unfortunately, the time taken for follicular maturation and ovulation tends to be inconsistent between animals (Savio *et al*, 1988; Sirois and Fortune, 1988; Ginther *et al*, 1989), causing the variation in fertility observed following fixed-time insemination after oestrus synchronisation treatments (Macmillan, 1978; Anderson *et al*, 1982; King *et al*, 1982; Dailey *et al*, 1983; Stevenson *et al*, 1984; Stevenson *et al*, 1987; Mikeska and Williams,

1988; Macmillan and Peterson, 1993). The variable rate of follicular development which occurs after a synchronous decline in plasma progesterone levels is the most limiting factor in achieving synchrony of oestrus and ovulation to allow for fixed-time insemination (Mikeska and Williams, 1988; Macmillan and Peterson, 1993).

Methods of controlling follicular wave development and ovulation

The achievement of similar stages of follicular development in heifers and cows at the cessation of synchrony treatments should result in a relatively synchronous ovulation in treated animals. The variability in timing of ovulation could be reduced by re-setting follicular wave patterns in different animals at the commencement of synchrony treatments, so that follicular wave emergence is synchronised. Methods available to control follicular wave dynamics in cattle include oestrogen treatment and mechanical ablation of the dominant follicle (Adams, 1994). Combinations of oestrogen and progestagen have been shown to cause regression of the dominant follicle present at the time of treatment, resulting in the synchronous emergence of the subsequent follicular wave (Adams, 1994; Bo *et al*, 1994; Bo *et al*, 1995). The inclusion of oestrogen treatment at the start of exogenous progestagen treatment programmes may help synchronise follicular wave development in different animals. Mechanical ablation of the dominant follicle via laparotomy or ultrasound-guided transvaginal follicle ablation hastens the emergence of the next follicular wave but is impractical as a method of synchronising wave emergence in a large number of animals (Adams, 1994).

The variability in timing of ovulation may be reduced by controlling the time of ovulation using exogenous oestrogen. Oestradiol benzoate, in the absence of progesterone, has a positive feedback effect on the release of LH (Hobson and Hansel, 1972; Beck and Convey, 1977; Swanson and McCarthy, 1978; Kesner *et al*, 1981; Schillo *et al*, 1983). The LH peak and associated oestrous behaviour occurs within a predictable period between 14 and 32 hours after treatment with oestradiol benzoate (Gonzalez-Padilla *et al*, 1975; Beck and Convey, 1977; Swanson and McCarthy, 1978; Zaied *et al*, 1981). The induced LH surge is capable of causing ovulation of follicles

when they are in their plateau growth phase in anoestrous cows (McDougall, 1994). Treatment with oestradiol benzoate after progesterone concentrations have declined, therefore offers the potential for controlling the time of ovulation in synchronised cows or heifers, allowing for successful fixed-time insemination.

Conclusion

The ultimate goal of oestrus synchronisation programmes is to provide a degree of oestrus and ovulatory synchrony which allows for the insemination of all treated animals at a single set-time after treatment. Current oestrus synchronisation methods are limited by their inability to control the rate of follicular development which occurs after treatment and therefore the success of set-time insemination is highly variable. Further development of oestrus synchronisation programmes which incorporate a method of controlling follicular development and/or ovulation is required if the goal of achieving fixed-time insemination with a consistently normal level of fertility is to be met.

This thesis investigates the use of exogenous oestrogen to improve the degree of oestrus synchrony by controlling follicular wave development and the timing of ovulation in heifers and cows treated with progesterone-containing intravaginal devices (CIDR-B™).

CHAPTER 2:

THE EFFECT OF OESTRADIOL BENZOATE ADMINISTRATION ON OESTROUS RESPONSE AND SYNCHRONISED PREGNANCY RATE IN DAIRY HEIFERS AFTER TREATMENT WITH EXOGENOUS PROGESTERONE

ABSTRACT

The objectives of this trial were to determine the effects of 0.5 mg oestradiol benzoate administered intramuscularly 24 hours after removal of progesterone containing intravaginal devices on the occurrence and timing of oestrus, synchronised pregnancy rate and synchronised conception rate in dairy heifers.

A clinical trial was conducted involving 750 dairy heifers in 13 herds. Within each herd heifers were randomly allocated to one of two oestrus synchronisation treatments. All heifers received a CIDR-B progesterone-containing intravaginal device with an attached 10 mg oestradiol benzoate capsule for 12 days. Twenty-four hours after CIDR-B removal one group received an intramuscular injection of 0.5 mg oestradiol benzoate and the other group received an intramuscular injection of a placebo. Heifers were inseminated to detected oestrus 48 and 72 hours after device removal. Administration of oestradiol benzoate 24 hours after removal of CIDR-B devices significantly increased the number of heifers exhibiting oestrus within the observation period (96.1% vs 90.5%, $p<0.01$). It also altered the onset of oestrus so that significantly more heifers were in oestrus (86.6% vs 72.3%, $p<0.01$) and conceived (47.1% vs 37.5%, $p<0.05$) by 48 hours after device removal. The synchronised conception rate was unaffected by treatment. The distribution of oestrus was such that it may be possible to recommend fixed-time insemination after oestrus synchronisation with the treatment programme described but further investigation is needed to establish the relationships between treatment, luteinising hormone concentrations, oestrous behaviour and ovulation, to see if the regime may be improved.

INTRODUCTION

Numerous pharmacological regimes to synchronise oestrus in cattle have been investigated but only some have shown acceptable results and are currently in use (Roche, 1976c; Odde, 1990; Larson and Ball, 1992; Wright and Malmo, 1992). The desirable features of a programme to synchronise oestrus in cattle should include: high response rates regardless of stage of cycle at which treatment is administered, tight synchrony in time of oestrus and ovulation, normal fertility at the synchronised ovulation and normal return to oestrus and fertility at subsequent services (Larson and Ball, 1992). The ability to perform a single insemination (appointment breeding) at a defined time after treatment would eliminate the need for oestrus detection (Larson and Ball, 1992). Low efficiency in oestrus detection is a major cause of poor reproductive performance in dairy herds (Williamson *et al*, 1972a; Roche, 1976c; Larson and Ball, 1992).

Current oestrus synchronisation regimes are based on synchronising the end of the progestational phase of the oestrous cycle so that ovulation occurs simultaneously in treated animals. At present, the end of the progestational phase can be synchronised using prostaglandin $F_{2\alpha}$ and its analogues or exogenous progesterone treatment. The time taken for subsequent follicular maturation and ovulation tends to be inconsistent between animals (Savio *et al*, 1988; Sirois and Fortune, 1988; Ginther *et al*, 1989), which may cause the variation in fertility obtained following fixed-time insemination after oestrus synchronisation treatments (Macmillan, 1978; Anderson *et al*, 1982; King *et al*, 1982; Dailey *et al*, 1983; Stevenson *et al*, 1984; Mikeska and Williams, 1988; Stevenson *et al*, 1987; Macmillan and Peterson, 1993). The variable rate of follicular development after a synchronous decline in plasma progesterone levels is the most limiting factor in achieving synchrony of oestrus and ovulation which would allow fixed-time insemination (Mikeska and Williams, 1988; Macmillan and Peterson, 1993).

Oestradiol benzoate, in the absence of progesterone, has a positive feedback effect on the release of luteinising hormone (LH) (Hobson and Hansel, 1972; Gonzalez-Padilla *et al*, 1975; Hausler and Malven, 1976; Beck and Convey, 1977; Cunningham *et al*,

1977; Swanson and McCarthy, 1978; Rajamahendran *et al*, 1979; Short *et al*, 1979; Kesner *et al*, 1981; Zaied *et al*, 1981; Schillo *et al*, 1983). The luteinising hormone peak and associated oestrous behaviour occurs predictably between 14 and 32 hours after treatment with oestradiol benzoate (Gonzalez-Padilla *et al*, 1975; Beck and Convey, 1977; Swanson and McCarthy, 1978; Zaied *et al*, 1981). The administration of oestradiol benzoate may overcome the variability in timing of LH peaks typically occurring in a herd of synchronised heifers due to different stages of follicular development at exogenous progesterone removal. Several workers have found that administration of oestradiol benzoate as a single intramuscular dose has enhanced the oestrous response following progesterone removal (Wiltbank *et al*, 1971; Nancarrow and Radford, 1975; Welch *et al*, 1975; Peters *et al*, 1977; Figueroa *et al*, 1988), whereas others have demonstrated no advantage from using oestradiol benzoate (Roche, 1974b; Miksch *et al*, 1978; Anderson *et al*, 1982; Dailey *et al*, 1983). Variation in the response to oestradiol benzoate following exogenous progesterone removal may be due to the timing of treatment relative to removal of progesterone and to the dose of oestradiol benzoate used. No significant effect on oestrus synchrony has been noted when oestradiol benzoate is given at the time of exogenous progesterone removal (Wiltbank *et al*, 1971; Miksch *et al*, 1978; Anderson *et al*, 1982). The LH and ovulation response following intramuscular administration of oestradiol benzoate depends on the stage of growth of the dominant follicle at the time of treatment. Cows treated with oestradiol benzoate when the dominant follicle was in the plateau phase of follicular development were more likely to have an LH surge and ovulate than cows with a dominant follicle in the growing phase (McDougall *et al*, 1994).

The CIDR-B (InterAg, Hamilton, New Zealand) intravaginal device provides controlled administration of exogenous progesterone to cattle (Macmillan *et al*, 1991) and is indicated for oestrus synchronisation and treatment of post-partum anoestrus (Anon., 1995b). As with other methods of oestrus synchronisation using exogenous progesterone, synchrony is enhanced when an oestrogen is administered at the start of the treatment period. In the case of the CIDR-B oestrogen is administered intravaginally as 10 mg of oestradiol benzoate in a gelatin capsule. Following a 12 day treatment period in yearling

heifers the overall oestrous response is approximately 94%, with the onset of oestrus occurring between 48 and 96 hours after device removal (Macmillan and Peterson, 1993). One recommended regime for using CIDR-B devices in dairy heifers is a 12 day treatment period with insemination to detected oestrus at 48 and 72 hours following device removal. Synchrony is not sufficient to allow the recommendation of a single set-time insemination. It is hypothesised that at approximately 48 hours after CIDR-B removal most cycling heifers will have a dominant follicle at or near the plateau growth phase. Administration of oestradiol benzoate at 24 hours after CIDR-B removal should result in an LH surge approximately 24 hours later, with heifers showing oestrus and ovulating at an earlier time, thereby increasing the degree of oestrus and ovulatory synchrony and allowing for fixed-time insemination.

The objectives of this clinical trial were to determine the effects of 0.5 mg oestradiol benzoate administered intramuscularly 24 hours after the removal of CIDR-B progesterone containing intravaginal devices on the occurrence and timing of oestrus, conception rate and synchronised pregnancy rate in dairy heifers.

MATERIALS AND METHODS

A clinical trial was conducted involving 750 yearling heifers in 13 intensively grazed dairy herds in the Manawatu region of New Zealand.

Farm Selection:

All of the farmers involved were clients of Massey University's Farm Service Veterinary Clinic. Selection of farms was based on farmers' willingness to participate, their perceived ability to keep accurate and complete records and the presence of adequate handling facilities on the farm.

Animals:

Individual heifer herd size ranged from 23 to 106. All animals were maiden Friesian heifers aged from 13 to 15 months at first insemination and an average weight of 282.7 $\pm$ 35.0 kg. Management of heifers during the project was under the direct control of the farm owner/manager utilising their usual farming practices. All herds grazed pastures consisting predominantly of ryegrass and clover for the duration of the project. The starting date for insemination for each herd of heifers was nominated by the herd owner and occurred between the 11th and 30th of October 1993.

Experimental Protocol:

The oestrus synchronisation regime used in this trial was based on a recommended method of using the CIDR-B progesterone-containing intravaginal device and is outlined in Table 2.1.

The CIDR-B consists of a silicone rubber elastomer impregnated with 1.9g progesterone. The CIDIROL (InterAg, Hamilton, New Zealand) capsule is a gelatin capsule containing 10 mg oestradiol benzoate and is inserted into a slot on the CIDR-B device. On the day of device insertion, all heifers were weighed using electronic scales for subsequent treatment group allocation and tailpaint was applied to facilitate oestrus detection.

Heifers that retained the CIDR-B for the entire treatment period were allocated to treatment groups. Based on the weights on the day of CIDR-B insertion, heifers were grouped in weight-matched pairs. For each pair of heifers within the herd one member was randomly allocated to the control group with the other member being allocated to the oestradiol benzoate treatment group. In herds with an uneven number of heifers, the last heifer was randomly allocated to one of the treatment groups. No animals were excluded from the trial on the basis of weight.

Table 2.1. Oestrus synchronisation protocol for dairy heifers treated with a progesterone containing intravaginal device (CIDR-B) for 12 days with or without intramuscular administration of 0.5 mg oestradiol benzoate 24 hours after device removal.

	TREATMENT	
	OESTRADIOL GROUP	CONTROL GROUP
Day 0	insert CIDR-B/CIDIROL[a] + tailpaint	
Day 12	remove CIDR-B + spray aerosol raddle over tailpaint	
Day 13	inject 0.5mg ODB[b] IM	inject placebo IM
Day 14	AI to detected oestrus	
Day 15	AI to detected oestrus	

[a] CIDIROL = 10 mg oestradiol benzoate in a gelatin capsule

[b] ODB = oestradiol benzoate

Both treatment groups involved intravaginal insertion of a CIDR-B and attached CIDIROL capsule for a 12 day period. Twenty-four hours after CIDR-B removal one group received an intramuscular injection of 0.5 mg oestradiol benzoate (Oestradiol Group) and the other group received an intramuscular injection of the same volume of a placebo (Control Group). Oestradiol benzoate (Intervet, Auckland, New Zealand) was commercially available at a concentration of 5 mg/ml. Dilution of this preparation was necessary to obtain a concentration compatible with an appropriate volume for injection. A diluent was prepared using the components of the vehicle in which the oestradiol benzoate is suspended, 90% v/v sterile medical grade peanut oil (Ancare New Zealand Ltd., New Zealand) and 10% v/v benzyl alcohol GPR (BDH Laboratory Supplies, England). This solution was used as the placebo and to dilute the oestradiol benzoate to give a final concentration of 0.25 mg/ml. All people involved in the trial were blinded

as to which treatment was being administered.

