STUDIES ON THE USE OF THE CIDR INTRAVAGINAL DEVICE FOR REPRODUCTIVE MANAGEMENT OF DAIRY CATTLE

ALBERTO RAUL DICK

1990

STUDIES ON THE USE OF THE CIDR INTRAVAGINAL DEVICE FOR REPRODUCTIVE MANAGEMENT OF DAIRY CATTLE

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF PHILOSOPHY

AT MASSEY UNIVERSITY

ALBERTO RAUL DICK

December, 1990

GENERAL ABSTRACT

This study was undertaken as a series of projects which involved selected studies with the CIDR[1] intravaginal device in dairy cattle.

In the first project, a CIDR device was inserted at different stages of the oestrous cycle to characterize the dynamic changes of the follicles in the ovaries of cycling cows, the associated changes in plasma progesterone concentrations (PPC), and the effects of progesterone from the device on cycle lengths. The results demonstrated that the progesterone released by the CIDR appeared to enhance the development and maintenance of a dominant follicle. The reproductive responses after device removal were influenced by the follicular population, and by the stage of the follicular wave even in the absence of a corpus luteum (CL). The PPCs during CIDR insertion or after removal were influenced by the type of animal and the stage of the cycle when treatment was initiated. The stage of the oestrous cycle in which the device was inserted also influenced the average cycle length.

In the second project, the tailpainting and raddle technique combined with scoring and re-raddling gave a precise correlation with visual oestrus detection and with patterns in the onset of oestrus in groups of heifers and cows after different synchrony treatments. The average interval to oestrus was concentrated between 30 and 120 h after using different treatments for oestrus synchronization.

In the third project, a controlled breeding programme for cows in herds with a daily milk quota was investigated, with a view to improving reproductive performance through the strategic use of the CIDR intravaginal device. The study showed that the mean interval from Planned Start of Mating (PSM) to first insemination was shorter in all treated groups in year-round herds, and treated cows had fewer days open than control. Conception rate to first insemination differed statistically between groups, but not for second inseminations. As a consequence, the mean number of services per conception also differed significantly between groups.

Oestrous responses in all farms differed significantly in the treatment group compared with the control group (76% vs 63%; $P < 0.001$, respectively). The return to oestrus in treated cows was synchronized in 85% of non-pregnant cows on days 22 to 25 after first insemination. The average responses to the two doses of prostaglandin at CIDR removal was similar for half dose vs full dose (72% vs 68%, respectively). However, this management advantage was partly lost because of lower fertility and because of mistakes relating to the interpretation of the tailpainting system which were frequently made by the owners.

---

[1] Controlled internal drug releasing device (Eazi-Breed CIDR™-B, Carter Holt Harvey Plastic Products, Hamilton, N.Z.

In the fourth project, the effectiveness of using a milk progesterone test to identify non-pregnant animals and consequently to improve oestrus detection rates was evaluated in a management system for dairy cows involving the use of CIDR devices for controlling return to service intervals. The average percentage of non-pregnant cows inseminated during the second period of artificial insemination (AI) was 63% and varied from 44% to 77.2% among individual herds. In general, the study showed that the identification of non-pregnant animals did not improve the percentage of animals inseminated over the second period of AI.

In the last project, the dynamic changes of follicles on the ovaries at two different post-partum periods was characterized as well as the post-treatment response rates in oestrus and ovulation in anoestrous dairy cows. The population of follicles in classes 1 (< 6 mm), 2 (6 to 9 mm) and 3 (> 9 mm) varied between cows in both post-partum periods, but the average number of follicles did not differ significantly between day 25 (early) and 50-55 (late) for treatment and control groups. In the late post-partum period, the average number of class 1 follicles increased in the animals of the treated groups ($P < 0.05$), and when the comparison in treatment groups was made between early and late post-partum period, the average number of class 2 follicles and the total number of follicles were both increased at CIDR removal ($P < 0.05$; $P < 0.01$, respectively). The average number of class 1 follicles in the early post-partum period increased significantly in the treatment group irrespective of whether or not animals displayed oestrus or ovulated with CL formation after treatment with CIDR/PMSG. The diameter of the largest unovulated luteinized follicle in treated cows which displayed oestrus and/or ovulated increased significantly during CIDR treatment, and its growth continued after the device was removed.

