

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**SYNCHRONIZATION OF FOLLICULAR DEVELOPMENT,
OESTRUS AND OVULATION USING OESTRADIOL
BENZOATE AND PROGESTERONE IN DAIRY CATTLE**

**A thesis presented in partial fulfillment
of the requirement for the
Degree of Master of Philosophy (Veterinary Clinical Science)
at Massey University**

Punimin Abdullah

2000

GENERAL ABSTRACT

The aim of oestrus synchronisation in cattle is to achieve a close synchrony of oestrus and ovulation with high submission rates. The status of follicular wave development at the time of treatment has been responsible for a large portion of the variability in ovarian response to treatments employed. The control of oestrus and ovulation require firstly that the life span of the corpus luteum is reduced, and secondly that follicular wave emergence is synchronized so that a healthy, oestrogen active dominant follicle is present at the end of the treatment.

A clinical trial was conducted to determine the effective dosage of oestradiol benzoate in combination with progesterone on follicular dynamics, oestrous behaviour and time of ovulation when treatment was administered intravaginally. Intravaginal treatment with 2 mg or 7 mg oestradiol benzoate and progesterone on day 3 of the oestrous cycle was effective in inducing atresia of the dominant follicle and a new cohort of follicles began to emerge, on average, 2.5 ± 0.93 days after treatment. However, the IBD Onsett12™ drug administration device failed to maintain the required progesterone output and plasma concentrations during the treatment period. This resulted in failure to synchronize oestrus and ovulation.

IBD Onsett12™, as a single application intravaginal drug delivery device for the purpose of controlling the oestrus cycle in cattle, was further evaluated in cycling and non-cycling cows and compared to the CIDR oestrus synchronization program. A total of 350 Friesian

or Friesian cross cows in five herds were involved in the trial. The retention rate for the IBD Onsett12™ was significantly lower than the CIDR (65.12% vs. 99.44%, $\chi^2 = 73.528$, $P = 0.001$), and the synchronized conception rate from the CIDR protocol was significantly higher than the IBD Onsett12™ among cycling and non-cycling cows ($\chi^2 = 15.087$, $P = 0.02$). The IBD Onsett12 oestrus synchronization program was effective in inducing fertile synchronized oestrus in some cycling and non-cycling cows, but resulted in a low synchronized conception rate.

Manipulation of follicular development and controlling the oestrous cycle length will synchronize oestrus more precisely and control the time of ovulation more exactly to allow a single fixed-time insemination. Controlling the time of new follicular wave emergence and synchronizing the follicular wave status in dairy cows at random stages of the oestrous cycle would provide a more practical and less variable method of synchronization than those of the past.

A clinical trial was conducted to control both follicular development and luteal function. Twenty randomly cycling, non-lactating dairy cows were randomly assigned to two treatments; 1) 2 mg oestradiol benzoate injected intramuscularly and 200 mg of progesterone subcutaneously, 9 days before prostaglandin (500 µg cloprostenol) and a second injection of 1 mg oestradiol benzoate 24 hours after prostaglandin treatment (ODB, $n = 10$). 2) 10 µg buserelin injected 7 days before prostaglandin (500 µg cloprostenol) and a second injection of 10 µg buserelin 48 hours after prostaglandin treatment (GnRH, $n = 10$). An acute short-acting treatment with progesterone and oestradiol benzoate or buserelin was effective in inducing atresia of the dominant follicle.

A new follicular wave emerged earlier in the GnRH treated group than in the ODB treated group (2.22 ± 0.15 vs. 3.60 ± 0.22 days, $P = 0.001$). An LH surge occurred earlier after a second buserelin treatment on day 9 than after a second oestradiol benzoate treatment on day 10 (4.0 ± 1.0 vs. 22.80 ± 1.20 hour, $P = 0.001$). The mean time of ovulation after the second oestradiol benzoate or buserelin treatment was not significantly different between the ODB and the GnRH group (1.70 ± 0.30 vs. 1.56 ± 0.18 , $P = 0.692$). The proportion of cows that were observed in oestrus was higher in the ODB group than the GnRH group (100% vs. 55.6%, $\chi^2 = 5.630$, $P = 0.018$).

In conclusion, progesterone and oestradiol treatment intravaginally or intramuscularly was effective in synchronizing follicular wave emergence. Administration of oestradiol benzoate 24 hours after prostaglandin given 9 days after an initial progesterone and oestradiol treatment produced the oestrus synchrony, induced an LH surge and provide a degree of synchrony in the time of ovulation. This program showed potential in manipulating follicular development and luteal function and has the possibility allowing fixed-time insemination. However, the efficacy of the IBD Onset12™ as a single application intravaginal drug delivery device to control the oestrous cycle or as progesterone-releasing device in cattle did not demonstrate satisfactory results when used in these trials. This might arise from the complexity of the drug delivery system. Nevertheless, the concept of delivering multiple drugs at different rates and times may have many benefits to the end user when current design and use problems are resolved.

ACKNOWLEDGEMENTS

I am very grateful to Prof. Norman B. Williamson, my main supervisor, for giving me the opportunity to complete a post-graduate study at Massey University. His guidance, assistance and supports make the learning process is more rewarding and enjoyable. Special thanks to Assoc. Prof. Tim J. Parkinson for his advice and suggestions regarding the study design and hormone analyses.

I am grateful to Nigel Perkins for the training and advice on the ultrasonography and to Sam Peterson for the encouragement and motivation. Thanks to Mahmud Fathalla and Claire Cooper for the time that they spent helping with the ultrasonography, blood collection and valuable friendship.

Special thanks to the Sabah state government for providing the scholarship and assistance and to the Department of Veterinary Services and Animal Industry, Sabah for giving me the opportunity to complete my postgraduate study.

Thanks also to Assoc. Prof. Keith Lapwood and Jane Candy for the assistance on the hormone assays, to Duncan Hedderley for the advice on the statistical analyses, and to Robin Whitson for helping in managing the cattle involved in the studies conducted at the Large Animal Teaching Unit.

The projects were partially funded by Plade Holdings Limited, Hamilton, and Institute of Veterinary, Animal and Biomedical Sciences Postgraduate Research Funds. My appreciation to Brian Cornish of Plade Holdings Limited for their generosity.

These studies would not have been possible without the help of Brigitte Slacek, Peter Jolly, Kevin Bird, Hugh Jellie and Manawatu dairy farmers and their staff who provided support. Thanks to David Glover, Tom Coppen, Bruce Martin, Rob Telfer and Tim Hopkins.

To my postgraduate colleagues Vicente, Ramon, Claudio and Alfredo; a big thank you for the rapport shown to me for the past two years.

Finally, I acknowledge the support and encouragement of my wife, Zaiton Baharun, and my three children, Khairulazahar, Suraidah and Mohd. Arief, during the two years of separation required completing the study.

TABLE OF CONTENTS

General Abstract	ii
Acknowledgements	v
Table of Contents	vii
List of Figures	viii
List of Tables	xi
List of Abbreviations	xiii
Chapter 1	Literature Review: Synchronization of follicular developments and ovulation in dairy cattle.....	1
Chapter 2	Control of oestrus and ovulation using intravaginal treatment with progesterone (IBD Onsett12™) and oestradiol benzoate in dairy cattle.....	38
Chapter 3	The efficacy of the IBD ONSETT12™ in inducing a fertile synchronized oestrus in cycling and non-cycling dairy cows.....	64
Chapter 4	Evaluation of a novel oestrus synchronization program in dairy cattle using oestradiol benzoate, progesterone and prostaglandin F _{2α}	87
General Discussion	115
References	120

LIST OF FIGURES

Figure 2.1	Diagram depicting the sequence of experimental procedures.....	44
Figure 2.2	Scatterplot of the number of cows and the amounts of progesterone dispensed from the progesterone-releasing devices (IBD Onsett12™) over the treatment periods of 12 days.....	50
Figure 2.3	The mean progesterone concentrations for the 12-day treatment periods according to the dose of progesterone dispensed by the IBD Onsett12™	51
Figure 2.4	The mean progesterone concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 gm) or low P4 (<1 gm).....	52
Figure 2.5	The mean FSH concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 gm) or low P4 (<1 gm).....	54
Figure 2.6	The mean LH concentrations after intravaginal treatment with oestradiol benzoate and progesterone (IBD Onsett12™) and according to the treatment groups.....	55
Figure 2.7	The mean LH concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 ng) or low P4 (<1 ng).....	56

Figure 2.8	Patterns of emergence and growth of individual cows' dominant follicles after intravaginal treatment with progesterone (IBD Onsett12™) and oestradiol benzoate (Groups ODB2 and ODB7) or benzyl alcohol (CON group).....	58
Figure 3.1	Diagrammatic representation of treatment protocol. The open rectangles represent either IBD ONSETT12™ or CIDR device inserted into cows from days 0 to either day 7 or 12 for CIDR or IBD ONSETT12™ respectively.	71
Figure 4.1	Diagram depicting the sequence of experimental procedures.....	94
Figure 4.2	The mean plasma FSH concentrations in cows treated with progesterone and oestradiol benzoate or buserelin treatment at day 0. Arrow indicates the time of treatment with either progesterone and oestradiol benzoate or buserelin.....	102
Figure 4.3	The mean plasma FSH concentrations in cows after treatment with prostaglandin, where the plasma FSH concentration in the ODB group was normalized to day 7 of the treatment period.....	102
Figure 4.4	Profiles of mean LH concentrations in heifers treated with acute progesterone and oestradiol benzoate or buserelin on day 0.....	103
Figure 4.5	Profiles of the mean LH concentration in cows after the prostaglandin treatment, where the mean LH concentration in the ODB group was normalized to day 7 of the treatment period.....	104

- Figure 4.6** The mean LH concentration in cows treated with 1 mg oestradiol benzoate (ODB group) one day after prostaglandin treatment and in cows treated with buserelin two days after prostaglandin treatment..... 104
- Figure 4.7** The mean plasma progesterone concentrations in the ODB and GnRH groups of cows, where prostaglandin treatment was administered on day 7 in the GnRH group and on day 9 in the ODB group..... 106

LIST OF TABLES

Table 2.1	The number of cows affected with vaginitis and its condition according to the treatment groups and the number of cows observed with nymphomania, in oestrus and not in oestrus according to the treatment groups.....	48
Table 2.2	The cows observed with nymphomania, oestrus and not in oestrus according to the second dose of intravaginal oestradiol benzoate in the ODB2 and ODB7 groups.....	49
Table 2.3	Day of emergence of the dominant follicle following treatment with intravaginal oestradiol benzoate and IBD Onsett12™ on Day 3 of the oestrous cycle.....	57
Table 3.1	The mean age, milk production and calving to treatment interval for the cows in the four treatment groups.....	75
Table 3.2	The average retention rate and synchronized conception rate among the treatment groups.....	77
Table 3.3	The reproductive performance for cycling and non-cycling cows treated with CIDR protocols.....	79
Table 4.1	The oestrus detection rate and the time of ovulation for the ODB and GnRH groups.....	98

Table 4.2	Size of the largest follicle at first treatment, PGF treatment, ovulation and the interval from progesterone and oestradiol benzoate or buserelin treatment to the emergence of the next follicular wave.....	99
------------------	---	----

LIST OF ABBREVIATIONS

CIDR	Controlled internal drug release
FSH	Follicle stimulating hormone
gm	Grams
GnRH	Gonadotrophin-releasing hormone
IBD	Intelligent breeding device
LH	Luteinizing hormone
mg	Milligrams
MHz	Megahertz
ml	Millilitres
ng	nanograms
No.	Number of
ODB	Oestradiol benzoate
P ₄	Progesterone
PGF _{2α}	Prostaglandin F _{2α}
PRID	Progesterone-releasing intravaginal device
SD	Standard deviation
SEM	Standard error of mean
μl	Microlitres

CHAPTER 1

LITERATURE REVIEW: SYNCHRONIZATION OF FOLLICULAR DEVELOPMENT AND OVULATION IN DAIRY CATTLE

INTRODUCTION

In New Zealand, most dairy herds have a single seasonally concentrated calving pattern (Macmillan et al., 1990), and 90% of the cows calve in spring (Holmes et al., 1987). Breeding and calving are restricted to a very limited period of the year in order to match cow feed requirements to the seasonal pattern of pasture growth and production. Therefore, in order to achieve this tight spring calving pattern there is an increased desire and need to utilise economically sound reproductive programs as part of dairy farm management to increase production efficiency of the herd. Reproductive performance is the most important determinant of production efficiency since it is central to dairy production systems, especially in large commercial dairy herds. In a compact breeding system, cows have to conceive by day 85 postpartum in order to maintain high reproductive performance of the herd and a calving interval of 365 days (Macmillan and Moller, 1977; Macmillan et al., 1990). In addition, reproductive performance affects the amount of milk produced per cow per day of herd life, the rates of involuntary and voluntary culling and the rate of genetic progress for traits of economic importance (Macmillan, 1979; Congleton and King, 1984). There is evidence to suggest that cow fertility in New Zealand, as measured by conception rate, has decreased significantly over the last decade (Burton et al., 1999).

The major factor limiting reproductive performance on many dairy farms is the failure to detect oestrus in a timely and accurate manner. Poor oestrous detection can limit optimal reproductive performance. Efficient and accurate oestrus detection is essential to

optimize the economic management of individual cows to yield profitable milk production. Undetected oestrus will limit the number of inseminations, resulting in increased days open, lower herd submission rates and economic loss. Cows not detected in oestrus by 60 days postpartum have a lower conception rate, longer calving intervals (Olds, 1990) and a higher risk of being removed from the herd for failing to conceive. Reimers et al. (1985) suggested that the low conception rates in dairy herds with many interoestral intervals between 10 to 15 and 30 to 35 days may be indicative of inaccurate detection of oestrus. It is generally agreed that detection efficiency is less than 50% in most dairy herds (Barr, 1975; Bosworth et al., 1972). Williamson et al. (1972) reported that only 56% of oestrus periods were detected by herdsmen and trained observers. Olds (1990) suggested that if only 50% of the oestrus are observed, then 13% of the cows will not be pregnant by 200-days postpartum. The ability of farmers to detect cows in oestrus accurately is highly variable and significantly affects conception rate (Reimers et al., 1985). The error rate in detecting oestrus varied from 0 to 60% among herds and resulted in more than 10% of the cows inseminated not being in oestrus in 30% of the herds (Heersche and Nebel, 1994).

In New Zealand, the most common practice was to use artificial breeding for five to six weeks followed by three weeks of hand-mating before allowing the bull to run freely with the herd during the breeding period (Macmillan and Moller, 1977). Nowadays, the period of hand mating is omitted. The key breeding objective is to achieve the highest pregnancy rate in the shortest period of time after the start of the breeding season in order to achieve a concentrated calving pattern during the following season (Macmillan and Watson,

1973). Fielden et al. (1973) reported that, in New Zealand, 14% of the inseminated by 4 weeks into the seasonal mating period. Anoestrus at the breeding season has been identified as the main cause of the high proportion of cows that were not bred (Xu and Burton, 1996; Rhodes et al., 1998b). Besides, between 1973 and 1996, the overall 21-day artificial breeding submission rate has decreased from 93.5% to 82.1% for Holstein-Friesian cows and from 93.7% to 88.7% for Jersey cows (Burton et al., 1999). Therefore, there is a need to increase conception rates, which usually are only about 40-60% after artificial insemination (Webb and Armstrong, 1999) in a compact breeding period. The ability to increase the submission rate in a compact breeding period will reduce the percentage of empty cows at the end of the breeding season.

Cows are inseminated at times when conception is likely to occur if oestrus detection is accurate. Insemination of cows not in oestrus results in unacceptably low fertility. Dairy cows may show a short duration of oestrus and in addition, several observations per day are required to detect a high percentage of cows that exhibit oestrus (Odde, 1990). Farmers may not have the time nor labour to implement good oestrous detection programs, especially in large herds (Larson and Ball, 1992). As herds increase in size, the problem of poor detection of oestrus will become amplified because the manpower input per cow will decrease (Senger, 1994). Undetected and falsely detected oestrus in cattle result in missed and untimely inseminations. The use of oestrus synchronization enables reproductive efficiency to be improved by improved demonstration and detection of oestrus and by giving non-oestrous but synchronously ovulating cows the opportunity of becoming pregnant.

FEATURES OF OESTRUS SYNCHRONIZATION PROGRAMS

The calving interval for optimal milk production lies between 12 and 13 months (Lucy et al., 1986). In New Zealand, to maintain the annual seasonal calving pattern, a calving interval of close to 12 months is required. To achieve shorter calving intervals and to increase reproductive efficiency, oestrous synchronization can be a useful aid to herd management, in order to optimise reproductive performance (Drew, 1978). Oestrus synchronization can increase the utilization of artificial insemination during a designated breeding period and contribute to improved reproductive efficiency (Ryan et al., 1995a). Oestrus synchronization can reduce management problems associated with daily monitoring to detect oestrus in herds of cows (Lehrer et al., 1992), especially in the presence of anoestrous cows (Odde, 1990). In addition, high-producing dairy cows may be difficult to detect in oestrus because behavioural interactions involving mounting behaviour are less obvious and intermittent (Lehrer et al., 1992).

The use of oestrus synchronization, as an aid in reproductive management, has its advantages and disadvantages. Hanlon (1995) outlined the benefits of using oestrous synchronization as follows:

1. To enable the use of artificial insemination and attain genetic progress.
2. To facilitate reproductive management in seasonally calving herds.
3. To facilitate reproductive management in non-seasonally calving herds.
4. To increase the efficiency of oestrus detection and reduce labour cost.
5. To facilitate embryo transfer programmes.

To make use of oestrous synchronization to its maximum advantage, oestrous synchronization programmes should have these features (Larson and Ball, 1992):

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

The main purpose of oestrous synchronization is to control the time of oestrus and ovulation, which then make^s it possible to increase the percentage of cows inseminated during the desired breeding period (Larson and Ball, 1992). Ovulation occurs close to the oestrous phase and in cows, the timing of ovulation after the end of the oestrus ranged from 10 to 12 hours (Gordon, 1996). Ideally, an oestrous synchronization system should elicit a fertile, tightly synchronized oestrus response in a high percentage of treated females (Odde, 1990). Breeding to synchronized oestrus can allow more control in artificial insemination programs and remove the dependence on oestrus detection before artificial insemination. If oestrus detection is not eliminated, at least the labour required for routine daily observations for oestrus could be reduced by grouping animals and concentrating observation efforts during periods of expected oestrus (Larson and Ball, 1992). Lucy et al. (1986) suggested that methods of oestrus synchronization that optimize first service conception and reduce variability in days to first service may be useful in reducing the variability and duration of calving interval for all cows in the herd.

Oestrus synchronization programs involve various protocols for administration of a single or combinations of hormones, but results have varied among programs and herds. The variation in ovulatory response to exogenous hormone treatments has been attributed to variations in follicular wave status at the time of treatment (Adams, 1998). Effective methods to synchronize oestrus should synchronize either the time of luteal regression, the end of a period of progestogen treatment, or ovarian follicular development. In addition, treatment should prevent prolonged and increased peripheral concentrations of oestradiol or an increase in the frequency of luteinizing hormone secretion and avoid prolonged periods of dominance of the ovulatory follicle (Cavalieri et al., 1998b). The duration of dominance of the ovulatory follicle was closely related to subsequent fertility. A short duration of dominance (1-4 days) results in pregnancy rates of more than 80%, and long duration of dominance (>9 days) results in abruptly decreasing pregnancy rates of less than 20% (Mihm et al., 1994).

FOLLICULAR DYNAMICS IN CATTLE

In cattle, ovarian function is regulated by the interaction between systemic and local feedback mechanisms involving gonadotrophins from the pituitary gland, steroids and proteins/peptides from the ovaries. This control system ensures that in more than 91% of females, only one follicle will ovulate per oestrous cycle (Rutledge, 1975).

Ovarian follicular development in cattle occurs in wave-like pattern and its dynamics proceeds through the integrated stages of follicular recruitment, selection and dominance

(Ireland, 1987), whereby the follicle undergoes phases of growth, maintenance and atresia. Once the growth of a follicle is initiated, it proceeds as a continuous process until the follicle either ovulates or becomes atretic (Gong and Webb, 1996). One or two anovulatory follicles develop before the ovulatory follicle (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Ginther et al., 1989b). Follicular development was greater on the corpus luteum-bearing ovary (Badinga et al., 1992). It has been estimated that in the lifetime of most mammalian species only 0.1 - 0.2 % of the primordial follicles will ever develop to the ovulatory stage (Ireland, 1987).

The dominant follicle of a wave is defined as the one that reaches the largest diameter and subordinate follicles are defined as those that appear to originate from the same follicular pool as the dominant follicle (Ginther et al., 1989b). The dominance concept was supported by the observation that the next wave of follicular growth cannot be detected until after the start of the regression of the previous dominant follicle (Webb and Armstrong, 1999). The selection of this dominant follicle is associated with a marked reduction in both the number and growth of subordinate follicles. The growth of smaller follicles occurs only when the larger follicle is either regressing or has been destroyed (Matton et al., 1981; Ko et al., 1991). This mechanism ensures that in cattle during each follicular wave, only a single follicle becomes dominant in more than 91% of occasions (Rutledge, 1975). The active life span of the dominant follicle during an oestrous cycle with two follicular waves is approximately 7 to 10 days (Lucy et al., 1992; Ginther et al., 1989a; Savio et al., 1988). However, the exact mechanisms involved are not fully understood.

The day of emergence of a follicular wave is defined as the day that the dominant follicle is retrospectively identified at a diameter of 4-5 mm (Ginther et al., 1989a) and is growing from a pool of smaller follicles. The day of cessation of growth of the dominant follicle is defined as the day when the progressive increase in diameter of the dominant follicle appears to cease (Ginther et al., 1989a). The first day on which a progressive decrease in diameter of the follicle appears to begin is defined as the day of onset of regression of the dominant follicle (Ginther et al., 1989a).

In cattle, follicular waves appear to be a constitutive characteristic since they are present prior to puberty (Adams et al., 1994), throughout most of the pregnancy period (Rajamahendran and Taylor, 1990; Savio et al., 1990), as well as during oestrous cycles (Ginther et al., 1989a). Transrectal real-time ultrasonography has confirmed that most bovine oestrous cycles have either two or three waves (Knopf et al., 1989; Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989b). However, oestrous cycles consisting of one or four waves of follicular growth have also been reported (Sirois and Fortune, 1988). The reason why some cattle have two waves of follicular development while others have three is unclear. Each wave of follicular development is characterized by simultaneous growth of between five and seven antral follicles from the growing follicle pool to >5 mm in diameter (recruitment) and then one of these follicles starts to grow rapidly (selection) while other cohort follicles regress (Gong and Webb, 1996). The onset of the first wave is detected as a group of 4 to 6 mm follicles just before the day of ovulation, the second wave emerges at about 10 days postovulation and is followed by another wave at 16 days for three wave cycles (Thatcher et al., 1996; Ginther et al.,

1996). Three-wave cycles have a longer luteal phase and a longer interovulatory interval (22.8 vs. 20.4 days) than do two-wave cycles (Ginther et al., 1989b; Taylor and Rajamahendran, 1991; Fortune, 1993). The ovulatory follicle originates from the final wave and maturation and ovulation of the dominant follicle occurs following luteal regression and a preovulatory gonadotrophin surge (Kastelic, 1994).

The future dominant follicle and largest subordinate follicle grow at a similar rate until deviation, a point in which the two follicles abruptly differ in growth rates (Ginther et al., 1997). The time from follicular emergence to deviation is about 2.5 days (Ginther et al., 1997). (Bao et al., (1997) demonstrated that the dominant follicle was selected about 36 to 48 hours following initiation of the follicular wave. On average, the future dominant follicle appears 6 and 10 hours earlier than do the largest and second largest subordinate follicles, respectively (Ginther et al., 1997). At the beginning of the deviation, the mean diameters of the dominant and largest subordinate follicle are 8.5 ± 0.2 mm and 7.2 ± 0.2 mm, respectively (Gibbons et al., 1997; Ginther et al., 1997). By day 4 to 5 (selection phase), the dominant follicle continues to grow while the subordinate follicles undergo atresia (Ginther et al., 1989a; Ginther et al., 1989b; Sirois and Fortune, 1988). It has been proposed that a dominant follicle inhibits subordinate follicles by preventing the reception of adequate gonadotrophin support (Driancourt, 1991). The selected dominant follicle continues to grow linearly for six days (growing phase) and reach maximum size of between 13 and 16 mm on day 6 to 7 of the oestrous cycle (Roche and Boland, 1991). Whereas, the size of first-wave subordinate follicle increases from day 1 to 3 and then decreases between day 6 and 11 (Ginther et al., 1989b; Badinga et al., 1992). The selected follicle exerts its dominance through inhibition of recruitment of additional

follicles for the next wave through the suppression of FSH (Adams et al., 1992a; Savio et al., 1993a). The dominant follicle remains active until approximately day 10-11 of the oestrous cycle (Ginther et al., 1989a) and then the dominance of the first-wave follicle is arrested. Thus, the recruitment phase of the second follicular wave begins.

The second dominant follicle is detected by day 10 when it subsequently ovulated (Knopf et al., 1989) or by day 12 when it was not the ovulatory follicle (Savio et al., 1988). The second dominant follicle of the oestrous cycle either ovulates after the occurrence of luteolysis or may undergo atresia and is followed by a third follicular wave that is initiated on day 16. When luteal regression occurs during the growth phase or early dominance phase, the second or third dominant follicle will continue to develop to preovulatory size (up to 20 mm in cattle) and will eventually trigger the hormonal cascade leading to ovulation (Webb and Armstrong, 1999).

The duration of dominance of the preovulatory dominant follicle affects pregnancy rate (Stock and Fortune, 1993; Mihm et al., 1994). Restricting the duration of dominance of the preovulatory follicle to 4 days resulted in a pregnancy rate of more than 70% in cyclic beef heifers (Austin et al., 1999). Mihm et al. (1994) reported a 30% decrease in pregnancy rate when the duration of dominance of the ovulatory follicle was increased from 4.1 to 8.6 days, and no pregnancies resulted when the duration of dominance was extended beyond 10 days. In addition, high pregnancy rates were reported in control beef cows and heifers and dairy heifers, following ovulation of follicles that did not persist on the ovary (Sanchez et al., 1993; Savio et al., 1993b). Austin et al. (1999) reported that the

relationship between duration of dominance of the preovulatory follicle and pregnancy rate is biphasic; the trend in pregnancy rate up to and including day 9 of dominance was negative, with a decrease in pregnancy rate of 10 to 25% during this period. After day 9 of dominance, the decline in pregnancy rate was more acute, declining by a further 35 to 75% in the next 3 days. The physiological reason for the decline in pregnancy rate as duration of dominance of the preovulatory follicle increases was unclear.

Ovarian follicles are classified according to size (Lucy et al., 1991). During the early oestrous cycle (day 1-5), the number of follicles in Class 1 (3-5 mm) decreases and then rises again by day 11 (Lucy et al., 1992; Badinga et al., 1992). The increased number of small-sized follicles detected after ovulation may have resulted from stimulatory effects of periovulatory discharges of FSH (Badinga et al., 1992). The number of follicles in Class 2 (6-9 mm) increases from day 1 to day 4 (Lucy et al., 1992) and then decreases between day 6 and 11 (Badinga et al., 1992). Average numbers of Class 3 (≥ 10 mm) follicles generally were low during the first four days of the oestrous cycle, and from day 5 to day 11, the average number of follicles in class 3 increases to one, as the selected follicle continues to increase in size (Badinga et al., 1992; Thatcher et al., 1996). The number of Class 2 follicles remains low until day 10 to 12 of the oestrous cycle as result of the inhibitory effect of the first-wave dominant follicle on recruitment of a new cohort of follicles (Thatcher et al., 1996).

ENDOCRINOLOGY OF FOLLICULAR DYNAMICS

In cows undergoing normal oestrous cycles, the presence of basal concentrations of gonadotrophins during the luteal phase results in regular waves of ovarian follicular development (Fortune, 1993). The pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), have long been recognised to provide the primary drive for ovarian follicle growth and development (Gong and Webb, 1996). FSH plays a critical role in recruiting follicles to undergo further development (Adams et al., 1992a) and LH may be involved in the selection of the dominant follicle (McNeilly et al., 1992; Campbell et al., 1995).