Oestrus Detection/Insemination Procedure:

Observation for oestrous activity was the responsibility of the herd owner/manager on each farm. Oestrus detection was aided by the use of blue tailpaint and yellow aerosol raddle sprayed over the tailpaint. This modified tailpainting technique has been shown to be an effective means of detecting oestrus in large groups of synchronised heifers (Macmillan *et al*, 1988). Forty-eight and 72 hours after removal of CIDR-B devices, heifers were yarded for insemination of those that had shown signs of tailpaint/raddle disturbance (day 14 and 15 of the programme). Only heifers with tailpaint/raddle removal were inseminated. Insemination of heifers was conducted by experienced, commercially-based AI technicians. Selection of semen was the farmer's choice. All inseminations utilised frozen-thawed semen processed and thawed following recommended industry standards. Semen was purchased through one of two commercial AI distribution centres, Livestock Improvement Corporation or Ambreed New Zealand Ltd. After each heifer was inseminated, red tailpaint was applied to the tailhead region. If heifers inseminated on day 14 had evidence of tailpaint removal on day 15, they were re-inseminated on day 15. On day 16, beef-breed herd bulls were run with all heifers and no further observations for oestrous activity were conducted.

Thirty-five days after the first insemination each heifer was pregnancy tested by palpation per-rectum and the pregnancy status recorded. All heifers were palpated again approximately 21 days later to confirm their pregnancy status.

Definitions:

For the purposes of this trial, methods of evaluating synchronisation systems as outlined by Odde (1990) were used, ie. oestrous response is the percentage of heifers showing oestrus of those treated, synchronised conception rate is the percentage of heifers conceiving of those inseminated and synchronised pregnancy rate is the

percentage of heifers conceiving of the total treated.

Statistical Analysis:

Results were analysed in terms of the effects of treatment group, body weight and herd on the occurrence and timing of oestrus, the synchronised conception rate and synchronised pregnancy rate. The analysis was conducted in two steps, a univariate and a multivariate analysis. For the univariate analysis the association between each dependent variable and the independent variables, treatment and herd, was examined for statistical significance. Two sample t-tests were used for weight data and chi-squared tests for the effect of treatment. In the multivariate analysis, logistic regression was used to assess the effect of confounding variables. A backward selection of variables was performed whereby those that were non-significant predictors (p value for Wald's test $>$ 0.05) were sequentially dropped from the logistic regression model. Analysis of data was performed using the computer software package Statistix Version 4.0 (Analytical Software, St. Paul, MN, USA).

RESULTS

Twelve of the 750 heifers lost the CIDR-B device before the planned time of removal and were subsequently not allocated to either treatment group. Retention rate of the CIDR-B device was therefore 98.4%.

Seven hundred and thirty-eight heifers were allocated to treatment groups but 24 were excluded from the analysis of results. At palpation 35 days after insemination, 8 heifers were found to be pregnant to a prior (unrecorded) mating and 4 were found to be freemartins. These 12 heifers and their matched partners were excluded.

Body weight had no statistically significant effect on any of the variables measured. There was a statistically significant effect of herd on the overall oestrous response and the distribution of oestrus but no effect on synchronised conception rate or

synchronised pregnancy rate.

Oestrous Response:

Seven hundred and fourteen heifers were included in the analysis of results, 666 (93.3%) exhibited oestrus as evidenced by tailpaint removal, at either 48 or 72 hours after CIDR-B removal (Table 2.2.). Of those heifers given the oestradiol benzoate injection, 96.1% showed oestrus compared to 90.5% in the control group ($X^2=8.9$, d.f. 1, $p=0.003$, Table 2.2.). There was considerable between-herd variation in terms of the occurrence of oestrus, 83.3% to 100% of heifers showing oestrus in different herds (Table 2.2.). Logistic regression was used to assess the effect of oestradiol benzoate treatment on the occurrence of oestrus, controlling for differences between herds. The odds ratio for treatment with oestradiol benzoate indicated that heifers treated with oestradiol benzoate were 2.83 (95% CI = 1.46 to 5.48) times more likely to show oestrus than heifers not so treated.

Distribution of Oestrus After CIDR-B Removal:

To test the hypothesis that treatment with oestradiol benzoate results in an earlier and more compact oestrous response, the distribution of oestrus after CIDR-B removal was examined. Significantly more of the oestradiol benzoate treated heifers showed oestrus by 48 hours (86.6%) compared to the control group (72.3%) ($X^2=22.3$, d.f. 1, $p<0.001$, Table 2.2.). Significantly fewer of the oestradiol benzoate treated heifers showed oestrus between 48 and 72 hours after device removal (17.1% vs 24.6%, $X^2=6.2$, d.f. 1, $p=0.01$, Table 2.2.). There was a wide range across herds in the proportion of heifers showing oestrus by 48 hours after device removal (57.1% to 100%, Table 2.2.). Logistic regression was used to assess the effect of oestradiol benzoate treatment on the occurrence of oestrus by 48 hours following device removal, controlling for differences between herds. The odds ratio for treatment with oestradiol benzoate indicated that heifers treated with oestradiol benzoate were 2.75 (95% CI = 1.84 to 4.11) times more likely to show oestrus by 48 hours following CIDR-B removal than

heifers not so treated. There was no significant difference between treatment groups in the number of heifers showing oestrus at both 48 and 72 hours after device removal (Table 2.2.).

Table 2.2. Number of heifers responding to treatment with a CIDR-B for 12 days with or without intramuscular administration of 0.5mg oestradiol benzoate 24 hours after device removal.

Response	Herd range %	Treatment		Total n=714 (%)
		Oestradiol n=357 (%)	Control n=357 (%)	
Total in oestrus	83.3-100	343 (96.1)[c]	323 (90.5)[d]	666 (93.3)
No oestrus	0-16.7	14 (3.9)[c]	34 (9.5)[d]	48 (6.7)
Bred at 48 hr	57.1-100	309 (86.6)[c]	258 (72.3)[d]	567 (79.4)
Pregnant at 48 hr	32.6-60.6	168 (47.1)[c]	134 (37.5)[d]	302 (42.3)
Bred at 72 hr	6.1-39.1	61 (17.1)[c]	88 (24.6)[d]	149 (20.9)
Pregnant at 72 hr	0-10.3	16 (4.5)[c]	31 (8.6)[d]	47 (6.6)
Bred at 48 & 72 hr	3.6-13.0	27 (7.6)	23 (6.4)	50 (6.7)
Pregnant at 48 & 72 hr	1.3-9.5	12 (3.4)	14 (3.9)	26 (3.6)
Pregnancy rate[a]	44.4-63.6	54.9	50.1	52.5
Conception rate[b]	48.5-66.7	57.1	55.4	56.3

[a] Number pregnant/total treated (%)

[b] Number pregnant/number in estrus (%)

[c,d] Figures within rows with different superscripts are significantly different ($p < 0.01$)

Synchronised Pregnancy Rate:

Of the 714 heifers included in the analysis of results, 375 became pregnant, a synchronised pregnancy rate of 52.5% (Table 2.2.). Treatment had no significant effect on the overall synchronised pregnancy rate, with 54.9% of the oestradiol benzoate treated and 50.1% of the control heifers becoming pregnant ($X^2=1.6$, d.f. 1, $p=0.20$, Table 2.2.). Synchronised pregnancy rate by 48 hours after device removal was significantly higher in the oestradiol benzoate treated heifers (47.1%) than the control heifers (37.5%) ($X^2=6.6$, d.f. 1, $p=0.01$, Table 2.2.). This pregnancy rate only includes those heifers inseminated once at 48 hours. Synchronised pregnancy rate at the 72 hour insemination was significantly lower in the oestradiol benzoate treated heifers (4.5%) than the control heifers (8.6%) ($X^2=5.1$, d.f. 1, $p=0.02$, Table 2.2.). This pregnancy rate only includes those heifers inseminated once at 72 hours.

Synchronised Conception Rate:

Of the 666 heifers inseminated, 375 became pregnant, an overall synchronised conception rate of 56.3% (Table 2.2). Treatment had no significant effect on synchronised conception rate with the oestradiol treated heifers having a 57.1% conception rate in comparison to 55.4% in the control group ($X^2=6.6$, d.f. 1, $p=0.65$, Table 2.2.).

DISCUSSION

Administration of 0.5 mg of oestradiol benzoate by intramuscular injection 24 hours after the removal of CIDR-B progesterone containing intravaginal devices significantly increased the number of heifers detected in oestrus and bred at 48 and 72 hours after device removal. The oestrous response in the control group is similar to that reported elsewhere following oestrus synchronisation with CIDR-B devices (Macmillan and Peterson, 1993). Although the modified tailpainting technique of oestrus detection relies on secondary signs of oestrus (mounting behaviour) rather than direct observation, it is

an effective method of detecting oestrus in synchronised heifers. Macmillan *et al* (1988) found that only 0.8% of synchronised heifers ovulated without being detected in oestrus using the modified tailpainting technique for oestrus detection.

The enhanced oestrous response following treatment with oestrogens after progesterone removal is in agreement with some workers (Wiltbank *et al*, 1971; Nancarrow and Radford, 1975; Welch *et al*, 1975; Peters *et al*, 1977; Figueroa *et al*, 1988), but in contrast to the findings of others (Roche, 1974b; Miksch *et al*, 1978; Anderson *et al*, 1982; Dailey *et al*, 1983). Timing of oestradiol benzoate injection relative to the withdrawal of exogenous progesterone sources or injection of $PgF_{2\alpha}$ appears to determine the degree to which the oestrous response is enhanced. Injection of oestradiol benzoate at the time of exogenous progesterone removal was associated with no increase in the occurrence of oestrus (Wiltbank *et al*, 1971; Miksch *et al*, 1978; Anderson *et al*, 1982) whereas treatment between 40 and 48 hours after $PgF_{2\alpha}$ injection (Welch *et al*, 1975; Peters *et al*, 1977; Figueroa *et al*, 1988) or 24 hours after exogenous progesterone removal (Wiltbank *et al*, 1971) resulted in an increase in the number of heifers or cows showing oestrus. In this trial the administration of oestradiol benzoate altered the distribution of the occurrence of oestrus with significantly more of the oestradiol benzoate treated heifers showing oestrus by 48 hours following CIDR-B removal than in the control group. This is similar to the findings of Peters *et al* (1977) who showed that 0.4 mg oestradiol benzoate given 48 hours after injection of a luteolytic dose of $PgF_{2\alpha}$ increased the occurrence of oestrus within a target period 56 to 86 hours after prostaglandin injection. McDougall *et al* (1994) showed that dominant follicles in the plateau growth phase were more likely to ovulate following oestradiol benzoate administration than those in the growing phase of development. Given the oestrous response obtained following a 12 day CIDR-B treatment protocol, it is likely that heifers that are normally in oestrus beyond 48 hours after device removal will have dominant follicles at or near the plateau growth phase by 48 hours after device removal. Injection of oestradiol benzoate at 24 hours after device removal is therefore likely to result in an LH surge approximately 24 hours later, associated oestrous behaviour and synchronous ovulation. The results of this trial support this hypothesis since the distribution of oestrus

was significantly altered by the administration of oestradiol benzoate. The potential for oestradiol benzoate administration to cause oestrous behaviour without ovulation has been documented in ovariectomised (Hobson and Hansel, 1972; Rajamahendran *et al*, 1979) and prepubertal heifers (Gonzalez-Padilla *et al*, 1975). Several workers have reported lowered conception rates following oestradiol administration to enhance the oestrous response (Wiltbank *et al*, 1971; Roche, 1974b; Anderson *et al*, 1982), whereas others have found no decrease in conception rates (Welch *et al*, 1975; Peters *et al*, 1977; Miksch *et al*, 1978; Dailey *et al*, 1983; Figueroa *et al*, 1988). Synchronised conception rates in this trial were not affected by treatment with oestradiol benzoate. As a result of the altered oestrous response, synchronised pregnancy rates were altered so that significantly more of the oestradiol benzoate treated heifers became pregnant at 48 hours after device removal.

Differences occurred between herds in the proportion of heifers showing oestrus and the distribution of oestrus in responding heifers. This could be explained by differences in oestrus detection efficiency which further highlights the need for oestrus synchronisation programmes which allow for fixed-time insemination. Interpretation of tailpaint status as an indicator of oestrous activity is a significant factor in determining the overall oestrous response following synchrony treatments (Dick, 1990). Non-specific stressors such as poor weather conditions are known to inhibit oestrous behaviour (Williamson *et al*, 1972b; Allrich, 1993) and may have affected the oestrous response on farms exposed to wind and rain. Weather conditions were not recorded for each farm but all herds were synchronised within a 19 day period during which weather conditions were typical for the season. Bodyweight had no significant effect on the oestrous response, thus removing the possibility that some herds may have had a number of prepubertal heifers presented for treatment.

In conclusion, intramuscular administration of 0.5 mg oestradiol benzoate 24 hours after the removal of CIDR-B progesterone containing intravaginal devices significantly increased the number of heifers exhibiting oestrus within the observation period. It also altered the time to oestrus so that significantly more heifers were in oestrus and

conceived by 48 hours after device removal. There was considerable variation between herds in the proportion of heifers showing oestrus and the distribution of oestrus, possibly due to differences in oestrus detection efficiency. The synchronised conception rate was unaffected by treatment. The distribution of oestrus was such that it may be possible to recommend fixed-time insemination after oestrus synchronisation with the described treatment programme. Further investigation into the relationships between treatment, luteinising hormone concentrations, oestrous behaviour and ovulation is needed to see if the treatment regime may be improved.

CHAPTER 3:

OVULATORY RESPONSES AND PLASMA LUTEINISING HORMONE CONCENTRATIONS IN DAIRY HEIFERS AFTER TREATMENT WITH EXOGENOUS PROGESTERONE AND OESTRADIOL BENZOATE

ABSTRACT

The objectives of this experiment were to determine the effects of 0.5 mg oestradiol benzoate, administered intramuscularly 24 hours after removal of CIDR-B progesterone containing intravaginal devices, on the time to oestrus, ovulation and peak LH concentration in dairy heifers.

Ovulatory responses and plasma LH concentrations were examined using 14 Friesian dairy heifers in a two-period crossover design. All heifers received a CIDR-B progesterone-containing intravaginal device with an attached 10 mg oestradiol benzoate capsule for 12 days. Within each period, 24 hours after CIDR-B removal one group received an intramuscular injection of 0.5 mg oestradiol benzoate and the other group received an intramuscular injection of a placebo. Blood samples for LH assay were collected at 0, 6 and 12 hours, and then every 4 hours for 60 hours after oestradiol injection. Detection of oestrus was conducted at 4 hourly intervals, and ultrasonographical examination to detect ovulation was conducted every 8 hours for 88 hours after device removal.

Treatment with oestradiol benzoate tended to reduce the time from device removal to LH peak in period 1 (median time to LH peak 40.1 hr vs 63.9 hr, $p=0.07$). In period 2, treatment with oestradiol had no significant effect on the time to LH peak, standing oestrus or ovulation. The period effect was likely due to the stage of cycle at the time of treatment. For heifers treated in period 1, the stage of cycle was random. However, because of the prior synchronisation of oestrus which was implicit in the cross-over design, heifers in period 2 tended to be in late dioestrus. The administration of oestradiol benzoate after treatment with exogenous progesterone tended to reduce the variability in timing of LH peaks which typically occur in a herd of synchronised heifers.