In both post-partum periods, normal and luteinized class 3 follicles were found in non-cycling cows, where some had large normal and others had large luteinized follicles. Only 25% and 33.3% of the treated cows in the early and late post-partum period displayed oestrus, respectively. However, 55% and 50% of the treated and control cows which had not displayed oestrus, actually ovulated and formed a CL in the early post-partum period.

From the results of this study, one can conclude that although insertion of a CIDR device into cycling cows synchronizes oestrus, there remains significant variation among animals in the precise time after device removal that oestrus commences. Moreover, some animals have reduced fertility at the synchronized oestrus. To overcome these two limitations on the benefits of synchronization, the duration of treatment programmes based on a progestagen will have to be adjusted to obtain high conception rates, and greater account will have to be taken of follicle wave patterns as a factor influencing precision of synchrony. These considerations apply also to the re-use of CIDRs after insemination, to reduce variation in return to oestrus and to improve fertility in cows which do not conceive to insemination at the first synchronized oestrus.

To ensure precise synchrony it is important to emphasize that the most common problem in breeding management in dairy cows is inadequate oestrus detection. The tailpainting system may be less satisfactory in herds with continuous calving patterns. It shows particular potential for further studies

between animals which may not display oestrus, and the associations of the tailpaint and raddle technique in animals with different coats and skeletal conformation.

In non-cycling cows, further studies have to be focused on the importance of identifying factors which affect the treatment response, the endocrinological characteristics of these animals, and the interactions of the nutritional status, body condition and ovarian function during the post-partum period.

ACKNOWLEDGEMENTS

This thesis is dedicated to the memory of my father Julian Alberto, who was my best friend and father. Thanks viejo (dad) I am very proud to be your son.

My project was unusual in that one of my objectives was to come to New Zealand to work with Dr K. L. Macmillan of Ruakura Agricultural Centre, because of his international reputation in dairy cattle reproduction. Since Dr Macmillan does not work in a University, the project was set up at Massey University under the joint supervision of Professor R. S. Morris (who has arranged my training and research activities) and Dr Macmillan (who has provided the specialist research guidance in reproduction). I therefore wish to express my sincere thanks and appreciation to Dr K. L. Macmillan for his continued interest, guidance, support and invaluable advice, not only in the completion of this work, but also in the development of my way of thinking in reproduction. I also wish to express my gratitude to Professor Morris for his invaluable guidance, advice and organizing my research to ensure the completion of this study.

I appreciate the help and guidance of Professor Fielden, who was co-supervisor during the first part of my training here. I also appreciate the rewarding discussions that I had with Professor N. Williamson during the final part of my studies here at Massey.

Special gratitude is extended to Mr Chockchai Chaimongkol of Thailand for valued friendship and willing assistance throughout the entire duration of my research in this country.

Special thanks is extended to Debbie Lovelock, Dirk Pfeiffer, Robert Sanson, Bill Tranter, Eugene Lañada, Brigitte Revol, Kerry Killorn and students of the 1989 fifth year class for their assistance with my experimental work. Also technical and farm staff of the Department of Veterinary Clinical Sciences, the Dairy Cattle Research Unit and Massey N° 4 Dairy Unit for their assistance in various ways during my work.

Dr K. Lapwood and Ms Jane Candy for the advice and assistance with the progesterone and LH assays.

Professor S. McCutcheon for my training in cannulation in cattle.

Fiona Dickinson for the assistance in computer programmes and her many helpful suggestions in my thesis preparation.

Special thanks are due to the dairy farmers in the Manawatu, for allowing me to work with their cows, in particular Mr and Mrs Johnston, Mr and Mrs Hislop, Mr and Mrs Cowen, Mr and Mrs McCarthy and Mr O'Connor and also for their help to complete my field research.

The financial support from the New Zealand government is also acknowledged.

All the other people, too numerous to mention, of the Department of Veterinary Clinical Sciences for their friendship and assistance.

A special thanks is extended to Emilio Ruz and J. Guillermo Cruz C. for their advice and help with the preparation of my thesis.

I have a deep respect for my Kiwi friend Sara Cotterall, who gave not only assistance and support, but also shared her knowledge and experiences with me which has helped make my stay in New Zealand a happy one.