There is an association between the FSH surge and emergence of a follicular wave (Bodensteiner et al., 1996; Sunderland et al., 1994). A periodic surge in circulating concentrations of FSH was responsible for eliciting the emergence of a follicular wave (Adams et al., 1992a; Badinga et al., 1992; Bergfelt et al., 1997). The peak of the FSH surge is reached when the largest follicles of the wave emerge at about 4 mm in diameter (Ginther et al., 1996; Ginther et al., 1997). In cattle, antral follicles up to 4 mm appear to be FSH-responsive, but gonadotrophin-independent (Gong and Webb, 1996). A surge in plasma FSH was detected one to two days before the emergence of the follicular wave. Two FSH surges were detected in two waves cycles and three surges in three wave cycles (Adams, 1994). On day 1 of the oestrous cycle, an increase in FSH secretion stimulates the emergence of the first wave of follicular development in association with the low circulating concentrations of oestradiol and progesterone (Adams et al., 1992a; Turzillo

and Fortune, 1990; Evans et al., 1994). There is an inverse relationship between concentrations of FSH and oestradiol in the plasma during follicular development at the early luteal phase (Kaneko et al., 1991). Oestradiol production by the dominant follicle increases during the growth of the follicle and decreases shortly after a plateau is reached (Xu et al., 1995a). As the follicle grows, there is a decreasing FSH secretion as result of negative feedback from increasing peripheral oestrogen and androgen concentrations (Kaneko et al., 1991). FSH declines rapidly about 2 days after wave emergence (Adams et al., 1993) and the subsequent decreases in FSH coincide with the time of divergence in rate of growth of the dominant and subordinate follicles. The low concentrations of circulating FSH prevent new wave emergence, but allow the dominant follicle to continue to grow (Adams, 1998). Factors controlling increasing and decreasing concentrations of circulating FSH during the FSH surge have not been defined (Ginther et al., 1996). About day 4 of the oestrous cycle, oestradiol secretion reaches a peak and the dominant follicles continue to grow in an environment of basal FSH (Adams et al., 1992a; Badinga et al., 1992; Sunderland et al., 1994; Rhodes et al., 1995) and decreasing LH concentrations and LH pulse frequency but increasing LH pulse amplitude (Evans et al., 1997). In response to the higher LH pulse amplitude, the dominant follicle continues to grow and survive without FSH (Ginther et al., 1996) because of a shift in gonadotrophin responsiveness from FSH to LH (Campbell et al., 1995; Ginther et al., 1998). Final growth and maturation of the follicle probably depends on increased pulse frequency of LH to act upon 1) thecal cells to provide androgen substrate for aromatization to oestradiol-17 β by granulosa cells and 2) granulosa cells to provide additional stimulus with FSH for increased aromatase activity when FSH concentrations


are declining with increasing size of the dominant follicle (Richards et al., 1987). Around day 8 of the oestrous cycle, the dominant follicle enters a static phase of growth under the influence of high androgen production (Evans et al., 1997). At the static phase of growth, androgen secretion decreases, allowing FSH secretion to increase which stimulates the emergence of the second wave of follicular development around day 11 (Adams et al., 1992a; Sunderland et al., 1994). LH pulse amplitude is reduced as oestradiol increases and increasing secretion of oestradiol as well as androgen secretion, again depress circulating FSH concentrations (Evans et al., 1997). A reduction in LH pulse frequency was associated with the atresia of dominant follicles (Savio et al., 1993a). Steroid and gonadotrophin concentration changes continue to maintain the pattern of second or third follicular dynamics until luteolysis. Upon luteolysis, the concentration of progesterone decreases and the large follicle produces oestradiol which triggers oestrus (Ireland, 1987; Stock and Fortune, 1988). This stimulates the release of GnRH-induced LH pulses and as the frequency of LH pulses increase, further growth of the dominant follicle is supported, resulting in higher circulating concentrations of oestradiol, which results in a surge of LH followed by ovulation (Adams, 1998). The primary effect of oestradiol on luteolysis is thought to be associated with the release of $\text{PGF}_{2\alpha}$ and oxytocin from the uterus and corpus luteum, respectively (McCracken et al., 1984). The time from the LH surge to ovulation ranged from 24 to 40 hours (Mann et al., 1999).

FACTORS AFFECTING FOLLICULAR DYNAMICS

The response of cattle to their environment is an important determinant of reproductive efficiency. The numbers of waves per oestrous cycle are affected by dietary intake (Murphy et al., 1991), heat stress (Wolfenson et al., 1995), parity and lactational status (Lucy et al., 1992). Such influences impinge on all facets of reproductive function (Lindsay, 1996).

Dietary Intake

The pattern of follicular development is affected by the status of energy balance in lactating cows (Lucy et al., 1992). Maurasse et al. (1985) reported that altering the plane of nutrition induced changes in ovarian follicle size distribution and increased follicle number in cattle. There is an increase in small follicle recruitment during the first follicular wave of the oestrous cycle with an increase in dietary intake (Gutierrez et al., 1997). The number of follicles within different size classes was altered and the diameter of large follicles was enhanced with improved energy balance during the postpartum period (Lucy et al., 1991). Undernourishment is also known to affect the growth of the dominant follicle in cattle (Murphy et al., 1991). Long-term periods of undernutrition induce anoestrus and decrease the number of small (≤ 6 mm) follicles recruited (Gutierrez et al., 1997). In New Zealand, the major form of infertility in dairy herds is postpartum anovulatory anoestrus through feeding fresh pasture not supplemented with grain (McDougall et al., 1995). Anoestrus was associated with reduced LH pulse



frequency in cattle on a restricted diet and nutritionally-induced anoestrus was associated with insufficient circulating LH to stimulate maturation of the ovulatory follicle (Rhodes et al., 1996).

The reproductive performance of postpartum cows is often limited by the intake of dietary energy (Butler and Smith, 1989). When cows are under the influence of negative energy balances, the ovarian follicles fail to reach mature size and ovulate (anoestrus) or develop cystic follicles (Bauman and Currie, 1980). Ovarian inactivity may result from insufficient LH secretion associated with inadequate energy intake (Lucy et al., 1991). To ensure follicular growth, recruitment and maturation, gonadotrophin secretion has to be adequate. Negative energy balances decrease LH secretion and delay return to oestrus in lactating, postpartum beef and dairy cows (Lucy et al., 1992). The amplitude of LH pulses as well as the diameter of the dominant follicle increased with positive energy balance (Roche and Boland, 1991). Lactating cows placed in negative energy balance before ovulation had preovulatory follicles that grew more slowly than follicles in cows that were in positive energy balance, and feeding of additional dietary energy stimulated the development of follicles (Lucy et al., 1990). This is associated with the movement of follicles into larger size classes (Lucy et al., 1991).

Heat Stress

Low fertility in cattle induced by heat stress is a multifactorial problem, because hyperthermia of various organs and tissues results in diverse functional alterations and

✓
 impairments (Wolfenson et al., 1988). Recent studies have indicated that ovarian follicles are susceptible to thermal stress (Badinga et al., 1993). The first-wave dominant follicle in heat-stressed lactating cows is found to be smaller in diameter and to contain less fluid on day 8 of the cycle (Badinga et al., 1993; Wilson, et al., 1998a). Heat-stressed cows have lower oestradiol when the first wave dominant follicle is establishing dominance (Wolfenson et al., 1995). The decrease in oestradiol may be related to a reduction in follicular size (Wilson et al., 1998a). Wilson et al. (1998a) postulated that low concentrations of oestradiol in heat-stressed cows caused a delay in luteolysis as result of follicular oestradiol failure to initiate the series of endocrine events leading to luteolysis. As result of failure in luteolysis, the luteal phase was extended, with multiple follicular waves (≥ 3) within the oestrous cycle (Wilson et al., 1998a). The mechanisms that lead to decreased oestradiol during heat stress are unknown.

Dominance of the first-wave follicle is altered by heat stress and the resulting changes included an enhancement in number of large follicles and earlier emergence of the second wave dominant follicle (Wolfenson et al., 1995). In heat-stressed cows, the number of class 3 follicles increased earlier, peaked on day 16, and failed to decrease subsequently (Wilson et al., 1998a). The number of class 3 follicles also failed to decrease late in the oestrous cycle of heat-stressed heifers (Wilson et al., 1998b).

Decreased follicular size (Badinga et al., 1993) or decreased dominant function (Wolfenson et al., 1995) occurred in lactating cows that were exposed to heat stress.

Developing small follicles that are damaged by heat stress may ovulate infertile oocytes or develop subfunctional corpora lutea (Howell et al., 1994).

The Post Partum Period

High milk production causes a decrease in energy balance, as cows require several weeks for energy intake to increase sufficiently to compensate for additional milk energy output due to the high levels of production. The development of preovulatory-sized dominant follicles is not a limiting factor in reproductive recrudescence in lactating dairy cows and the initiation of a follicular wave, including recruitment and dominant follicle selection, occurs regardless of typical early postpartum negative energy balance (Beam and Butler, 1997).

The initiation of follicular growth in the early postpartum period is not well defined (Roche and Boland, 1991). Dufour and Roy (1985) reported growth of antral follicles between day 15 and 35, while Spicer et al. (1986) reported the presence of 8 mm follicles by day 7 postpartum with an increase in their number between day 7 and 42 in beef suckler cows.

Follicular development in the early postpartum periods of dairy cows is characterized by the development of follicles <8 mm in diameter, and one of these follicles continues development and becomes the dominant follicle, on average on day 11 (range 5 - 39 days) postpartum (Savio et al., 1990). The first dominant follicle after parturition either

ovulates, or develops into a cyst (Savio et al., 1990). In addition to developing an ovulatory or cystic follicle, 40% of cows may experience multiple waves of nonovulatory dominant follicle growth prior to first ovulation (Beam and Butler, 1997). The first wave of dominant follicles in ovulating cows has a greater steroidogenic output than dominant follicles of nonovulating cows both during early follicle growth (day 8 to 14) and at the peak of levels reached prior to ovulation or atresia (Beam and Butler, 1997). The first dominant follicle ovulated in 70-80% (Roche and Mihm, 1996) of dairy cows on day 27 (range 10-55 days; Savio et al., 1990) and first oestrus occurred on day 59 (range 17-139 days; Adams, 1998) in cows with reasonable body condition. In New Zealand, first ovulation was much earlier in cows >4 years old than in 2 or 3 years old cows (day 35 vs. day >50, Moller, 1970). First ovulation is not accompanied by oestrous behaviour in 94% of postpartum cows (Savio et al., 1990).

The duration of the first ovarian cycle was affected by postpartum interval (Savio et al., 1990). Cows that ovulated within 9 days of calving had either normal or long first cycles (>25 days) and cows first ovulating after day 20 had short (9 - 13 days) first cycles (Roche and Boland, 1991). Cows with short cycles developed only one dominant follicle, which became the ovulatory follicle, whereas cows with normal cycles had mainly 2 dominant follicles while those with long cycles had either 3, 4 or more dominant follicles (Savio et al., 1990). The association between short cycles and a longer postpartum anovulatory period may be attributable to a long period of low progesterone compared to cows with normal-length cycles in which the anovulatory period is short (Adams, 1998).

METHODS OF CONTROLLING FOLLICULAR WAVE DEVELOPMENT AND OVULATION

Ablation of ovarian follicles ≥ 5 mm, altering the endogenous release of LH and FSH, or administration of exogenous steroids or gonadotrophins can cause regression of a dominant follicle and be followed with the emergence of a new follicular wave (Roche et al., 1998). Controlling the time of emergence of a new follicular wave and synchronizing the follicular wave status of animals within a group to be synchronized could improve the synchrony of oestrus and ovulation and ensure that the ovulatory follicle has the optimum potential for fertilization and embryo development (Beal, 1998).

The primary consideration in synchrony of ovulation should be a uniform recruitment and development of ovulatory follicles among cows. Control of follicular wave development and ovulation can be accomplished by the exogenous administration of various hormones either given alone or in combination. Destruction of the dominant follicle on day 3 after ovulation delayed the regression of the largest subordinate follicles of the first wave (Ko et al., 1991), increased plasma FSH concentrations immediately and hastened the emergence of the second wave (Adams et al., 1992a). Atresia of dominant follicles has been induced by administration of exogenous oestrogen (Rajamahendran and Manikkam, 1994; Bo et al., 1993; Bo et al., 1994; Bo et al., 1995; Cavalieri et al., 1997), progesterone (Anderson and Day, 1994; Rajamahendran and Manikkam, 1994), or gonadotrophin-releasing hormone antagonist (Manikkam et al., 1995). In addition, luteinization or ovulation of dominant follicles has been achieved by administering a

gonadotrophin-releasing hormone agonist (Macmillan and Thatcher, 1991; Wolfenson et al., 1994; Schmitt et al., 1996).

Gonadotrophin-releasing hormone and prostaglandin

Prostaglandin $F_{2\alpha}$, a natural luteolysin (Manns and Hafs, 1976; McCracken, 1972), does not have a direct influence on the dominant follicle. The luteolytic response was influenced by the maturity of the corpora lutea at the time of the prostaglandin treatment (Wiltbank et al., 1995). Exogenous prostaglandin is only effective in inducing luteolysis between day 5 and 16 of the oestrous cycle (Rowson et al., 1972) and more consistent in inducing luteolysis in heifers compared to lactating cows (Macmillan et al., 1977; Macmillan et al., 1978). Synchronized oestrus response was lowest (67%) following treatment on day 5 through 9, moderate (77%) when treated on day 9 through 12 and highest (>91%) when treated after day 12 of the cycle (Beal, 1996). Effective prostaglandin-induced luteolysis will ensure oestrus in 90% to 95% of cows, occurring over a three- to seven-day period (Rosenberg et al., 1990; Folman et al., 1990). The dominant follicle present at the onset of prostaglandin-induced luteolysis will become the ovulatory follicle and the time of ovulation will depend on the follicular status at the time of prostaglandin treatment (Ireland and Roche, 1982; Macmillan, 1983). Prostaglandin treatment at the late growing or early static phase of the dominant follicle will result in ovulation of that follicle within 2 to 3 days, whereas treatment after the mid- to late-static phase will result in ovulation of the dominant follicle of the next wave 4 to 5 days after treatment (Kastelic and Ginther, 1991; Kastelic et al., 1990; Savio et al., 1990). Recent

studies demonstrated that the timing of ovulation after the onset of oestrus following prostaglandin treatment ranged from 20 to 48 hours (Walker et al., 1996; Mann et al., 1999).

Gonadotrophin-releasing hormone (GnRH) is a decapeptide believed to stimulate or cause the release of LH and FSH from the anterior pituitary (Chenault et al., 1990) into the circulation within two to four hours (Stevenson et al., 1993; Thatcher et al., 1993). The treatment effects of GnRH on follicular status lasted for 4-6 days (Thatcher et al., 1993). GnRH was found to induce ovulation or luteinization of the largest follicle present at the time of treatment in lactating cattle (Macmillan and Thatcher, 1991; Pursley et al., 1995), and a new follicular wave was recruited approximately two days later (Thatcher et al., 1993; Twagiramungu et al., 1995; Pursley et al., 1995). However, in contrast, GnRH (Follitropin) treatment on day 5 of the oestrous cycle elicited the emergence of new follicular wave within a mean of 26 to 32 hours after treatment (Bodensteiner et al., 1996). Following GnRH treatment, ovulation occurred in 63% to 80% of cycling cows (Vasconcelos et al., 1997; Pursley et al., 1995), most consistently when a dominant follicle was in its growing phase (Prescott et al., 1992; Silcox et al., 1993). Nevertheless, ovulation did not occur when the dominant follicle was in its regressing phase at the time of treatment (Kastelic et al., 1998). The GnRH-induced ovulation of the large follicle is associated with a decrease in oestradiol concentration in the peripheral circulation (Twagiramungu et al., 1994) and thus occurrence of spontaneous oestrus is inhibited (Thatcher et al., 1989; Twagiramungu et al., 1992). However, GnRH does not always result in ovulation or luteinization of the dominant follicle in heifers and hence, it does

not consistently induce the emergence of a new follicular wave (Martinez et al., 1997). Heifers did not respond to synchronization of luteal function by exogenous GnRH (Pursley et al., 1997), possibly because of inconsistent follicular wave patterns (Nebel and Jobst, 1998).

A new oestrus synchronization protocol has been described in recent studies. This program involves synchronization of ovulation and has been called Ovsynch (Wiltbank, 1998). This incorporates GnRH into the oestrus synchronization protocol (Pursley et al., 1995; Stevenson et al., 1996; Twagiramungu et al., 1992). GnRH is administered 6 to 7 days prior to prostaglandin treatment. The first administration of GnRH is given at a random stage of the oestrous cycle, causing either ovulation or luteinization of the largest follicle. A new follicular wave is recruited approximately two days later (Thatcher et al., 1993; Twagiramungu et al., 1995; Pursley et al., 1995). Administration of prostaglandin regresses any corpus luteum that is present and the new dominant follicle formed is available for ovulation. Ovulation is synchronized by a second injection of GnRH given two days after prostaglandin and ovulation occurs within an eight hour period from 24 to 32 hour after the second GnRH treatment (Pursley et al., 1995; Taponen et al., 1999). After the second injection of GnRH the cows are bred without regard to behavioural oestrus and the optimum time for insemination is found to be at 16 hours after GnRH (Pursley et al., 1998). This synchronized ovulation is possible because preovulatory follicles are at a similar stage of growth and are responsive to GnRH-induced LH released at the time of the second GnRH injection (Wolfenson et al., 1994). However, this program only synchronizes ovulation in about 60-70% of heifers as compared to

about 90% of lactating dairy cows (Wiltbank, 1998). Therefore, this program was not designed for heifers as it does not effectively synchronize ovulation in heifers (Wiltbank, 1998).

Progesterone and Oestradiol

Progesterone and progestogens, with or without a luteolytic treatments have been effectively used to synchronize oestrus in cattle. The mode of delivery of progesterone or progestogens has been via daily injections, oral feeding of progestogens, subcutaneous implants and intravaginal hormone releasing devices (such as the PRID™ and CIDR™). Progesterone treatment alters ovarian function in cattle and, if given for long enough (>14 days) to allow normal regression of the corpus luteum, induces a synchronous oestrus which is associated with lowered fertility (Odde, 1990; Peters, 1986). The lowered fertility has been attributed to ageing of the oocyte within the longlived oversized follicles (Custer et al., 1994; Mihm et al., 1994). Shorter (<14 days) periods of treatment in combination with a luteolytic treatment have resulted in greater fertility (Wiltbank et al., 1961; Roche, 1974). Acute treatment with progesterone has been used to induce atresia of persistent dominant follicles, in order to induce a new wave of follicular development and to improve fertility at a synchronized oestrus (Anderson and Day, 1994; Cavalieri et al., 1997; Cavalieri et al., 1998a; Murray et al., 1998). Anderson and Day (1994) showed that a single injection of progesterone caused a new follicular wave to emerge within 4 days. Exogenous progesterone suppressed the dominant follicle in a dose-dependent manner when given during the growing phase, but had no effect on

static- or regressing-phase follicles (Adams et al., 1992b; Sawyer et al., 1995). The suppressive effects of progesterone on the growth of the dominant follicle were mediated by suppression of LH (Savio et al., 1993b; Stock and Fortune, 1993).

Oestrogen treatment disrupts the normal pattern of follicular growth in cycling cows (Engelhart et al., 1989; Nadaraja and Hansel, 1976; Rajamahendran and Walton, 1990), and has been recognised for its capacity to induce atresia of ovarian follicles (Rajamahendran and Manikkam, 1994; Bo et al., 1995; Cavalieri et al., 1997). Treatment with oestradiol alone suppresses the growth of the dominant follicle (Bo et al., 1993; Bo et al., 1994) and results in early emergence of the next follicular wave when the treatment is given early in the growing phase but not at the middle or end of the growing phase of follicular development (Bo et al., 1993; McDougall, 1994). However, treatment with short-acting oestradiol during the mid-growing phase did not delay the emergence of the next wave (Bo et al., 1994). Whereas, oestrogen treatment at the static-growing phase (day 6 of the oestrous cycle) did not alter follicle dominance and the emergence of the next wave was delayed (Bo et al., 1993). In heifers implanted with norgestomet for 17 days, treatment with oestradiol alone on day 10 also resulted in the consistent emergence of a new cohort of follicles approximately 4.5 days later, but induced an LH surge and ovulation before the removal of the norgestomet implant in 30% of the heifers (Murray et al., 1998). In addition, Burke et al. (1999) reported that intravaginal treatment with 10 mg oestradiol benzoate during the mid-luteal phase (day 13 of the oestrous cycle) was effective in inducing atresia of the dominant follicle and a new follicular wave emerged 4 days after treatment.

Treatment with oestradiol will cause luteal regression (Wiltbank et al., 1961) and induce atresia of ovarian follicles (Rajamahendran and Manikkam, 1994; Bo et al., 1995). It is for these reasons that oestradiol was incorporated in progesterone-based oestrus synchronization programs. Progesterone treatment over a short period (9-10 days) when combined with oestrogen at the beginning of treatment to induce luteolysis, was an effective method of oestrous cycle control (Wiltbank and Kasson, 1968) by promoting follicle wave turnover and preventing the development of persistent follicles (Burke et al., 1997). Oestrogen treatment at various stages of follicular development (growing, early static, late static or early regressing phase) in progestogen-implanted cows was followed consistently by the emergence of a new follicular wave, on average, 4.3 ± 0.2 days later (Adams, 1998; Bo et al., 1995; Cavalieri et al., 1997). In anoestrous beef cows, follicular wave emergence occurred on average 3.1 days after progesterone and oestradiol treatment (Rivera et al., 1998). In heifers implanted with norgestomet for 17 days, treatment with a combination of oestradiol and progesterone on day 10, resulted in the emergence of a new follicular wave approximately 4.5 days later which followed the atresia of large follicles in a variety of stages of follicular growth and dominance (Murray et al., 1998). But by contrast, in other studies (Sawyer et al., 1995; Yaakub et al., 1998), there was a lack of synchronization of follicular wave emergence when PRIDs or CIDRs were used in conjunction with oestradiol benzoate. The interval from oestradiol treatment to follicular wave emergence in these studies varied from 1 to 7 days.

Following the synchronization of follicular wave emergence using a combination of oestrogen and prolonged progesterone treatment, a new dominant follicle will develop

during the period of progesterone treatment (Burke et al., 1997). Prostaglandin treatment at or before termination of progesterone treatment increased the oestrus synchrony and fertility was normal (Roche, 1976b). In addition, oestradiol benzoate, administered 24 hour after prostaglandin treatment, reduced the interval to oestrus and ovulation (Lammoglia et al., 1998; Ryan et al., 1995b). Treatment with oestradiol benzoate alone 24 hour after removal of the progesterone caused 98 to 100% of the cows to show oestrus in a 48-hour period and 100% to ovulate within a 36-hour period (Fike et al., 1997; Johnson et al., 1997; Lane et al., 1999; McDougall et al., 1992; Wiltbank et al., 1971).

Progestogen and progesterone

Treatment with progestogens suppress oestrus behaviour and prevent the pre-ovulatory LH surge and ovulation (Roche et al., 1981). The progestin-based systems of oestrus synchronization provide good control of the oestrous cycles in cattle, however the fertility at the synchronized oestrus has been variable (Spitzer et al., 1978b; Brown et al., 1988; Odde, 1990). The reduction in fertility associated with the use of progestogens, has been attributed to abnormal oocyte development (Kinder et al., 1996). Follicular waves were interrupted and a large, dominant follicle developed and persisted on the ovary throughout the treatment period in 80% of the progestin-treated cows (Beal et al., 1990; Custer et al., 1994). When ovulation of persistent dominant follicles is prevented and a new dominant ovarian follicle is recruited and ovulates, fertility is improved (Cavalieri et al., 1998b; Wehrman et al., 1993). Acute progesterone treatment either by an intravaginal controlled drug release (CIDR, Cavalieri et al., 1998a) or by intramuscular administration

(Anderson and Day, 1994; Rajamahendran and Manikkam, 1994; Cavalieri et al., 1998c) has been used to induce atresia of persistent dominant ovarian follicles, to induce a new wave of ovarian follicular development and to improve fertility at a synchronized oestrus (Anderson and Day, 1994; Cavalieri et al., 1998b). Acute treatment with progesterone was more effective in follicles that had reached the plateau phase in growth, while the development and maintenance of dominant follicles was less likely to be arrested by exogenous treatment with progesterone during the growing stages of ovarian follicular growth (Cavalieri et al., 1998b). Dominant follicles were critically dependent on LH for continued survival (Campbell et al., 1995). Acute treatments with progesterone reduced the frequency of pulsatile LH secretion (Rajamahendran and Manikkam, 1994; McDowell et al., 1998), and a reduction in LH pulse frequency is associated with the atresia of dominant follicles (Savio et al., 1993b). Therefore, acute treatments of progesterone rendered anovulatory those follicles that had achieved a functional state of dominance at the time of treatment, as these follicles are more susceptible to a reduction in LH secretion (Anderson and Day, 1994). A single 100 mg dose of progesterone is sufficient to cause atresia of dominant follicles and synchronize the emergence of a new dominant follicle in heifers implanted with progestogens for 17 days (Cavalieri et al., 1998c).

In heifers implanted with norgestomet for 17 days, acute progesterone treatment on day 10 of the implantation period caused atresia of dominant follicles (Cavalieri et al., 1998b, 1998c) and synchronized the emergence of the ovulatory follicle about 2.6 days later (Cavalieri et al., 1998c). However, the synchrony of ovulation is affected by the method

of progesterone administration. Administration of progesterone in a saline/alcohol vehicle significantly delayed the timing of ovulation and reduced the synchrony of ovulation compared with heifers treated with progesterone in a CIDR or in an oil base (Cavalieri et al., 1998c) in heifers implanted with norgestomet for 17 days. The delay in the timing of ovulation following treatment with progesterone in a saline/alcohol vehicle may be due to the less frequent LH release and thus, delayed maturation of the preovulatory ovarian follicle (Cavalieri et al., 1998c). The time interval from implant removal to ovulation was 60, 75.2 and 97.6 hours in heifers treated with progesterone using a CIDR, an oil base and a saline/alcohol vehicle respectively (Cavalieri et al., 1998c).

The timing and synchrony of ovulation is affected by the time when progesterone treatment is administered, relative to the end of a period of norgestomet treatment (Cavalieri et al., 1998b). Progesterone treatment 7 days before the removal of norgestomet implants, provided the best balance between achieving a precise time of ovulation and avoiding the ovulation of the aged oocyte (Cavalieri et al., 1998b). The time interval from the end of the norgestomet treatment to ovulation occurred between 60 to 80 hours (Cavalieri et al., 1997; Cavalieri et al., 1998a; Murray et al., 1998).

Progesterone and gonadotrophin-releasing hormone

The occurrence of two or three wave cycles has been linked to differences in fertility, especially during the 21 days following insemination when cows with three waves of follicles in this period had higher conception rates (Ahmed et al., 1997). Progesterone

treatment alone starting at day 2 of the oestrous cycle for 10 days resulted in shorter oestrus cycles (16 days), premature luteolysis and ovulation of the second dominant follicle (Clark et al., 1998; Lynch et al., 1999). However, progesterone treatment at metoestrus followed by gonadotrophin given at dioestrus extended the cycle length to 20 days (Lynch and Macmillan, 1996; Clark et al., 1998). An additional treatment with GnRH resulted in the turnover of the second wave dominant follicle and initiation of a third follicle that emerged about two days following the second gonadotrophin treatment. This extended cycle length was due to the conversion of two wave cycles to three wave cycles (Lynch and Macmillan, 1996). The conception rates following this oestrus synchronization program was high (68.3%) after the first insemination when compared to controls (56.1%) (Lynch et al., 1999).

In another study, gonadotrophin was given at the time of starting a 10 day CIDR treatment and prostaglandin was administered 24 hours prior to withdrawal of the CIDR device (Ryan, 1994). Buserelin treatment at the onset of progesterone treatment increased follicle turnover with recruitment of a new dominant follicle (Thatcher et al., 1989), which grew to the preovulatory stage after the injection of the prostaglandin. The pregnancy rate to first service was reduced by 11% with the omission of the gonadotrophin treatment. In further work, an additional treatment with 1 mg oestradiol benzoate 10 hour post-CIDR withdrawal reduced the interval to oestrus and increased the oestrus detection rate to 95% in the 48 hours after CIDR withdrawal (Ryan et al., 1999).

METHODS OF PROGESTERONE OR PROGESTOGEN DELIVERY

Progesterone has a short biological half-life, which makes repeated or continuous administration necessary to achieve an effective physiological action (Gordon, 1996). However, the development of long-term progesterone or progestogen delivery devices has facilitated the use of progesterone or progestogen for oestrus synchronization in cattle. The development of these devices has eliminated the management problems associated with daily injection and oral applications of progesterone.

Progesterone-releasing intravaginal device (PRID™)

PRID™ (Abbott Laboratories) is a stainless-steel spiral coated with progesterone-impregnated silicone elastomer usually containing 1.55 g or 2.25 g progesterone. The plasma progesterone concentrations rise sharply following insertion to the vagina and then decline gradually over the next 14 days (Munro, 1989). The PRID is a satisfactory means of controlling oestrus and ovulation in cattle (Roche, 1976c; Munro and Moore, 1985). Following removal of the PRID device, plasma progesterone concentrations fall within hours to basal concentrations (Rathbone et al., 1998). This device is used on its own, or in combination with oestradiol benzoate or prostaglandin (Roche, 1974; Roche, 1978). The retention rate is greater than 95% (Rathbone et al., 1998). PRID is not recommended for use in maiden heifers due to the size of the implanting device (Penny, 1998)

Controlled internal drug release (CIDR-B™)

The CIDR-B (InterAg, Hamilton, New Zealand) was designed and developed in New Zealand for the controlled administration of progesterone (Macmillan and Peterson, 1993). This T-shaped nylon device is covered with a 1.9 g progesterone-containing silicone-base elastomer. The amount of hormone released during a 15-day treatment period was highly repeatable and adequately maintained during treatment to suppress oestrus (Macmillan et al., 1991). Plasma progesterone concentrations are rapidly elevated within 6 hours of insertion (Macmillan and Petterson, 1993). The average plasma progesterone concentration for a 12-day treatment in ovariectomized heifers was 5.6 ± 0.1 ng/ml and ranged from 8.7 ± 0.3 ng/ml 6 hours after insertion to 2.5 ± 0.2 ng/ml at device removal (Macmillan et al., 1991). In ovariectomized heifers, post-treatment concentrations 6 hours and 24 hours after device removal were 0.12 ng/ml and 0.10 ng/ml respectively. The changes in the average plasma progesterone concentrations following insertion or removal of CIDR-B were influenced by the duration of treatment, the stage of oestrous cycle when treatment was initiated or terminated and concurrent treatment with oestrogen or prostaglandin (Macmillan et al., 1991, Macmillan and Peterson, 1993). The retention rate of the device was 99% in heifers with treatment periods of 4 to 15 days, and 98% in dairy cows treated for periods of 4 to 7 days (Macmillan et al., 1988; Macmillan et al., 1991).