INTRODUCTION

Oestrus synchronisation in cattle aims to provide a degree of synchrony which allows a single insemination (appointment breeding) at a defined time after treatment which achieves a normal level of fertility. Current oestrus synchronisation regimes are based on achieving a synchronous decline in plasma progesterone concentrations using prostaglandins or an exogenous progesterone source. The time taken for follicular maturation and ovulation tends to be inconsistent between animals (Savio *et al*, 1988; Sirois and Fortune, 1988; Ginther *et al*, 1989), which may cause the variation in fertility obtained following fixed-time insemination after oestrus synchronisation treatments (Macmillan, 1978; Anderson *et al*, 1982; King *et al*, 1982; Dailey *et al*, 1983; Stevenson *et al*, 1984; Stevenson *et al*, 1987; Mikeska and Williams, 1988; Macmillan and Peterson, 1993). The variable rate of follicular development after a synchronous decline in plasma progesterone levels is the most limiting factor in achieving synchrony of oestrus and ovulation which would allow fixed-time insemination (Mikeska and Williams, 1988; Macmillan and Peterson, 1993).

One possible method of controlling the time of ovulation is through the use of oestradiol benzoate at or near the end of oestrus synchronisation treatment periods. Oestradiol benzoate, in the absence of progesterone, has a positive feedback effect on the release of luteinising hormone (LH) (Hobson and Hansel, 1972; Gonzalez-Padilla *et al*, 1975; Hausler and Malven, 1976; Beck and Convey, 1977; Cunningham *et al*, 1977; Swanson and McCarthy, 1978; Rajamahendran *et al*, 1979; Short *et al*, 1979; Kesner and Convey, 1981; Zaied *et al*, 1981; Schillo *et al*, 1983).

In a previous study involving 750 dairy heifers (Chapter 2), the intramuscular administration of 0.5 mg oestradiol benzoate 24 hours after CIDR-B removal significantly increased the number of heifers exhibiting oestrus within the observation period. It also altered the onset of oestrus so that significantly more heifers were in oestrus and conceived by 48 hours after device removal. Conception rate was unaffected by treatment. It is unknown whether the earlier onset of oestrus was associated with an

earlier LH peak and ovulation.

The objectives of this experiment were to determine the effects of 0.5 mg oestradiol benzoate, administered intramuscularly 24 hours after removal of CIDR-B progesterone containing intravaginal devices, on the time to oestrus, ovulation, and peak LH concentration in dairy heifers.

MATERIALS AND METHODS

Animals:

Fourteen Friesian dairy heifers, with an average age of 15 months and an average weight of 322.7 $\pm$ 6.5 kg, were used for this experiment. They were selected from 75 replacement heifers of the Massey University No. 1 dairy herd. Seven days prior to the experiment all 75 heifers were palpated per-rectum for evidence of ovarian cyclicity and weighed. Fourteen heifers with evidence of ovarian activity, based on palpation of a corpus luteum, were randomly selected for the experiment. Heifers were grazed at pasture on the Massey University Veterinary Farm as one group for the duration of the experiment. Pastures were predominantly ryegrass and clover. Pasture hay was made available ad lib. The experiment was conducted in the Autumn of 1994.

Experimental Protocol:

The experiment consisted of a two-period crossover design. Each heifer received two oestrus synchronisation treatments with a 16 day period between removal of the first CIDR-B device and re-insertion of the second device. Heifers were initially randomly allocated to one of the two treatment groups (Period 1), then to the opposite treatment group for Period 2. The oestrus synchronisation regimes used in this experiment are outlined in Table 3.1.

Treatment of both groups involved insertion of a CIDR-B device (InterAg,

Hamilton, New Zealand) and an attached CIDIROL capsule (InterAg, Hamilton, New Zealand) for a 12 day period. On the day of device insertion, tailpaint was applied to facilitate oestrus detection. Twenty-four hours after CIDR-B removal the "Oestradiol group" received an intramuscular injection of 0.5 mg oestradiol benzoate and the "Control group" received an intramuscular injection of a placebo. The placebo was prepared using the components of the vehicle in which the oestradiol benzoate is suspended, 90% v/v sterile medical grade peanut oil (Ancare New Zealand Ltd., New Zealand) and 10% v/v benzyl alcohol GPR (BDH Laboratory Supplies, England). All people involved in the experiment were blinded as to which treatment was being administered.

Table 3.1. Oestrus synchronisation protocol for dairy heifers treated with a progesterone containing intravaginal device (CIDR-B) for 12 days with or without intramuscular administration of 0.5 mg oestradiol benzoate 24 hours after device removal.

	TREATMENT	
	OESTRADIOL GROUP	CONTROL GROUP
Day 0	insert CIDR-B/CIDIROL[a] + tailpaint	
Day 12	remove CIDR-B + spray aerosol raddle over tailpaint	
Day 13	inject 0.5mg ODB[b] IM	inject placebo IM
Day 14	oestrus detection, ultrasonography, blood collection	
Day 15	oestrus detection, ultrasonography, blood collection	

[a] CIDIROL = 10 mg oestradiol benzoate in a gelatin capsule

[b] ODB = oestradiol benzoate

Oestrus Detection:

Detection of oestrus was aided through the use of tailpaint and aerosol raddle applied prior to CIDR-B removal (Macmillan *et al*, 1988). Heifers were observed for oestrus prior to yarding for blood collection/ultrasonography, ie. at 4 hourly intervals.

Blood Collection:

A jugular venous catheter was placed in each heifer on the afternoon preceding CIDR-B removal to enable whole blood collection after oestradiol benzoate/placebo injection. Blood was collected from the jugular catheter at 0, 6, and 12 hours, and at 4 hourly intervals until 60 hours after the oestradiol benzoate/placebo injection. Blood was placed into Vacutainers (Becton Dickinson, New Jersey, USA) containing lithium heparin and centrifuged within 30 minutes of collection for 15 minutes at 4000 rpm to enable removal of plasma. Plasma was stored at -20°C until assayed for luteinising hormone content.

Hormone Assay:

LH concentration was determined using a double antibody radioimmunoassay. The standards and LH for iodination were of ovine origin (NIH Ovine LH-1-2, IOD#AFP-7071B). The within assay coefficients of variation were 6.04, 10.9 and 6.61 for three quality control plasma's with mean concentrations of 0.9, 2.6 and 10.4 ng/ml respectively, each assayed 5 times in duplicate in the assay. The sensitivity upper 95% confidence interval of the zero standard was 0.273 ng/ml. All samples were processed in one assay. Time of LH peak was defined as the time at which maximum LH concentration occurred in heifers which had an identifiable LH peak.

Ultrasonography:

The ovaries of all heifers were examined at 8 hourly intervals from the time of

oestradiol benzoate/placebo injection for a period of 88 hours to detect the time of ovulation. A transrectal real time linear array ultrasound (Aloka SSD - 210 DXII) with a 5 megahertz probe was used for each examination. Before insertion of the probe faecal material was removed from the rectum and the ovaries located. Each ovary was scanned with the probe in at least two planes. When an image which depicted follicles at their widest diameter was obtained, the screen was frozen and the image recorded on videotape. Ovulation was detected when a previously dominant follicle was not present at the next examination (Pierson and Ginther, 1988). When this occured, heifers were examined again 8 hours later to confirm that ovulation had occurred and then no further examinations were conducted.

Statistical Analysis:

Results were analysed in terms of the effects of treatment on the time from CIDR-B removal to: peak LH concentration, standing oestrus and ovulation. The effect of treatment on the magnitude of the LH surge was also examined. Since not all animals showed the measured responses, survival analysis was used as it allows their inclusion as right censored observations.

The objective of a two-period crossover design is to enable within-animal comparisons of two different treatments, the order of treatments being decided randomly (Pocock, 1983). With crossover designs the potential exists for carry-over effects whereby treatment status in the first period influences the outcome in the second period. If these "period-effects" are significant then the data cannot be pooled (Pocock, 1983). The presence of a "period-effect" was assessed by including period of treatment and it's interaction with treatment as variables in a Cox's proportional hazards regression model. The effect of treatment was examined for statistical significance by comparing survival functions using log-rank tests. Means were compared using Student's t-test and categorical effects using Fisher's exact test. Analysis of data was performed using the computer software package Number Cruncher Statistical System Version 5.03 (Utah, USA).

RESULTS

Analysis of Period-effect:

There was a significant period-effect on the time to reach peak LH concentration after CIDR-B removal ($p=0.008$). The interaction between period and treatment on the time to reach peak LH concentration was not significant ($p=0.14$). There was no period effect or interaction with treatment on any of the other variables measured. Data was analysed separately for each period.

Time to LH Peak:

In Period 1, all 7 oestradiol-treated heifers had an LH peak, whereas only 4 of 7 control heifers had an LH peak, however this difference was not significant (Fisher Exact test, $p=0.19$). One control heifer was observed in standing oestrus and ovulated, therefore it is likely that her LH peak occurred between two blood collections and was missed. The median time to LH peak tended to be shorter in heifers treated with oestradiol benzoate (40.1 hr vs 63.9 hr, $p=0.07$), (Table 3.2., Figs.3.1. and 3.3.) in Period 1. In Period 2, treatment with oestradiol benzoate had no effect on the median time to LH peak (35.9 hr vs 35.9 hr, $p=0.38$), (Table 3.2., Figs. 3.2. and 3.3.).

Time to Standing Oestrus:

There was no significant effect of treatment on the time to standing oestrus. In Period 1, the median time to standing oestrus was 40.0 hr in those treated with oestradiol, and 44.0 hr in control heifers ($p=0.30$)(Table 3.2., Fig. 3.4.). Only 5 of 7 control heifers exhibited standing oestrus whereas all 7 heifers treated with oestradiol were observed in standing oestrus, however this difference was not significant (Fisher's Exact test, $p=0.46$). In Period 2, the median time to standing oestrus was 32.0 hr in those treated with oestradiol, and 40.0 hr in control heifers ($p=0.67$)(Table 3.2., Fig. 3.4.).

Table 3.2. Ovulatory, oestrous and LH responses in dairy heifers treated with a CIDR-B, with or without 0.5 mg oestradiol benzoate intramuscularly 24 hours after CIDR-B removal.

	Period 1		Period 2	
	Oestradiol (n=7)	Control (n=7)	Oestradiol (n=7)	Control (n=7)
Median time to LH peak (hrs)(no. responding)	40.1[a] (7)	63.9[b,c] (4)	35.9 (7)	35.9[d] (7)
Median time to standing oestrus (hrs) (no. responding)	40.0 (7)	44.0 (5)	32.0 (7)	40.0 (7)
Median time to ovulation (hrs)(no. responding)	64.0 (6)	72.0 (5)	64.0 (7)	64.0 (7)
Time from LH peak to ovulation (mean ± SD hr)[e]	22.1 ±14.5	10.8 ±15.1	24.1 ±7.3	29.9 ±3.9
Magnitude of LH peak (mean ± SD ng/ml)[e]	7.9 ± 4.2	11.7 ± 6.0	14.3 ± 9.5	13.7 ± 6.9

[a,b] Figures with different superscripts are significantly different at $p < 0.10$

[c,d] Figures with different superscripts are significantly different at $p < 0.05$

[e] Means are calculated from those heifers which responded

Time to Ovulation:

There was no significant effect of treatment on the time to ovulation. In Period 1, the median time to ovulation was 64.0 hr in those treated with oestradiol and 72.0 hr in control heifers ($p=0.55$)(Table 3.2., Fig. 3.5.). Five of 7 control heifers ovulated within the observation period, whereas 6 of 7 heifers treated with oestradiol were observed to

ovulate, however this difference was not significant (Fisher's Exact test, p=1.0). In Period 2, the median time to ovulation was 64.0 hr in those treated with oestradiol, and 64.0 hr in control heifers (p=0.21)(Table 3.2., Fig. 3.5.).

Time From LH Peak to Ovulation:

There was no significant effect of treatment on the mean time from LH peak to ovulation. In Period 1, the mean time from LH peak to ovulation was 22.1 ± 14.5 hr in those treated with oestradiol, and 10.8 ± 15.1 hr in control heifers (p=0.31)(Table 3.2.). In Period 2, the mean time from LH peak to ovulation was 24.1 ± 7.3 hr in those treated with oestradiol, and 29.9 ± 3.9 hr in control heifers (p=0.09)(Table 3.2.).

Magnitude of LH Peak:

There was no significant effect of treatment on the magnitude of the LH peak. The mean LH peak in Period 1 in heifers receiving oestradiol was 7.9 ± 4.2 ng/ml, and 11.7 ± 6.0 ng/ml in control heifers (p=0.29), (Table 3.2.). In Period 2, the mean LH peak was 14.3 ± 9.5 ng/ml in those treated with oestradiol, and 13.7 ± 6.9 ng/ml in control heifers (p=0.89)(Table 3.2.).

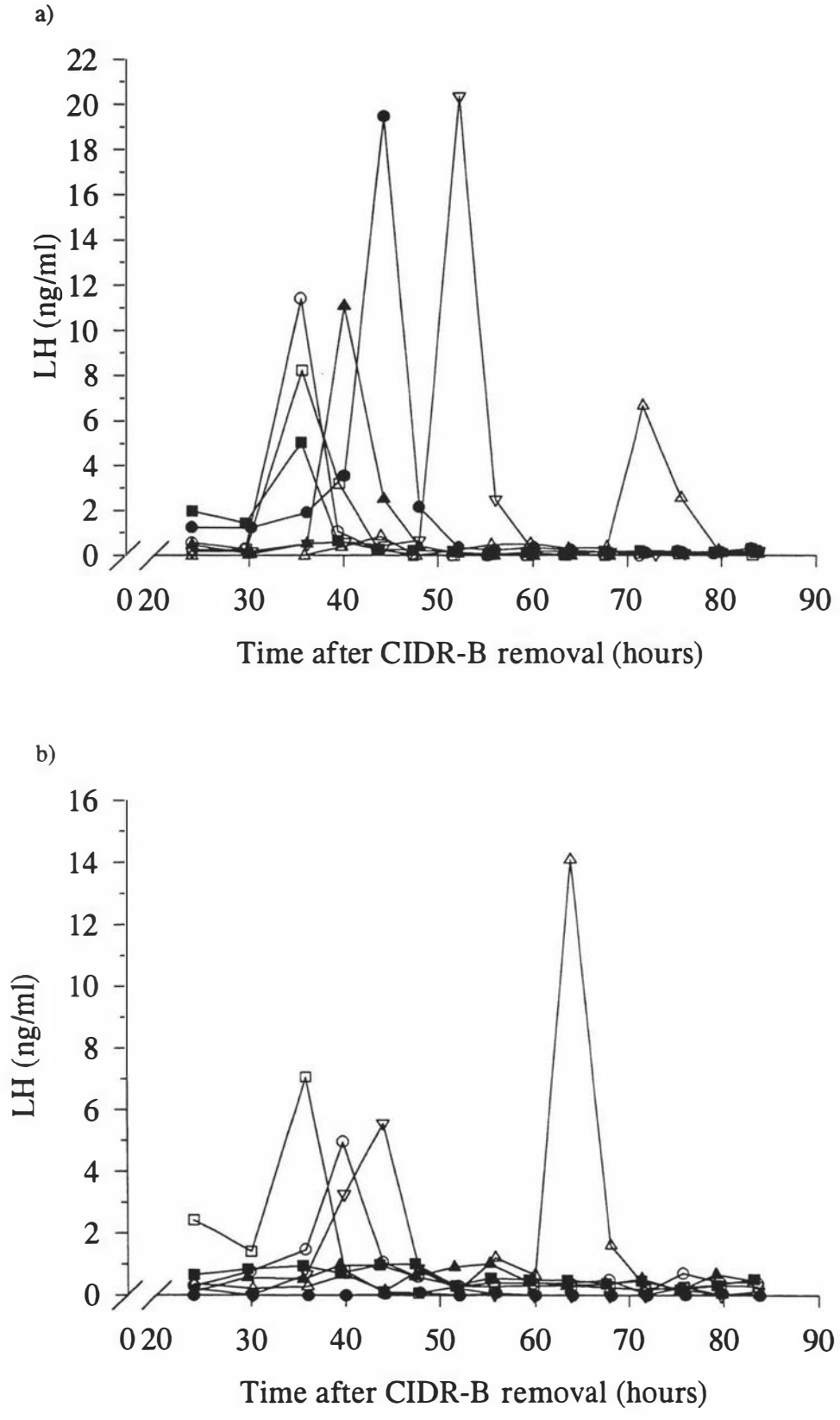

Figure 3.1. Profiles of concentration of LH in plasma for dairy heifers treated with a CIDR-B progesterone containing intravaginal device for 12 days in Period 1, with (n=7) (a) or without (n=7) (b) 0.5mg oestradiol benzoate intramuscularly 24 hours after device removal.