A very special thanks is also extended to my dear mother, my Argentinian friends and relatives for their love, their faith in me, and their moral support during my studies here at Massey.

Finally, my wife Diana, and children, Martin, Mariana and Laura for always having the patience, tolerance, love and understanding while I completed this thesis, without which it would never had been written.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Page

GENERAL INTRODUCTION

The present thesis was undertaken as a series of five projects in which selected studies were undertaken on the use of the CIDR intravaginal device in dairy cattle.

The first study was designed to characterize the interactions involving the dynamic changes of follicles in the ovaries of cycling cows, measured by ultrasound during and after CIDR insertion and until oestrus, and the associated changes in plasma progesterone concentrations at the different stages of the oestrous cycle in entire cows, and in ovariectomized animals.

The second study concerns an evaluation of the effectiveness of tailpainting combined with the use of an aerosol raddle technique in detecting oestrus in synchronized animals. Other aims were to compare the patterns of onset of oestrus with different synchrony treatment, duration of oestrus, and some observations of oestrous behaviour in animals forming sexually active groups.

The third study evaluated a reproductive programme in dairy herds with a daily quota, and its effects on oestrus detection rates and return to service intervals, oestrous responses to two dose rates of prostaglandin combined with CIDR, and the effect of progesterone supplementation on fertility.

The fourth study was designed to evaluate the effectiveness of using milk progesterone tests to identify non-pregnant animals, and consequently improve oestrus detection rates in cows previously synchronized in a controlled breeding management system involving the use of CIDR devices for controlling return to service intervals.

The fifth study concerns a study of dynamic changes of follicles in the ovaries by using ultrasonography during two different phases of the post-partum period (early and late), an examination of the effects of CIDR insertion and injection of PMSG, and measurement of post-treatment response rates in oestrus and ovulation in anoestrous dairy cows.

CHAPTER 1

OVARIAN FOLLICULAR POPULATION OESTROUS RESPONSE PLASMA PROGESTERONE CONCENTRATIONS AND LENGTH OF CYCLES IN COWS TREATED WITH AN INTRAVAGINAL DEVICE AT DIFFERENT STAGES OF THE OESTROUS CYCLE

ABSTRACT

This study was designed to characterize the interactions involving the dynamic changes of the follicles in the ovaries of cycling cows treated with CIDR devices inserted for 10 days when animals were at day -2 (CD, day 0 = day of oestrus) of the oestrous cycle (Pro-oestrous CIDR insertion group [PCI]), CD 3 (Metoestrous CIDR insertion group [MCI], CD 7 (Early-dioestrous CIDR insertion group [E-DCI], CD 13 (Late-dioestrous CIDR insertion group [L-DCI] and Late-dioestrous + prostaglandin [PGF] during CIDR insertion group [L-DCI + PGF]), and the associated changes in plasma progesterone concentrations (PPC) at the different stages of the oestrous cycle in the same cows, and in ovariectomized animals. The effect of supplemental progesterone from a CIDR device on cycle lengths in cycling animals treated at different stages of the cycle also has been studied. The same animals were used in the treatment group, as well as in a control group when they were studied throughout one entire normal oestrous cycle. Some were used in the ovariectomized group. Ovarian function was measured by ultrasound during and after CIDR insertion and until oestrus. Blood samples were collected at different stages of the cycle, and treatment sequences were chosen to best reflect PPC patterns associated with device use, and during a normal cycle.

The follicular population in the ovaries was affected by the presence or absence of a corpus luteum (CL). The size of the largest follicle at CIDR removal was smaller in cows with a CL, and the number of class 3 (large; > 9 mm diameter of clear antral fluid) follicles was increased ($P < 0.10$). In cows without a CL, follicle diameter increased steadily until day of device removal when it averaged 15.2 ± 0.8 vs 12.7 ± 0.8 mm in cows with a CL ($P < 0.05$).