Silicone Ear Implant (Crestar™)

Crestar (Intervet) is a silicone implant containing 3 mg norgestomet. The implant is placed subcutaneously in an ear and an intramuscular injection of 3 mg norgestomet and 5 mg oestradiol valerate is given at implant insertion. Norgestomet release from the implant is consistent and linear over a 9-day period (Kesler et al., 1995). The rate of loss of the ear implant is less than 1% (Drew et al., 1979) but it was reported that high proportion of heifers (18%) treated with implants were infected at the site of implantation (Tregaskes et al., 1994). In a 9-day treatment regimen, Crestar effectively synchronizes oestrus. The mean interval from the removal of the device to the onset of oestrus was 45 hours (Tregaskes et al., 1994).

Hydron Ear Implant (Synchro-Mate-B™)

Synchro-Mate-B is a hydron ear implant with 6 mg norgestomet. As with Crestar, the treatment regimen of Synchro-Mate-B includes an intramuscular injection of 5 mg oestradiol valerate and 3 mg of norgestomet. The release of norgestomet is very rapid over the first 2 days and then is substantially slower over the next 7 days (Kesler et al., 1995). The retention rate over a 9-day insertion period is high, and been reported to exceed 99% (Spitzer et al., 1978a; Drew et al., 1979; Wishart, 1977b).

Intravaginal Progesterone Device (Cue-Mate™)

Cue-Mate™ (Dairs-PfarmAg Ltd., Hamilton New Zealand) is a new intravaginal progesterone-releasing device developed in New Zealand and only recently licensed in

New Zealand for lactating cows but not heifers. The device consists of two main components, the carrier body and two treatment pods. The carrier body is used to hold the treatment pods in place inside the vaginal cavity. The two treatment pods are made from silicone and the two pods contain a total of 1.4 g progesterone. It is claimed by the manufacturer that the retention rate of the device was 99% and it induced oestrus in 95% of treated cows between day 2 and 4 after device removal.

Intelligent Breeding Device (IBD Onsett12™)

IBD Onsett12™ (Plade Holding Ltd., Hamilton, New Zealand) is a single application intravaginal drug delivery device, developed in New Zealand and used to stimulate oestrus in anoestrous or synchronize oestrus in cycling cows. It is claimed that the device is able to provide a complex hormonal treatment regime in a single application. The device consists of multiple body parts moulded from specialised plastics. Contained within the body are four individual drug reservoirs, a microprocessor, printed circuit board, power source and pump mechanism. Hormone release is achieved through the electronic computer control of a unique pumping system. The hormones are dissolved in alcoholic solution and the hormone delivery sequence is turned on at device insertion. At insertion, 7 mg oestradiol benzoate is delivered as spike release, and a total of 2 g of progesterone is released at a constant rate from device insertion for 10 days. On day 6 of the insertion period, 250 µg of sodium cloprostenol is released and 1 mg of oestradiol benzoate is delivered on day 11 and the device is removed on day 12. The device is recommended as a controlled breeding device for single fixed-time insemination in

cycling and non-cycling cows. The product was introduced to the New Zealand market after rapid development in the 1996 season, but resulted in product failure due to unforeseen delivery problems arising from the complexity of the device (Rathbone et al., 1998). Subsequent prototypes of the device have been developed and field tested including the one further investigated in this thesis.

CONCLUSION

The aim of oestrus synchronization is to control the timing of oestrus by controlling the length of the oestrous cycle. The methods used to control oestrous cycle length are: 1) to regress the corpus luteum before the time of natural luteolysis and thereby shorten the cycle, or 2) to administer exogenous progestins to delay the time of oestrus following natural or induced luteolysis that may extend the length of the oestrous cycle (Beal, 1998). The development of a better understanding of follicular development including methods to interrupt or manipulate the wave-like pattern of follicular growth and control ovulation have recently been developed with the aid of trans-rectal ultrasound examination of the ovaries. The objective of synchronization of follicular wave emergence is to synchronize oestrus and ovulation, thus allowing for the effective use of a fixed-time artificial insemination associated with a high pregnancy rate.

The objectives of this thesis were to investigate:

1. The effective intravaginal dose of oestradiol benzoate to induce suppression or atresia of the dominant follicle and to evaluate the efficiency of IBD Onsett12™ as an intravaginal progesterone delivery device.
2. The efficacy of the IBD Onsett12™, as a single application drug delivery device, to induce a fertile and synchronous oestrus in cycling and non-cycling dairy cows.
3. The evaluation of a novel oestrus synchronization program using an acute treatment with progesterone and oestradiol benzoate followed by prostaglandin treatment 9 days later and 1 mg of oestradiol benzoate 24 hours after the prostaglandin treatment.

CHAPTER 2

CONTROL OF OESTRUS AND OVULATION USING INTRA VAGINAL TREATMENT WITH PROGESTERONE (IBD Onsett12™) AND OESTRADIOL BENZOATE IN DAIRY CATTLE

Abstract

Non-lactating and cycling Friesian cows ($n = 15$) were studied to evaluate the efficiency of the IBD Onsett12™ device in regulating progesterone, and to determine the effective intravaginal dosage of oestradiol benzoate in combination with progesterone on follicular dynamics, oestrous behaviour and time of ovulation. The cows were presynchronized with two injections of prostaglandin given 11 days apart. On day 3 of the oestrous cycle, all cows were treated with IBD Onsett12™ devices for 12 days and were randomly allocated to three treatments: 1) 7 mg oestradiol benzoate (ODB7, $n = 5$) infused intravaginally; 2) 2 mg oestradiol benzoate (ODB2, $n = 5$) infused intravaginally; and 3) 0 mg (62% benzyl alcohol) oestradiol benzoate (CON, $n = 5$) infused intravaginally. One day prior to device removal, all cows were treated with 1 mg oestradiol benzoate infused intravaginally. Blood sera collected daily for three days before treatment and every six hours on day zero and day one, and daily thereafter following the treatment were analysed for progesterone, FSH, and LH. Ovaries were monitored daily by transrectal ultrasonography to assess changes in ovarian follicles. Erroneously, four cows in the ODB7 and CON group, and two cows in the ODB2 were infused with 7 mg, 0 mg and 2 mg of oestradiol benzoate respectively one day prior to device removal. All cows developed vaginitis a day after treatment and persisted for the 12-day treatment period. The IBD Onsett12™ failed to dispense consistent progesterone and the amount dispensed ranged from 350 mg to 2250 mg. The proportion of cows observed in oestrus was low in all groups but 50% of the cows in the ODB2 and ODB7 were observed with nymphomania. Intravaginal treatment with oestradiol benzoate and progesterone on day 3 of the oestrous cycle was effective to arrest the growth of the dominant follicle. A new cohort of follicles began to emerge, on average, 2.5 days after treatment in both the ODB2 and the ODB7 groups and 5.7 days in the CON group. A second post-treatment wave emerged on average at day 9, 7.6 and 8.8 in the CON, ODB2 and ODB7 groups respectively and the dominant follicle became persistent and failed to ovulate after device removal.

INTRODUCTION

In the pastoral and seasonally calving dairy-farming system practised in New Zealand, cows are bred during a restricted breeding period of approximately 12 weeks (Macmillan and Moller, 1977). To achieve high conception rates, compact breeding in dairy herds is dependent on high oestrus detection rates. However, the major factor limiting reproductive performance on many dairy farms is the failure to detect oestrus in a timely and accurate manner. The error rate varied from 0 to 60% among herds and resulted in $\geq 10\%$ of the cows inseminated being not in oestrus in 30% of the herds (Heersche and Nebel, 1994). Therefore, oestrus synchronization can reduce management problems associated with daily monitoring to detect oestrus in herds of cows (Lehrer et al, 1992). The aim of oestrus synchronisation in cattle is to achieve a close synchrony of oestrus and ovulation with high submission rates. But, ovarian asynchrony and variability in response to treatments remain the most limiting factors to the widespread implementation of advanced reproductive technologies in cattle (Adams, 1998). The status of follicular wave development at the time of treatment was responsible for a large portion of the variability in ovarian response to synchronization treatments employed (Adams, 1998).

Short-term progestogen treatment was effective in synchronizing oestrus in cattle and conception rates were not reduced (Wiltbank and Kasson, 1968). Nevertheless, for this system to be effective, a luteolytic agent must be incorporated (Odde, 1990). Oestrogens are claimed to be luteolytic when administered during the early part of the oestrous cycle (Wiltbank et al., 1961). Oestrogen treatment disrupts the normal pattern of follicular

growth (Nadaraja and Hansel 1976; Engelhart et al. 1989; Rajamahendran and Walton 1990), and suppresses the growth of the dominant follicle when administered early in the oestrous cycle (Bo et al. 1993; Bo et al. 1994). Oestrogen treatment resulted in early emergence of the next follicular wave when the treatment was given early in the growing phase but not at the middle or end of the growing phase (Bo et al. 1993; Bo et al., 1995; McDougall, 1994). Oestrogen treatment during the growing phase did not alter follicle dominance but the emergence of the next wave was delayed (Bo et al. 1993). Oestrogens plus progestogen given in combination during the early growing phase was however effective in inducing follicle regression and did not delay the emergence of the second follicular wave (Bo et al. 1994). On average the emergence of a new wave occurred 4.3 ± 0.2 days later with oestradiol treatment in progestogen-implanted cows (Bo et al. 1995).

Oestrogens employed in the current treatment regimes of oestrus synchronization programs with oestrogen-progesterone in combination were administered either intramuscularly or per vaginum from a gelatin capsule. Therefore, it was hypothesized that oestradiol benzoate administered per vaginum, in combination with progesterone, would cause atresia of the dominant follicle when given early in the oestrous cycle and enhance the emergence of new follicular wave.

13

The aim of this clinical trial was to investigate the effective intravaginal dosage of oestradiol benzoate (ODB) when administered concurrently with progesterone and to evaluate the efficacy of the IBD Onsett12™ in regulating progesterone. This study examined the effects on dominant follicle regression and establishment of the subsequent

follicular wave emergence after intravaginal treatment with oestradiol benzoate at two doses in combination with IBD Onsett12™ treatment.

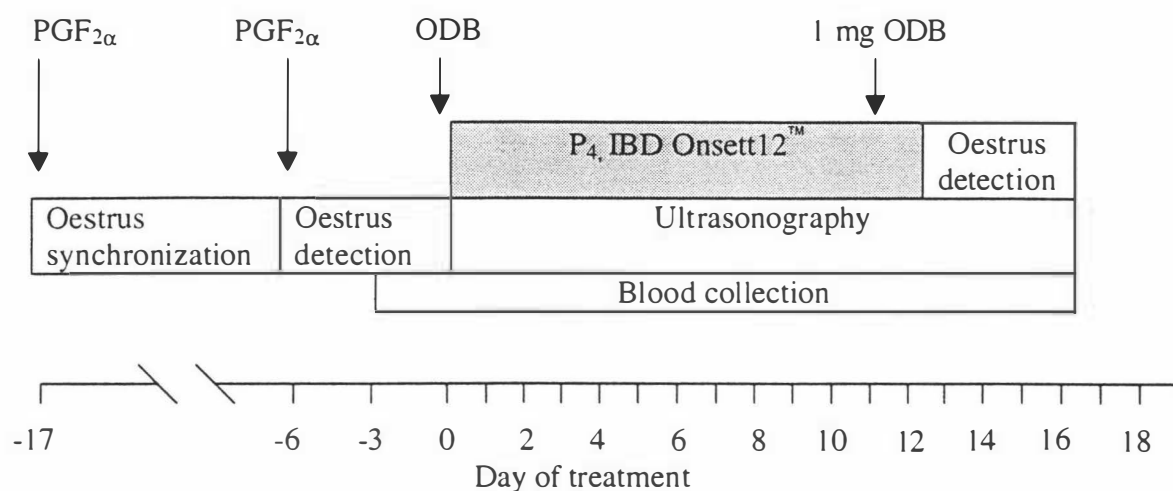
MATERIALS AND METHODS

Animals and design

Twenty-three non-lactating and cycling Friesian cows, grazed at the Large Animal Teaching Unit, Massey University, were synchronized utilizing two injections of 500 µg sodium cloprostenol (PGF_{2α}, estoPLAN®, Parnell Laboratories New Zealand Ltd., East Tamaki, New Zealand) by intra-muscular injections, given 11 days apart. Tails heads were painted as an aid for oestrus detection (Macmillan et al. 1988) and cows were observed for oestrus, twice daily at 0800 hours and 1600 hours for 30 minutes. On day 1 to day 4 after the second injection of prostaglandin, any cow which was observed to stand to be ridden by another cow (Williamson et al. 1972a) or which had more than 75% of its tail paint removed was defined as being in oestrus. Fifteen cows observed in oestrus, on the second and third day after the second prostaglandin injection, were assigned randomly to one of three treatments groups. Treatment groups were treated with the progesterone-releasing device (IBD Onsett12™, Plade Holdings Limited, Hamilton, New Zealand) for 12 days. The progesterone-releasing device (IBD Onsett12™) contains 3 grams (8 ml) of progesterone in 38% benzyl alcohol solution. The device was programmed to deliver the progesterone over 10-day periods for 750 doses at an average of 10 µl per dose or 4 mg of progesterone per dose. After device insertion, the control group (CON, n=5) was

treated with 0 mg (62% benzyl alcohol) oestradiol benzoate (Plade Holdings Limited, Hamilton, New Zealand), manually infused intravaginally using a syringe and pipette. Another group (ODB2, n=5) was treated with 2 mg (0.4 ml) oestradiol benzoate in benzyl alcohol (Plade Holdings Limited, Hamilton, New Zealand), and the final group (ODB7, n=5) had 7 mg (0.4 ml) oestradiol benzoate infused in a similar way. On day 11, all treatment cows were to be given 1 mg (0.4 ml) oestradiol benzoate in 62 per cent benzyl alcohol infused intravaginally using syringe and pipette. Erroneously, four cows in the ODB7 group were infused with 7 mg oestradiol benzoate while only one cow was infused with 1 mg oestradiol benzoate. Similarly, four cows in the CON group were infused with only 62% benzyl alcohol solution and one cow was infused with 1 mg oestradiol benzoate. Whereas, in the ODB2 group, two cows were infused with 1 mg oestradiol benzoate and three cows were infused with 2 mg oestradiol benzoate. The progesterone-releasing device, IBD Onsett12™, was withdrawn on day 12. The devices were then sent to Plade Holdings Limited for progesterone analysis. The progesterone solution left in the progesterone-releasing devices was measured and progesterone dispensed by the device was determined by determining the difference between the initial volume and the volume after withdrawal. Those conducting the experiment were blinded as to the oestrogen treatment employed. The time of insertion of the progesterone-releasing device and infusion of ODB is designated as day 0.

Figure 2.1 Diagram depicting the sequence of experimental procedures.



Blood collection and hormone assays

Blood samples were collected by jugular venipuncture into lithium heparin tubes (Vacutainers; Becton-Dickson, Rutherford, NJ) daily for three days before treatment and every six hours on day zero and day one, and daily thereafter following the treatment of oestradiol benzoate and progesterone intravaginally. Within 30 minutes of blood collection, plasma was harvested and stored at -20°C before analysis for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P_4).

Concentrations of LH and FSH were measured in plasma using double antibody radioimmunoassays that were modified from methods described for the assay of ovine gonadotrophins by Parkinson and Follett (1994). Antibodies and standards were supplied by NIDDK; the characterisation of the antibodies was supplied by Dr. A. F. Parlow, Director, Pituitary Hormones and Antiserum Centre, Hubor-UCLA Medical Centre.

Samples were dispensed in duplicate 50 µl aliquots into 4 ml polystyrene assay tubes. Standards (FSH: 0.04 to 10 ng/ml; LH 0.02 to 10 ng/ml) were similarly dispensed in triplicate. Standards were prepared in assay buffer (0.05M phosphate buffer, containing 0.0375% EDTA, 0.0875% sodium chloride and 0.05% bovine serum albumen; pH 7.05). To these, 20 µl each of normal rabbit serum (1:400 dilution in assay buffer) and antiserum (FSH: 1:25,000; LH: 1:100,000; both diluted in assay buffer) were added and, after vortex mixing, the tubes were incubated overnight at 4°C. On the following day, tracer ($[^{125}\text{I}]$ FSH or $[^{125}\text{I}]$ LH: chloramine /sodium metabisulphite iodination) was added (5,000 cpm in 20 µl assay buffer), the tubes were then vortex mixed and incubated overnight at 4°C. A second antibody (Donkey anti-rabbit; IDS, Boldon, UK) was then added to all tubes (20 µl of a 1:60 dilution in assay buffer) and, after a further overnight incubation (4°C), the free and bound counts were separated by centrifugation. Immediately prior to centrifugation, 200 µl of buffer (assay buffer, to which 0.5% egg albumen had been added) was added, then all tubes were centrifuged at 2,000 g for 45 minutes. After aspiration of the supernatant, the pellets were counted for 2 min in a gamma counter.

The limit of sensitivity was determined as twice the standard deviation of the binding in blank tubes. For FSH, this was 0.1 ng/ml, while for LH the figure was 0.07 ng/ml. The assay was validated by (i) demonstrating parallelism between standards prepared in serum and others prepared in gonadotrophin-free (anoestrous cow) plasma and (ii) parallelism between standards and serial dilutions of a plasma containing known high

concentrations of gonadotrophin in anoestrous cow plasma. Inter- and intra-assay coefficients of variation were 22.56% and 9.58% for LH and 13.06% and 9.26% for FSH, respectively.

Concentrations of progesterone in plasma were measured using a commercial enzyme immunoassay (EIA) kits (Ridgeway Science Limited, Gloucestershire, UK). The standard curve ranged from 0.41 to 8.05 ng/ml. All samples were processed in duplicate in one assay that had intra- and interassay coefficients of variation of 8.53% and 23.8% respectively.

Ultrasonography

The ovaries of each animal were examined by transrectal ultrasonography using an ultrasound with a 7.5 MHz linear array transducer (Pie Medical 200, Pie Medical Equipment B.V., Maastricht, Holland) daily from day 0 to day 16. Daily ultrasound examinations were recorded on videotape (VHS Video Cassette, SKC Ltd., Korea) for analysis. The tape was subsequently reviewed with video recorder (Panasonic NV-HS880 G-mode VHS, Japan) on high-resolution colour monitor (Panasonic MT-M2080, Japan). Follicles were measured with a ruler calibrated against the scale provided by the ultrasound unit. The examinations were conducted to individually identify and monitor the dominant and largest subordinate follicles and to detect the day of wave emergence. Ovulation was taken as the sudden disappearance of an ovarian follicle greater than 8.0 mm in diameter (Cavalieri et al. 1997).

Statistical Analysis

Statistical analysis was not performed on all data due to the variations of the progesterone treatment within the treatment groups.

RESULTS

One cow in the CON group was found to have a cystic ovary and one cow each in the CON and ODB2 groups had rectal inflammation induced by the examination, which were then stopped. Data from these animals were excluded from analyses pertaining to follicular growth, but were included in the analyses of endocrine concentrations.

On day 11, the protocol was to administer a second intravaginal infusion of 1 mg oestradiol benzoate to all the cows. Erroneously, four cows in the ODB7 group were infused with 7 mg oestradiol benzoate while one cow was infused with 1 mg oestradiol benzoate. Similarly, four cows in the CON group were infused with only 62% benzyl alcohol solution and one cow was infused with 1 mg oestradiol benzoate. Whereas, in the ODB2 group, two cows were infused with 1 mg oestradiol benzoate and three cows were infused with 2 mg oestradiol benzoate.

All cows in the treatment groups developed vaginitis a day after initial treatment, which persisted for the duration of treatment with the IBD Onset¹²[™]. The condition of vaginitis was graded (Table 2.1) as mild, moderate and significant based on the viscosity, colour

and smell of the vaginal discharges. From fifteen cows used in this trial, seven cows showed mild vaginitis and four cows with moderate and significant vaginitis respectively. The cause of vaginitis was not diagnosed and antibiotic treatment was not given during the trial period.

Table 2.1 The number of cows affected with vaginitis and its condition according to the treatment groups and the number of cows observed with nymphomania, in oestrus and not in oestrus according to the treatment groups.

	Treatment Groups			TOTAL
	CON	ODB2	ODB7	
1. Vaginitis Condition:				
a. Mild	2	2	3	7
b. Moderate	1	2	1	4
c. Significant	2	1	1	4
2. Oestrous detection:				
a. Nymphomania	0	2	2	4
b. Oestrus	2	1	1	4
c. Not in oestrus	3	2	2	7

Oestrous detection

One cow in the ODB7 and two cows in the ODB2 group (Table 2.2) showed repeated signs of oestrus (nymphomania) starting one day after the withdrawal of the progesterone-releasing device (IBD Onsett12™). In addition, one cow in ODB7 group showed signs of nymphomania two days after the withdrawal of the progesterone-releasing device (IBD Onsett12™). The nymphomania lasted for three to four days.

Two cows in the CON group and one cow each in the ODB2 and ODB7 group were observed in oestrus one day after device removal. In the CON group, one cow was observed in oestrus three days after withdrawal of the progesterone-releasing device (IBD Onsett12™) and another cow was observed one day later.

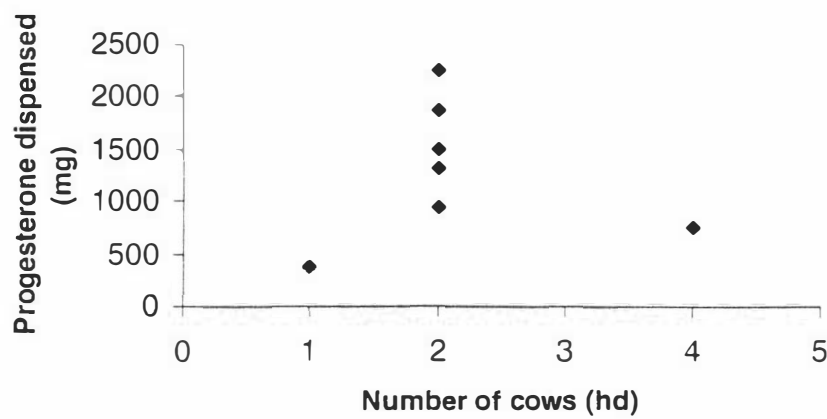
Table 2.2 The cows observed with nymphomania, oestrus and not in oestrus according to the second dose of intravaginal oestradiol benzoate in the ODB2 and ODB7 groups.

Group	Dose of Oestradiol (mg)	No. of cows observed		
		No oestrus	In oestrus	Nymphomania
ODB2	1	1	1	-
	2	-	1	2
ODB7	1	-	1	-
	7	1	1	2

Progesterone-releasing device (IBD Onsett12™)

The progesterone-releasing device (IBD Onsett12™) was designed to release 3 gm of progesterone over a 10-day period but it was found that the device was not dispensing perpetually this amount of progesterone, as it was programmed to do. Although the treatment period had been extended to 12 days, the amounts of progesterone released by most of the devices were below the expected amounts. The amounts of progesterone dispensed ranged from 375 mg to 2250 mg (Figure 2.2). The amount of progesterone dispensed by the device ranged from 750 mg to 2250 mg in CON and ODB2 groups, and 375 mg to 1500 mg in ODB7 group. The retention of the devices was 100%.

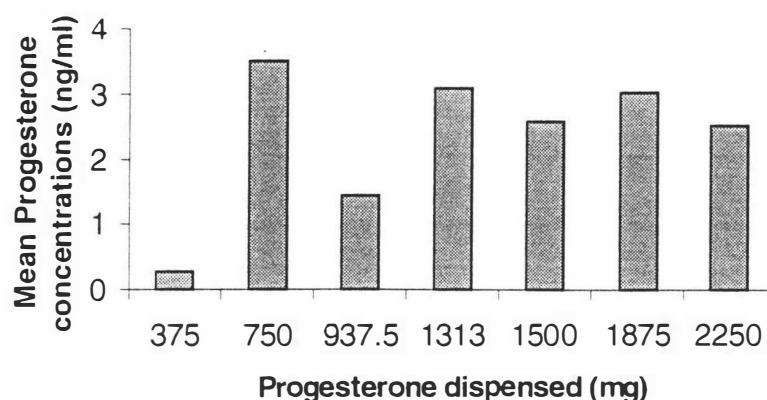
Figure 2.2 Scatterplot of the number of cows and the amounts of progesterone dispensed from the progesterone-releasing devices (IBD Onsett12™) over the treatment periods of 12 days.



Progesterone concentrations

The individual cows' mean progesterone concentrations for the 12-day treatment periods with IBD Onsett 12™ varied from 0.28 to 4.94 ng/ml (mean $2.35 \pm \text{STD } 1.12$ ng/ml). The mean progesterone concentrations were aberrant as to the dose of progesterone dispensed by the IBD Onsett12™ (Figure 2.3). Cows, which received 750 mg of progesterone, have the highest mean progesterone concentrations (3.51 ng/ml) for the 12-day treatment periods, and a cow which received the lowest dose (375 mg, n=1) had the lowest mean progesterone concentrations (0.28 ng/ml).

Figure 2.3 The mean progesterone concentrations for the 12-day treatment periods according to the dose of progesterone dispensed by the IBD Onsett12™.



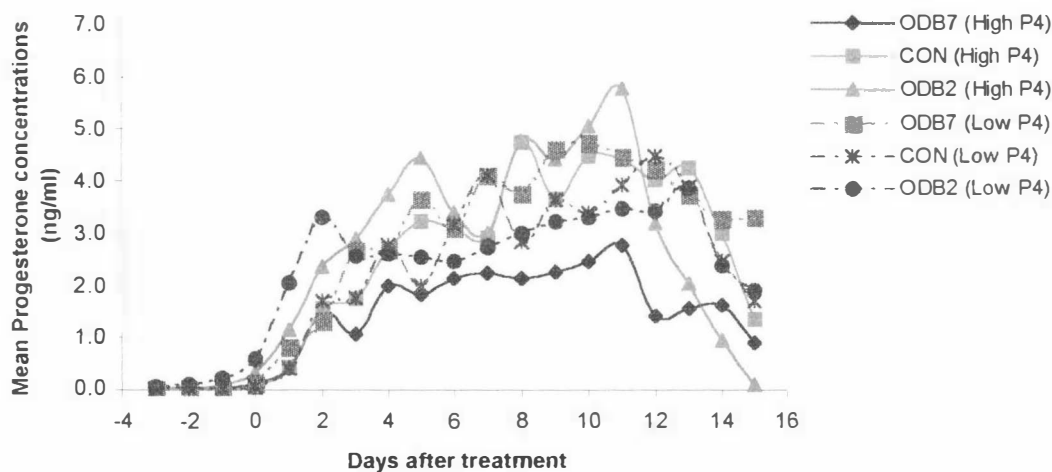
During the three days prior to treatment, the progesterone concentrations remained at basal concentrations (<1 ng/ml) in all groups (Figure 2.4). This showed that all the cows used in this study had undergone luteolysis at least three days prior to treatment.

In the CON group, four cows had a gradual increase in progesterone concentrations from day 4 to 15 except in two cows, where it dropped to basal concentration (<1 ng/ml) on days 14 to 15. One cow had a gradual increase in plasma progesterone concentrations after treatment until day 3, then the concentrations decreased to basal concentrations on day 4 and then increased thereafter. Daily mean progesterone concentrations were not much different between cows that received either a low or high progesterone dose from the IBD Onsett12™ (Figure 2.4).

In the ODB2 group, one cow had basal progesterone concentrations (<1 ng/ml) from the

day of treatment until day 6 and another cow had plasma progesterone concentrations below the basal concentration (< 1 ng/ml) from day 3 to day 12. Three cows showed gradual increases in progesterone concentrations after treatment and then the concentrations decreased to basal concentrations either on day 13, 14 or 15.

Figure 2.4 The mean progesterone concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 ng) or low P4 (<1 ng).



In the ODB7 group, one cow which received a low progesterone dose (375 mg) supplied by the IBD Onsett 12™ had a basal progesterone concentration (< 1 ng/ml) from the beginning to end of the experimental period. The progesterone concentrations in three cows were gradually increased to 4 ng/ml after treatment until day 4 or 5 and then the concentrations remained at this level until day 15 except in one cow where the progesterone concentrations dropped to basal level (<1 ng/ml) on days 14 to 15. The cow,

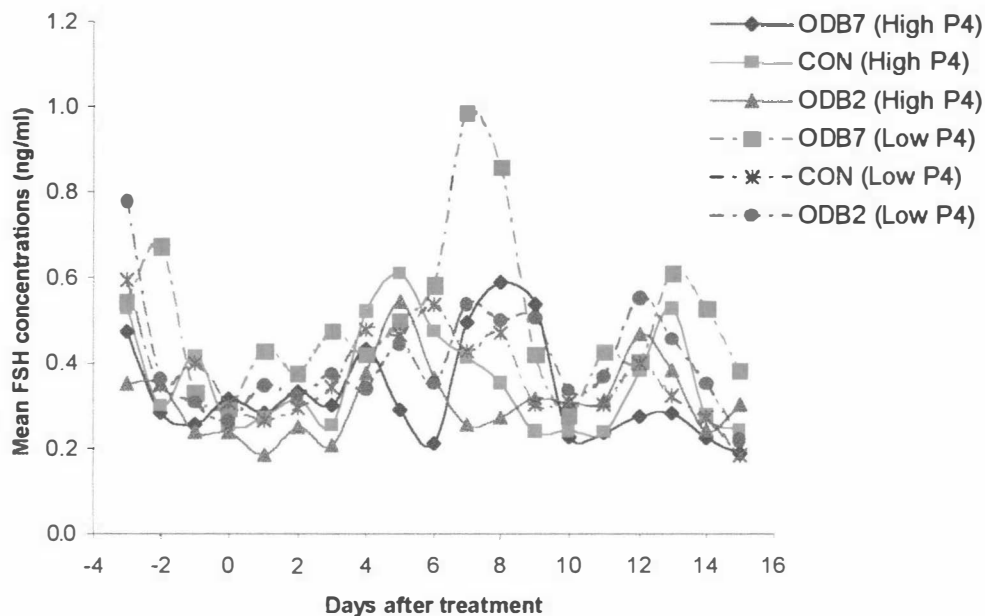
which received a high progesterone dose (1.5 g) supplied by the IBD Onsett12™, had a basal concentration between day 0 to 1, between day 3 to 5, and between day 12 to 15 indicating that the progesterone payout may have been intermittent.