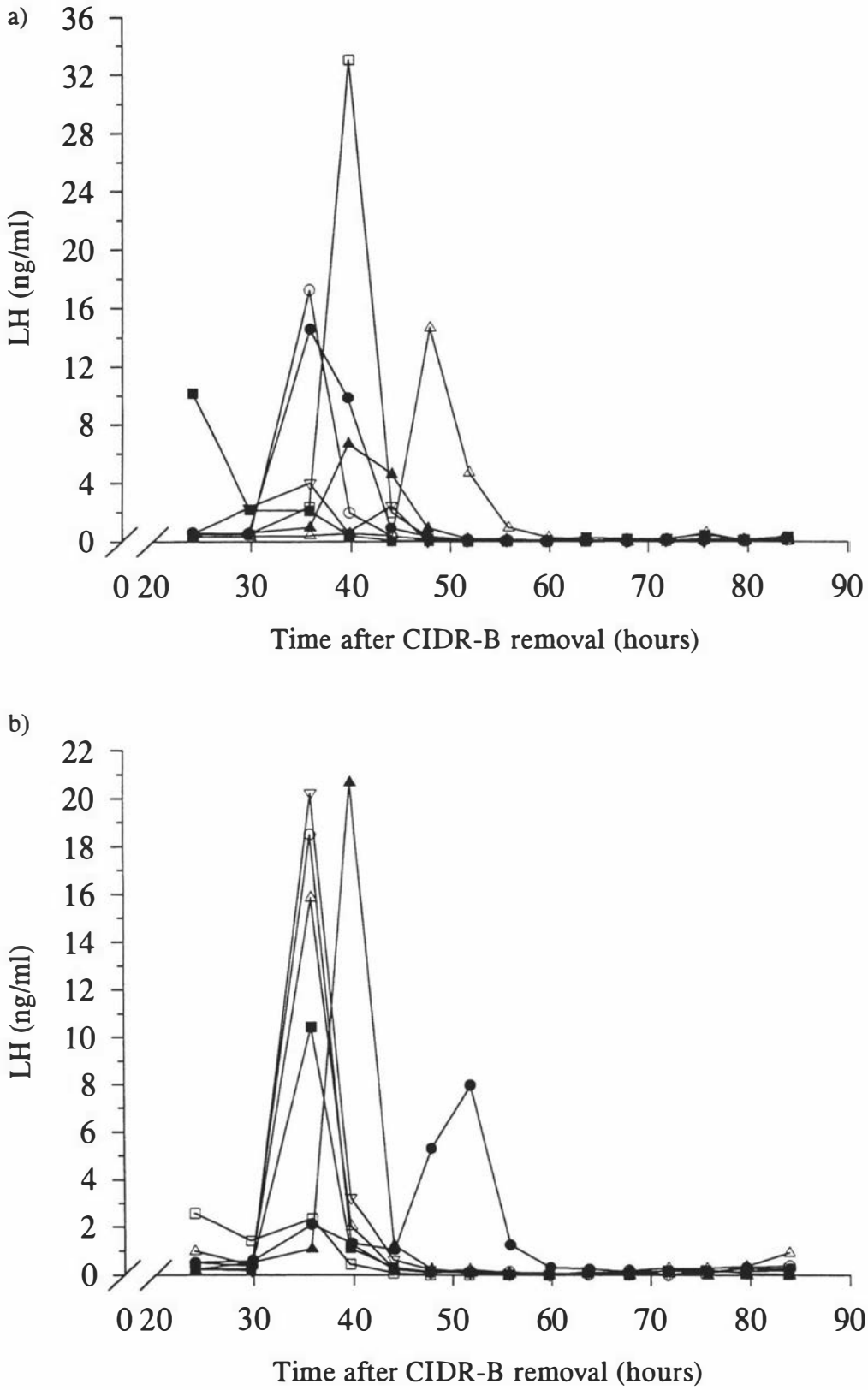

Figure 3.2. Profiles of concentration of LH in plasma for dairy heifers treated with a CIDR-B progesterone containing intravaginal device for 12 days in Period 2, with (n=7) (a) or without (n=7) (b) 0.5mg oestradiol benzoate intramuscularly 24 hours after device removal.

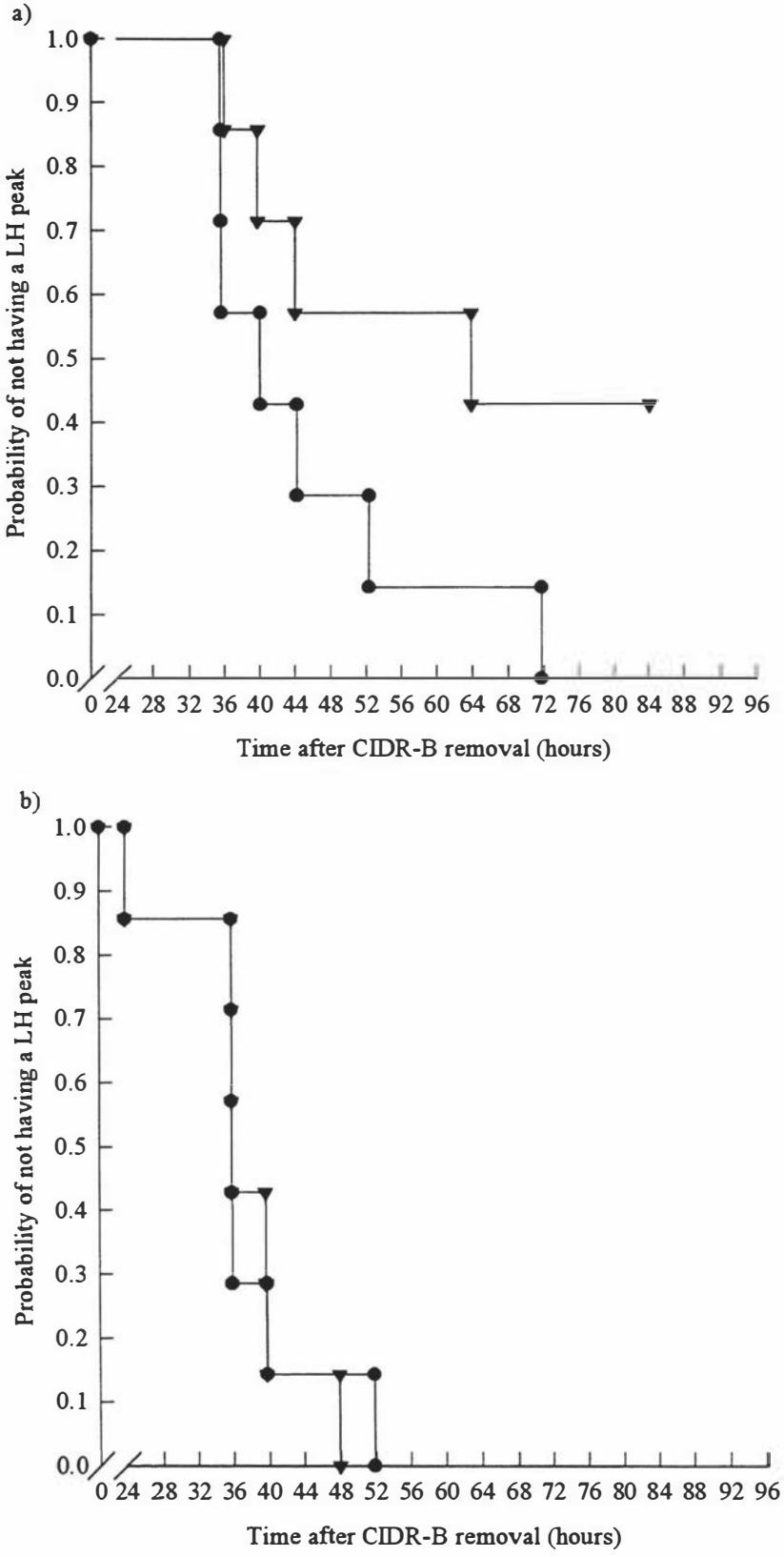

Figure 3.3. Survival function of time to LH peak in dairy heifers treated with a CIDR-B in Period 1 (n=14) (a) and Period 2 (n=14) (b) with or without 0.5mg oestradiol benzoate intramuscularly 24 hours after device removal (●Oestradiol, ▼ Control).

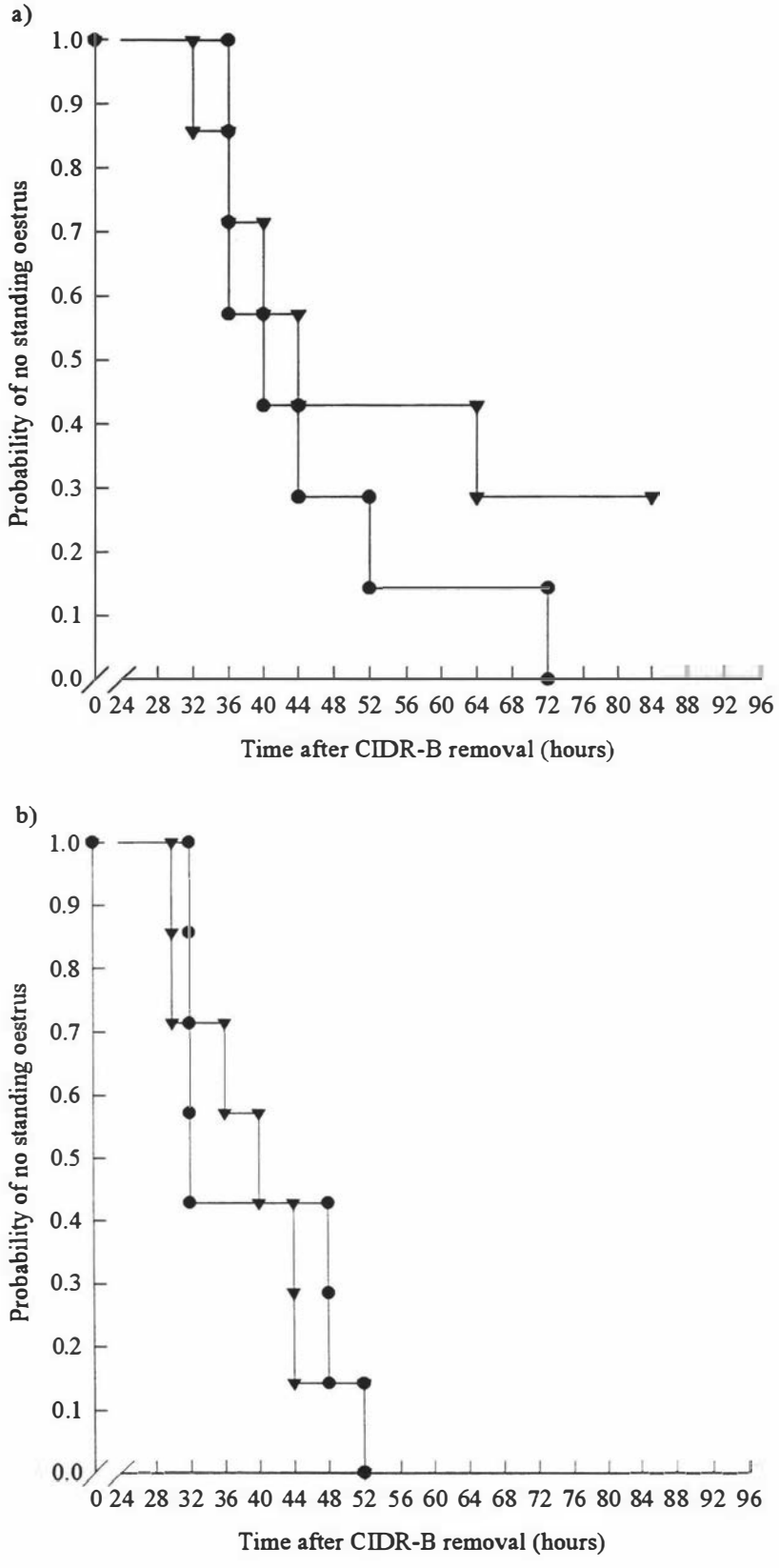

Figure 3.4. Survival function of time to standing oestrus in dairy heifers treated with a CIDR-B in Period 1 (n=14) (a) and Period 2 (n=14) (b) with or without 0.5mg oestradiol benzoate intramuscularly 24 hours after device removal (●Oestradiol, ▼ Control).

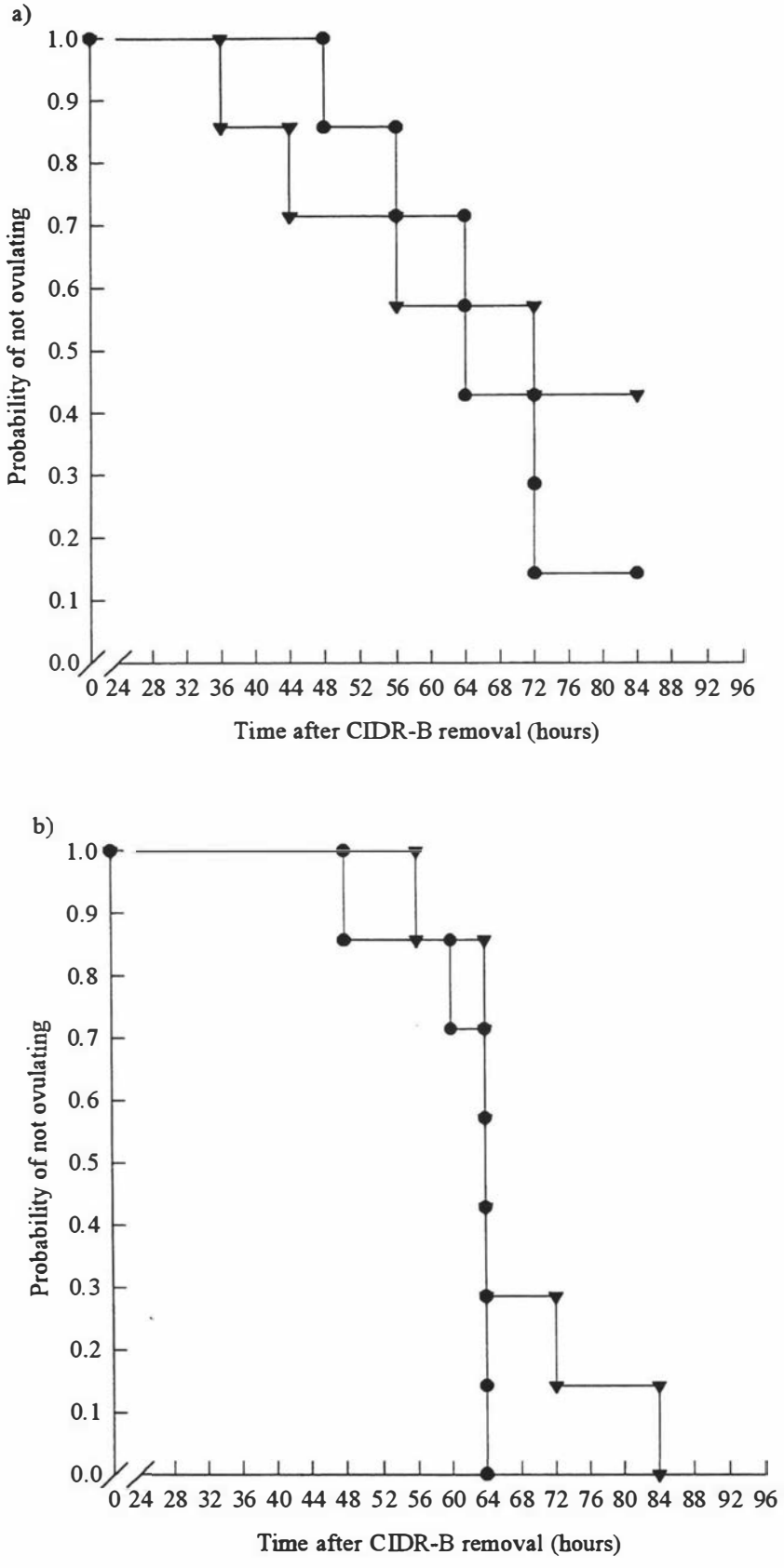

Figure 3.5. Survival function of time to ovulation in dairy heifers treated with a CIDR-B in Period 1 (n=14) (a) and Period 2 (n=14) (b) with or without 0.5mg oestradiol benzoate intramuscularly 24 hours after device removal (●Oestradiol, ▼ Control).

DISCUSSION

In this two-period crossover experiment, the administration of oestradiol benzoate 24 hours after removal of CIDR-B devices tended to reduce the time between device removal and LH peak in cycling dairy heifers in Period 1 but not in Period 2. Given the experimental design, heifers were at random stages of the oestrous cycle when CIDR-B devices were inserted in Period 1. Any heifers ovulating as a result of treatment in Period 1 would be in late dioestrus (day 13 or 14 of the oestrous cycle) when CIDR-B devices were inserted in Period 2. This effectively means that even though the same animals were used, the two Periods represent two different populations of heifers, with the stage-of-cycle at CIDR-B insertion being random for the first group and in most cases during late dioestrus for the second group.

The fact that the administration of oestradiol benzoate 24 hours after removal of CIDR-B devices tended to reduce the time between device removal and LH peak in randomly cycling heifers is in agreement with observations of others (Hobson and Hansel, 1972; Nancarrow and Radford, 1975; Welch *et al*, 1975) who found that oestradiol benzoate administration, 12-48 hr after corpus luteum removal decreased the time to LH peak. In the present study heifers which were treated with oestradiol benzoate and had an LH peak also ovulated. This is an important finding because oestradiol benzoate administration can cause behavioural oestrous without ovulation in ovariectomised (Hobson and Hansel, 1972; Rajamahendran *et al*, 1979) and pre-pubertal heifers (Gonzalez-Padilla *et al*, 1975). This confirms results of a previous field trial using this regime, where conception rates were unaffected by oestradiol treatment (Chapter 2). The failure of this regime to significantly decrease the time to standing oestrus and ovulation is in contrast to the findings of Nancarrow and Radford (1975), but in agreement with others (Welch *et al*, 1975). In the light of previous work where it was observed that treating heifers with oestradiol benzoate 24 hours after removal of CIDR-B devices resulted in an earlier onset of oestrus, the failure of oestradiol benzoate administration to alter the time of onset of oestrus or ovulation in this study is probably an artifact due to the small number of control animals which exhibited oestrus and ovulated. The times from LH peak to ovulation for control heifers synchronised in Period

1 were unusually short and highly variable compared to other heifers in the experiment. This may reflect a lack of control over the rate of follicular development following oestrus synchronisation using progesterone-containing devices, and highlights the need for regimes which synchronise LH peaks and ovulation. The times from LH peak to ovulation for the other three groups of heifers were similar to those reported in normal cycling heifers (Bernard *et al*, 1983). Ovulation has been consistently detected 22 to 26 hours after the LH peak (Duchens *et al*, 1994), and the results from this study support this finding. LH peak and the onset of oestrous behaviour in this study were closely associated, which agrees with the findings of others (Bernard *et al*, 1983).

When devices were inserted in Period 2, treatment with oestradiol benzoate had no significant effect on the time to reach peak LH concentrations, standing oestrus or ovulation. In Period 1, 10 of the 14 heifers ovulated and were therefore in late dioestrus when CIDR-B devices were inserted in Period 2. Control heifers in Period 2 had a significantly shorter time to LH peak than control heifers in Period 1. This is probably due to the presence of a more uniform stage of follicular development amongst these heifers, synchronised at a similar stage of the oestrous cycle. Asynchrony of ovulations is attributed to differing stages of follicular development at the time of the decline of plasma progesterone concentrations (Adams, 1994; Bergfelt *et al*, 1994). Treatment with oestradiol valerate at the start of synchrony treatments is known to cause atresia of the dominant follicle at the time of insertion of an exogenous progestagen source (Adams, 1994; Bo *et al*, 1993; Bo *et al*, 1994). Insertion of a CIDR-B with attached oestrogen capsule is likely to cause atresia of the dominant follicle and re-set follicular wave patterns. This effect may occur more reliably in animals that are at the same stage of the oestrus cycle. The effect of CIDR-B/oestrogen treatment on follicular wave dynamics at various stages of the oestrous cycle has not been investigated.

The results of the present study suggest that treatment with 0.5 mg oestradiol benzoate, 24 hours after removal of CIDR-B device, reduces the time from device removal to LH peak in randomly cycling heifers. Stage-of-cycle at the time of treatment may have a significant effect on the time to LH peak, standing oestrus and ovulation. When heifers were treated in late dioestrus, treatment with oestradiol had no significant

effect on the time to LH peak, standing oestrus or ovulation. The findings of this study support the hypothesis that the degree of synchrony of oestrus and ovulation following oestrus synchronisation treatments, largely depends on follicular wave dynamics. The administration of oestradiol benzoate appears to overcome the variability in timing of LH peaks which typically occur in a herd of synchronised heifers. This variability is likely due to different stages of follicular development being present after a synchronous decline in plasma progesterone concentrations.

CHAPTER 4:

THE EFFECT OF OESTRADIOL BENZOATE ON OVARIAN DYNAMICS IN DAIRY COWS TREATED WITH PROGESTERONE

ABSTRACT

The objective of this experiment was to examine the effects of exogenous oestradiol on the dominant follicle and corpus luteum of cows treated with progesterone at different stages of the oestrous cycle.

Thirty non-pregnant, non-lactating, multiparous dairy cows that were cycling normally were used in this experiment. The cows were treated intravaginally with progesterone (CIDR-B) for 12 days, with or without oestradiol. A completely randomised design was employed using treatment with oestradiol (0 or 10 mg) and day of cycle at the onset of treatment (3, 6, 9, 12 or 15 days after oestrus) in a 2×5 factorial arrangement. The ovaries of all cows were examined by trans-rectal ultrasonography every second day starting on the day of treatment and continuing for 4 days after device removal. Treatment with oestradiol on day 3 of the oestrous cycle caused atresia of the dominant follicle present at device insertion and resulted in the early emergence of the subsequent follicular wave. Treatment with oestradiol on days 6, 9, 12 and 15 of the oestrous cycle had no effect on follicular characteristics or the emergence of the subsequent follicular wave. Treatment with oestradiol benzoate had no effect on the day of onset of regression of the corpus luteum regardless of the stage of the oestrous cycle at device insertion.

INTRODUCTION

Current oestrus synchronisation regimes are based on synchronising the end of the progestational phase of the oestrous cycle so that ovulation occurs simultaneously in treated animals. The end of the progestational phase can be synchronised using prostaglandin $F_{2\alpha}$ and its analogues or exogenous progesterone. Oestrus synchronisation programmes using exogenous progestagens routinely incorporate oestrogens at the start of the treatment period (Odde, 1990; Larson and Ball, 1992). The addition of oestrogen at the start of the period of progesterone treatment enhances the oestrus response by increasing the number of animals observed in oestrus at an earlier time after device removal (Miksch *et al*, 1978; Cumming *et al*, 1982; Sprott *et al*, 1984; Munro and Moore, 1985; Macmillan and Peterson, 1993). The mechanism whereby oestrogen treatment enhances the oestrous response may involve luteolysis, although evidence supporting this is inconclusive, with many conflicting reports (Wiltbank and Kasson, 1968; Roche, 1974b; Lemon, 1975; Thimonier *et al*, 1975; Cumming *et al*, 1982; Munro and Moore, 1985). Recent studies utilising ultrasonography indicate that oestrogens are capable of altering the emergence of follicular waves (Adams, 1994; Bo *et al*, 1993; Bo *et al*, 1994; Bo *et al*, 1995) and therefore the enhanced oestrous response may be due to increased synchrony of follicular development. No studies have been reported which examine the ovarian dynamics of cows treated intravaginally with progesterone and oestradiol.

The objectives of this experiment were to examine the effects of exogenous oestradiol on the dominant follicle and corpus luteum of cows receiving a CIDR-B progesterone-containing intravaginal device on days 3, 6, 9, 12 or 15 of the oestrous cycle.

MATERIALS AND METHODS

Animals:

Thirty non-pregnant, non-lactating multiparous dairy cows were used for this experiment. All cows had tailpaint applied 6 weeks prior to commencing the experiment to ensure that they were cycling normally. Breed representation was as follows: Friesian n=16, Jersey n=10, and Ayrshire n=4. For the duration of the experiment cows were grazed at pasture, consisting predominantly of ryegrass and clover, and had access to fresh water.

Experimental Protocol:

A completely randomised design was employed using treatment with oestradiol and day of cycle at the onset of treatment in a 2×5 factorial arrangement. Five groups, with 6 cows in each, corresponded to insertion of devices on day 3, 6, 9, 12 or 15 of the oestrous cycle. Within each group, cows were randomly assigned to either receive (treatment = $+E_2$) or not receive (treatment = $-E_2$) a gelatin capsule containing 10 mg oestradiol benzoate (CIDIROL, InterAg, Hamilton, New Zealand) attached to the CIDR-B (InterAg, Hamilton, New Zealand) at insertion.

Ultrasonography:

The ovaries of all cows were examined by trans-rectal ultrasonography every second day starting on the day of treatment and continuing for 4 days after CIDR-B removal. At each examination the diameter of visible follicles and/or corpora lutea were measured and recorded. All cows had tailpaint applied at device removal to facilitate oestrus detection and were examined twice daily for signs of oestrous activity for 8 days following device removal.

The dominant follicle was defined as the follicle that grew to at least 10 mm and exceeded the diameter of all other follicles in the wave. A wave of follicular activity was

retrospectively identified by the presence of a dominant follicle. The day of first detection of a 5 mm follicle that was retrospectively identified as a dominant follicle was taken as the first day of a wave. Onset of regression of the corpus luteum was the day on which the corpus luteum appeared to begin a progressive decrease in diameter. The "treatment wave" was defined as the wave of follicular activity present at the time of device insertion. The "post-treatment wave" was defined as the wave of follicular activity subsequent to the treatment wave. It was expected that Day 3 would correspond to the mid-growing phase, Day 6 to the late growing to early static phase, and Day 9 to the late static phase of the dominant follicle of the first wave. It was expected that Day 12 would correspond to the mid-growing phase, and Day 15 to the late growing to early static phase of the second dominant follicle. For illustration of the follicular profiles, data for each wave were normalised to begin on the mean day of emergence for the group represented (Ginther *et al*, 1989).

Progesterone Assay:

Blood was collected for progesterone assay from cows which had a corpus luteum visible on ultrasonography 96 hours after device removal. Progesterone concentration was determined using solid phase ^{125}I-labelled radioimmunoassay (RIA Coat-a-Count, Diagnostic Products Corporation, Los Angeles, California, USA). Three quality control pools were run in triplicate in the assay and the intra-assay coefficient of variations (%) were 2.17, 2.79, 7.33 for mean concentrations of 0.328, 1.783 and 3.771 ng/ml respectively. The sensitivity was 0.198 ng/ml.

Statistical Analysis:

Two-way analysis of variance was used to assess the effect of treatment, day and treatment x day interaction on follicular characteristics, wave emergence, regression of the corpus luteum and the time to standing oestrus. When analysis of variance indicated a significant effect of day, treatment or of the interaction term between them, a planned comparison within groups was conducted to determine treatment and/or day effects. Analysis of data was performed using the statistical computer software package Statistix

Version 4.1(Analytical Software, Tallahassee, FL, USA).