Follicle stimulating hormone (FSH) concentrations

Generally the concentrations of FSH were low (<1 ng/ml) in all treatment groups throughout the experimental periods. In the CON group, two cows have slightly increased concentrations (0.6-0.8 ng/ml) on day 4 and another two cows have increased concentrations on day 6 after intravaginal treatment with progesterone alone. Another increase in the FSH concentrations occurred between day 12 and 13 in four cows and on day 8 in one cow. The mean FSH concentrations by day were similar between cows that received high (>1 gm) and low (<1 gm) dose of progesterone from the IBD Onsett12™ (Figure 2.5).

For the ODB2 group, two cows show a slight increase in concentrations (0.6 ng/ml) on day 5 and another two cows have an increase in concentration (0.6 ng/ml) on day 7. Another increase in the FSH concentrations (0.6 ng/ml) occurred between day 12 and 13 in four cows. Whereas, one cow had an increase in FSH concentrations which occurred on day 5, 9 and 12. The mean FSH concentrations were generally similar in cows that received high (>1 gm) or low (<1 gm) doses of progesterone from the IBD Onsett12™ (Figure 2.5). Between day 7 to 9, the mean concentrations were higher in cows that received a low dose of progesterone from the IBD Onsett12™.

Figure 2.5 The mean FSH concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 ng) or low P4 (<1 ng).



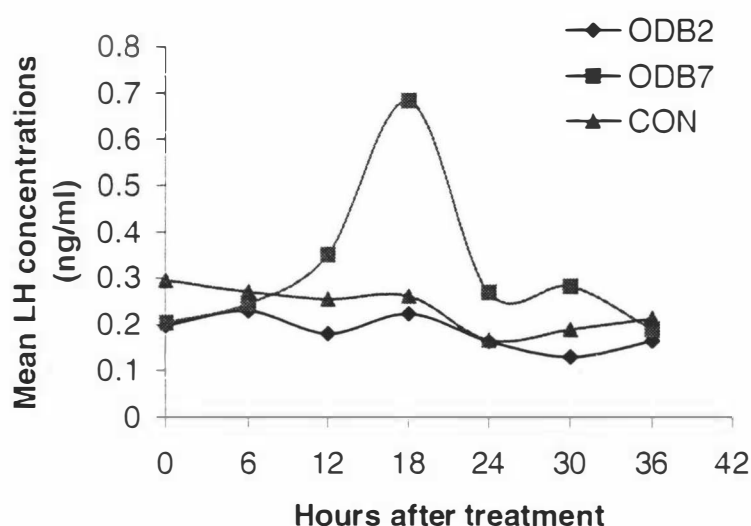
Three cows in the ODB7 group have a single increase in FSH concentration either on day 7 (0.8 ng/ml, n=1), or 8 (0.7-1.2 ng/ml, n=2). One cow had double increases in FSH concentrations on day 4 (0.6 ng/ml) and on day 7 (0.8 ng/ml). One cow had low daily FSH concentrations (<0.5 ng/ml) from the beginning of the treatment to end of the experimental period. The mean FSH concentrations were generally higher in cows that received low progesterone doses (< 1gm) especially on day 7 and day 13 (Figure 2.5).

Luteinizing hormone (LH) concentrations

The mean LH profiles following intravaginal treatment with oestradiol benzoate and progesterone are shown in Figure 2.6. In the ODB7 group, the cows have an apparent

increase in plasma LH after 18 hours and a subsequent decrease following the intravaginal treatment with oestradiol benzoate and progesterone. No changes in LH concentrations were detected in the cows of the ODB2 and CON groups following the treatment.

Figure 2.6 The mean LH concentrations after intravaginal treatment with oestradiol benzoate and progesterone (IBD Onsett12™) and according to the treatment groups.

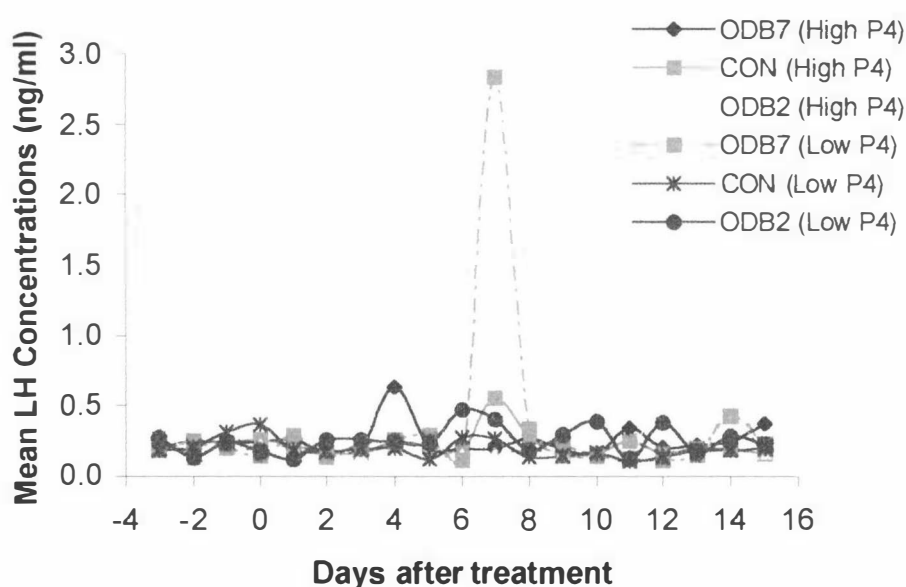


Two of the cows in the CON group had an apparent increase in LH concentrations (0.6 ng/ml) on day 6 or 7 respectively while there are no changes in the daily LH concentrations of the other three cows during the experimental period. In the ODB2 group, two of the cows have daily LH concentrations of less than 0.3 ng/ml following treatment until end of the experimental period. One cow has basal LH concentrations until day 12 when there is a significant increase in concentration (1.02 ng/ml). Another two cows have apparent increases in LH concentrations on day 6 (0.83 ng/ml) and 10

6

(0.66 ng/ml), and on day 7 (0.56 ng/ml) and 12 (0.48 ng/ml) respectively. In the ODB7 group, two cows have a significant increase in LH concentrations on day 4 (1.23 ng/ml) and on day 7 (5.44 ng/ml) respectively. Two cows have apparent increases in LH concentrations on day 11 (0.65 ng/ml) and on day 15 (0.73 ng/ml) respectively. In another cow, there are no noticeable changes in daily LH concentrations.

Figure 2.7 The mean LH concentrations by day according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™, either high P4 (>1 ng) or low P4 (<1 ng).



The mean LH concentrations according to treatment groups and according to the dose of progesterone dispensed by the IBD Onsett12™ device are shown in Figure 2.7. Generally, there are no remarkable differences in the mean LH concentrations between treatment groups except on day 7 of treatment. On day 7, there is a significant increase in LH concentrations in cows in the ODB7 group with a low dose of progesterone dispensed by the IBD Onsett12™.

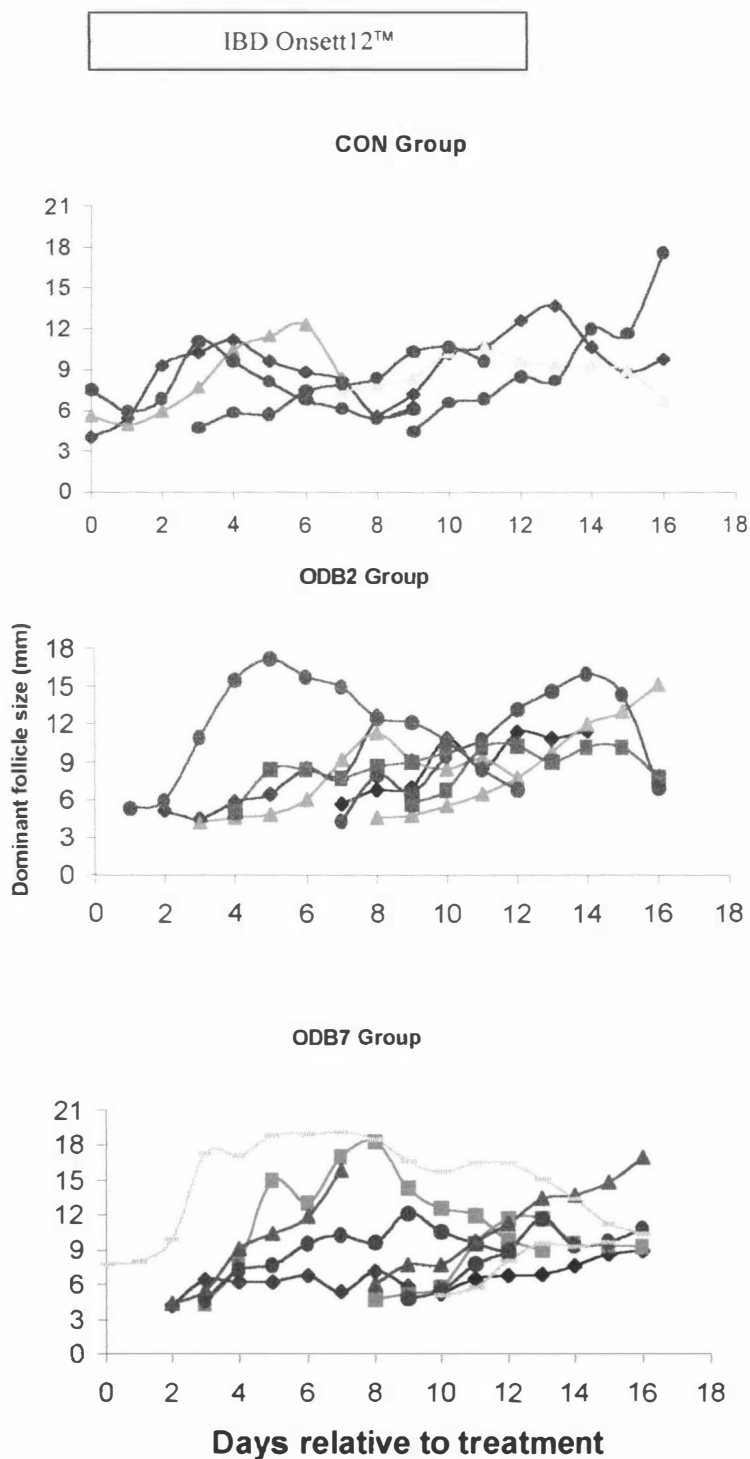
Growth of follicles

Patterns of emergence and growth of follicles are shown in Figure 2.8. Intravaginal treatment with oestradiol benzoate and progesterone on day 3 of the oestrous cycle arrested growth of the dominant follicle present at the time of treatment. A new cohort of follicles emerged, on average, 2.5 days after treatment in both the ODB2 and the ODB7 groups (Table 2.2). Whereas, the dominant follicle present at the time of treatment continued growing in the control cows (CON). The post-treatment wave in the control cows emerged on average 5.7 days after treatment. The dominant follicle formed following treatment with oestradiol benzoate and progesterone ceased growth, began to regress and was followed by emergence of a second post-treatment wave on average at day 9, 7.6 and 8.8 in the CON, ODB2 and ODB7 groups respectively. However, the second post-treatment dominant follicle became persistent in all the cows in the CON, ODB2 and ODB7 groups.

Table 2.3 Day of emergence of the dominant follicle following treatment with intravaginal oestradiol benzoate and IBD Onsett12™ on day 3 of the oestrous cycle.

	Group		
	CON	ODB2	ODB7
Day of post-treatment wave emergence:			
Mean ± SD	5.7 ± 2.1 (n=3)	2.5 ± 1.12 (n=4)	2.5 ± 0.5 (n=4)
Range	3 - 8	1 - 4	2 - 3
Day of second post-treatment wave emergence:			
Mean ± SD	9 (n=1)	7.6 ± 0.8 (n=4)	8.8 ± 0.8 (n=4)
Range		7 - 9	8 - 10
No. of cows with persistent follicle	5	5	5

Figure 2.8 Patterns of emergence and growth of individual cows' dominant follicles after intravaginal treatment with progesterone (IBD Onsett12™) and oestradiol benzoate (Groups ODB2 and ODB7) or benzyl alcohol (CON group).



DISCUSSION

Procedures to synchronize oestrus and ovulation in cycling cattle were based on synchronizing the end of the progestational phase of the oestrous cycle (Wright and Malmo, 1992). The aim of administering an exogenous source of progesterone for a time is to control plasma progesterone levels, which allows natural regression and then termination of the progestational phase to occur when progesterone treatment ceases. In this study, the IBD Onset12™ device failed to dispense a consistent dose of progesterone (Figure 2.2). As result, the plasma progesterone levels were irregular between and within cows among and between the treatment groups (Figures 2.3 and 2.4). The plasma progesterone concentration in most cows between day 10 and 15 was still above the basal concentrations (Figure 2.4). These high concentrations of plasma progesterone after device removal explained the failure to synchronize oestrus and ovulation in all the cows. The progesterone suppresses oestrus and ovulation by inhibiting the releasing of LH and the maturation of the Graaffian follicles (Peters, 1986).

The number of cows detected in oestrus was low in all treatment groups (Table 2.1). Nevertheless, the number of cows observed in oestrus was high when compared to the number of cows that ovulated (Figure 2.8). The high oestrous response was associated with oestrus induced as result of the oestradiol benzoate treatment given a day before device removal. This was further explained by the presence of progesterone concentrations above the basal concentrations during the period of observed oestrus. In

22 7

normal oestrous cycling, oestrus occurred, on average, 69 hours after the fall of the plasma progesterone concentrations to below 1 ng/ml (Mann et al., 1999).

Intravaginal treatment with either 2 mg or 7 mg oestradiol benzoate in combination with progesterone suppressed follicular growth and resulted in the emergence of a new follicular wave. There was no difference of effect on the onset of new follicular wave emergence between the ODB2 and ODB7 group but the follicular emergence was more precise in the ODB7 group (2.5 ± 1.12 vs. 2.5 ± 0.5 , Table 2.3). The combined mean time to follicular wave emergence was 2.5 ± 0.93 days. There was no difference in the time of follicular wave emergence ($P = 0.05$) between the ODB2 and the ODB7 group and there was no variation in the time of follicular emergence ($P = 0.05$). The follicular wave emergence in this study was earlier than reported previously (4.3 ± 0.2 days; Bo et al., 1995). There is no clear explanation for the earlier follicular wave emergence.

The dominant follicle of the control cows (CON group) did not undergo regression after the treatment with the IBD Onset12™ alone. A new follicular wave was only detected in the control cows, on average, at day 5.7 ± 2.1 which is equivalent to about day 7 to 10 of the oestrous cycle as treatment was commenced on day 3 in this study. This result was similar with findings observed in a normal oestrous cycle. In a normal oestrous cycle, the second follicular wave emerges, on average, at day 9 or 10 in two-wave cycles or day 8 to 9 for three-wave cycles (Savio et al., 1988; Ginther et al., 1989a).

A second post-treatment follicular wave emerged on day 9, 7.6 and 8.8 for the CON, ODB2 and ODB7 groups respectively. This may be due to the long treatment with the IBD Onset12™ device, which was programmed to deliver the progesterone over a 10-day period but was left in place for 12 days in this study. With the irregularities of the device in delivering the progesterone (Figure 2.3), a tendency for the device to dispense the progesterone in small doses over the 12-day period may have occurred. In normal oestrous cycling cows, follicular wave growth was reported to occur every 7 to 10 days during the oestrous cycle (Savio et al., 1988; Ginther et al., 1989). Since new follicular wave emergence occurred 2.5 days after the treatment, the dominant follicle might undergo regression before the removal of the device and be replaced with a new follicular wave.

After treatment using exogenous progesterone such as the PRID (Rathbone et al., 1998) and CIDR (Macmillan et al., 1991), the plasma progesterone concentrations fall within hours following device removal to basal concentrations. As progesterone decreases, this stimulates oestradiol production which triggers oestrus (Ireland, 1987; Sirois and Fortune, 1988), and the release of GnRH-induced LH which results in a surge of LH followed by ovulation (Adams, 1998). In the present study, IBD Onset12™ was programmed to cease dispensing progesterone on day 10. However, following device removal on day 12, the plasma progesterone levels in all cows were still above 2 ng/ml between days 10 and 14 (Figure 2.4). The high plasma progesterone concentrations during this period inhibited an LH surge (Figure 2.7) in all the cows. The absence of LH surge explains the reason for the high proportion of the cows that did not show oestrus and ovulation (Figure 2.8).

Treatment with 10 mg oestradiol valerate during the late luteal phase resulted with decreasing plasma progesterone concentrations within 43 ± 0.5 hours. LH reached peak concentrations within 41 ± 11 hours and this treatment also caused premature luteolysis (Rajamahendran and Walton, 1990). However, in this study, treatment with 1, 2 or 7 mg of intravaginal oestradiol benzoate, given a day before device removal, did not decrease the plasma progesterone concentration and an LH surge was not observed in all cows. This difference could be due to the form of oestradiol used, route of administration or as result of the vaginitis, which could have prevented the absorption of the oestradiol benzoate intravaginally. Due to the failure of the device to synchronize the end of the progestational phase, the second treatment with oestradiol benzoate was given at a time when the plasma progesterone concentrations remained high. In addition, according to Engelhardt et al. (1989), the administration of oestradiol valerate to cows in dioestrus has been shown to cause failure of ovulation and the formation of ovarian cysts. This may explain the reason for the ovulation failure in most cows, and the observed nymphomania.

CONCLUSION

Intravaginal treatment with oestradiol benzoate in combination with progesterone effectively induce atresia of the dominant follicles and induced emergence of a new follicular wave. The results demonstrated that a low dose (2 mg) of oestradiol benzoate was effective to cause these effects. However, the IBD Onset12 device failed to control the plasma progesterone concentration during the treatment period. As result, the oestrus

synchronization program failed to synchronize oestrus and ovulation. Further improvement of the capability of the IBD Onset12 device to deliver progesterone consistently and to control the plasma progesterone level was required.

CHAPTER 3

THE EFFICACY OF THE IBD ONSETT12™ IN INDUCING A FERTILE SYNCHRONIZED OESTRUS IN CYCLING AND NON-CYCLING DAIRY COWS

Abstract

The aim of this study was to evaluate the efficacy of the IBD Onsett12™ to induce fertile oestrus in cycling and non-cycling dairy cows. A total of 350 Friesian or Friesian cross cows in five herds were involved in the trial. Within each herd, the cows were randomly allocated to one of four treatment groups; 1) Cycling cows were treated with the IBD ONSETT12™ device for 12 days and inseminated at device removal (IBD-C, n = 65); 2) Cycling cows were treated with the CIDR for 7 days plus 2 mg oestradiol benzoate, and prostaglandin was given at device removal (CIDR-C, n = 68). Cows were bred on display of oestrus. An additional 1 mg of oestradiol benzoate was given to cows which were not detected in oestrus two days after device removal and cows were bred again on display of oestrus; 3) Non-cycling cows (IBD-NC, n = 107) received the same treatment as in group (1); 4) Non-cycling cows (CIDR-NC, n = 110) were treated with a CIDR for 7 days and bred on display oestrus. An additional 1 mg of oestradiol benzoate was given to cows, which were not detected in oestrus two days after the device removal, and cows were bred again on display of oestrus.

The retention rate for the IBD Onsett12™ was significantly lower than the CIDR (65.12% vs. 99.44%, $\chi^2 = 73.528$, $P = 0.001$), and the synchronized conception rate from the CIDR protocol was significantly higher ($\chi^2 = 15.087$, $P = 0.02$) than the IBD Onsett12™ among the cycling (36.76% vs. 9.23%) and non-cycling (27.27% vs. 13.08%) cows. The IBD Onsett12™ oestrus synchronization program was effective in inducing fertile synchronized oestrus in some cycling and non-cycling cows, but resulted in a low synchronized conception rate. The causes for the low retention rate and low synchronized conception rate require further investigation.

INTRODUCTION

Oestrus synchronization can reduce management problems associated with daily oestrus detection (Lehrer et al., 1992) and thus improve reproductive efficiency. However, variability in response has continued to be one of the most frustrating problems in oestrus synchronization programs (Adams, 1998). Much of the variation in the synchronized oestrus response, as well as variation in fertility associated with the synchronized oestrus, has been related to differences in the development of the follicle that ovulate following the synchronized oestrus (Beal, 1996). The variation in ovulatory response to exogenous hormone treatments has been attributed to variations in follicular wave status at the time of treatment (Adams, 1998).

Progesterone has been widely used in oestrus synchronization protocols. Progesterone suppresses oestrus and ovulation (Christian and Cassida, 1948) by inhibiting the release of luteinizing hormone and thus the maturation of Graaffian follicles (Peters, 1986). Effective oestrous cycle regulation has been obtained with various regimens of progesterone, oestrogen and gonadotrophins (Hansel and Convey, 1983). Longer progestogen treatment periods of 14 to 21 days, were effective in synchronizing oestrus, however, fertility at the synchronized oestrus was reduced (Peters, 1986; Odde, 1990). Lowered fertility after prolonged progestogen treatment has been attributed to aging of the oocyte within the long-lived, oversized follicles (Custer et al., 1994; Mihm et al., 1994). Whereas short progestogen treatments of less than 14 days do not reduce conception rate (Roche, 1976a), but synchrony of oestrus was lower (Macmillan and

Asher, 1990). Therefore, it was found necessary to incorporate a luteolytic agent with the short-term progestogen treatment to obtain both oestrus synchrony and normal fertility (Odde, 1990; Larson and Ball, 1992). Oestrogen was reported to be luteolytic when administered early in the oestrous cycle (Wiltbank et al., 1961). However, the synchrony and fertility from progestogen-oestrogen combination treatment tended to be variable because the administered oestradiol had different effects at different stages of the cycle (Bo et al., 1994; Bo et al., 1995).

Postpartum anoestrus is the most common form of infertility in New Zealand dairy cows and 33% were diagnosed as anovulatory anoestrus (McDougall et al., 1993). Progesterone has been used to induce oestrous cycles in anoestrous cows and prepubertal heifers (Short et al., 1976; Miksch et al., 1978; Anderson and Day, 1994). The current hormonal treatment for postpartum anoestrous cows in New Zealand involves a 5 to 7 days period of progesterone followed 24-48 hours later by intramuscular injection of 1 mg oestradiol benzoate (Macmillan et al., 1995; Rhodes et al., 1998a). Injections of oestradiol benzoate improve not only oestrus and ovulation induction but also synchrony (Peters et al., 1977; Macmillan and Burke, 1996). An acceptable conception rate to first insemination (45.2%) is achieved in anoestrous cows treated using this regime (Xu and Burton, 1997; Rhodes et al., 1998a).

IBD ONSETT12™ is a single application drug delivery device developed at Plade Holdings Limited, Hamilton, New Zealand. The device has four individual drug reservoirs that retain secluded volumes of progesterone, oestradiol benzoate and

prostaglandin. Hormone release is achieved through the electronic computer control of a unique pumping system for each hormone. The hormones are dissolved in benzyl alcohol solution and their delivery sequence is turned on at device insertion. The device can be programmed to dispense these hormones at specific phases of the treatment period. The progesterone (2.0 gm of progesterone in benzyl alcohol) was programmed to be continually and uniformly dispensed over a 10-day period. Upon insertion, the device also dispensed a dose of 7 mg oestradiol benzoate in benzyl alcohol two hours after insertion. On day 6 of the treatment period, 240 µg of prostaglandin (Sodium Cloprostenol) was dispensed by the device. Another dose of 1 mg of oestradiol benzoate in benzyl alcohol was dispensed on day 11 of the treatment periods. The above describes the production model of the prototype device that was current at the time of conducting this experiment.

The current recommended oestrus synchronization programs for lactating dairy cows using CIDR devices at the time of the experiment involved a 7-day treatment period. At CIDR insertion, 2 mg of oestradiol benzoate was administered intramuscularly and a prostaglandin injection was given at CIDR removal. An additional 1 mg oestradiol benzoate was injected intramuscularly to any cows not showing oestrus two days after device removal.

The aim of this clinical study was to evaluate the efficacy of the IBD ONSETT12 to induce fertile oestrus in cycling and non-cycling dairy cows, and to compare the efficacy of this device with the CIDR protocols current at the time. The comparison was made in

terms of the retention rate, the induction of fertile oestrus and the synchronized conception rate in non-cycling and cycling dairy cows.

MATERIALS AND METHODS

Farm Selection

The trial was conducted in five commercial grazed dairy herds in the Manawatu and Rangitikei region of New Zealand. Lactating spring-calving Friesian or Friesian cross cows ($n = 350$) were involved in the trial. The farmers involved were selected by participating local veterinarians in the region. The herd size for the farms involved in the trial ranged from 220 to 680 head. The clinical trial was conducted from 13 November 1998 (day of first treatment) to 9 February 1999 (pregnancy diagnosis).

Animals

Normal healthy milking cows, calved at least 40 days, with ages ranging from 2 to 12 years were used in the trial. Management of the cows during the trial was under the direct control of the farm owner/manager utilizing their usual farming practices. All herds grazed pastures consisting predominantly of perennial ryegrass and white clover. Water was freely available for the duration of the trial. Cows were milked twice daily, as per normal farming practice.

Cows used in the trial were not treated with any reproductive treatments for the two months prior to the trial. In addition, cows selected for the trial had no visible vaginal discharge, pyometra and no history of milk fever.

Experimental Protocol

The lactating cows in each herd were examined per rectum on the day of treatment. The cows were classified as either (a) cycling at random stages of the oestrous cycle, when a palpable corpus luteum or other evidence of cycling was found, or (b) non-cycling, when the ovaries had no corpus luteum present or evidence of cycling. The cows were then allocated at random, to four groups with respect to the ovarian status.

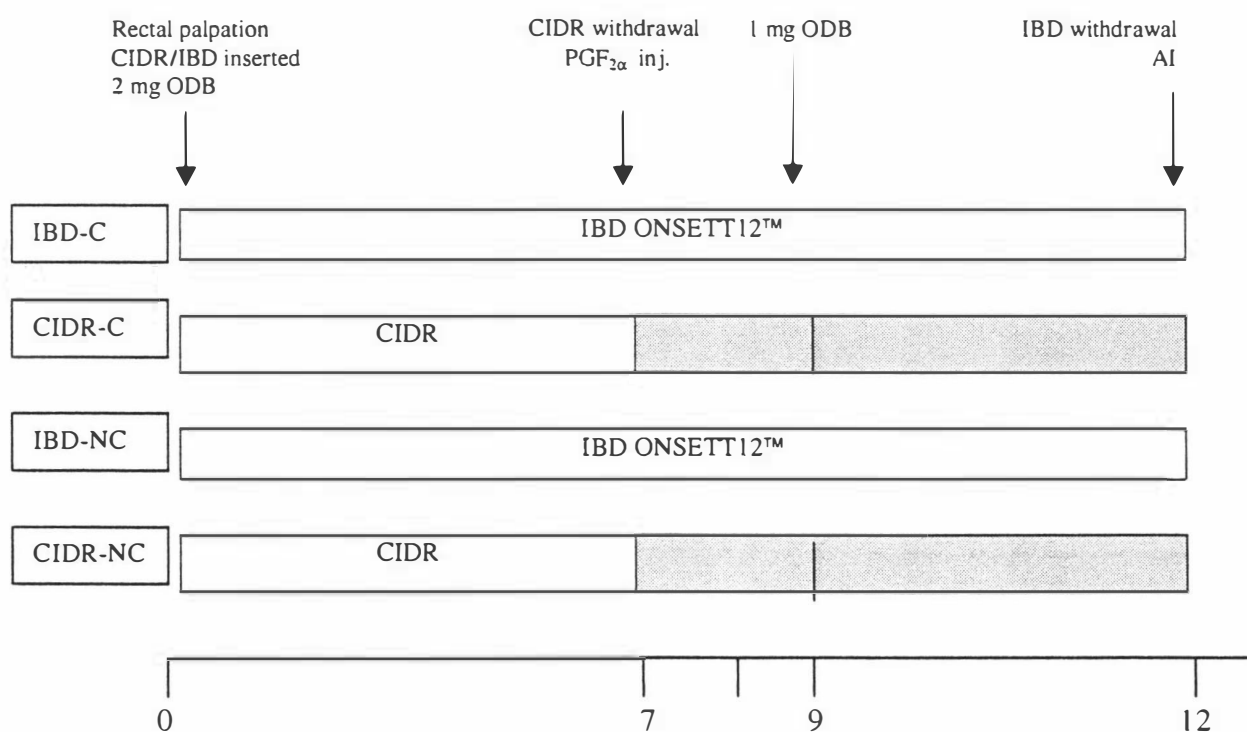
The four groups in each herd received the following treatments:

- 1) A single IBD ONSETT12™ (Plade Holdings Ltd., Hamilton, New Zealand) inserted per vaginum for 12 days to cycling cows (IBD-C, n = 65). All cows were bred using a single fixed insemination at the time of device removal;
- 2) A controlled intravaginal drug releasing device (CIDR®, Livestock Improvement Corp. Ltd., Hamilton, New Zealand) inserted per vaginum for 7 days plus 2 mg oestradiol benzoate (CIDIROL®, InterAg, Hamilton, New Zealand) administered intramuscularly at the time of insertion to cycling cows (CIDR-C, n = 68). At CIDR removal, Prostaglandin (Estrumate®, Schering-Plough Animal Health, Upper Hutt, New Zealand) was injected intramuscularly. Cows were bred on display of oestrus. An additional 1 mg of oestradiol benzoate was given on day 9 to cows which were

not detected in oestrus by day 9 (day 0 = day of CIDR insertion), and cows were bred again on display of oestrus;

- 3) Non-cycling cows (IBD-NC, n = 107) received the same treatment as described for the IBD-C group (1);
- 4) Non-cycling cows (CIDR-NC, n = 110) were treated per vaginum with a CIDR for 7 days and an additional 1 mg oestradiol benzoate was given on day 9 to cows which were not detected in oestrus by day 9 (day 0 = day of CIDR insertion). Cows were inseminated to detected oestrus.