RESULTS

The effects of treatment with oestradiol benzoate at CIDR-B insertion on characteristics of the dominant follicle, corpus luteum and the time taken to standing oestrus are presented in Table 4.1., and Figures 4.1. to 4.4. Treatment with oestradiol benzoate on day 3 was followed by regression of the dominant follicle present at device insertion, resulting in the early emergence of the post-treatment wave ($p=0.008$). As a result of follicular regression, the maximum diameter of the dominant follicle of the treatment wave was smaller in $+E_2$ cows ($p=0.005$). The maximum diameter of the dominant follicle of the post-treatment wave was similar for both groups ($p=0.70$). Characteristics of the dominant follicle of the treatment and post-treatment wave were not different between $+E_2$ cows and $-E_2$ cows after device insertion on days 6, 9, 12 or 15. In the day 9 group only 1 $+E_2$ cow and 2 $-E_2$ cows had a post-treatment wave, therefore a comparison of post-treatment wave characteristics was not performed.

Onset of regression of the corpus luteum was not different between $+E_2$ cows and $-E_2$ cows in any group. In 5 cows treated on day 3 (3 $+E_2$ cows and 2 $-E_2$ cows) the corpus luteum was still visible ultrasonographically 96 hours after removal of CIDR-B devices. Three of these 5 cows had plasma progesterone concentrations above 1 ng/ml, indicating luteal activity.

Treatment with oestradiol had no significant effect on the time to oestrus after device removal ($p=0.38$). Cows in the day 3 group took significantly longer to come into oestrus than cows in other groups ($p<0.05$).

Table 4.1. Characteristics (mean ± SEM) of the dominant follicle and corpus luteum and the time taken to standing oestrus in cows receiving a CIDR-B progesterone-containing intravaginal device with (+ E_2) or without (- E_2) 10mg oestradiol benzoate intravaginally at device insertion on days 3, 6, 9, 12 and 15 of the oestrous cycle (n=3 per group).

End Point	Treatment Group									
	Day 3		Day 6		Day 9		Day 12		Day 15	
	- E_2	+ E_2	- E_2	+ E_2	- E_2	+ E_2	- E_2	+ E_2	- E_2	+ E_2
Treatment wave										
Maximum diameter of dominant follicle (mm)	16.3±1.5[a]	10.6±0.7[b]	15.3±1.8	14.3±0.3	16.0±1.0	16.0±0.6	11.7±1.7	11.3±0.7	15.0±1.5	14.7±1.8
Post-treatment wave										
Emergence (days after CIDR insertion)	6.6±1.0[c]	2.0±0[d]	5.3±0.7	4.7±1.8	-	-	2.0±1.2	3.3±0.7	1.0±1.0	3.3±1.8
Maximum diameter of dominant follicle (mm)	16.3±0.7	15.3±0.9	18.3±2.4	17.0±1.5	-	-	16.7±0.9	19.3±1.5	20.0±6.0	22.7±1.5
Regression of CL (days after CIDR insertion)	-	-	13.3±1.2	14.0±2.0	10.0±0	9.3±3.0	7.3±0.7	6.7±0.7	4.6±1.8	3.3±1.3
Number in oestrus	3	2	2	3	3	3	3	2	3	3
Time to oestrus (hours after CIDR removal)	144±36.7[e]	150±18.0[e]	96±36.0[f]	72±20.8[f]	84±42.0[f]	52±4.0[f]	56±8.0[f]	54±6.0[f]	56±10.6[f]	44±4.0[f]

[a, b, c, d, e, f] Figures within rows with different superscripts are significantly different ($p<0.05$)

- Insufficient observations to allow a comparison

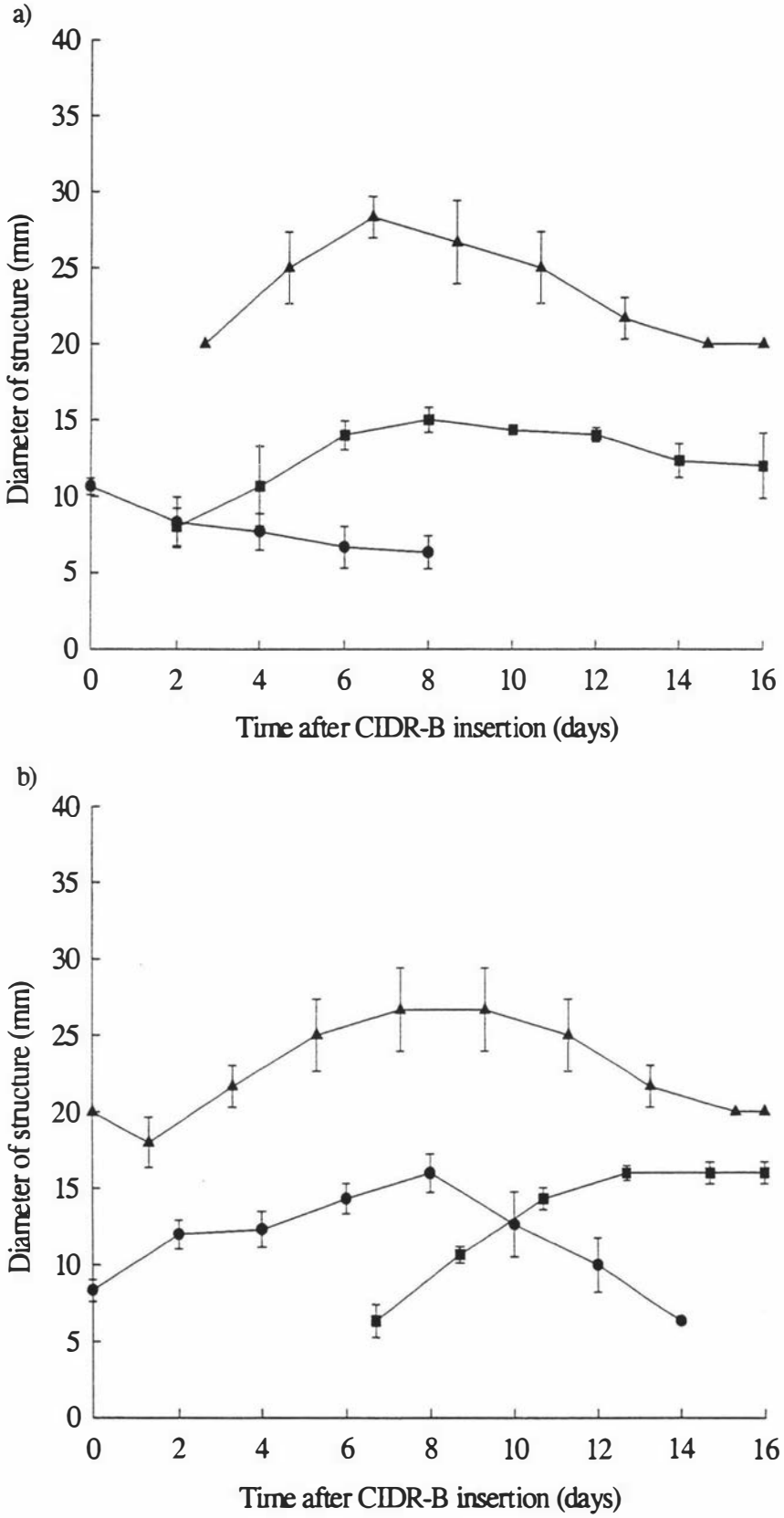

Figure 4.1. Profiles of the treatment dominant follicle (●), post-treatment dominant follicle (■), and corpus luteum (▲) in cows treated with a CIDR-B for 12 days commencing on day 3 of the oestrous cycle, with (a) or without (b) 10mg oestradiol benzoate intravaginally at CIDR-B insertion.

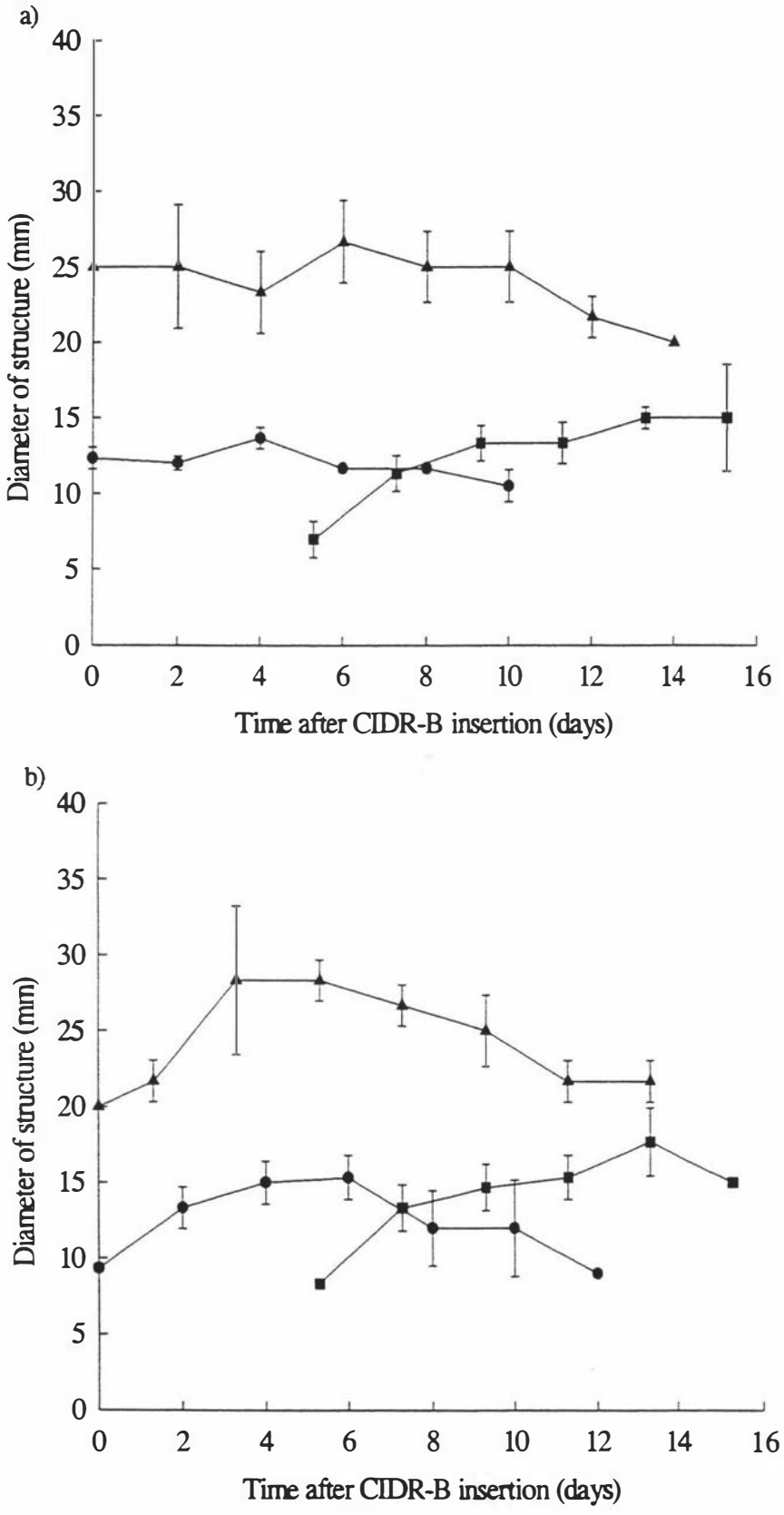

Figure 4.2. Profiles of the treatment dominant follicle (●), post-treatment dominant follicle (■), and corpus luteum (▲) in cows treated with a CIDR-B for 12 days commencing on day 6 of the oestrous cycle, with (a) or without (b) 10mg oestradiol benzoate intravaginally at CIDR-B insertion.

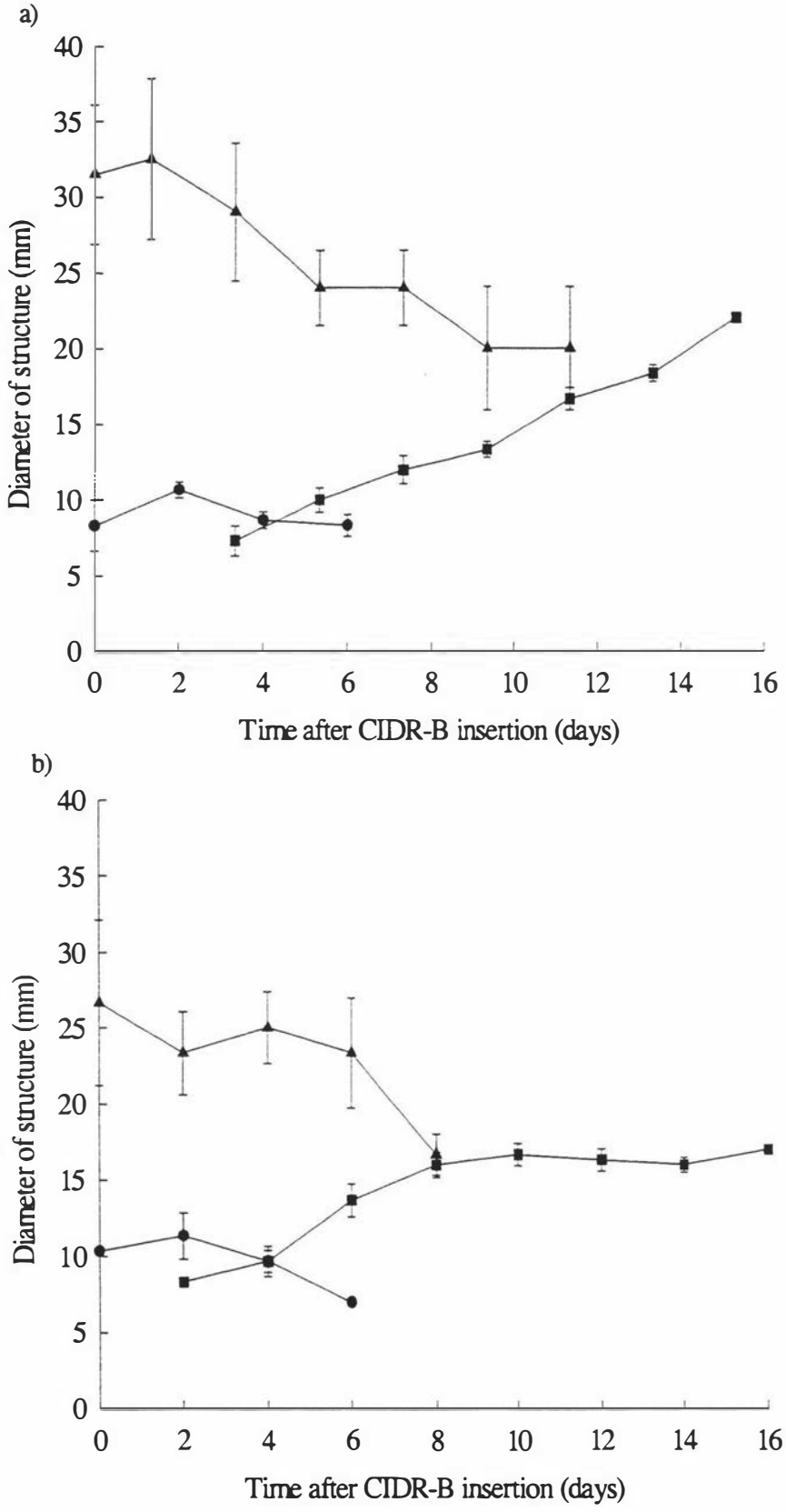

Figure 4.3. Profiles of the treatment dominant follicle (●), post-treatment dominant follicle (■), and corpus luteum (▲) in cows treated with a CIDR-B for 12 days commencing on day 12 of the oestrous cycle, with (a) or without (b) 10mg oestradiol benzoate intravaginally at CIDR-B insertion.

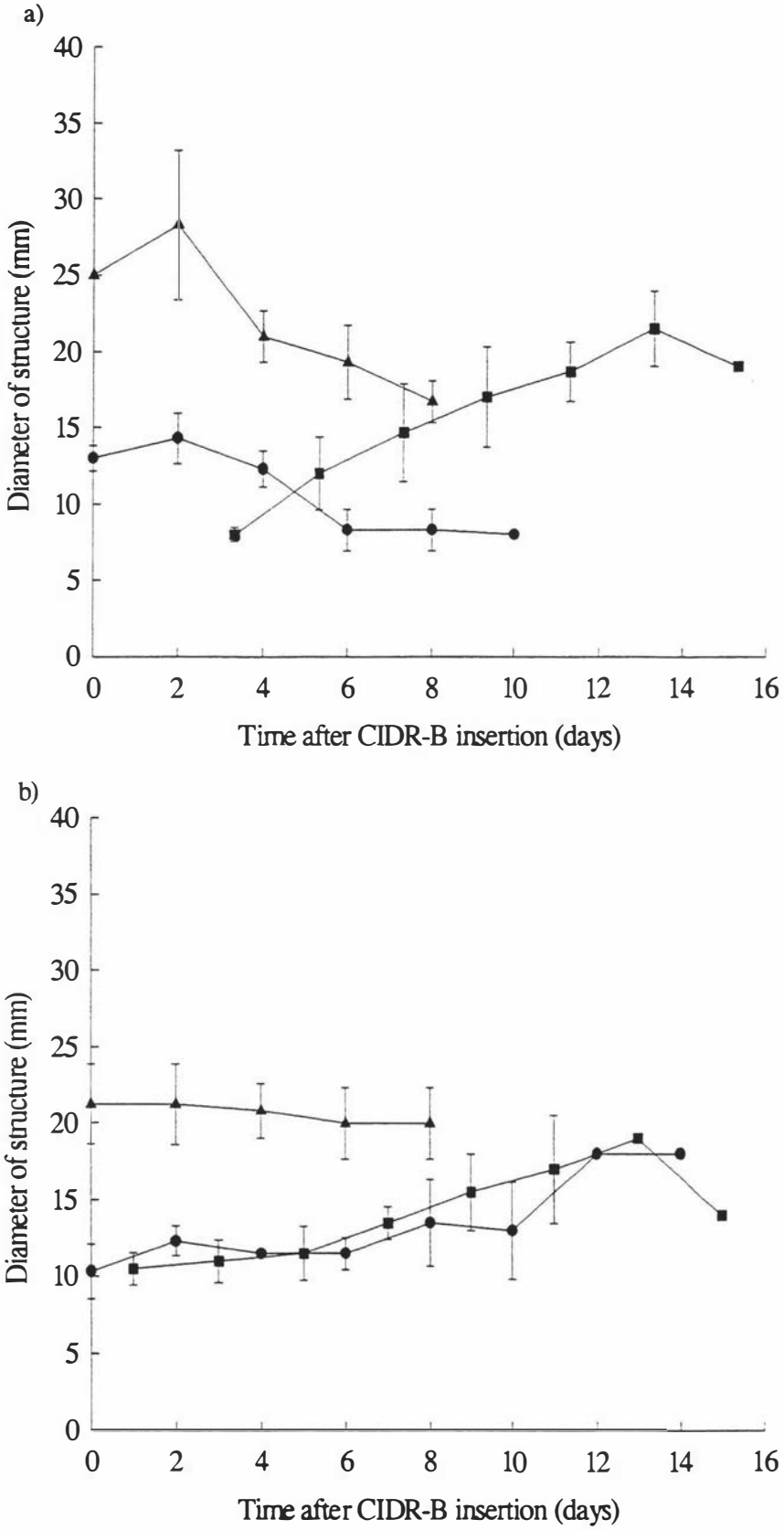

Figure 4.4. Profiles of the treatment dominant follicle (●), post-treatment dominant follicle (■), and corpus luteum (▲) in cows treated with a CIDR-B for 12 days commencing on day 15 of the oestrous cycle, with (a) or without (b) 10mg oestradiol benzoate intravaginally at CIDR-B insertion.

DISCUSSION

Treatment on day 3 of the oestrous cycle with 10 mg oestradiol benzoate intravaginally in association with a CIDR-B progesterone containing intravaginal device caused atresia of the dominant follicle present at device insertion and resulted in the early emergence of the subsequent follicular wave. This supports the findings of others using oestradiol valerate (Bo *et al*, 1993) or oestradiol-17β (Bo *et al*, 1994; Bo *et al*, 1995) in combination with an ear-implant containing 6 mg norgestomet. Treatment with oestradiol benzoate on days 6, 9, 12 and 15 of the oestrous cycle had no effect on follicular characteristics or the emergence of the subsequent follicular wave. This is in contrast to the findings of Bo *et al* (1995) who found that treatment with a 6mg norgestomet ear implant and 5 mg oestradiol-17β intramuscularly on days 6 and 9 of the oestrous cycle resulted in the delayed emergence of the next follicular wave. The two studies differed however in terms of the route of administration of progestagen and oestradiol and the type of progestagen.

Treatment with oestradiol benzoate had no effect on the day of onset of regression of the corpus luteum regardless of the stage of the oestrous cycle at device insertion, thus agreeing with the findings of Bo *et al* (1995). The effectiveness of oestrogens as luteolysins has been inconsistent (Wiltbank and Kasson, 1968; Roche, 1974b; Lemon, 1975; Thimonier *et al*, 1975; Cumming *et al*, 1982; Munro and Moore, 1985) and the results of this study indicate that 10 mg oestradiol benzoate administered intravaginally does not cause early regression of the corpus luteum nor prevent corpus luteum development in the metoestrus cow.

Treatment with oestradiol benzoate had no effect on the time to standing oestrus following removal of CIDR-B devices. The stage of the oestrous cycle at device insertion significantly affected the time to standing oestrus with cows in the day 3 group taking longer to come into oestrus than any other group. The most likely reason for this is the persistence of a corpus luteum after device removal and explains why the onset of oestrus is earlier following CIDR-B treatment when prostaglandins are injected at or near the end of the treatment period (Macmillan and Peterson, 1993).

In conclusion, treatment with intravaginal oestradiol benzoate and progesterone on day 3 of the oestrous cycle caused atresia of the dominant follicle present at the time of device insertion. As a result, the emergence of the subsequent follicular wave occurred earlier. This effect was not observed with insertion on days 6, 9, 12 or 15 of the oestrous cycle. Treatment had no effect on the day of onset of regression of the corpus luteum.

CHAPTER 5:

RE-INSERTION OF THE CIDR-B PROGESTERONE-CONTAINING INTRAVAGINAL DEVICE TO SYNCHRONISE RETURNS TO OESTRUS IN DAIRY HEIFERS

ABSTRACT

Recommendations for oestrus synchronisation of dairy heifers using the CIDR-B device suggest re-insertion of used devices 16 days after first insemination, for a period of 5 days to allow a second opportunity for AI. Controlled studies on the effectiveness of re-using CIDR-B devices to synchronise returns to oestrus in non-pregnant dairy heifers are lacking. A clinical trial was conducted involving 750 maiden Friesian heifers in 13 herds. After an initial CIDR-B synchronisation programme heifers were grouped within-herd, in weight-matched pairs. For each pair of heifers, one member was randomly allocated to receive a CIDR-B device and the other member to not receive a device. The used CIDR's were re-inserted at either 14 or 16 days after first insemination, for a period of 5 days. Re-insertion of used CIDR-B devices significantly increased the number of non-pregnant heifers detected in oestrus and inseminated by 48 hours after device removal (45.2% vs 27.3%, $p<0.05$, in herds where CIDR's were re-inserted on day 14; 48.8% vs 13.6%, $p<0.05$, in herds where CIDR's were re-inserted on day 16). Re-insertion at 14 or 16 days after first insemination was equally effective in increasing visible returns to service. Conception rate was unaffected by CIDR-B treatment.

INTRODUCTION

Synchronisation of oestrus in dairy heifers concentrates oestrus detection and insemination into a narrow, pre-determined time period and enables the practical use of artificial insemination (AI). Current oestrus synchronisation regimes are based on synchronising the end of the progestational phase of the oestrous cycle so that ovulation occurs simultaneously in treated animals. At present, the end of the progestational phase can be synchronised using prostaglandins or exogenous progesterone treatment. The CIDR-B (InterAg, Hamilton, New Zealand) intravaginal device provides controlled administration of exogenous progesterone to cattle (Macmillan *et al*, 1991) and is indicated for oestrus synchronisation and treatment of post-partum anoestrus (Anon., 1995b). Synchrony is enhanced when 10 mg of oestradiol benzoate in a gelatin capsule is administered at the start of the treatment period. Regimes that produce the highest degree of synchrony incorporate treatment with prostaglandin prior to CIDR-B removal (Jellie, 1993; Macmillan, 1993).

Oestrus synchronisation programmes which provide an extremely high oestrous response and high degree of synchrony mean that animals not conceiving to the synchronised insemination will be in oestrus approximately 21 days later. A problem can arise if there is an inadequate number of bulls to serve previously synchronised heifers returning to oestrus, particularly in large herds. Re-synchrony of returns to oestrus in non-pregnant heifers allows for a second round of AI, increases the total number of heifers conceiving to artificial breeding and reduces the need for large numbers of bulls.

Current oestrus synchronisation regimes using the CIDR-B device include the recommendation of re-inserting previously used devices, 16 days after first insemination, for a period of 5 days (Jellie, 1993). Forty-eight hours after device removal heifers are inseminated to detected oestrus. Large scale, controlled studies on the effectiveness of synchronising returns to oestrus in non-pregnant dairy heifers with used CIDR-B devices are lacking.

The objective of this clinical trial was to determine the effectiveness of re-using

CIDR-B devices to synchronise returns to oestrus in dairy heifers.

MATERIALS AND METHODS

A clinical trial was conducted involving 750 yearling heifers in 13 intensively grazed dairy herds in the Manawatu region of New Zealand.

Farm Selection:

All of the farmers involved were clients of Massey University's Farm Service Veterinary Clinic. Selection of farms was based on farmers' willingness to participate, their perceived ability to keep accurate and complete records and the presence of adequate handling facilities on the farm.

Animals:

Individual heifer group size ranged from 23 to 106. All animals were maiden Friesian heifers with an average age at first insemination of 15 months and an average weight of 282.7 $\pm$ 35.0 kg. Management of heifers during the project was under the direct control of the farm owner/manager utilising their usual farming practices. All herds grazed pastures, consisting predominantly of ryegrass and clover, for the duration of the project. The date of first insemination for each herd of heifers was nominated by the herd owner and occurred between 11 and 30 October 1993.

Experimental Protocol:

The oestrus synchronisation regime used was based on a recommended method of using the CIDR-B progesterone-containing intravaginal device (Anon., 1995b). A CIDR-B with CIDIROL capsule (InterAg, Hamilton, New Zealand) was inserted into the vagina of each heifer for a period of 12 days. The CIDR-B consists of a silicone rubber elastomer impregnated with 1.9g progesterone. The CIDIROL capsule is a gelatin capsule containing 10 mg oestradiol benzoate and is inserted into a slot on the CIDR-B

device. On the day of device insertion all heifers were weighed using electronic scales. Detection of oestrus was the responsibility of the herd owner/manager and was aided by the use of tailpaint and aerosol raddle (Macmillan *et al*, 1988). Forty-eight and 72 hours after removal of CIDR-B devices, heifers were yarded and if they had shown evidence of tailpaint removal they were inseminated. Insemination of heifers was conducted by experienced, commercially-based AI technicians. Selection of semen was the farmer's choice. All inseminations utilised frozen-thawed semen, processed and thawed following recommended industry standards. Semen was purchased through one of two commercial AI distribution centres, Livestock Improvement Corporation or Ambreed New Zealand Ltd. After each heifer was inseminated, tailpaint of a different colour was applied to the tailhead region. If heifers inseminated at 48 hours had evidence of tailpaint removal at 72 hours, they were re-inseminated at that time.

After CIDR-B devices were removed they were washed thoroughly in disinfectant solution (Hibiclens®; ICI Australia, Melbourne), dried and stored in a dark, dry place. CIDR-B devices were re-inserted only into heifers from the same herd. In 7 herds (371 heifers) CIDR-B devices were re-inserted 16 days after first insemination, and in 5 herds (379 heifers) they were inserted 14 days after first insemination. Within each herd, heifers were grouped in weight-matched pairs. For each pair of heifers one member of the pair was randomly allocated to receive a CIDR-B device and the other to not receive a device. CIDR-B devices were inserted for a period of 5 days. Tailpaint was applied at CIDR-B removal so that only heifers that were in oestrus between CIDR-B removal and 48 hours after CIDR-B removal would be inseminated. Forty-eight hours after device removal heifers were yarded and inseminated to detected oestrus. Oestrus detection and insemination was the same as the first synchronised round of mating.

All heifers were pregnancy tested by palpation per-rectum 35 days after each of the synchronised rounds of insemination.

Statistical Analysis:

Results were analysed in terms of the effects of: 1) day of CIDR-B re-insertion,

2) treatment group and 3) herd, on a) the occurrence of oestrus by 48 hours after device removal, and b) the conception rate. The analysis was conducted in two steps, a univariate and a multivariate analysis. For the univariate analysis the association between each dependent variable and the independent variables (treatment and day of CIDR-B re-insertion), was examined for statistical significance using Chi-square tests. In the multivariate analysis, logistic regression was used to assess the effect of confounding variables such as herd. A backward selection of variables was performed whereby those that were non-significant predictors (p value for Wald's test > 0.05) were sequentially dropped from the logistic regression model. Regression coefficients were converted into odds ratios indicating strength of association between the independent and dependent variable, controlling for other independent variables in the regression model. Analysis of data was performed using the computer software package Statistix Version 4.0 (Analytical Software, St. Paul, MN, USA).

RESULTS

During the first period of insertion, 12 of the 750 heifers lost the CIDR-B device prior to the planned time of removal and were subsequently removed from the trial. Retention rate of the CIDR-B device was therefore 98.4%. No heifers lost the CIDR-B device during the 5 day period of re-insertion.