Figure 3.1 Diagrammatic representation of treatment protocol. The open rectangles represent either IBD ONSETT12™ or CIDR device inserted into cows from day 0 to either day 7 or 12 for CIDR or IBD ONSETT12™ respectively.



The trial design was reviewed and approved by the Animal Ethics Committee, Massey University (AEC No 98/156), and the Animal Welfare Act(s) and Regulation of New Zealand were complied with in relation to animal management.

Oestrus Detection and Insemination Procedure

Observation for oestrous activity was the responsibility of the herd owner/manager on each farm. As for the CIDR protocols, the number of cows detected in oestrus within 6 days of CIDR removal and inseminated was defined as a proportion of the number of cows submitted for oestrus synchronization. Insemination of cows was carried out once a day and conducted by the farm's usual AI technician. Selection of semen was the farmer's choice.

Each cow was pregnancy tested by palpation per-rectum and/or by ultrasound between 78 and 82 days after the last insemination to the synchronized oestrus. The proportion of cows observed in oestrus and artificially inseminated or inseminated at a fixed-time that became pregnant was defined as the synchronized conception rate. The individuals involved in assessing the pregnancy status were blinded to the treatment allocations.

Statistical Analysis

The analysis was conducted in two steps, a univariate and a multivariate analysis. Data analysis was performed using GLM and FREQ procedures of SAS (1996) for a univariate

analysis and GENMOD procedures of SAS for a multivariate analysis. In the univariate analysis, the association between all dependent and independent variables, treatment and herd, were examined for statistical difference. A two-way ANOVA was used for age, daily milk production, and calving-to-treatment interval data. Any significant difference on these data was tested by Least Significant Difference. The retention rate and synchronized conception rate was analysed using chi-square tests for the effect of treatment. In the multivariate analysis, logistic regression was used to assess the effect of confounding variables on conception.

RESULTS

Eight cows were withdrawn from the trial after the oestrus synchronization phase; three cows each from group IBD-C and CIDR-C and one cow each from group IBD-NC and CIDR-NC. Three cows of these eight died and another five were culled from the herds due to mastitis and lameness. Data from these cows were excluded from analyses pertaining to conception rate, but were included in the analyses for retention rate. In addition, 51 cows from one herd were not herd-tested for milk production and thus, data on milk production was not available from these cows. The distribution of the cows, which were not herd-tested for milk production, were 13 cows each from the IBD-C, CIDR-C and CIDR-NC groups and 12 cows from the IBD-NC group. These cows were excluded from the analyses pertaining to milk production but were included for the other parameters.

Age, Milk Production, Calving-to-Treatment Intervals and Induced Cows

There was no difference in average age between the CIDR-treated cows and IBD-treated cows (4.23 ± 0.18 vs. 4.17 ± 0.16 , Table 3.1), but the cows were slightly older for cycling cows compared to non-cycling cows (4.71 ± 0.20 vs. 3.89 ± 0.14 , $P = 0.001$). There was a significant difference ($P < 0.05$) in age for cows in the IBD-C group compared to both the IBD-NC and the CIDR-NC group, but no difference with the CIDR-C group. The CIDR-C and the IBD-NC groups also differed in age ($P < 0.05$). The average age was highest in the IBD-C group (4.89 ± 0.30) and lowest in the IBD-NC group (3.76 ± 0.18). Although there was significant difference in age of the cows between the treatment groups, on logistic regression analysis, age does not effect the synchronized conception rate between the treatment groups ($\chi^2 = 5.805$, $P = 0.445$).

Milk production was higher in cycling cows than non-cycling cows (19.07 ± 0.58 vs. 17.67 ± 0.36 , $P < 0.032$), but there was no difference in daily milk production between the IBD-treated cows and the CIDR-treated cows (18.46 ± 0.48 vs. 17.89 ± 0.41). There was no significant difference in milk production between the cows in the CIDR-C, IBD-NC and CIDR-NC groups, but the milk production was higher in IBD-C group compared to these three groups ($P < 0.05$). Logistic regression analysis showed that milk production did not effect the synchronized conception rate between the treatment groups ($\chi^2 = 2.256$, $P = 0.133$).

Table 3.1 The mean age, milk production and calving to treatment interval for the cows in the four treatment groups.

Treatment Group	Age (yr, mean \pm SEM)	Milk Production (L/day, mean \pm SEM)	Calving to treatment interval (days, mean \pm SEM)	No. of induced calving cows n (%)
IBD-C	4.89 \pm 0.30 ^{ab*}	20.16 \pm 0.88 ^{d±}	94.71 \pm 12.32 ^{i#}	7 (11.48)
CIDR-C	4.55 \pm 0.28 ^{ab*}	18.16 \pm 0.73 ^{e±}	85.66 \pm 6.25 ^{g#}	9 (14.29)
IBD-NC	3.76 \pm 0.18 ^{c*}	17.53 \pm 0.54 ^{e±}	72.25 \pm 1.87 ^{g#}	18 (16.98)
CIDR-NC	4.03 \pm 0.23 ^{bc*}	17.73 \pm 0.50 ^{e±}	80.01 \pm 5.18 ^{g#}	11 (10.28)
IBD	4.17 \pm 0.16	18.46 \pm 0.48	80.45 \pm 4.71	25 (14.97)
CIDR	4.23 \pm 0.18	17.89 \pm 0.41	82.20 \pm 3.98	20 (11.63)
Significance	NS	NS	NS	NS

^{abcdedfg} Indicates means within a column with different letters are significantly different ($P < 0.05$)

* Cows without age data were excluded from analysis (4 in group IBD-C, 2 in group CIDR-C, 1 in group IBD-NC)

± Cows which were not herd-tested were excluded from analysis (13 in group IBD-C, 13 in group CIDR-C, 12 in group IBD-NC and 13 in group CIDR-NC)

Cows without a recorded calving date were excluded from analysis (4 in group IBD-C, 2 in group CIDR-C, 1 in group IBD-NC)

NS not significant ($P > 0.05$)

There was a significant difference ($P < 0.05$) in calving-to-treatment interval for the IBD-C compared to the CIDR-C, IBD-NC and the CIDR-NC groups (94.71 \pm 12.32 vs. 85.66 \pm 6.25, 72.25 \pm 1.87, and 80.01 \pm 5.18 respectively, Table 3.1). Nevertheless, there was no difference in calving-to-treatment interval among the CIDR-C, IBD-NC and the

CIDR-NC group and between the IBD and the CIDR protocol group ($P > 0.05$). Logistic regression analysis shows that the calving-to-treatment intervals did not affect the synchronized conception rate between the treatment groups ($\chi^2=3.50$, $P = 0.061$).

There was no significant difference ($P = 0.515$) for the number of cows that were induced to calve and used in this study between the four treatment groups (Table 3.1). Similarly, the number of cows that were induced to calve was not different between the IBD protocol and the CIDR protocol group ($P = 0.365$). Logistic regression analysis shows that induced cows did not effect the synchronized conception rate between the four treatment groups ($\chi^2 = 1.752$, $P < 0.186$).

Retention Rate

The average retention rate for the IBD Onsett12™ was significantly lower than the CIDR (65.12% vs. 99.44%, $\chi^2 = 73.528$, $P = 0.001$, Table 3.2). However, there was no difference in retention rates among the IBD Onsett12™ ($\chi^2 = 1.204$, $P = 0.273$) or the CIDR groups ($\chi^2 = 0.622$, $P = 0.430$) between the cycling and non-cycling groups. Between groups within the herds, the retention rate was significantly different between the IBD Onsett12™ and the CIDR in 3 herds, marginally significant in one herd and no different in another herd. Since fixed-time insemination was the protocol used in this study for the groups using the IBD Onsett12™, cows which did not retain the device ($n = 60$) were excluded from the analyses pertaining to the synchronized conception rates.

Table 3.2 The average retention rate and synchronized conception rate among the treatment groups.

Group	Retention Rate			Synchronized Conception Rate [#]			Total Conception Rate [*]
	Total	Retained	%	Total	Conceived	%	%
IBD-C	65	39	60 ^a	36	6	16.67 ^c	9.23 ^c
CIDR-C	68	68	100 ^b	65	25	38.46 ^d	36.76 ^d
IBD-NC	107	73	68.22 ^a	72	14	19.44 ^c	13.08 ^c
CIDR-NC	110	109	99.09 ^b	108	30	27.78 ^d	27.27 ^d

^{abcd} Indicates means within a column with different letters are significantly different ($\chi^2 = 73.528$, $P < 0.000$ for retention rate, and $\chi^2 = 15.087$, $P < 0.002$ for synchronized conception rate).

[#] Number conceived/Total that retained the IBD Onsett12 or CIDR device (%)

^{*} Number conceived/total treated (%)

Submission Rate

The submission rate for the IBD-C and IBD-NC groups was 100% for those cows that retained the device for the 12-day treatment period. The high submission rate in these groups was due to the fixed-time insemination employed for this protocol. Whereas, the number of cows that were inseminated to the synchronized oestrus for CIDR protocols within 5 days of device removal were 90.77% and 86.32% for CIDR-C and CIDR-NC group respectively (Table 3.3). The submission rate was not different between these two groups ($\chi^2 = 3.03$, $P = 0.582$). More than 80% of the cows were inseminated within 72 hours after the CIDR removal for both treatment groups. However, there was no difference in the number of cows observed in oestrus and inseminated over time within

the 5-day period after CIDR removal between these two groups. The mean time of insemination after CIDR removal was 64 ± 2 hour and 66 ± 2 hour for the CIDR-C and CIDR-NC group respectively ($P = 0.418$). The number of cycling (CIDR-C) and non-cycling cows (CIDR-NC) that conceived from the synchronized oestrus was not different over time within the 5-days after CIDR removal (Table 3.3). The mean time of conception after CIDR removal was 65 ± 3 hour and 66 ± 4 hour for the CIDR-C and CIDR-NC groups respectively ($P > 0.05$).

Synchronized Conception Rate

The synchronized conception rates for the cows that retained the IBD Onset12 or CIDR device were low in all treatment groups (Table 3.2). The synchronized conception rate was highest in the CIDR-C group (38.5%) and lowest in the IBD-C group (16.67%). The synchronized conception rate from the CIDR protocol was significantly higher than the IBD Onset12™ protocol ($\chi^2 = 15.087$, $P = 0.02$, Table 3.2). Nevertheless, there was no difference in synchronized conception rate (16.67% vs. 19.44%, $\chi^2 = 0.123$, $P = 0.726$) between cycling and non-cycling cows using the IBD Onset12 protocol. For the CIDR programs also, the synchronized conception rate was 38.5% in cycling cows and 27.8% in non-cycling cows which was not significantly different ($P = 0.144$) between these groups.

Table 3.3 The reproductive performance for cycling and non-cycling cows treated with CIDR protocols.

Response	Treatment Group		Total n = 173 (%)
	CIDR-C n = 65 (%)	CIDR-NC n = 108 (%)	
No inseminated	59 (90.77)	93 (86.32)	152 (87.86)
Bred at 24 hour	0	1 (0.93)	1 (0.58)
Conceived at 24 hour	0	1 (0.93)	1 (0.58)
Bred at 48 hour	27 (41.54)	37 (34.26)	64 (36.9)
Conceived at 48 hour	9 (13.85)	12 (11.11)	21 (12.14)
Bred at 72 hour	26 (40)	40 (37.04)	66 (38.15)
Conceived at 72 hour	15 (23.08)	13 (12.03)	28 (16.19)
Bred at 96 hour	5 (7.69)	13 (12.03)	18 (10.41)
Conceived at 96 hour	0	2 (1.85)	2 (1.16)
Bred at 120 hour	1 (1.54)	2 (1.85)	3 (1.73)
Conceived at 120 hour	1 (1.54)	2 (1.85)	3 (1.73)
Time at insemination (h, Mean \pm SEM)	64 \pm 2	66 \pm 2	65 \pm 1.5
Time at Conception (h, Mean \pm SEM)	65 \pm 3	66 \pm 4	64 \pm 2
Conception rate ^a	38.46	27.78	31.79

^a Number conceived/total treated (%)

There was no significant difference ($P > 0.05$) in the total conception rates, compared to the synchronized conception rates, for cows treated with the CIDR protocols both in the cycling and non-cycling cows (Table 3.2). However, the total conception rates were much lower in the IBD-C group (9.23%) and IBD-NC group (13.08%), compared with the synchronized conception rates.

DISCUSSION

The present study compared the reproductive performance of cycling and non-cycling cows where treatment varied between the IBD Onsett12 oestrus synchronization program, inseminated at fixed-time, and the CIDR oestrus synchronization program, with cows bred at detected oestrus. The efficacy of the IBD Onsett12 oestrus synchronization program in inducing a fertile synchronized oestrus in cycling and non-cycling dairy cows has not been reported previously. However, it was not possible in the present study to compare the oestrus detection rate between the two oestrus synchronization programs because oestrus was not recorded in cows that were treated with the IBD Onsett12, as they were inseminated at a fixed-time. Nevertheless, the IBD Onsett12 oestrus synchronization program resulted in significantly fewer conceptions in cycling and non-cycling cows compared with the CIDR protocols for these groups. This study supports the conclusion of Roche et al. (1998), that the insemination of animals once, at a specific time, without recourse to oestrous detection, to obtain repeatable high pregnancy rates is a practical but not yet attainable goal in farm animals using hormones.

Based on the results of this study, the device tested in this project can not be recommended for commercial farm use at this stage, as the loss rate of the IBD Onsett12 device for the 12-day treatment period was high (average 34.88%) and furthermore, the synchronized conception rate was also low in cycling or in non-cycling cows (average 18.52%, Table 3.2). Oestrus synchronization programs are only likely to be of relevance on commercial farms if they are safe, easy to administer, effective on the majority of

animals and economically beneficial (Drew, 1978). The low IBD Onsett12 retention rate observed will inherently reduce the synchrony of oestrus and has implications for choosing an oestrus synchronization program. There was no clear explanation for the high loss rate of the IBD Onsett12 device. The high proportion of the devices that was lost may have been due to the improper bonding between the IBD Onsett12 device's attachment and the hair of the cow, or the cows may have endured vaginal irritation which may lead to rubbing and subsequent loss of the device. The causes for the low retention rate require further investigation and testing, as retention rate has critical implications for choosing an oestrus synchronization program.

The IBD Onsett12 oestrus synchronization program with fixed-time insemination induced a fertile synchronized oestrus and conception in some cycling and non-cycling dairy cows but the synchronized conception rate was low (18.52% overall). The low conception rate observed was in compliance with the observation of Roche et al. (1998), which stated that regulation of oestrous cycle to allow farm species to be bred with a single insemination without regard to oestrous behaviour has met with limited success. Previous reviews (Odde, 1990; Larson and Ball, 1992) also noted that there was no oestrus synchronization program, which consistently synchronized oestrus in the lactating dairy cow, allowing for fixed-time insemination. Fixed-timed insemination requires both the functional life span of the corpus luteum to be regulated and follicle wave status to be controlled in all animals, irrespective of their stage of cycle or follicle waves (Roche et al., 1998). The low conception rate obtained using the IBD Onsett12 oestrus synchronization program may be due to the failure of the program to synchronize follicular

wave emergence, and thus the preovulatory follicle was not homogeneous at the end of the treatment period. Yaakub et al. (1998) reported the lack of synchronization of follicular wave emergence with oestradiol and progesterone treatment given to heifers at random stages of the oestrous cycle. The interval from treatment to follicular wave emergence in that study varied from 1 to 7 days. Controlling the time of emergence of a new follicular wave and synchronizing the follicular wave status could improve the synchrony of oestrus and ovulation and ensure that the ovulatory follicle has optimum potential for fertilization (Beal, 1998).

Another possible explanation for the low conception rate may be due to the longer duration of dominance of the preovulatory dominant follicle as result of the 12-day treatment period given to cows at random stages of the oestrous cycle. Therefore, the preovulatory dominant follicles may be at different stages of growth and dominance at the end of the treatment period. It was reported that the duration of dominance of the preovulatory dominant follicle affects pregnancy rate in progesterone-treated cattle (Stock and Fortune, 1993; Mihm et al., 1994; Cooperative Regional Research Project NE-161, 1996). Restricting the duration of dominance of the preovulatory follicle to 4 days resulted in a pregnancy rate of more than 70% in cyclic beef heifers (Austin et al., 1999). In addition, Mihm et al. (1994) reported a 30% decrease in pregnancy rate when the duration of dominance of the ovulatory follicle was increased from 4.1 to 8.6 days, and no pregnancies resulted when duration of dominance was extended beyond 10 days. Reductions in fertility of 26 to 40% were also reported following progestogen-induced

extended dominance of the first dominant follicle when compared to controls (Sanchez et al., 1993; Savio et al., 1993b; Cooperative Regional Research Project NE-161, 1996).

The synchronized conception rate was low in cycling cows following treatment with the IBD Onsett12 protocol as compared to a previous report (16.67% vs. 51.2%), using the same hormone treatment regime administered using different devices and with a fixed-time insemination (Xu and Burton, 1999). There was no clear explanation for the observed difference in conception rate. There is a possibility that the IBD Onsett12 may have failed to deliver the hormones at the appropriate treatment time, arising from the complexity of the device (Rathbone et al., 1998; Chapter 2) and, thus, it failed to synchronize the oestrus and ovulation. Nevertheless, in another study, using a similar hormone treatment regime, the conception rate was slightly improved (52.9%) when cows were inseminated at detected oestrus (Xu et al., 1995b). Therefore, the conception rate of the IBD Onsett12 oestrus synchronization program could possibly be improved by inseminating cows at detected oestrus rather than by using fixed-time insemination.

Anoestrous cows, which were not ovulating, could not be synchronized reliably (Mauleon, 1974; Odde, 1990). These anoestrous cows were more likely to exhibit behavioural oestrus after they had been injected with a low dose of oestradiol benzoate given 24 to 48 hours after a 5-day period of progesterone priming (McDougall et al., 1992). Macmillan et al. (1994) reported that 90% of anoestrous cows treated with progesterone and then injected with 1 mg oestradiol benzoate could be detected in oestrus and inseminated within a period of 4 days. Therefore, the low synchronized conception

rate (19.44%) following oestrus synchronization program with the IBD Onset¹² oestrus synchronization program in the non-cycling cows may be due to an inappropriate timing of insemination. The conception rate probably could be improved if the cows were inseminated at detected oestrus, although possibly fewer cows would be inseminated, and the proportion pregnant of those treated may be reduced.

During the first 5 days after CIDR removal, the numbers of cows observed in oestrus and inseminated was comparable to those reported from the previous studies in cycling cows (Armer et al., 1993; Macmillan et al., 1993; Xu et al., 1996; Lammoglia et al., 1998), and in non-cycling dairy cows (McDougall et al., 1992; Fike et al., 1997; Lammoglia et al., 1998; Verkerk et al., 1998). However, the synchronized conception rate in cycling cows (CIDR-C group) was lower (38.46% vs. 52.9%) than reported previously using the same oestrus synchronization program (Xu et al., 1996), but in comparable (33% to 42%) with the findings of others (Armer et al. 1993; Lammoglia et al. 1998). It was reported that prostaglandin treatment at or before the termination of progesterone treatment for 7- to 9-days increased the synchrony of oestrus and fertility was normal (Roche, 1976b). Also in beef heifers, after treatment with a norgestomet implant for 7 days and prostaglandin on day 6 or 7 after implantation, 93% showed oestrus within 5 days, and the pregnancy rate was 62%, which was similar to the 60% pregnancy rate observed in controls (Heersche et al., 1979). In contrast, an 8-day CIDR plus prostaglandin treatment on the day before CIDR removal was associated with a reduced pregnancy rate (Ryan et al., 1995b; Xu et al., 1996). In addition, short-term progesterone treatment beginning later than day 13 of the oestrous cycle was also associated with reduced fertility (Beal et al., 1988; Xu et al.,

1996). In the present study, the synchrony of oestrus was high but with a reduction in synchronized conception rate. The cause of the reduction in fertility was not known and could be due to persistence of dominant follicle in cows that were in the late stages of their cycles when treatment was initiated, or the treatment was initiated later than day 13 of the oestrous cycle. It has been shown that the progesterone concentrations maintained by the CIDR in the absence of a functional corpus luteum resulted in persistence of dominant follicle and this is associated with a reductions in conception rate to ovulation from these persistent dominant follicles (Savio et al., 1993b; Stock and Fortune, 1993).

Despite the high oestrous synchrony (86.32%) in the non-cycling cows, the synchronized conception rate to first insemination following the CIDR oestrus synchronization program was lower (27.78%) than reported previously (Macmillan et al., 1995; Xu and Burton, 1997; Verkerk et al., 1998). The high oestrous response may have been associated with a false oestrus. However, Rhodes et al. (1999) demonstrated that less than 10% of previously anoestrous cows displaying oestrus after treatment had not ovulated after treatment with progesterone and oestradiol benzoate. Therefore, the low conception rate recorded would be expected to be associated only to a limited extent with a failure of ovulation.

CONCLUSION

The present study demonstrated that the IBD Onsett12 oestrus synchronization program was effective in inducing a fertile synchronized oestrus in some cycling and non-cycling

cows, but resulted in a low synchronized conception rate compared to the CIDR oestrus synchronization program. The causes for the low retention rate and low synchronized conception rate require further investigation. The IBD Onset12 oestrus synchronization program has potential for commercial use, as the device was safe, easy to administer and has the potential for artificial insemination of a large proportion of the herd at the same time. The single application of the device will reduce the cost for veterinary visits and reduce the need for repeatedly handling cattle to treat them. These factors would be beneficial for farmers.

CHAPTER 4

EVALUATION OF A NOVEL OESTRUS SYNCHRONIZATION PROGRAM IN CATTLE USING OESTRADIOL BENZOATE, PROGESTERONE AND PROSTAGLANDIN F_{2α}

Abstract

This study was designed to control both the follicular development and luteal function in cows in order to achieve synchronized oestrus and ovulation. Twenty randomly cycling, non-lactating dairy cows were randomly assigned to two treatments; 1) 2 mg oestradiol benzoate injected intramuscularly and 200 mg of progesterone subcutaneously, 9 days before prostaglandin (500 μ g cloprostenol) and a second injection of 1 mg oestradiol benzoate 24 hours after prostaglandin treatment (ODB, $n = 10$). 2) 10 μ g buserelin injected 7 days before prostaglandin (500 μ g cloprostenol) and a second injection of 10 μ g buserelin 48 hours after prostaglandin treatment (GnRH, $n = 10$). The ovaries were examined using 8 MHz trans-rectal ultrasonography from day -5 until ovulation. Cows were observed for oestrus, three times daily for 7 days after the prostaglandin treatment. Blood sera collected were analysed for progesterone, FSH and LH. The proportion of cows that were observed in oestrus was higher in the ODB group than the GnRH group (100% vs. 55.6%, $\chi^2 = 5.630$, $P = 0.018$). Acute treatment with progesterone and oestradiol benzoate or buserelin was effective in inducing atresia of the dominant follicle and a new follicular wave emerged earlier in the GnRH group than the ODB group (2.22 ± 0.15 vs. 3.60 ± 0.22 days, $P = 0.001$). There was no difference in the variation of the follicular wave emergence around the mean between the ODB and the GnRH group ($P > 0.05$). There was no difference in the size of the largest follicles between the ODB and the GnRH group at initial treatment (10.77 ± 1.38 vs. 11.10 ± 0.81 , $P = 0.845$), at prostaglandin treatment (13.66 ± 0.69 vs. 13.97 ± 1.25 , $P = 0.824$) and at ovulation (15.41 ± 0.66 vs. 17.57 ± 1.07 , $P = 0.097$) respectively. The LH surge occurred earlier

after the second buserelin than the second oestradiol benzoate treatment (4.0 ± 1.0 vs. 22.80 ± 1.20 hour, $P = 0.001$). The mean time of ovulation after the second oestradiol benzoate or buserelin treatment was not significantly different between the ODB and the GnRH group (1.70 ± 0.30 vs. 1.56 ± 0.18 , $P = 0.692$). Treatment with either the ODB regimen or the GnRH regimen used, can influence the pattern of follicular development and ovulation in cattle, however, the mechanism of action of the two treatments was different. The mean time to of synchronized ovulation was not different between groups but the ODB group was more variable than the GnRH group. However, the ODB group had a high oestrus occurrence.

INTRODUCTION

The timing of the onset of oestrus can be synchronized by controlling the length of the oestrous cycle. The approaches for controlling cycle length are either by regressing the corpus luteum before the time of natural luteolysis or by administering exogenous progestins to delay the time of oestrus following natural or induced luteolysis (Beal, 1998). However, abbreviation or prolongation of the oestrous cycle length results in a wide range of oestrus synchrony and ovulation response (Adams, 1998). These variations were due to the follicular wave status at the time of treatment (Adams, 1998). Trans-rectal ultrasonography on bovine ovaries has allowed a better understanding of follicular development (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Ginther et al., 1989b). Methods of interrupting or manipulating follicular growth and controlling ovulation have been developed by administration of exogenous oestrogen (Rajamahendran and Manikkam, 1994; Bo et al., 1993; Bo et al., 1994; Bo et al., 1995;

Cavalieri et al., 1997), progesterone (Anderson and Day, 1994; Rajamahendran and Manikkam, 1994), or gonadotrophin-releasing hormone (Macmillan and Thatcher, 1991; Wolfenson et al., 1994; Schmitt et al., 1996). Manipulation of follicular development and control of cycle length provide the possibility of precise oestrus synchrony and control of the time of ovulation more exactly, to permit fixed-time insemination without the need for detection of behavioural oestrus (Adams, 1998; Beal, 1998).

Anderson and Day (1994) showed that a single injection of progesterone caused a new follicular wave to emerge within 4 days after the progesterone treatment. Progesterone suppressed LH pulse frequency (Savio et al., 1993b; Stock and Fortune, 1993) which in turn induced the suppression of growth of the dominant follicle (Adams et al., 1992a; Sirois and Fortune 1990). Progesterone, when given in combination with oestradiol, has a more profound suppressing effect on growth of the dominant follicle (Bo et al., 1995). The combination treatment of progesterone and oestradiol suppressed LH and was followed by the atresia of large follicles in a variety of stages of follicular growth and dominance (Burke et al., 1996; Murray et al., 1998; Rajamahendran and Manikkam, 1994).

The current study was undertaken to test the hypothesis that acute treatment with progesterone and oestradiol benzoate will suppress LH release and induce the atresia of large follicles. It was hypothesized that a new follicular wave would emerge 4-5 days later in order to increase the likelihood for a dominant follicle to be present at a similar stage of growth in all treated cows at the time of prostaglandin injection. It was predicted

that prostaglandin treatment, given 9 days after the initial oestradiol and progesterone treatment, would lyse any corpus luteum that was present. Thus, the newly formed dominant follicle present at the time of luteolysis would be suitable for ovulation. It was postulated that the time of prostaglandin treatment would be at the stage where the dominant follicles were at the late growing or early static phase. Previous reports have shown that treatment with prostaglandin when the dominant follicle was in its late growing or early static phase resulted in ovulation within 2 to 3 days (Kastelic and Ginther, 1991; Kastelic et al., 1990; Savio et al., 1990), whereas treatment after the mid- to late-static phase resulted in ovulation of the dominant follicle of the next wave 4 to 5 days after treatment (Kastelic and Ginther, 1991; Kastelic et al., 1990; Savio et al., 1990). Oestradiol benzoate administered 24 hours after prostaglandin treatment has been previously shown to reduce the interval to oestrus (Lammoglia et al., 1998; Ryan et al., 1995b), and reduce the variation in the interval to ovulation by inducing an LH release.

This novel oestrus synchronization program was designed to control both the luteal duration and follicular development. The potential advantages of this program, if it can be developed to be effective, are a reduction in cost by eliminating the use of progesterone-releasing device and, thus, preventing progesterone residues in the cows and the environment. As progesterone was administered as a single injection, this program utilized the endogenous progesterone supply in the presence of corpus luteum where present, to prevent oestrus and ovulation until the time of induced luteolysis. In the absence of corpus luteum, the regime relied on the absence of a follicle at a suitable stage of development to prevent cows from coming into oestrus and ovulating. This program

was also designed as a potential alternative treatment regime using the IBD Onsett12™ (Plade Holdings Limited, Hamilton, New Zealand), as each of the hormones used in this regime are present in the IBD Onsett12™. The IBD Onsett12™ was designed as a single application intravaginal drug delivery device.

The aim of this study was to determine the effects on follicular atresia, follicular wave emergence, and the occurrence of oestrus and ovulation of an oestrus synchronization program involving oestradiol, progesterone and prostaglandin treatments, and to compare this with a program that recommends utilizing fixed-time insemination (Pursley et al., 1997).

MATERIALS AND METHODS

Fourty cows, grazed at the Large Animal Teaching Unit, Massey University, were observed for oestrus for about 21 days and cows that were observed in oestrus over the period were examined rectally by an experienced veterinarian one day prior to the first treatment. The cows were classified as cycling when a palpable corpus luteum was found on the ovaries or when there were genital findings consistent with recent or impending ovulation. Twenty non-lactating Friesian or Friesian-cross cows with recorded oestrus in the preceeding three weeks, no palpable abnormality and at the various stages of oestrous cycle were used. The study design was a completely randomized block experiment. There were two treatments:

- 1) 2 mg oestradiol benzoate (Oestradiol Benzoate™, Chemavet Division Pharmaco (NZ) Ltd., Auckland, New Zealand) was injected intramuscularly and 200 mg of progesterone (Progestin™, Chemavet Division Pharmaco (NZ) Ltd., Auckland, New Zealand) was injected subcutaneously 9 days before prostaglandin treatment (500 µg sodium cloprostenol, estroPLAN®, Parnell Laboratories New Zealand Ltd., East Tamaki, New Zealand) was injected intramuscularly, followed by a second intramuscular injection of 1 mg oestradiol benzoate 24 hours after prostaglandin treatment (ODB, n = 10),
- 2) The cows were treated according to Ovsynch protocols (Pursley et al., 1997a; Pursley et al., 1997b), in which 10 µg buserelin (Receptal®, Bomac Laboratories Ltd., Auckland, New Zealand) was injected intramuscularly 7 days before prostaglandin treatment (500 µg sodium cloprostenol, estroPLAN®, Parnell Laboratories New Zealand Ltd., East Tamaki, New Zealand) and second injection of 10 µg buserelin was given 48 hours after prostaglandin treatment (GnRH, n = 10).