Of the 738 heifers remaining in the trial, 24 were excluded from the analysis of results for the following reasons; at palpation 35 days after the first insemination 8 heifers were found to be pregnant to a prior (unrecorded) mating and 4 were found to be freemartins. These 12 heifers and their matched partners were excluded.

Six hundred and sixty-six heifers (93.3%) were detected in oestrus after the first synchrony treatment. Three-hundred and seventy-five heifers conceived to the first round of insemination, a synchronised pregnancy rate of 52.5% and a conception rate of 56.3%. There were therefore 339 (47.5%) non-pregnant heifers after the first round of insemination. The re-insertion of a used CIDR-B device for 5 days significantly increased the number of non-pregnant heifers showing oestrus by 48 hours after device removal

(Table 5.1.). When CIDR-B devices were re-inserted 14 days after insemination, 45.2% of non-pregnant heifers showed oestrus and were inseminated, compared to 27.3% of heifers not receiving CIDR-B devices (X^2 = 6.0, d.f. 1, p=0.01). When CIDR-B devices were re-inserted 16 days after insemination, 48.8% of non-pregnant heifers showed oestrus and were inseminated, compared to 13.6% of heifers not receiving CIDR devices (X^2 = 23.9, d.f. 1, p=0.00) (Table 5.1.).

Table 5.1. Oestrous response and conception rates for non-pregnant heifers either receiving or not receiving a CIDR-B device for 5 days, 14 or 16 days after first insemination, to synchronise returns to oestrus

Day of re-insertion	CIDR-B		No CIDR-B	
	Oestrous response[a] (%)	Conception Rate[b] (%)	Oestrous response (%)[a]	Conception rate (%)[b]
Day 14	45.2[c]	57.9	27.3[d]	45.8
Day 16	48.8[c]	50.0	13.6[e]	36.4

[a] Percentage of non-pregnant heifers in oestrus by 48 hours after device removal.

[b] Number pregnant/number inseminated

[c,d,e] Values with different superscripts are significantly different ($p < 0.05$).

There was no significant effect of the day of re-insertion on the proportion of non-pregnant heifers exhibiting oestrus after re-insertion of CIDR-B devices at either 14 or 16 days (p=0.64), or on the conception rate to the re-synchronised oestrus (p=0.60). For heifers which did not receive a CIDR-B, significantly more were in oestrus when their herd mates had CIDR-B devices re-inserted on day 14 rather than day 16 (27.3% vs 13.6%, X^2 = 4.8, d.f. 1, p=0.03).

There was considerable variation between herds in the occurrence of oestrus when CIDR-B devices were re-inserted 16 days after first insemination, with between 27.2% and 68.8% of CIDR-B treated heifers exhibiting oestrus by 48 hours after device

removal. Logistic regression was used to assess the effect of CIDR-B re-insertion on the occurrence of oestrus, controlling for differences between herds. The odds ratio for CIDR-B re-insertion indicated that heifers which received a CIDR-B 16 days after first insemination were 6.29 (95% CI = 2.83 to 13.98) times more likely to exhibit oestrus 48 hours after device removal than heifers not receiving a CIDR-B.

There was no significant effect of herd on the occurrence of oestrus in heifers which had CIDR-B devices re-inserted 14 days after first insemination. Logistic regression analysis indicated that CIDR-B re-insertion was the only significant predictor of the occurrence of oestrus. The odds ratio for CIDR-B re-insertion indicated that heifers which received a CIDR-B 14 days after first insemination were 2.20 (95% CI = 1.17 to 4.16) times more likely to show oestrus than heifers not receiving a CIDR-B. There was no significant effect of herd or CIDR-B re-insertion on conception rate.

DISCUSSION

Re-insertion of used CIDR-B devices for a period of 5 days, 14 or 16 days after first insemination, significantly increased the number of non-pregnant heifers detected in oestrus by 48 hours after device removal. Re-insertion at either 14 or 16 days after first insemination was equally effective in increasing visible returns to service. The results of this trial are in agreement with the findings of others using lactating dairy cows (Macmillan and Peterson, 1993), suckling beef cows (Munro and Bertram, 1990) or maiden dairy heifers (Van Cleeff *et al*, 1989). In lactating dairy cows, new CIDR-B devices were inserted from 14 to 17 days after first insemination and removed on day 21 after insemination. This significantly increased the percentage of second inseminations made 23 or 24 days after first insemination, with 67.4% of inseminations occurring on these two days (Macmillan and Peterson, 1993). In suckling beef cows, used CIDR-B devices were inserted on days 6 or 10 after first insemination and removed on day 21 after first insemination. Fifty-five percent of non-pregnant cows were detected in oestrus between 21 and 24 days after the first insemination (Munro and Bertram, 1990). Van Cleeff *et al* (1989) inserted used CIDR's into heifers 17 days after first insemination and removed them 5 days later. This resulted in 81.3% of non-pregnant heifers being

detected in oestrus and inseminated by 48 hours after device removal.

Conception rates were unaffected by CIDR re-insertion and were similar for both rounds of insemination in CIDR-B treated heifers. In contrast to these findings, Munro and Bertram (1990) found that conception rates to a second synchronised insemination in beef cows was 30%.

In herds in which CIDR's were re-inserted 16 days after first insemination there was considerable variation in terms of the number of heifers detected in oestrus. This may be due to differences in oestrus detection efficiency. Interpretation of tailpaint status as an indicator of oestrus activity is a significant factor in determining the overall oestrous response following synchrony treatments (Dick, 1990).

Given the results of this trial, there appears to be sufficient residual progesterone in used CIDR-B devices to synchronise returns to oestrus in non-pregnant heifers. Previous work investigating plasma progesterone concentrations in animals treated with new and used CIDR-B devices agrees with this conclusion (Duirs *et al*, 1986; Macmillan *et al*, 1991).

Significantly more non-pregnant control heifers were detected in oestrus in herds where CIDR-B devices were re-inserted on day 14 after insemination compared to herds re-inserted on day 16. The lower number of control heifers inseminated in the 16 day herds indicates that most returns to oestrus occur on or prior to 21 days after first insemination and agrees with the findings of Macmillan and Peterson (1993).

Results from this and other trials show that the number of non-pregnant animals which are synchronised for a second insemination and are detected in oestrus is lower than would be expected. A possible reason for this is that progesterone supplementation, without oestrogen at the start of the treatment regime, is producing no control over follicular wave patterns. Variation in the rate of follicular development after a synchronous decline in plasma progesterone levels is the most limiting factor in achieving a desirable degree of synchrony of oestrus and ovulation (Mikeska and Williams, 1988;

Macmillan and Peterson, 1993). Oestrogens synchronise follicular waves by causing atresia of the dominant follicle (Bo *et al*, 1994) and are routinely incorporated into oestrus synchrony treatments which utilise progestagens. Incorporating oestrogens into re-synchrony treatments may offer a method of enhancing the number of non-pregnant heifers detected in oestrus. As oestrogen treatment would occur before the pregnancy status was known, the effect of oestrogen on bovine early embryonic survival needs to be determined. Single doses of 20 to 40 mg of oestradiol valerate are capable of causing abortion in cows between days 60 and 120 of gestation (Roberts, 1986). The number of non-pregnant heifers inseminated to a synchronised second oestrus may be increased by detecting oestrus and inseminating heifers on two consecutive days, ie. 48 and 72 hours after device removal. Suckling beef cows synchronised for a second insemination were observed for oestrus and inseminated between 21 and 24 days after first insemination (Munro and Bertram, 1990). This resulted in 55% of non-pregnant cows being inseminated.

Re-insertion of previously used CIDR-B devices for 5 days at either 14 or 16 days after first insemination is equally effective in synchronising returns to oestrus. The earlier re-insertion regime is preferable in that it decreases the time between first and second insemination. Some degree of flexibility is now available for remating in that the day of re-insertion can be selected to suit management requirements.

In conclusion, re-insertion of used CIDR-B devices for a period of 5 days, 14 or 16 days after first insemination, significantly increased the number of non-pregnant heifers detected in oestrus by 48 hours after device removal. Conception rate was unaffected by CIDR-B treatment.

GENERAL DISCUSSION

Current oestrus synchronisation regimes for cattle are effective in producing a synchronous decline in blood progesterone levels in different animals after treatment. Unfortunately, the time taken for follicular maturation and ovulation tends to be inconsistent between animals (Savio *et al*, 1988; Sirois and Fortune, 1988; Ginther *et al*, 1989), which may cause the variation in fertility observed following fixed-time insemination after oestrus synchronisation treatments (Macmillan, 1978; Anderson *et al*, 1982; King *et al*, 1982; Dailey *et al*, 1983; Stevenson *et al*, 1984; Stevenson *et al*, 1987; Mikeska and Williams, 1988; Macmillan and Peterson, 1993). The variable rate of follicular development occurring after a synchronous decline in plasma progesterone levels is the most limiting factor in achieving a degree of synchrony of oestrus and ovulation to allow fixed-time insemination (Mikeska and Williams, 1988; Macmillan and Peterson, 1993). Development of oestrus synchronisation programmes which incorporate a method of controlling follicular development and/or ovulation is desirable if the goal of achieving fixed-time insemination with a consistently normal level of fertility is to be met.

The variability in timing of ovulation may be reduced by re-setting follicular wave patterns in different animals at the commencement of synchrony treatments, so that follicular wave emergence is synchronised. Combinations of oestrogen and progestagen have been shown to be capable of causing atresia of the dominant follicle present at the time of treatment, resulting in the synchronous emergence of the subsequent follicular wave (Adams, 1994; Bo *et al*, 1994; Bo *et al*, 1995).

Treatment on day 3 of the oestrous cycle with 10 mg oestradiol benzoate intravaginally, in association with a CIDR-B progesterone containing intravaginal device caused atresia of the dominant follicle which was present at device insertion. Early emergence of the subsequent follicular wave then followed (Chapter 4). This agrees with the findings of others who used oestradiol valerate (Bo *et al*, 1993) or oestradiol-17β (Bo *et al*, 1994; Bo *et al*, 1995) in combination with an ear-implant containing 6 mg norgestomet. Treatment with oestradiol benzoate on days 6, 9, 12 and 15 of the oestrous cycle had no effect on follicular characteristics or the emergence of the subsequent follicular wave. This is in contrast to the findings of Bo *et al* (1995) who found that

treatment with a 6mg norgestomet ear implant and 5 mg oestradiol-17β intramuscularly on days 6 and 9 of the oestrous cycle resulted in the delayed emergence of the next follicular wave. The two studies differed however in terms of the type, dose and route of administration of progestagen and oestradiol. Given the results of this study, there appears to be little benefit in including oestradiol benzoate at the commencement of CIDR-B treatment programmes. In a herd of randomly cycling heifers or cows it is unlikely that oestradiol benzoate as a 10 mg intravaginal capsule will effectively re-set follicular waves and synchronise subsequent wave emergence. This may explain why a 12-day CIDR-B treatment with 10 mg oestradiol benzoate intravaginally at device insertion does not provide sufficient oestrus synchrony to allow for fixed-time insemination. If the synchronous emergence of follicular waves is a necessary component of CIDR-B synchrony programmes, then the effects of alternative routes of administration of oestradiol benzoate, alterations in dose rate and different forms of oestrogen such as oestradiol valerate need to be investigated.

Treatment with 10 mg oestradiol benzoate intravaginally at CIDR-B insertion had no effect on the time to standing oestrus following removal of devices. The stage of the oestrous cycle at device insertion significantly affected the time to standing oestrus, with cows treated on day 3 of the oestrous cycle taking longer to come into oestrus than cows treated on days 6, 9, 12 or 15. The most likely reason for this was the persistence of a corpus luteum after device removal and explains why the onset of oestrus is earlier following CIDR-B treatment when prostaglandins are injected at or near the end of the treatment period (Macmillan and Peterson, 1993).

The variability in the time of ovulation which occurs after oestrus synchronisation may be reduced by exogenous oestrogen. Administration of 0.5 mg oestradiol benzoate intramuscularly 24 hours after removal of CIDR-B devices (Chapter 2) significantly increased the number of heifers which exhibited oestrus within the observation period (96.1% vs 90.5%, $p<0.01$). It also altered the onset of oestrus so that significantly more heifers were detected in oestrus (86.6% vs 72.3%, $p<0.01$) and conceived (47.1% vs 37.5%, $p<0.05$) by 48 hours after device removal. The synchronised conception rate was unaffected by treatment. Treatment with oestradiol benzoate tended to reduce the

time from device removal to LH peak in randomly cycling heifers (median time to LH peak 40.1 hr vs 63.9 hr, p=0.07), but treatment with oestradiol had no significant effect on the time to LH peak, standing oestrus or ovulation in heifers synchronised during late dioestrus (Chapter 3). The administration of oestradiol benzoate after treatment with exogenous progesterone appears to overcome the variability in timing of LH peaks typically occurring in a herd of synchronised heifers due to different stages of follicular development. The failure of this treatment regime to significantly decrease the time to standing oestrus and ovulation contrasts with the findings of Nancarrow and Radford (1975), but agrees with others (Welch *et al*, 1975). The failure of oestradiol benzoate administration to alter the time of onset of oestrus or ovulation in this study may be an artifact due to the small number of control animals which were in oestrus and ovulated. The times from LH peak to ovulation for control heifers synchronised at random stages of the oestrous cycle were unusually short and highly variable compared to other heifers in the experiment. This may reflect a lack of control over the rate of follicular development following oestrus synchronisation using progesterone-containing devices, and highlights the need for regimes which synchronise LH peaks and ovulation. Asynchrony of ovulations is attributed to differing stages of follicular development at the time of the decline of plasma progesterone concentrations (Adams, 1994; Bergfelt *et al*, 1994).

Concluding Remarks

This thesis investigated the use of exogenous oestrogen to improve the degree of oestrus synchrony by controlling follicular wave development and the timing of ovulation in heifers and cows treated with progesterone-containing intravaginal devices (CIDR-B). Results indicated that inclusion of 10 mg oestradiol benzoate intravaginally at the commencement of CIDR-B treatment had no significant effect on synchronising follicular wave emergence in different animals. Persistence of the corpus luteum after the cessation of exogenous progesterone treatment is responsible for causing a delay in the time from CIDR-B removal to oestrus in cows treated in metoestrus. The inclusion of prostaglandin treatment towards the end of a CIDR-B treatment period is therefore recommended to ensure that there is no endogenous source of progesterone remaining after device

removal. Administration of 0.5 mg oestradiol benzoate intramuscularly, 24 hours after removal of CIDR-B devices, increases the degree of oestrus synchrony. These findings indicate that a high degree of oestrus synchrony could be achieved with a 10 day CIDR-B treatment period, with prostaglandin on day 6, removal of devices on day 10, and 0.5 mg oestradiol benzoate intramuscularly on day 11. Further research is required to identify the time of ovulation after such a treatment programme, to determine whether fixed-time insemination would be successful.

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