Subcutaneous and intramuscular injections were applied in the cranial part of the neck. Tail heads were painted as an aid for oestrus detection. Cows were observed for oestrus, thrice daily at 0800 hours, 1200 hours and 1700 hours for 30 minutes for each oestrus observation for 7 days after the prostaglandin treatments in both treatment groups. Any cow which was observed to stand to be ridden by another cow (Williamson et al., 1972) or which had more than 75% of its tail paint removed was defined as being in oestrus. The time of oestradiol benzoate and progesterone or first buserelin treatments was designated as day 0.

Figure 4.1 Diagram depicting the sequence of experimental procedures.

ODB Group



GnRH Group



Blood Collection and Hormone Assays

Blood samples were collected by jugular venipuncture into lithium heparin tubes (Vacutainers; Becton-Dickson, Rutherford, NJ) daily from three days prior to treatment until the second oestrogen or buserelin treatment, and then every six hours for 36 hours after the second oestrogen or buserelin treatment. Beyond this 36 hours, blood was collected daily until ovulation. Within 30 minutes of blood collection, the blood samples were placed in refrigeration and then plasma was harvested and stored at -20°C before subsequent analysis for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P₄).

Concentrations of LH and FSH were measured in plasma using double antibody radioimmunoassays that were modified from methods described for the assay of ovine gonadotrophins (Parkinson and Follet, 1994, Chapter 2). All samples were processed in duplicate in one assay which had inter- and intra-assay coefficients of variation of 19.1% and 9.46% for LH and 13.89% and 7.88% for FSH, respectively.

Concentrations of progesterone in plasma were determined using commercial enzymeimmunoassay kits (Ridgeway Science Limited, Gloucestershire, UK). All samples were processed in duplicate in one assay that had intra- and interassay coefficients of variation of 13.61% and 10.19% respectively. In addition, for validation, 15 samples were assayed in duplicate samples using radioimmunoassay methods.

Ultrasonography

The ovaries of each animal were examined by transrectal ultrasonography using an ultrasound with an 8.0 MHz linear array (Scanner 100LC, Pie Medical Equipment B.V., Maastricht, Holland) daily from day -5 until three days after detected oestrus to record ovulation. Daily ultrasound examinations were recorded on videotape for subsequent analysis. The tape was subsequently reviewed with video recorder (Panasonic NV-HS880 G-mode VHS, Japan) on a high-resolution colour monitor (Panasonic MT-M2080, Japan). Follicles were measured with a ruler calibrated against the scale provided by the ultrasound unit. The examinations were made to identify and monitor the dominant and

subordinate follicles and to detect the day of follicular wave emergence and confirm the occurrence of ovulation.

A new wave of follicular development always began before regression of the dominant follicle had been detected. The day of emergence of the ovulatory follicle was defined, retrospectively, as the day on which the ovulatory follicle was first detected at a size of > 5 mm in diameter. In addition, ovulation is defined as the sudden disappearance of an ovarian follicle greater than 8.0 mm in diameter (Cavalieri et al., 1997).

Statistical Analysis

Data analysis was performed using GLM and FREQ procedures of SAS (1996). Analysis of variance (ANOVA) was used to compare the means on: (i) time from second treatment of oestradiol benzoate or buserelin to onset of oestrus, (ii) time from second treatment of oestradiol benzoate or buserelin to ovulation, (iii) interval from oestrus to ovulation, (iv) day of emergence of the ovulatory follicle. Follicular dynamics were compared by ANOVA and differences in means were compared for the equality of two variances using Fisher's exact test. Chi-Square analysis was used to compare the occurrence of oestrus between the two treatments groups. Plasma hormones (FSH, LH, and P₄) concentrations were analyzed for effects of group, day and group-by-day interaction using repeated measures analysis of variance.

RESULTS

One cow (GnRH group) developed multiple follicular cysts two days after the first buserelin treatment and this cow was excluded from data analyses pertaining to follicle growth and endocrine concentrations.

Oestrus detection and ovulation rates

The proportion of cows that were observed in oestrus was higher in the ODB group than the GnRH group (100% vs. 55.6%, Table 4.1, $\chi^2 = 5.630$, $P = 0.018$). Nevertheless, the observed synchronized oestrus among the cows in the GnRH group was tighter and less variable compared to the ODB group. Oestrus that was observed in the GnRH group cows occurred 2 days after the prostaglandin treatment. These cows were observed in oestrus within 12-hour period after the second treatment of gonadotrophin. In the ODB group, three cows were in oestrus 1 day before the prostaglandin treatment and another two cows were in oestrus 12 hours after the prostaglandin treatment. However, three of these five cows were again observed in oestrus together with the other five cows, 2 days after the prostaglandin treatment. Of the five cows in the ODB group that were observed in oestrus either a day before or on the same day of the prostaglandin treatment, one cow was at metoestrous and two cows each were at the dioestrous and proestrous stages of the oestrous cycle at the time of the progesterone and oestradiol benzoate treatment (day 0).

Table 4.1 The oestrus detection rate and the time of ovulation for the ODB and GnRH groups.

		Days relative to treatment	
Response	%	Mean \pm SEM (days)	Range (days)
Oestrus detection rate [#] :			
ODB	100 [*]	0.7 \pm 0.45 ^{**}	-1 to 2
GnRH	55.6	2.0 \pm 0	0
Day at ovulation ^{##} :			
ODB	100	1.70 \pm 0.30	1 to 4
GnRH	100	1.56 \pm 0.18	1 to 3

* Indicates the percentage within a column was significantly different ($\chi^2 = 5.630$, $P = 0.018$)

** Oestrus that was observed for the second time in three cows were not included in the calculation of the mean.

Days relative to prostaglandin treatment

Days relative to second oestradiol benzoate or buserelin treatment

In the ODB group, two cows that were observed in oestrus one day prior to the prostaglandin treatment ovulated two days after prostaglandin treatment, whereas the other cow ovulated one day after the prostaglandin treatment. Furthermore, the two cows that were observed in oestrus on the day of the prostaglandin treatment ovulated two days after the prostaglandin treatment. Nevertheless, the mean time of ovulation after the second oestradiol benzoate or buserelin treatment was not significantly different between the ODB and the GnRH group (1.70 \pm 0.30 vs. 1.56 \pm 0.18, $P = 0.692$).

Follicle growth

Based on the oestrus detection record and trans-rectal examination of ovaries prior to the initial treatment, there was no difference in the proportion of cows according to the stage of oestrous cycle at the time of the initial treatment (day 0) between the groups ($\chi^2 = 0.281$, $P = 0.869$). There were 2, 5, and 3 cows (ODB group) and 2, 4, and 3 cows (GnRH) at the metoestrous, dioestrous, and proestrous stage of the oestrous cycle respectively.

Table 4.2 Size of the largest follicle at first treatment, PGF treatment, ovulation and the interval from progesterone and oestradiol benzoate or buserelin treatment to the emergence of the next follicular wave.

	Treatment group	
	ODB	GnRH
Size of follicle (mm)		
At treatment (day 0)	10.77 ± 1.38	11.10 ± 0.81
At PGF treatment	13.66 ± 0.69	13.97 ± 1.25
At ovulation	15.41 ± 0.66	17.57 ± 1.07
Day of follicular wave emergence (mean ± SEM)		
	3.60 ± 0.22	2.22 ± 0.15**

** Indicates the mean within a row was significantly different ($P = 0.001$)

26

Acute treatment with progesterone and oestradiol benzoate was effective in inducing suppression or atresia of the dominant follicle. A new follicular wave emerged, on average, 3.60 ± 0.22 days later (Table 4.2). However, the mean time of follicular wave emergence was significantly earlier in the GnRH group than the ODB group (2.22 ± 0.15

vs. 3.60 ± 0.22 days, $P = 0.001$). There was no difference in the time of follicular wave emergence ($P > 0.05$) between the two groups, and the variation in time to emergence was also similar. In the GnRH group, the buserelin treatment (day 0) induced ovulation of the largest follicle in four cows (44.44%) while in another five cows, the buserelin treatment induced atresia of the largest follicles. The largest follicles that underwent ovulation in the four cows were at the growing stage of follicular growth at the time of buserelin treatment. The size of follicle that underwent ovulation, on average, was 11.25 mm. Follicles that underwent atresia were at early (2 cows), late (1 cow) and regressing (2 cows) stages of follicular development respectively. In the ODB group, 5, 3 and 2 cows were at the early growing, late growing and regressing stage of follicular growth respectively at the time of progesterone and oestradiol treatment (day 0). There was no difference in size of the largest follicles between the ODB and the GnRH group at initial treatment (10.77 ± 1.38 vs. 11.10 ± 0.81 , $P = 0.845$), at prostaglandin treatment (13.66 ± 0.69 vs. 13.97 ± 1.25 , $P = 0.824$) and at ovulation (15.41 ± 0.66 vs. 17.57 ± 1.07 , $P = 0.097$) respectively.

Hormonal profiles

For the purpose of statistical analysis, changes in FSH and LH concentrations over time were divided into two periods; i) days after the progesterone and oestradiol benzoate or buserelin treatment and ii) days after the prostaglandin treatment. The plasma FSH and LH concentrations in the ODB group were normalized to day 7 of the treatment period for comparing the interaction between the treatment groups.

FSH and LH concentrations

There was a significant effect of day ($P = 0.004$) and group-by-day ($P = 0.0001$) but no group ($P = 0.528$) interaction on plasma FSH concentrations. The mean FSH concentrations increased within 24 hour after treatment with buserelin and then gradually declined thereafter (Figure 4.2). In contrast, acute treatment with progesterone and oestradiol benzoate decreased the mean FSH concentrations for the next 24 hours which then gradually increased and reached a high amplitude three days after treatment. The mean FSH concentrations between the treatment group was statistically difference on day 1 ($P = 0.002$) and day 3 ($P = 0.012$) after the treatment but no significance difference occurred at the other days.

There was a significant effect of day ($P = 0.004$) but no group ($P = 0.65$) or group-by-day ($P = 0.189$) interaction on the plasma FSH concentrations after the prostaglandin treatment (Figure 4.3). The mean plasma FSH concentration was higher in the ODB group at the time of prostaglandin treatment ($P = 0.001$) and at one day after the prostaglandin treatment ($P = 0.05$).

Figure 4.2 The mean plasma FSH concentrations in cows treated with progesterone and oestradiol benzoate or buserelin treatment at day 0. Arrow indicates the time of treatment with either progesterone and oestradiol benzoate or buserelin.

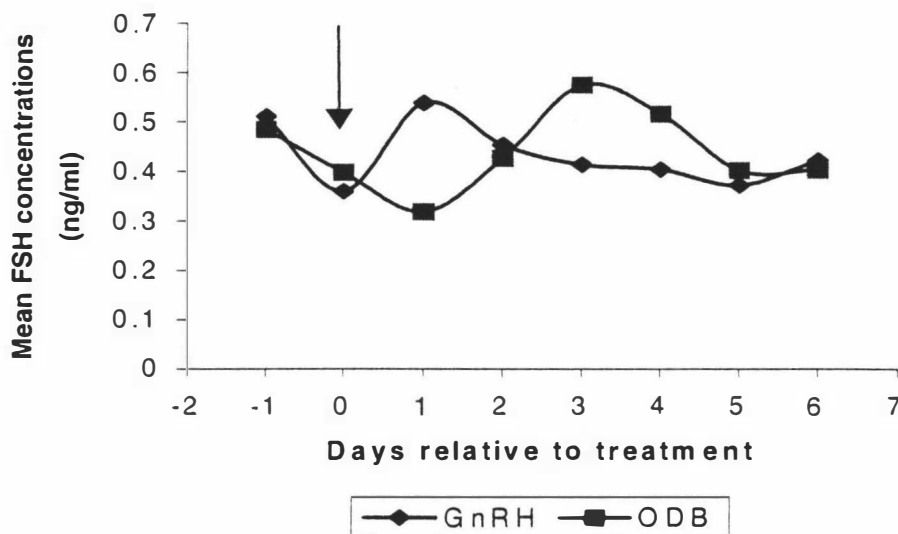
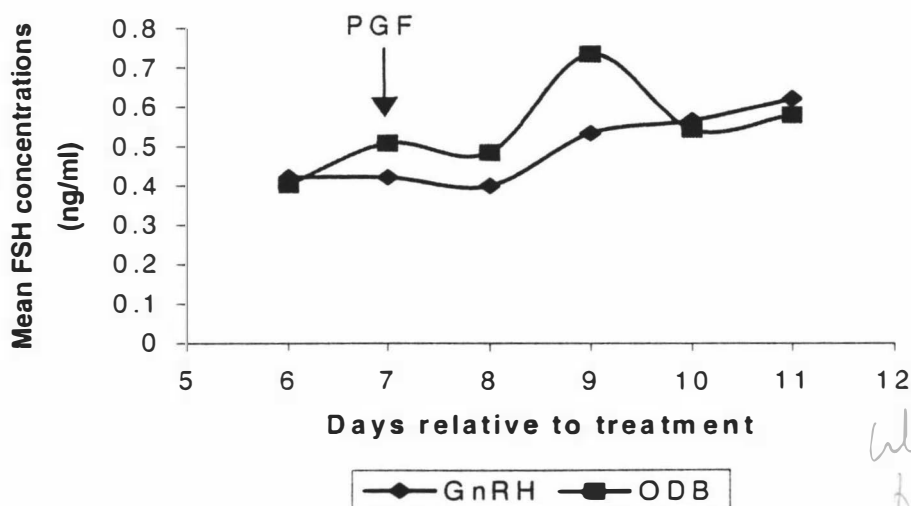


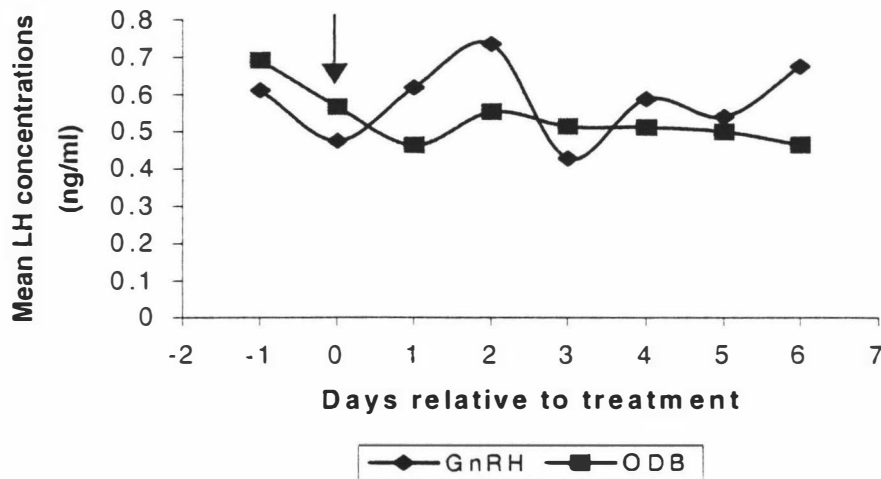
Figure 4.3 The mean plasma FSH concentrations in cows after treatment with prostaglandin, where the plasma FSH concentration in the ODB group was normalized to day 7 of the treatment period.



When was treatment?
Day 2 or day 7?

There was no significant effect of day ($P = 0.227$) and group ($P = 0.148$) but there was a significant difference on group-by-day ($P = 0.043$) interaction on plasma LH concentrations (Figure 4.4). Acute treatment with progesterone and oestradiol benzoate (ODB group) resulted in a decrease in LH concentration on day 1, while treatment with buserelin (GnRH) resulted in a gradual increase in LH concentration on day 1 ($P = 0.029$) and day 2 ($P = 0.197$).

Figure 4.4 Profiles of mean LH concentrations in heifers treated with acute progesterone and oestradiol benzoate or buserelin on day 0. Arrow indicates the time of treatment with either progesterone and oestradiol benzoate or buserelin.



There was no significant effect of group ($P = 0.703$), day ($P = 0.299$) and group-by-day ($P = 0.727$) interaction on mean LH concentrations after the prostaglandin treatment. The average time of the LH surge was similar in both groups, which occurred, on average, two days after the prostaglandin treatment (Figure 4.5).

Figure 4.5 Profiles of the mean LH concentration in cows after the prostaglandin treatment where, the mean LH concentration in the ODB group was normalized to day 7 of the treatment period.

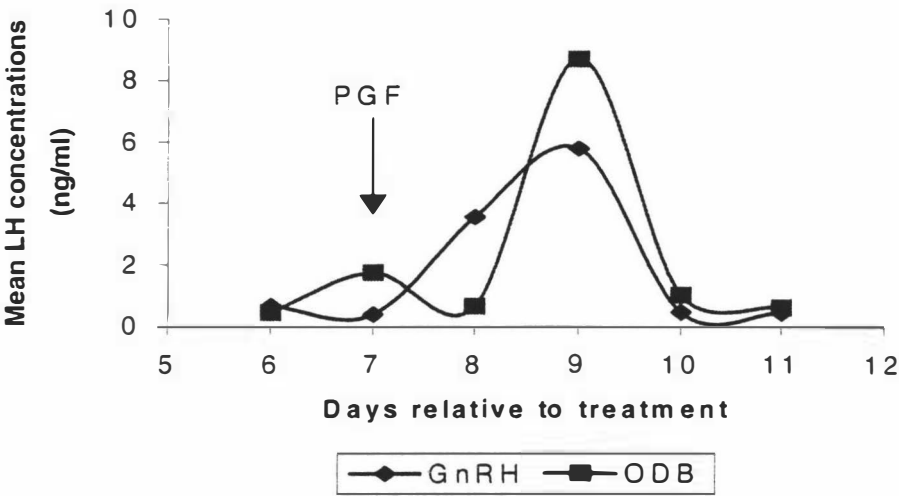
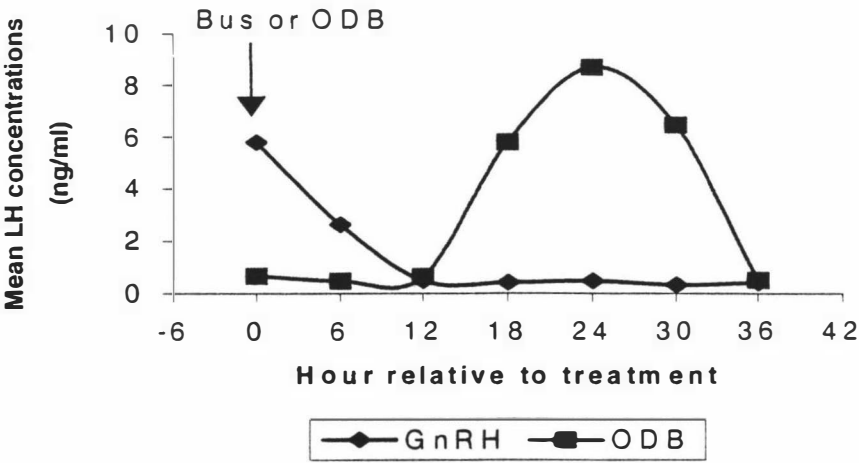


Figure 4.6 The mean LH concentration in cows treated with 1 mg oestradiol benzoate (ODB group) one day after prostaglandin treatment and in cows treated with buserelin two days after prostaglandin treatment.

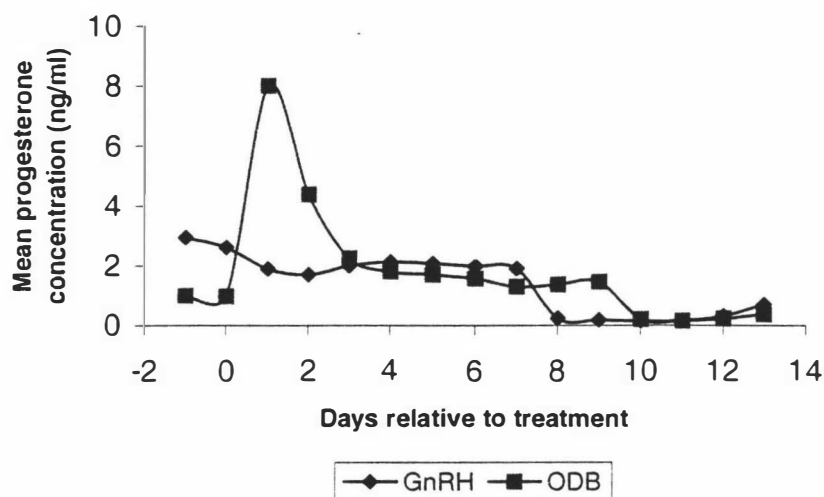


A significant group-by-hour ($P = 0.034$) and group ($P = 0.05$) interaction was detected for plasma LH concentrations (Figure 4.6) in cows treated with a second injection of 1 mg oestradiol benzoate or buserelin after the prostaglandin treatment. At the time of treatment with 1 mg oestradiol benzoate (ODB group), the cows have a low mean LH concentrations ($P = 0.003$) compared to cows in the GnRH group. The LH concentration increased 12 hours after treatment and a LH surge occurred at 22.80 ± 1.20 hours after treatment with a second injection of oestradiol benzoate. In contrast, the cows in the GnRH group had a LH surge at 4.0 ± 1.0 hour after the second buserelin treatment and the concentration decreased gradually to basal levels thereafter. The mean LH concentrations were significantly higher in the GnRH group at 0 ($P = 0.003$) and 6 hour ($P = 0.01$), but higher in cows in the ODB group at 18 ($P = 0.004$), 24 ($P = 0.006$) and 30 hour ($P = 0.036$).

Progesterone concentrations

There was no significant effect of group ($P = 0.139$) and day ($P = 0.099$) but there was a significant effect of group-by-day interaction ($P = 0.0001$) on plasma progesterone concentrations in cows between the ODB and the GnRH group (Figure 4.6). Acute treatment with progesterone and oestradiol benzoate (ODB group) significantly elevated the plasma progesterone concentration in all cows between day 1 and day 2 ($P = 0.0001$). On day 0, cows that were in the metoestrous (2 cows) and proestrous (3 cows) stages of the oestrous cycle had progesterone concentrations less than 1 ng/ml. Two of these metoestrous cows had progesterone concentrations less than 1 ng/ml from day 3 onward.

Figure 4.7 The mean plasma progesterone concentrations in the ODB and GnRH groups of cows, where prostaglandin treatment was administered on day 7 in the GnRH group and on day 9 in the ODB group.



While the two proestrous cows had progesterone concentrations above 1 ng/ml between day 1 and 4 or 6 they then declined to less than 1 ng/ml on days 5 and 7 respectively. In addition, one cow in the dioestrus stage of the oestrous cycle had a luteolytic fall in progesterone on day 7 of the treatment period. In the GnRH group, cows that were at the metoestrous (2 cows) and proestrous (3 cows) stage of the oestrous cycle had progesterone concentrations less than 1 ng/ml at day 0. Subsequently both of the metoestrous cows had progesterone concentrations more than 1 ng/ml from day 2 onward. The progesterone concentrations increased gradually in the proestrous cows and the concentration at day 7 was between 1 and 2 ng/ml. After prostaglandin treatment on day 7 (GnRH group) or on day 9 (ODB group), all cows had progesterone concentrations less than 1 ng/ml on day 8 or 10 respectively, except the two metoestrous cows in the

ODB group which had had progesterone concentrations less than 1 ng/ml from day 3 onward.

DISCUSSION

The present study demonstrated that the use of acute progesterone and oestradiol benzoate treatment in cows at random stages of the oestrous cycle, was effective in inducing atresia or suppression of large follicles and consequently induced a new follicular wave to emerge, on average, 3.60 ± 0.22 days later (Table 4.2). This result shows that even in the absence of chronic progesterone, treatment with short-acting progesterone and acute oestradiol treatment, at random stages of the oestrous cycle, induced atresia of dominant follicles and caused the emergence of a new follicular wave at 4.3 ± 0.2 days later (Adams, 1998; Bo et al., 1995; Cavalieri et al., 1997). In addition, in heifers implanted with norgestomet for 17 days, progesterone and oestradiol treatment on day 10 resulted in the emergence of a new follicular wave approximately 4.5 days later which followed the atresia of large follicles in a variety of stages of follicular growth and dominance (Murray et al., 1998). The day of follicular wave emergence corresponded to the day FSH concentrations increased, on day 3 (Figure 4.2). It was reported that a periodic surge in circulating concentrations of FSH was responsible for eliciting the emergence of a follicular wave (Adams et al., 1992a; Badinga et al., 1992; Bergfelt et al., 1997).

Synchronization of follicular emergence makes it possible for preovulatory follicles at a similar stage of growth to occur and these were responsive to an LH surge induced by the second oestradiol benzoate injection. The results show that 1 mg oestradiol benzoate administered 24 hours after prostaglandin injection was effective in inducing a preovulatory LH surge which occurred, on average, 22.80 ± 1.20 hour after the oestradiol benzoate treatment (Figure 4.6) and lead to ovulation. The present study shows that the time of ovulation was, on average, 1.70 ± 0.30 days after the second oestradiol benzoate treatment. It had been shown previously that oestradiol benzoate, administered 24 hour after prostaglandin treatment, had reduced the interval between oestrus and ovulation (Lammoglia et al., 1998; Ryan et al., 1995b). Previous reports demonstrated that treatment with oestradiol benzoate alone, 24 hours after the removal of progesterone-releasing device caused 100% of the cows to ovulate within a 36-hour period (Fike et al., 1997; Johnson et al., 1997; Lane et al., 1999; Wiltbank et al., 1971), which is a comparable result to the present study.

In the GnRH group, the present study shows that buserelin treatment either induced ovulation or luteinized the largest follicle in cows at random stages of the oestrous cycle and a new follicular wave was recruited 2.22 ± 0.15 days later. This is in agreement with previous findings (Macmillan and Thatcher, 1991; Pursley et al., 1995; Thatcher et al., 1993; Twagiramungu et al., 1995). In the present study, the proportion of the largest follicles that underwent ovulation was 44.44% and these follicles were at the growing stage of follicular development. This proportion is lower than was reported in previous studies (Pursley et al., 1995; Vasconcelos et al., 1997), but is in agreement with the

observation that ovulation of the largest follicle occurred when a dominant follicle was in its growing phase (Prescott et al., 1992; Silcox et al., 1993). Following the second buserelin treatment, a LH surge occurred on average at 4.0 ± 1.0 hour and ovulation occurred, on average, at 1.56 ± 0.18 days. The time of ovulation in this study was comparable to previous reports (Peters et al., 1999; Pursley et al., 1995; Taponen et al., 1999).

Following luteolysis in normal oestrous cycles, the maturing dominant follicle develops increased numbers of LH receptors on granulosa cells, resulting in increased oestradiol concentrations that stimulate a further increase in GnRH release (Hansel and Convey, 1983; Moenter et al., 1991), which induces the gonadotrophin (LH) surge and ovulation. However in the present study, acute progesterone and oestradiol benzoate (3 cows) or buserelin (3 cows) treatment in cows at the proestrous stage of oestrous cycle, induced atresia of the large follicle and prevented oestrus, the natural LH surge and ovulation in these cows. The demise of the large follicle probably reduced oestradiol production and prevented oestrus in these cows. Furthermore, the reduction in oestradiol production probably prevented the GnRH pulse frequency that was required for a LH surge and ovulation.

However, two of these proestrous cows in the ODB group had a luteolytic fall in progesterone on day 5 and day 7 of the treatment period and both of these cows were observed in oestrus one day before the time of prostaglandin treatment. In addition, one cow in the dioestrous stage of the oestrous cycle on day 0 had fall in progesterone

concentration on day 7 and this cow was observed in oestrus at the time of prostaglandin treatment. The cause for luteolytic falls in progesterone on day 5 and 7 in these cows was unknown, but was likely to be a result of normal regression process. Nevertheless, both of these proestrous cows were again observed in oestrus one day after the second oestradiol benzoate treatment. The second oestrus observed in these cows was probably associated with an oestrus induced due to the second injection of oestradiol benzoate. The cow that had luteolytic fall in progesterone on day 5 ovulated on the day of the prostaglandin treatment, whereas the other cow ovulated two days after prostaglandin treatment. Furthermore, two metoestrous cows that had progesterone concentrations less than 1 ng/ml from day 3 onward were observed in oestrus one at one day prior to prostaglandin treatment and the other at the time of prostaglandin treatment. This demonstrates the large variation in oestrus response for these five cows.

The proportion of cows in the ODB group that were observed in oestrus was higher than in the GnRH group. The protocol used in the GnRH group was directed at synchronizing the time of ovulation but not at synchronizing oestrus (Pursley et al., 1995; Pursley et al., 1996), therefore the low proportion of cows observed in oestrus was expected. Nevertheless, the proportion of cows observed in this study was higher (55.6% vs. 7%) than reported previously (Stevenson et al., 1996; Stevenson et al., 1999). In the ODB group, 8 cows were observed in oestrus after the second oestradiol benzoate injection given 24 hour after prostaglandin. However, this includes a repeat oestrus in the three cows that were observed in oestrus prior^{to} or at the time of prostaglandin treatment. This result indicates that oestradiol benzoate treatment given 24 hour after prostaglandin was

effective in increasing oestrus synchrony. In lactating beef cows and heifers, it was reported that intramuscular injection of oestradiol benzoate increased the precision of synchronization of oestrus (Peters et al., 1977). Since oestrogen is the hormone that induces the behavioural signs of oestrus and is capable of doing so even in ovariectomized cattle (Carrick and Shelton, 1969; Lefebvre and Block, 1992; Stewart and Stevenson, 1991), it is not surprising that there was a high occurrence of oestrus in this group.

In the absence of a functional corpus luteum, progestogen (norgestomet implants, melengestrol acetate in feed, progesterone releasing intravaginal devices) administered for 9 to 14 days, at doses used commercially to synchronize oestrus resulted in an increase in LH release pulse frequency from the anterior pituitary gland, a prolonged increase in peripheral concentrations of oestradiol and the development of persistent ovarian follicles, all of which were associated with a reduction in fertility at the synchronized oestrus (Cooperative Regional Research Project NE-161, 1996; Cupp et al., 1992; Kinder et al., 1996; Lucy et al., 1990; Sirois and Fortune, 1990; Savio et al., 1993b). In the present study, in the ODB group, there were five cows (2 cows at metoestrus and 3 cows at proestrus) at the time of acute progesterone and oestradiol benzoate treatment (day 0). In these cows, acute progesterone and oestradiol benzoate treatment decreased LH concentration (Figure 4.4) and persistent ovarian follicles were not observed in these cows following a single injection of progesterone. Therefore, the single and acute progesterone treatment used in the oestrus synchronization regime tested in this study (ODB group) has the advantage over other progestin-based oestrus

synchronization regimes when treatment is administered in the absence of a functional corpus luteum, that it appears to avoid the development of persistent follicles.

The ovulation response from the oestrus synchronization program tested (ODB group) was not significantly different from the GnRH group that was directed at synchronizing the time of ovulation and recommended to achieve fixed-time insemination (Pursley et al., 1995; Pursley et al., 1996). The results shows that this oestrus synchronization program has the potential not only to synchronize oestrus but also has the potential to synchronize ovulation. Therefore, this program, possibly after further refinement, has the potential to be used with a fixed-timed insemination. However, further investigations need to be implemented, as the present study does not investigate the fertility response. The advantage of this program was that it utilized the endogenous progesterone supply when present, for the support of follicular development and to withhold cows from oestrus before induced luteolysis. Where a corpus luteum was not present, the absence of a suitably developed primary follicle was relied upon to delay the occurrence of oestrus and ovulation until the desired time of synchrony. This will reduce the cost of the program as compared to progestin-based oestrus synchronization programs, which depend prolonged on administration of exogenous progesterone. Another advantage of this program was that it could prevent environmental progesterone residues in the cows as the program employed a single injection of short-acting progesterone. Reduction in cost of the program and minimal progesterone residues will benefits the farmers. In addition, the need for a persistent progesterone administration is removed.

Bad phrase

CONCLUSIONS

Acute progesterone treatment with oestradiol benzoate was effective in synchronizing follicular wave emergence and a relatively synchronized ovulation was induced when prostaglandin was administered 9 days later with a second injection of 1 mg oestradiol benzoate 24 hour after prostaglandin. However, there was a large variation in the time of oestrus, and relatively poor synchrony. A reduction in the interval between initial treatment with progesterone and oestradiol benzoate and prostaglandin to 7 or 8 days might improve the oestrus synchrony achievable using this program. The reduction in the treatment interval might prevent the cows from coming in oestrus before the induction of luteolysis. Further investigation is needed to examine this postulation. 33

IBD Onsett12™ (Plade Holdings Ltd., Hamilton, New Zealand) is a single application intravaginal drug delivery device, developed in New Zealand. The device is able to provide a complex hormonal treatment regime with a single application. Therefore, it might be possible to utilize this device for the drug delivery of the treatment regime used in this study. If the effectiveness of the treatment regime via this device is possible, then handling stress on animals could be avoided, although the benefit of not needing a delivery device would obviously disappear.

GENERAL DISCUSSION

Previously, oestrus synchronization was achieved by controlling the timing of the onset of oestrus by controlling the length of the oestrous cycle. The methods used to control oestrous cycle length were: 1) to regress the corpus luteum before the time of natural luteolysis and thereby shorten the cycle, or 2) to administer exogenous progestins to delay the time of oestrus following natural or induced luteolysis and to extend the length of the oestrous cycle (Beal, 1998). Prostaglandin $F_{2\alpha}$ has become the most commonly used treatment for elective induction of oestrus in cattle by shortening the oestrous cycle (Inskeep, 1973; Odde, 1990). However, in cows in which luteolysis is effectively induced by prostaglandin treatment, the ensuing oestrus is distributed over three to seven days period (Rosenberg et al., 1990; Folman et al., 1990). This variation is due to follicular status at the time of treatment (Adams, 1998).

Progestin-based oestrus synchronization programs (norgestomet implants, melengestrol acetate in feed, progesterone releasing intravaginal devices) are associated with development of oversized persistent dominant follicle (Cupp et al., 1992; Lucy et al., 1990; Sirois and Fortune, 1990) and result in lowered fertility. Development of the persistent follicle is caused by increasing LH frequency (Custer et al., 1994; Kojima et al., 1995; Savio et al., 1993b; Stock and Fortune, 1993). The treatment of cyclic cows or heifers with exogenous progestin preceded by an injection of oestradiol is usually followed by a high incidence (>90%) of oestrus during the 5 days following progestin removal (Armer et al., 1993; Lammoglia et al., 1998; Macmillan et al., 1993; Xu et al., 1996; Chapter 3). However, the synchronized conception rates varied between 33% and 55% (Armer et al., 1993; Lammoglia et al., 1998; Xu et al., 1996; Chapter 3). The variations in fertility response following progestin-based oestrus synchronization programs lead to the development of the **IBD Onsett 12™** as a single intravaginal drug delivery device. The **IBD**

Onset12™ oestrus synchronization program has the potential for commercial use to synchronize oestrus. However, further improvement of the device is required to improve the low retention rate (65.12%) and synchronized conception rate (17-19%; Chapter 3).

The development of a better understanding of follicular development (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Ginther et al., 1989b) including the recognition and development of methods to interrupt or manipulate the wave-like pattern of follicular growth (Anderson and Day, 1994; Bo et al., 1995; Cavalieri et al., 1997; Macmillan and Thatcher, 1991; Rajamahendran and Manikkam, 1994; Schmitt et al., 1996; Twagiramungu et al., 1995; Wolfenson et al., 1994) and control ovulation (Pursley et al., 1995; Pursley et al., 1997) have recently been elucidated with the aid of trans-rectal ultrasound examination of the ovaries. These developments make it possible to develop oestrus synchronization by combining methods to control cycle length with the manipulation of follicular development. Controlling the time of emergence of a new follicular wave and synchronizing the follicular wave development of animals within a group to be synchronized is now more practical in dairy cows that are randomly distributed throughout the oestrous cycle (Beal, 1998). The timing of an induced oestrus and ovulation has previously been variable in uncontrolled follicular growth such as in a mixed group of randomly cycling cows.

Treatment with progesterone and oestradiol benzoate was effective in inducing atresia of large follicles (Cavalieri et al., 1998b; Cavalieri et al., 1998c; Bo et al., 1995). The combination treatment of progesterone and oestradiol suppressed LH and was followed by the atresia of large follicles in a variety of stages of follicular growth and dominance (Burke et al., 1996; Murray et

al., 1998; Rajamahendran and Manikkam, 1994). Intravaginal treatment with progesterone and oestradiol benzoate on day 3 of the oestrus cycle was effective in inducing atresia of large follicles and followed with a new follicular wave that emerged, on average, 2.5 ± 0.93 days later (Chapter 2). In the following study (Chapter 4), acute treatment with progesterone and oestradiol benzoate was effective in inducing atresia of large follicles in cows at random stages of oestrous cycle. This was followed with a new follicular wave emergence, on average, 3.60 ± 0.22 days later. Following induced luteolysis with prostaglandin on day 9 of the treatment period, subsequently the newly formed dominant follicle ovulated, on average, 1.70 ± 0.30 days after the second oestradiol benzoate injection given 24 hours after the prostaglandin treatment. The treatment with the second oestradiol benzoate 24 hours after prostaglandin, induced an LH surge (22.80 ± 1.20 hour), increased the synchrony of oestrus and the time of ovulation. This result indicates that it is possible to control both luteal function and follicular development in cows at random stages of the oestrous cycle at the time of treatment. Interestingly, the mean time of ovulation following this oestrus synchronization regime was no different to the oestrus synchronization regime that recommended fixed-time insemination (1.70 ± 0.30 vs. 1.56 ± 0.18 , 0.692). However, for this regime to be practical, less variation in the time until ovulation will need to be achieved.


Concluding Remarks

This thesis investigated the efficacy of the IBD Onsett12™ as a single intravaginal drug delivery device, as well as the use of exogenous progesterone and oestradiol benzoate to improve the degree of oestrus synchrony by controlling follicular wave development. Results indicated that

the efficacy of the IBD Onsett12™ as a single intravaginal drug delivery device tested was doubtful at this stage as the retention rate and synchronized conception rate was low. The causes for the low retention rate require further investigation and testing.

Study results indicated that it was possible to control follicular wave development at random stages of the oestrus cycle with acute progesterone and oestradiol benzoate treatment, and to control luteal function with prostaglandin given 9 days later. The administration of 1 mg oestradiol benzoate 24 hours after the prostaglandin, achieved same degree of oestrus synchrony and ovulation. Further research is required to improve the synchrony of ovulation, then to determine the fertility response from this program. It might be possible to utilize the IBD Onsett12™ device as the delivery mode for the drugs used in this oestrus synchronization regime since drugs currently used in the device are similar to those used in this program. If this is feasible, handling stress on animals will be minimized. Further research also required to explore this possibility to determine the effectiveness of the programs tested using intravaginal treatment and the efficacy of the device to deliver the treatment regimes.

REFERENCES

 ✓
Adams, G. P. (1994). Control of ovarian follicular wave dynamics in cattle: Implications for synchronization and superstimulation. Theriogenology, 41, 19-24. ✓

Adams, G. P. (1998). Control of ovarian follicular wave dynamics in mature and prepubertal cattle for synchronization and superstimulation. Proceedings of the XX World Association for Buiatrics Congress, Sydney, Australia. 595-605 ✓

Adams, G. P., Kot, K., Smith, C. A., and Ginther O. J. (1993). Selection of a dominant follicle and suppression of follicular growth in heifers. Animal Reproduction Science, 30, 259-271. ✓

Adams, G. P., Matteri, R. L., and Ginther, O. J. (1992b). The effect of progesterone on growth of ovarian follicles, emergence of follicular waves and circulating FSH in heifers. Journal of Reproduction and Fertility, 95, 617-640. ✓

Adams, G. P., Matteri, R. L., Kastelic, J. P., Ko, J. C. H., and Ginther, O. J. (1992a). Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. Journal of Reproduction and Fertility, 94, 177-188. ✓

Ahmed, N., Townsend, E. C., Dailey, R. A., and Inskeep, E. K. (1997). Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. Animal Reproduction Science, 49, 13-28. ✓

Anderson, L. H., and Day, M. L. (1994). Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. Journal of Animal Science, 72, 2955-2962. ✓

- Armer, C. C., Macmillan, K. L., and Jellie, H. P. (1993). The application of controlled calving and breeding programmes to the management of large dairy herds. Proceedings of the New Zealand Society of Animal Production, 53, 87-90. ✓
- Austin, E. J., Mihm, M., Ryan, M. P., Williams, D. H., and Roche, J. F. (1999). Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. Journal of Animal Science, 77, 2219-2226. ✓
- Badinga, L., Driancourt, M. A., Savio, J. D., Wolfenson, D., Drost, M., De La Sota, R. L., and Thatcher, W. W. (1992). Endocrine and ovarian responses associated with the first wave dominant follicle in cattle. Biology of Reproduction, 47, 871-883. ✓
- Badinga, L., Thatcher, W. W., Diaz, T., Drost, M., and Wolfenson, D. (1993). Effect of environmental heat stress on follicular steroidogenesis and development in lactating Holstein cows. Theriogenology, 39, 797-810. ✓
- Bao, B., and Garverick, H. A. (1998). Expression of steroidogenic enzyme and gonadotrophin receptor genes in bovine follicles during ovarian follicles waves: a review. Journal of Animal Science, 76, 1903-1921.
- Barr, L. H. (1975). Influence of estrous detection on days open in dairy herds. Journal of Dairy Science, 58, 246-247. ✓
- Bauman, D. E., and Currie, W. B. (1980). Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science, 63, 1514-1529. ✓

Beal, W. E. (1996). Application of knowledge about corpus luteum function in control of estrus and ovulation in cattle. Theriogenology, 45, 1399-1411. ✓

Beal, W. E. (1998). Current estrus synchronization and artificial insemination programs for cattle. Journal of Animal Science, 76(Suppl. 3), 30-38. ✓

Beal, W. E., Chenault, J. R., Day, M. L., and Corah, L. R. (1988). Variation in conception rates following synchronization of estrus with melengesterol acetate and prostaglandin $F_{2\alpha}$. Journal of Animal Science, 66, 599-602. ✓

Beal, W. E., Perry, R. C., and Corah, L. R. (1990). Follicular development in cows fed melengesterol acetate to synchronize estrus. Journal of Animal Science, 68(Suppl. 1), 12(Abstr.). ✓

Beam, S. W., and Butler, W. R. (1997). Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biology of Reproduction, 56, 133-142. ✓

Bergfelt, D. R., Smith, C. A., Adams, G. P., and Ginther, O. J. (1997). Surges of FSH during the follicular and early luteal phases of the estrous cycle in heifers. Theriogenology, 48, 757-768. ✓

Bo, G. A., Adams, G. P., Caccia, M., Pierson, R. A., and Mapletoft, R. J. (1995). Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Animal Reproduction Science, 39, 193-204. ✓

Bo, G. A., Adams, G. P., Nasser, L. F., Pierson, R. A., and Mapletoft, R. J. (1993). Effect of ✓

estradiol on ovarian follicles, emergence of follicular waves and circulating gonadotrophins in heifers. Theriogenology, **40**, 225-239.

Bo, G. A., Adams, G. P., Pierson, R. A., Tribulo, H. E., Caccia, M., and Mapletoft, R. J. (1994). Follicular wave dynamics after estradiol-17 β treatment of heifers with or without a progestogen implant. Theriogenology, **41**, 1555-1569.

Bodensteiner, K. J., Kot, K., Wiltbank, M. C., and Ginther, O. J. (1996). Synchronization of emergence of follicular waves in cattle. Theriogenology, **45**, 1115-1128.

Bosworth, R. W., Ward, G., Call, E. P., and Bonewitz, E. R. (1972). Analysis of factors affecting calving intervals of dairy cows. Journal of Dairy Science, **55**, 334-338.

Brown, L. N., Odde, K. G., King, M. E., LeFever, D. G., and Neubauer, C. J. (1988). Comparison of melengesterol acetate-prostaglandin F_{2a} to syncro-mate B for estrus synchronisation in beef heifers. Theriogenology, **30**, 1-12.

Burke, C. R., Boland, M. P., and Macmillan, K. L. (1999). Ovarian responses to progesterone and oestradiol benzoate administered intravaginally during dioestrus in cattle. Animal Reproduction Science, **55**, 23-33.

Burke, C. R., Day, M. L., Clark, B. A., Bunt, C. R., Rathbone, M. J., and Macmillan, K. L. (1997). Effect of luteolysis on follicle wave control using oestradiol benzoate in cattle. Proceedings of the New Zealand Society of Animal Production, **40**, 134.

Burke, C. R., Macmillan, K. L., and Boland, M. P. (1996). Oestradiol potentiates a prolonged progesterone-induced suppression of LH release in ovariectomised cows. Animal

Reproduction Science, 45, 13-28.

Burton, L. J., Harris, B. L., Winkelman, A. M., and Xu, Z. Z. (1999). Reproductive performance and genetic improvement of fertility in dairy cattle. Dairyfarming Annual, 51, 59-67.

Butler, W. R., and Smith, R. D. (1989). Interrelationships between energy balance and postpartum reproductive function in dairy cattle. Journal of Dairy Science, 72, 767-783.

Campbell, B. K., Scaramuzzi, R. J., and Webb, R. (1995). Control of antral follicle development and selection in sheep and cattle. Journal of Reproduction and Fertility, 49(Suppl.), 335-350.

Carrick, M. J. and Shelton, J. N. (1969). Oestrogen-progesterone relationships in the induction of oestrus in spayed heifers. Journal of Endocrinology, 45, 99-109

Cavalieri, J., Coleman, C., Kinder, J. E., and Fitzpatrick, L. A. (1998c). Comparison of three methods of acute administration of progesterone on ovarian follicular development and the timing and synchrony of ovulation in *Bos indicus* heifers. Theriogenology, 49, 1331-1343.

Cavalieri, J., Kinder, J. E., and Fitzpatrick, L. A. (1998a). Effect of acute treatment with progesterone on the timing and synchrony of ovulation in *Bos indicus* heifers treated with a norgestomet implant for 17 days. Journal of Reproduction and Fertility, 112, 249-258.

Cavalieri, J., Kinder, J. E., De'ath, G., and Fitzpatrick, L. A. (1997). Effect of 48 h treatment with 17-oestradiol or progesterone on follicular wave emergence and synchrony of ovulation in *Bos indicus* cows when administered at the end of a period of progesterone treatment.

Animal Reproduction Science, 46, 187-201.

Cavalieri, J., Kinder, J. E., De'ath, G., and Fitzpatrick, L. A. (1998b). Effects of short-term treatment with progesterone superimposed on 11 or 17 days of norgestomet treatment on the interval to oestrus and fertility in *Bos indicus* heifers. Animal Reproduction Science, 51, 169-183. ✓

Chenault, J. R., Kratzer, D. D., Rzepkowski, R. A., and Goodwin, M. C. (1990). LH and FSH response of Holstein heifers to fertirelin, gonadorelin and buserelin. Theriogenology, 34, 81-98. ✓

Christian, R. E., and Casida, L. E. (1948). The effects of progesterone in altering the estrus cycle of the cow. Journal of Animal Science, 7, 540(Abstr.).

Clark, B. A., Rhodes, F. M., Burke, C. R., Morgan, S. R., and Macmillan, K. L. (1998). Manipulating patterns of ovarian follicle development in cattle with progesterone and gonadotrophin releasing hormone to produce oestrous cycles with two or three follicles waves. Proceedings of the New Zealand Society of Animal Production, 58, 85-87. ✓

Congleton, W. R. Jr., and King, L. W. (1984). Profitability of dairy cow herd life. Journal of Dairy Science, 67, 661-674. ✓

Cooperative Regional Research Project NE-161. (1996). Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. Journal of Animal Science, 74, 1943-1952. ✓

Custer, E. E., Beal, W. E., Wilson, S. J., Meadows, A. W., Berardinelli, J. G., and Adair, R.

(1994). Effect of melengestrol acetate (MGA) or progesterone-releasing intravaginal device (PRID) on follicular development, concentrations of estradiol-17 β and progesterone, and LH release during an artificially lengthened bovine estrous cycle. Journal of Animal Science, 72, 1282-1289.

Drew, B. (1978). Management factors in oestrous cycle control. In: Sreenan, J. M (Ed). Control of Reproduction in the cow. The Hague/Boston/London, Martinus Nijhoff.

Drew, S. B., Wishart, D. F., and Young, I. M. (1979). Fertility of norgestomet treated suckler cows. Veterinary Record, 104, 523-525.

Driancourt, M. A., Thatcher, W. W., and Andrieu, T. M. (1991). Dynamics of ovarian follicular development in cattle during the estrous cycle, early pregnancy and in response to PMSG. Domestic Animal Endocrinology, 8, 209-221.

Dufour, J. J., and Roy, G. L. (1985). Distribution of ovarian follicular populations in the dairy cows within 35 days after parturition. Journal of Reproduction and Fertility, 73, 229-235.

Engelhart, H., Walton, J. S., Miller, R. B., and King, G. J. (1989). Estradiol-induced blockade of ovulation in the cow: effect of luteinizing hormone release and follicular fluids steroids. Biology of Reproduction, 40, 1287-1297.

Evans, A. C. O., Adams, G. P., and Rawlings, N. C. (1994). Endocrine and ovarian follicular changes leading up to the first ovulation in prepubertal heifers. Journal of Reproduction and Fertility, 100, 187-194.

Evans, A. C. O., Komar, C. M., Wandji, S.-A., and Fortune, J. E. (1997). Changes in androgen

secretion and luteinizing hormone pulse amplitude are associated with the recruitment and growth of ovarian follicles during the luteal phase of the bovine estrous cycle. Biology of Reproduction, **57**, 394-401.

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

Fike, K. E., Day, M. L., Inskeep, E. K., Kinder, J. E., Lewis, P. E., Short, R. E., and Hafs, H. D. (1997). Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. Journal of Animal Science, **75**, 2009-2015.

Folman, Y., Kaim, M., Herz, Z., and Rosenberg, M. (1990). Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. Journal of Dairy Science, **73**, 2817-2825.

Fortune, J. E. (1993). Follicular dynamics during the bovine estrous cycle: a limiting factor in improvement of fertility? Animal Reproduction Science, **33**, 111-125.

Gibbons, J. R., Wiltbank, M. C., and Ginther, O. J. (1997). Functional interrelationships between follicles greater than 4 mm and the follicle-stimulating hormone surge in heifers. Biology of Reproduction, **57**, 1066-1073.

Ginther O. J., Wiltbank, M. C., Fricke, P. M., Gibbons, J. R., and Kot, K. (1996). Selection of the dominant follicle in cattle. Biology of Reproduction, **55**, 1187-1194.

Ginther, O. J., Bergfelt, D. R., Kulick, L. J., and Kot, K. (1998). Pulsatility of systemic FSH and LH concentrations during follicular-wave development in cattle. Theriogenology, 50, 507-519. ✓

Ginther, O. J., Kastelic, J. P., and Knopf, L. (1989a). Composition and characteristics of follicular waves during the bovine estrous cycle. Animal Reproduction Science, 20, 187-200. ✓

Ginther, O. J., Knopf, L., and Kastelic, J. P. (1989b). Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. Journal of Reproduction and Fertility, 87, 223-230. ✓

Ginther, O. J., Kot, K., Kulick, L. J., and Wiltbank, M. C. (1997). Emergence and deviation of follicles during the development of follicular waves in cattle. Theriogenology, 48, 75-87. ✓

Gong, J. G., and Webb, R. (1996). Control of ovarian follicle development in domestic ruminants: its manipulation to increase ovulation rate and improve reproductive performance. Animal Breeding Abstracts, 64, 195-204. ✓

Gordon, I. (1996). Controlled reproduction in cattle and buffaloes. CAB International. Oxon, UK. ✓

Gutierrez, C. G., Oldham, J., Bramley, T. A., Gong, J. G., Campbell, B. K., and Webb, R. (1997). The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. Journal of Animal Science, 75, 1876-1884. ✓

*
Hanlon, D. W. (1995). The use of oestradiol benzoate and progesterone to synchronise oestrus in ✓

dairy cattle. MVS thesis, Massey University, Palmerston North, New Zealand.

Hansel, W., and Convey, E. M. (1983). Physiology of the estrous cycle. Journal of Animal Science, 57(Suppl. 2), 404-424. ✓

Heersche, G. Jr., and Nebel, R. L. (1994). Measuring efficiency and accuracy of detection of estrus. Journal of Dairy Science, 77, 2755-2761. ✓

Heersche, G. Jr., Kiracofe, G. H., DeBenedetti, R. C., Wen, S., and McKee, R. M. (1979). Synchronization of estrus in beef heifers with a norgestomet implant and prostaglandin- $F_{2\alpha}$. Theriogenology, 11, 197-208. ✓

Holmes, C. W., Wilson, G. F., Mackenzie, D. D. S., Flux, D. S., Brookes, I. M., and Davey, A. W. F. (1987). Milk production from pasture. Butterworths of New Zealand (Ltd), Wellington, New Zealand. ✓

Howell, J. L., Fuquay, J. W., and Smith, A. E. (1994). Corpus luteum growth and function in lactating Holstein cows during spring and summer. Journal of Dairy Science, 77, 735-739. ✓

Inskeep, E. K. (1973). Potential uses of prostaglandins in control of reproductive cycles of domestic animals. Journal of Animal Science, 36, 1149-1157. ✓

Ireland, J. J. (1987). Control of follicular growth and development. Journal of Reproduction and Fertility, 34, 39-54. ✓

Ireland, J. J., and Roche, J. F. (1982). Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, ✓

and gonadotropin receptors. Endocrinology, 111, 2077-2086.

Johnson, S. K., Day, M. L., Lynch, J. M., Kinder, J. E., Rasby, R., Short, R. E., Wettemann, R. P., and Hafs, H. D. (1997). Onset of estrus and luteal function in peripubertal heifers given an intravaginal progesterone releasing insert with or without a subsequent injection of estradiol benzoate. Journal of Animal Science, 75(Suppl. 1), 231(Abstr.).

Kaneko, H., Terada, T., Taya, K., Watanabe, G., Sasamoto, S., Hasegawa, Y., and Igarashi, M. (1991). Ovarian follicular dynamics and concentrations of oestradiol-17, progesterone, luteinizing hormone and follicle stimulating hormone during the periovulatory phase of the oestrous cycle in the cow. Journal of Reproduction, Fertility and Development, 3, 529-535.

Kastelic J. P. (1994). Understanding ovarian follicular development in cattle. Veterinary Medicine, 89, 64-71.

Kastelic, J. P., and Ginther, O. J. (1991). Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. Animal Reproduction Science, 26, 13-24.

Kastelic, J. P., and Mapletoft, R. J. (1998). Ovarian follicular responses in dairy cows treated with GnRH and cloprostenol. Canadian Veterinary Journal, 39, 107-109.

Kastelic, J. P., Knopf, L., and Ginther, O. J. (1990). Effect of day of prostaglandin $F_{2\alpha}$ treatment on selection and development of the ovulatory follicle in heifers. Animal Reproduction Science, 23, 169-180.

Kesler, D. J., and Favero, R. J. (1995). Estrus synchronization in beef females with norgestomet

and estradiol valerate: Part 1. Mechanism of action. Agri-Practice, 16, 6-11.

Kinder, J. E., Kojima, F. N., Bergfeld, E. G. M., Wehrman, M. E., and Peter, K. E. (1996).

Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. Journal of Animal Science, 74, 1424-1440.

Knopf, L., Kastelic, J. P., Schallenberger, E., and Ginther, O. J. (1989). Ovarian follicular

dynamics in heifers: Test of two wave hypothesis by ultrasonically monitoring individual follicles. Domestic Animal Endocrinology, 6, 111-119.

Ko, J. C. H., Kastelic, J. P., Del Campo, M. R., and Ginther, O. J. (1991). Effects of a dominant

follicle on ovarian follicular dynamics during the oestrous cycle in heifers. Journal of Reproduction and Fertility, 91, 511-519.

Kojima, F. N., Chenault, J. R., and Wehrman, M. E., Bergfeld EG, Cupp AS, Werth LA,

Mariscal V, Sanchez T, Kittok RJ, Kinder JE (1995). Melengestrol acetate at greater doses than typically used for estrus synchrony in bovine females does not mimic endogenous progesterone in regulation of secretion of LH and estradiol-17 β . Biology of Reproduction, 52, 455-463.

Lammoglia, M. A., Short, R. E., Bellows, S. E., Bellows, R. A., MacNeil, M. D., and Hafs, H. D.

(1998). Induced and synchronized estrus in cattle: Dose titration of estradiol benzoate in peripubertal heifers and postpartum cows after treatment with an intravaginal progesterone-releasing insert and prostaglandin F_{2 α} . Journal of Animal Science, 76, 1662-1670.

Lane, E., Austin, E. T., Ryan, M. P., and Roche, J. F. (1999). The effect of oestradiol benzoate

post PRID removal on synchrony of oestrus and fertility in cattle. Journal of Reproduction and Fertility, 23, 41(Abstr.).

Larson, L. L., and Ball, P. J. H. (1992). Regulation of estrous cycles in dairy cattle: A review. Theriogenology, 38, 255-267. ✓

Lefebvre, D. M. and Block, E. (1992). Effect of recombinant bovine somatotropin on estradiol-induced estrous behavior in ovariectomized heifers. Journal of Dairy Science, 75, 1461-1464. ✓

Lehrer, A. R., Aizinbud, E., and Lewis, G. S. (1992). Oestrus detection in cattle: recent developments. Animal Reproduction Science, 28, 355-362. ✓

Lindsay, D. R. (1996). Environment and reproductive behaviour. Animal Reproduction Science, 42, 1-12. ✓

Lucy, M. C., Beck, J., Drost, M., and Thatcher, W. W. (1990). Plasma metabolites, growth factor (GF), and IGF-I binding proteins (BP) during altered follicular growth induced by shifting energy balance (EB) in lactating Holstein cows. Journal of Dairy Science, 73(Suppl. 1), 451(Abstr.). ✓

Lucy, M. C., Savio, J. D., Badinga, L., De la Sota, R. L., and Thatcher, W. W. (1992). Factors that affect ovarian follicular dynamics in cattle. Journal of Animal Science, 70, 3615-3626. ✓

Lucy, M. C., Staples, C. R., Michel, F. M., and Thatcher, W. W. (1991). Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin $F_{2\alpha}$, luteinizing ✓

hormone, and follicular growth. Journal of Dairy Science, 74, 483-489.

Lucy, M. C., Stevenson, J. S., and Call, E. P. (1986). Controlling first service and calving interval by prostaglandin $F_{2\alpha}$, gonadotrophin-releasing hormone, and timed insemination. Journal of Dairy Science, 69, 2186-2194. ✓

Lynch, P. R., and Macmillan, K. L. (1996). Progesterone and a GnRH agonist interact to affect oestrous cycle length and fertility in cattle. New Zealand Society of Endocrinology, 26, P4-32. ✓

Lynch, P. R., Macmillan, K. L., and Taufa, V. K. (1999). Treating cattle with progesterone as well as a GnRH analogue affects oestrous cycle length and fertility. Animal Reproduction Science, 56, 189-200.

Macmillan, K. L. (1979). Calving patterns and herd production in seasonal dairy herds. Proceedings of the New Zealand Society of Animal Production, 39, 168-174. ✓

Macmillan, K. L. (1983). Prostaglandin responses in dairy herd breeding programmes. New Zealand Veterinary Journal, 31, 110-113. ✓

Macmillan, K. L., and Asher, G. W. (1990). Developments in artificial insemination and controlled breeding in dairy cattle and deer in New Zealand. Proceedings of the New Zealand Society of Animal Production, 50, 123-133. ✓

Macmillan, K. L., and Burke, C. R. (1996). Effects of oestrous cycle control on reproductive efficiency. Animal Reproduction Science, 42, 307-320. ✓

Macmillan, K. L., and Moller, K. (1977). Aspects of reproduction in New Zealand dairy herds. 2. ✓

Calving interval, breeding period and non-pregnancy rates. New Zealand Veterinary Journal, 25, 220-224.

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

Macmillan, K. L., and Thatcher, W. W. (1991). Effects of an agonist gonadotrophin-releasing hormone on ovarian follicles in cattle. Biology of Reproduction, 45, 883-889.

Macmillan, K. L., and Watson, J. D. (1973). AB in New Zealand dairy herds: II. Interactions between conception rate and submission rate on the proportion of the herd reported in calf to AB. New Zealand Journal of Experimental Agriculture, 1, 309-314.

Macmillan, K. L., Curnow, R. J., and Morris, G. R. (1977). Oestrus synchronization with a prostaglandin analogue: 1. Systems in lactating dairy cattle. New Zealand Veterinary Journal, 25, 366-372.

Macmillan, K. L., Curnow, R. J., and Morris, G. R. (1978). Oestrus synchronization with a prostaglandin analogue: 1. Systems in maiden heifers. New Zealand Veterinary Journal, 26, 96-103.

Macmillan, K. L., Henry, R. I., Taufa, V. K., and Phillips, P. (1990). Calving patterns in seasonal dairy herds. New Zealand Veterinary Journal, 38, 151-155.

Macmillan, K. L., McDougall, S., Taufa, V. K., and Day, A. M. (1994). Ovulation and oestrus among dairy cows with anovulatory anoestrus following progesterone pre-treatment.

Proceedings of Australian Society of Reproductive Biology, 26, 74(Abstr.).

Macmillan, K. L., Taufan, V. K., and Day, A. M. (1993). Combination treatments for synchronising oestrus in dairy heifers. Proceedings of the New Zealand Society of Animal Production, 53, 267-270. ✓

Macmillan, K. L., Taufan, V. K., Barnes, D. R., and Day, A. M. (1991). Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Animal Reproduction Science, 26, 25-40. ✓

Macmillan, K. L., Taufan, V. K., Barnes, D. R., Day, A. M., and Henry, D. R. (1988). Detecting oestrus in synchronised heifers using tailpaint and an aerosol raddle. Theriogenology, 30, 1099-1114.

Macmillan, K. L., Taufan, V. K., Day, A. M., and McDougall, S. (1995). Some effects of using progesterone and oestradiol benzoate to stimulate oestrus and ovulation in dairy cows with anovulatory anoestrus. Proceedings of the New Zealand Society of Animal Production, 55, 239-241. ✓

Manikkam, M., Rajamahendran, R., and Railings, N. C. (1995). Effects of bovine follicular fluids and luteinizing-hormone-releasing hormone antagonist on the dominant follicle maintained by a norgestomet ear implant in heifers. Canadian Journal of Animal Science, 75, 71-78. ✓

Mann, G. E., Starbuck, G. S., Benboulaid, M., Peters, A. R., and Lamming, G. E. (1999). Variation in the timing of ovulation relative to other follicular phase events following natural and induced luteal regression in dairy cattle. Proceedings of the British Society of ✓

Animal Science, Scarborough, 63.

Manns, J. G., and Hafs, H. D. (1976). Controlled breeding in cattle: A review. Canadian Journal of Animal Science, 56, 121-131. ✓

Martinez, M. F., Bergfelt, D. R., Adams, G. P., Kastelic, J. P., and Mapletoft, R. J. (1997). Synchronization of follicular wave emergence and its use in an estrus synchronization program. Theriogenology, 47, 145(Abstr.). ✓

Matton, P., Adelakoun, V., Couture, Y., and Dufour, J. J. (1981). Growth and replacement of the bovine ovarian follicles during the oestrous cycle. Journal of Animal Science, 52, 813-820. ✓

Mauleon, P. (1974). New trends in the control of reproduction in the bovine. Livestock Production Science, 1, 117-131. ✓

Maurasse, C., Matton, P., and Dufour, J. J. (1985). Ovarian follicular populations at two stages of an estrous cycle in heifers given high energy diets. Journal of Animal Science, 61, 1194-1200. ✓

McCracken, J. A. (1972). Prostaglandins and luteal regression - A review. Prostaglandins, 1, 1-4. ✓

McCracken, J. A., Schramm, W., and Okulicz, W. C. (1984). Hormone receptor control of pulsatile secretion of $\text{PGF}_{2\alpha}$ from the ovine uterus during luteolysis and its abrogation in early pregnancy. Animal Reproduction Science, 7, 31-55. ✓

*
McDougall, S. (1994). Postpartum anoestrus in the pasture grazed New Zealand dairy cow. PhD thesis, Massey University, Palmerston North, New Zealand. ✓

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

McDougall, S., Burke, C. R., Macmillan, K. L., and Williamson, N. B. (1995). Follicle patterns during extended periods of postpartum anovulation in pasture-fed dairy cows. Research in Veterinary Science, 58, 212-216. ✓

McDougall, S., Leijnse, P., Day, A. M., Macmillan, K. L., and Williamson, N. B. (1993). A case control study of anoestrus in New Zealand dairy cows. Proceedings of the New Zealand Society of Animal Production, 53, 101-103. ✓

McDowell, C. M., Anderson, L. G., Kinder, J. E., and Day, M. L. (1998). Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. Journal of Animal Science, 76, 850-855. ✓

McNeilly, A. S., Crow, W., Brooks, J., and Evans, G. (1992). Luteinizing hormone pulses, follicle-stimulating hormone and control of follicle selection in sheep. Journal of Reproduction and Fertility, 45(Suppl.), 5-19. ✓

Mihm, M., Baguisi, A., Boland, M. P., and Roche, J. F. (1994). Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. Journal of Reproduction and Fertility, 102, 123-130. ✓

Miksch, E. D., LeFever, D. G., Mukembo, G., Spitzer, J. C., and Wiltbank, J. N. (1978). Synchronization of estrus in beef cattle. II. Effect of an injection of norgestomet and an ✓

estrogen in conjunction with a norgestomet implant in heifers and cows. Theriogenology, 10, 201-221.

Moenter, S. M., Caraty, A., Locatelli, A., and Karsch, F. J. (1991). Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. Endocrinology, 129, 1175-1182.

Moller, K. (1970). Uterine involution and ovarian activity after calving. New Zealand Veterinary Journal, 18, 140-145.

Munro, R. K. (1989). The effects of duration and concentration of plasma progesterone on the fertility of post-partum cows treated with pregnant mare serum, gonadotrophin and intravaginal progesterone. Australian Veterinary Journal, 66, 43-45.

Munro, R. K., and Moore, N. W. (1985). Effects of progesterone, oestradiol benzoate and cloprostenol on luteal function in the heifer. Journal of Reproduction and Fertility, 73, 353-359.

Murphy, M. G., Enright, W. J., Crowe, M. A., McConnell, K., Spicer, L. J., Boland, M. P., and Roche, J. F. (1991). Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. Journal of Reproduction and Fertility, 92, 333-338.

Murray, A. J., Cavalieri, J., D'Occhio, M. J., Whyte, T. R., Maclellan, L. J., and Fitzpatrick, L. A. (1998). Treatment with progesterone and 17-oestradiol to induce emergence of a newly-recruited dominant ovulatory follicle during oestrus synchronisation with long-term use of norgestomet in Brahman heifers. Animal Reproduction Science, 50, 11-26.

- Nadaraja, R., and Hansel, W. (1976). Hormonal changes associated with experimentally produced cystic ovaries in the cow. Journal of Reproduction and Fertility, 47, 203-208. ✓
- Nebel, R. L., and Jobst, S. M. (1998). Evaluation of systemic breeding programs for lactating dairy cows: A review. Journal of Dairy Science, 81, 1169-1174. ✓
- Odde, K. G. (1990). A review of synchronization of estrus in postpartum cattle. Journal of Animal Science, 68, 817-830. ✓
- Olds, D. (1990). Viewpoints on dairy herd fertility. Journal of American Veterinary Medical Association, 196, 726-727. ✓
- Parkinson, T. J., and Follett, B. K. (1994). Effect of thyroidectomy upon seasonality in rams. Journal of Reproduction and Fertility, 101, 51-58.
- Penny, C. (1998). Controlled breeding in cattle. In Practice, 10, 351-357. ✓
- Peters, A. R. (1986). Hormonal control of the bovine oestrous cycle. II. Pharmacological principles. British Veterinary Journal, 142, 20-29. ✓
- Peters, A. R., Ward, S. J., Warren, M. J., Gordon, P. J., Mann, G. E., and Webb, R. (1999). Ovarian and hormonal responses of cows to treatment with an analogue of gonadotrophin releasing hormone and prostaglandin $F_{2\alpha}$. Veterinary Record, 144, 343-346. ✓
- Peters, J. B., Welch, J. A., Lauderdale, J. W., and Inskeep, E. K. (1977). Synchronization of estrus in beef cattle with $PGF_{2\alpha}$ and estradiol benzoate. Journal of Animal Science, 45, 230-235. ✓

- Prescott, R. E., Silcox, R. W., and Byerley, D. J. (1992). Effect of GnRH on the dominant follicle of the first follicular wave in beef cows. Journal of Animal Science, 70(Suppl. 1), 254(Abstr.).
- Pursley, J. R., Kosorok, M. R., and Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. Theriogenology, 44, 915-923.
- Pursley, J. R., Kosorok, M. R., and Wiltbank, M. C. (1997). Reproductive management of lactating dairy cows using synchronization of ovulation. Journal of Dairy Science, 80, 301-306.
- Pursley, J. R., Silcox, R. W., and Wiltbank, M. C. (1998). Effect of time of artificial insemination of pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. Journal of Dairy Science, 81, 2139-2144.
- Pursley, J. R., Wiltbank, M. C., Stevenson, J. S., Ottobre, J. S., Garverick, H. A., and Anderson, L. L. (1996). Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. Journal of Dairy Science, 80, 295-300.
- Rajamahendran, R., and Manikkam, M. (1994). Effects of exogenous steroid hormones on the dominant follicle maintained by a norgestomet implant in heifers. Canadian Journal of Animal Science, 74, 457-464.
- Rajamahendran, R., and Taylor, C. (1990). Characterization of ovarian activity in postpartum dairy cows using ultrasound imaging and progesterone profiles. Animal Reproduction

Science, 22, 171-180.

Rajamahendran, R., and Walton, J. S. (1990). Effect of treatment with estradiol valerate on endocrine changes and ovarian follicle populations in dairy cows. Theriogenology, 33, 441-452. ✓

Rathbone, M. J., Macmillan, K. L., Jöchle, W., Boland, M. P., and Inskeep, E. K. (1998). Controlled-release products for the control of the estrus cycle in cattle, sheep, goats, deer, pigs, and horse. Critical Reviews in Therapeutic Drug Carrier Systems, 15, 285-380. ✓

Reimers, T. J., Smith, R. D., and Newman, S. K. (1985). Management factors affecting reproductive performance of dairy cows in the northeastern United States. Journal of Dairy Science, 68, 963-972. ✓

Rhodes, F. M., Clark, B. A., McDougall, S., and Macmillan, K. L. (1999). Insemination at the second of two induced oestrous periods in anoestrous dairy cows increases conception rates to first service. New Zealand Veterinary Journal, 47, 39-43. ✓

Rhodes, F. M., Clark, B. A., Nation, D. P., Taufu, V. K., Day, M. L., Day, A. M., Macmillan, K. L., and McDougall, S. (1998a). Treatment of postpartum anoestrus in New Zealand dairy Cows with progesterone and oestradiol benzoate. Proceedings of the XX World Association for Buiatrics Congress, Sydney, Australia, 607-611. ✓

Rhodes, F. M., Clark, B. A., Nation, D. P., Taufu, V. K., Macmillan, K. L., Day, M. L., and Day, A. M. (1998b). Factors influencing the prevalence of postpartum anoestrus in New Zealand dairy cows. Proceedings of the New Zealand Society of Animal Production, 58, 79-81. ✓

- Rhodes, F. M., Entwistle, K. W., and Kinder, J. E. (1996). Changes in ovarian function and gonadotropin secretion preceding the onset of nutritionally induced anestrus in *Bos indicus* heifers. Biology of Reproduction, 55, 1437-1443. ✓
- Rhodes, F. M., Fitzpatrick, L. A., Entwistle, K. W., and Kinder, J. E. (1995). Hormone concentrations in the caudal vena cava during the first ovarian follicular wave of the oestrous cycle in heifers. Journal of Reproduction and Fertility, 104, 33-39. ✓
- ~~Richards~~ Richards, J. S., Jahnsen, T., Hedlin, L., Lifka, J., Ratoosh, S., Durica, J. M., and Goldring, N. B. (1987). Ovarian follicular development: From physiology to molecular biology. Recent Progress in Hormone Research, 43, 231-276. ✓
- Rivera, G. M., Goñi, C. G., Chaves, M. A., Ferrero, S. B., and Bo, G. A. (1998). Ovarian follicular wave synchronization and induction of ovulation in postpartum beef cows. Theriogenology, 49, 1365-1375. ✓
- Roche, J. F. (1974). Effect of short-term progesterone treatment on oestrous response and fertility in heifers. Journal of Reproduction and Fertility, 40, 433-440. ✓
- Roche, J. F. (1976a). Calving rate of cows following insemination after a 12-day treatment with silastic coils impregnated with progesterone. Journal of Animal Science, 43, 164-169. ✓
- Roche, J. F. (1976b). Fertility in cows after treatment with a prostaglandin analogue with or without progesterone. Journal of Reproduction and Fertility, 46, 341-345. ✓
- Roche, J. F. (1976c). Synchronization of oestrus in cattle. World Review of Animal Production, 12, 79-88. ✓

- Roche, J. F. (1978). Control of oestrus in cattle using progesterone coils. Animal Reproduction Science, 1, 145-154. ✓
- Roche, J. F., and Boland, M. P. (1991). Turnover of dominant follicles in cattle of different reproductive states. Theriogenology, 35, 81-90. ✓
- Roche, J. F., and Mihm, M. (1996). Physiology and practice of induction and control of oestrus in cattle. Proceedings of the XIV World Association for Buiatrics Congress, Edinburgh, Scotland, 1, 157-163. ✓
- Roche, J. F., Ireland, J. J., and Mawhinney, S. (1981). Control and Induction of ovulation in cattle. Journal of Reproduction and Fertility, 30(Suppl. 1), 211-222.
- Roche, J. F., Mihm, M., Diskin, M. G., and Ireland, J. J. (1998). A review of regulation of follicle growth in cattle. Journal of Animal Science, 76(Suppl. 3), 16-29. ✓
- Rosenberg, M., Kaim, M., Herz, Z., and Folman, Y. (1990). Comparison of methods for the synchronization of estrous cycles in dairy cows. 1. Effects on plasma progesterone and manifestation of estrus. Journal of Dairy Science, 73, 2807-2816.
- Rowson, L. E. A., Tervit, R., and Brand, A. (1972). The use of prostaglandins for synchronization of oestrus in cattle. Journal of Reproduction and Fertility, 29, 145(Abstr.). ✓
- Rutledge, J. J. (1975). Twinning in cattle. Journal of Animal Science, 40, 803-815. ✓
- Ryan, D. O., Snijders, S., Yaakub, H., and O'Farrell, K. J. (1995a). An evaluation of estrus synchronization programs in reproductive management of dairy herds. Journal of Animal ✓

Science, 73, 3687-3695.

Ryan, D. P. (1994). Heat Synchronization for compact calving. Irish Farmers' Journal, 46(48), 30. ✓

Ryan, D. P., Galvin, J. A., and O'Farrell, K. J. (1999). Comparison of oestrous synchronization regimens for lactating dairy cows. Animal Reproduction Science, 56, 153-168. ✓

Ryan, D. P., Snijders, S., Aarta, A., and O'Farrell, K. J. (1995b). Effect of estradiol subsequent to induced luteolysis on development of the ovulatory follicle and interval to estrus and ovulation. Theriogenology, 43, 310(Abstr.).

Sanchez, T., Wehrman, M. E., Bergfeld, E. G., Peters, K. E., Kojima, F. N., Cupp, A. S., Mariscal, V., Kittok, R. J., Rasby, R. J., and Kinder, J. E. (1993). Pregnancy rate is greater when the corpus luteum is present during the period of progestin treatment to synchronize time of estrus in cows and heifers. Biology of Reproduction, 49, 1102-1107. ✓

SAS. (1996). SAS Release 6.12 Cary, North Carolina, USA.: SAS Institute Inc. ✓

Savio, J. D., Boland, M. P. H. N., and Roche, J. F. (1990). Resumption of follicular activity in the early post-partum period of dairy cows. Journal of Reproduction and Fertility, 88, 569-579. ✓

Savio, J. D., Kennan, L., Boland, M. P., and Roche, J. F. (1988). Pattern growth of dominant follicles during the oestrous cycle in heifers. Journal of Reproduction and Fertility, 83, 663-671. ✓

Savio, J. D., Thatcher, W. W., Badinga, L., de la Sota, R. L., and Wolfenson, D. (1993a). ✓

Regulation of dominant follicle turnover during the oestrous cycle in cows. Journal of Reproduction and Fertility, 97, 197-203.

Savio, J. D., Thatcher, W. W., Morris, G. R., Entwistle, K., Drost, M., and Mattiacci, M. R. (1993b). Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. Journal of Reproduction and Fertility, 98, 77-84.

Sawyer, G. J., Broadbent, P. J., and Dolman, D. F. (1995). Ultrasound-monitored ovarian responses in normal and superovulated cattle given exogenous progesterone at different stages of the oestrous cycle. Animal Reproduction Science, 38, 187-201.

Schmitt, E. J. P., Drost, M., Diaz, T., Roomes, C., and Thatcher, W. W. (1996). Effect of gonadotrophin-releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. Journal of Animal Science, 74, 154-161.

Senger, P. L. (1994). The estrus detection problem: New concepts, technologies, and possibilities. Journal of Dairy Science, 77, 2745-2753.

Short, R. E., Bellows, R. A., Carr, J. B., Staigmiller, R. B., and Randel, R. D. (1976). Induced or synchronized puberty in heifers. Journal of Animal Science, 43, 1254-1258.

Silcox, R. W., Powell, K. L., and Kiser, T. E. (1993). Ability of dominant follicles (DF) to respond to exogenous GnRH administration is dependent on their stage of development. Journal of Animal Science, 71(Suppl. 1), 219(Abstr.).

Sirois, J., and Fortune, J. E. (1988). Ovarian follicular dynamics during the estrous cycle in

heifers monitored by real-time ultrasonography. Biology of Reproduction, 39, 308-317.

Sirois, J., and Fortune, J. E. (1990). Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology, 127, 916-925.

Spicer, L. J., Leung, K., Convey, E. M., Gunther, J., Short, R. E., and Tucker, H. A. (1986). Anovulation in postpartum suckled beef cows. I. Associations among size and numbers of ovarian follicles, uterine involution and hormones in serum and follicular fluid. Journal of Animal Science, 62, 734-741.

Spitzer, J. C., Burrell, W. C., LeFever, D. G., Whitman, R. W., and Wiltbank, J. N. (1978a). Synchronization of estrus in beef cattle. I. Utilization of a norgestomet implant and injection of estradiol valerate. Theriogenology, 10, 181-200.

Spitzer, J. C., Jones, D. L., Miksch, E. D., and Wiltbank, J. N. (1978b). Synchronization of estrus in beef cattle. III. Field trials in heifers using a norgestomet implant and injections of norgestomet and estradiol valerate. Theriogenology, 10, 223-229.

Stevenson, J. S., Kobayashi, Y., and Thompson, K. E. (1999). Reproductive performance of dairy cows in various programmed breeding system including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$. Journal of Dairy Science, 85, 506-515.

Stevenson, J. S., Kobayashi, Y., Shipka, M. P., and Rauchholz, K. C. (1996). Altering conception of dairy cattle by gonadotrophin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. Journal of Dairy Science, 79, 402-410.

- Stevenson, J. S., Phatak, A. P., Rettmer, I., and Stewart, R. E. (1993). Postinsemination administration of receptal: follicular dynamics, duration of cycle, hormonal responses and pregnancy rates. Journal of Dairy Science, 76, 2536-2547. ✓
- Stewart, R. E. and Stevenson, J. S. (1991). Induction of estrus in thyroidectomized and ovariectomized nonlactating Holstein cows. Journal of Animal Science, 69(Suppl. 1), 397(Abstr.). ✓
- Stock, A. E., and Fortune, J. E. (1993). Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology, 132, 1108-1114. ✓
- Sunderland, S. J., Crowe, M. A., Boland, M. P., Roche, J. F., and Ireland, J. J. (1994). Selection, dominance and atresia of follicles during the oestrous cycle of heifers. Journal of Reproduction and Fertility, 101, 547-555. ✓
- Taponen, J., Katila, T., and Rodríguez-Martínez, H. (1999). Induction of ovulation with gonadotropin-releasing hormone during proestrus in cattle: influence on subsequent follicular growth and luteal function. Animal Reproduction Science, 55, 91-105. ✓
- Taylor, C., and Rajamahendran, R. (1991). Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. Canadian Journal of Animal Science, 71, 61-68. ✓
- Thatcher, W. W., De la Sota, R. L., Schmitt, E. J. P., Diaz, T. C., Badinga, L., Simmen, F. A., Staples, C. R., and Drost, M. (1996). Control and management of ovarian follicles in cattle to optimize fertility. Reproduction Fertility Development, 8, 203-217. ✓

Thatcher, W. W., Drost, M., Savio, J. D., Macmillan, K. L., Entwistle, K. W., Schmitt, E. J., De la Sota, R. L., and Morris, G. R. (1993). New clinical uses of GnRH and its analogues in cattle. Animal Reproduction Science, 33, 27-49. ✓

Thatcher, W. W., Macmillan, K. L., Hansen, P. J., and Drost, M. (1989). Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology, 31, 149-164. ✓

Tregaskes, L. D., Broadbent, P. J., Dolman, D. F., Grimmer, S. P., and Franklin, M. F. (1994). Evaluation of Crestar, a synthetic progestogen regime, for synchronising oestrus in maiden heifers used as recipients of embryo transfers. Veterinary Record, 134, 92-94. ✓

Turzillo, A. M., and Fortune, J. E. (1990). Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. Journal of Reproduction and Fertility, 89, 643-653. ✓

Twagiramungu, H., Guilbault, L. A., and Dufour, J. J. (1995). Synchronization of ovarian follicular waves with a gonadotrophin-releasing hormone agonist to increase the precision of estrus in cattle: A review. Journal of Animal Science, 73, 3141-3151. ✓

Twagiramungu, H., Guilbault, L. A., Proulx, J. G., Ramkumar, R., and Dufour, J. J. (1994). Histological populations and atresia of ovarian follicles in postpartum cattle treated with an agonist of gonadotrophin-releasing hormone. Journal of Animal Science, 72, 192-200. ✓

Twagiramungu, H., Guilbault, L. A., Proulx, J., Villeneuve, P., and Dufour, J. J. (1992). Influence of an agonist of gonadotrophin-releasing hormone (Buserelin) on estrus synchronization and fertility in beef cows. Journal of Animal Science, 70, 1904-1910. ✓

- Vasconcelos, J. L. M., Silcox, R. W., Rosa, G. J., Pursley, J. R., and Wiltbank, M. C. (1997). Synchronization rate, size of the ovulatory follicle, and conception rate after synchronization of ovulation with GnRH on different days of the estrous cycle. Journal of Dairy Science, 80(Suppl. 1), 178(Abstr.). ✓
- Verkerk, G. A., Taufa, V. K., Morgan, S., Clark, B. A., and Macmillan, K. L. (1998). Effects of oestradiol benzoate by injection at CIDR insertion for the treatment of postpartum anovulatory anoestrus in dairy cows. Proceedings of the New Zealand Society of Animal Production, 58, 82-84. ✓
- Walker, W. L., Nebel, R. L., and McGilliard, M. L. (1996). Time of ovulation relative to mounting activity in dairy cattle. Journal of Dairy Science, 79, 1555-1561. ✓
- Webb, R., and Armstrong, D. G. (1999). Control of ovarian function: effect of local interactions and environmental influences on follicular turnover in cattle: a review. Livestock Production Science, 53, 95-112. ✓
- Wehrman, M. E., Roberson, M. S., Cupp, A. S., Kojima, F. N., Stumpf, T. T., Werth, L. A., Wolfe, M. W., Kittok, R. J., and Kinder, J. E. (1993). Increasing exogenous progesterone during synchronization of estrus decreases endogenous 17-estradiol and increases conception in cows. Biology of Reproduction, 49, 214-220. ✓
- Williamson, N. B., Morris, R. S., Blood, D. C., Cannon, C. M., and Wright, P. J. (1972). A study of oestrous behaviour and oestrous detection methods in a large commercial dairy. II. Oestrous signs and behaviour patterns. Veterinary Record, 91, 58-62. ✓
- Wilson, S. J., Kirby, C. J., Koenigsfeld, A. T., Kuisler, D. H., and Lucy, M. C. (1998b). Effects ✓

of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. Journal of Dairy Science, 81, 2132-2138.

Wilson, S. J., Marion, R. S., Spain, J. N., Spiers, D. E., Keisler, D. H., and Lucy, M. C. (1998a). Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. Journal of Dairy Science, 81, 2124-2134.

Wiltbank, J. N., and Kasson, C. W. (1968). Synchronisation of oestrus in cattle with an oral progestational agent and an injection of an oestrogen. Journal of Animal Science, 27, 113-116.

Wiltbank, J. N., Ingalls, J. E., and Rowden, W. W. (1961). Effects of various forms and levels of estrogens alone or in combination with gonadotrophins on the estrous cycle of beef heifers. Journal of Animal Science, 20, 341-346.

Wiltbank, J. N., Sturges, J. C., Wideman, D., LeFever, D. G., and Faulkner, L. C. (1971). Control of estrus and ovulation using subcutaneous implants and estrogens in beef cattle. Journal of Animal Science, 33, 600-606.

Wiltbank, M. C. (1998). Improving reproductive efficiency in high producing dairy cattle. Proceedings of the XX World Association for Buiatrics Congress, Sydney, Australia, 571-583.

Wiltbank, M. C., Shiao, T. F., Bergfelt, D. R., and Ginther, O. J. (1995). Prostaglandin $F_{2\alpha}$ receptors in the early bovine corpus luteum. Biology of Reproduction, 52, 74-78.

Wishart, D. F. (1977a). Fertility of norgestomet treated dairy heifers. Veterinary Record, 100,

417-420.

Wishart, D. F. (1977b). Synchronisation of oestrus in heifers using steroid (SC 5914, SC 9880 and SC 21009) treatment for 21 days. 1. The effect of treatment on pregnancy rate to artificial insemination. Theriogenology, 8, 233-247.

Wolfenson, D., Flamenbaum, I., and Berman, A. (1988). Hyperthermia and body energy score effects on fertility and ovarian function in dairy cows. Journal of Dairy Science, 71, 3497-3504.

Wolfenson, D., Thatcher, W. W., Badinga, L., Savio, J. D., Meidan, R., Lew, B. J., Brawtal, R., and Berman, A. (1995). Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biology of Reproduction, 52, 1106-1113.

Wolfenson, D., Thatcher, W. W., Savio, J. D., Badinga, L., and Lucy, M. C. (1994). The effect of GnRH analogue on the dynamics of follicular development and synchronisation of estrus in lactating cyclic dairy cows. Theriogenology, 42, 633-644.

Wright, P. J., and Malmo, J. (1992). Pharmacologic manipulation of fertility. Veterinary Clinics of North America - Food Animal Practice, 8, 57-89.

Xu, Z. Z., and Burton, L. J. (1996). Reproductive efficiency in lactating dairy cows. Proceedings of the New Zealand Society of Animal Production, 56, 34-37.

Xu, Z. Z., and Burton, L. J. (1997). Reproductive performance of post-partum anoestrous dairy cows treated with progesterone and oestradiol benzoate. New Zealand Veterinary Journal, 45, 213-214.

- Xu, Z. Z., and Burton, L. J. (1999). Effects of oestrus synchronisation and fixed-timed artificial insemination on the reproductive performance of dairy heifers. New Zealand Veterinary Journal, 47, 101-104.
- Xu, Z. Z., Burton, J. R., Burton, L. J., and Macmillan, K. L. (1995b). Reproductive performance of synchronised lactating dairy cows. Proceedings of the New Zealand Society of Animal Production, 55, 242-244.
- Xu, Z. Z., Burton, L. J., and Macmillan, K. L. (1996). Reproductive performance of lactating dairy cows following oestrus synchronisation with progesterone, oestradiol and prostaglandin. New Zealand Veterinary Journal, 44, 99-104.
- Xu, Z. Z., Garverick, H. A., Smith, G. W., Smith, M. E., Hamilton, S. A., and Youngquist, R. S. (1995a). Expression of messenger ribonucleic acid encoding cytochrome P450 side-chain cleavage, cytochrome P450 17-hydroxylase, and cytochrome P450 aromatase in bovine follicles during the first follicular wave. Endocrinology, 136, 981-989.