Most cows (60%) came into oestrus within three days of device removal. Cows having a short interval to oestrus (< 72 h) had a lower number of medium sized follicle (class 2; from 6 to 9 mm diameter) on the day of device removal. There was a tendency for these cows to have fewer class 1 (small; < 6 mm diameter) and more class 3 follicles. This tendency was also observed between stages of the cycle. Moreover, cows having shorter intervals to oestrus had a higher number of class 3 follicles on the ovulatory ovary. There were significant interactions between treatments initiated at different stages of the oestrous cycle and the interval to oestrus, number of follicles in each class, and CL status of the ovary, especially when CIDR devices were inserted during pro-oestrus when all cows had a short post-treatment interval to oestrus, and with early dioestrus insertion when all cows had a long interval to oestrus.

Most cows which were in oestrus soon after device removal and then ovulated, had a large follicle which developed or was maintained from the early stages of the CIDR treatment period. Cows with longer intervals (> 72 h) had more follicular waves during CIDR treatment, and the ovulatory follicle was detected later during treatment, or during the post-treatment period ($P < 0.05$).

Overall, the average PPC 1 h after device insertion in cycling cows was 2.1 ng/ml compared with 6.2 ng/ml in the ovariectomized cows ($P < 0.01$). Changes in average PPC 1 hour after insertion also differed between animals treated at different stages of their cycles, being 2.4 ng/ml for the PCI group, no difference in the MCI group, 4.5 ng/ml for the E-DCI group, and 2.2 and 1.6 ng/ml for L-DCI and L-DCI + PGF groups, respectively.

The average length of the oestrous cycle in the metoestrous group was 16.6 ± 1.5 days compared to 21.4 ± 2.1 days for the control group ([NC] $P < 0.01$). Dioestrous groups and the early dioestrous group had similar average length cycles, but values differed from the untreated control group, (26.0 ± 1.7, 25.5 ± 1.9, 24.4 ± 2.3 days respectively, compared with the control group at 21.4 ± 2.1 days).

These results demonstrate that the progesterone released by the CIDR appeared to enhance the development and maintenance of a dominant follicle. The post-treatment responses after CIDR treatment were influenced by the follicular population, and by the stage of the follicular wave, even in the absence of a CL. PPC during the CIDR insertion phase or after removal were influenced by the type of animal (ovariectomized, cycling), and the stage of the cycle when treatment was initiated.

INTRODUCTION

Ultrasonography has opened several new lines of research for clinicians and reproductive biologists. When first introduced into theriogenology, ultrasound scanners were used primarily for early pregnancy diagnosis and detection of twins in mares and ewes (Ginther, 1986). Transrectal real-time ultrasound scanning of the bovine reproductive tract and ovarian structures allows the operator to view images of structures which can sometimes be palpated (Pierson and Ginther, 1984).

Images of the ovaries via ultrasound scanning can provide information about the follicle population. Changes in the growth and development and regression of the follicles in the ovary of cattle have been studied with the aid of daily scanning (Pierson and Ginther, 1988a; Savio et al., 1988; Sirois and Fortune, 1988). Follicular populations were studied in cattle when a luteal phase was artificially lengthened with exogenous progesterone (Sirois and Fortune, 1990), and during the growth and regression of follicles in the late luteal phase of the oestrous cycle in heifers undergoing spontaneous or prostasglandin F 2 alpha (PGF) induced luteolysis (Quirk et al., 1986). Dynamic changes in follicles have not been studied during on oestrous cycle associated with a treatment with CIDR-B device.

For the last 35 years, direct research contributions have been made towards a general goal of developing effective methods for the control of the oestrous cycle in cattle. There are several programmes involving the use of hormonal compounds. Oestrous cycles have been synchonized in cattle with progestagens, PGF, progestagen and oestrogen combinations, progestagen and PGF combinations, and progestagen and PGF combined with gonadotrophin releasing hormones (GnRH).

Ideally, a synchrony system will be one which can at least maintain normal fertility, produce a high degree of synchrony, and be economically used to control the oestrous cycle before the first insemination, as well as before subsequent inseminations among those animals which return to service (Macmillan, 1988c; Odde, 1990).

Daily injections of progesterone or injecting and feeding potent orally active progestagens for 14 to 20 days produced effective synchrony, but had low fertility (Zimbelman et al., 1970; Wiltbank et al., 1967). Other forms of treatment with a progestagen have involved the use of a subcutaneous ear implant containing norgestomet (Wiltbank and Gonzalez Padilla, 1975), or an intravaginal device releasing progesterone (PRID), with a capsule of oestradiol benzoate applied to the surface (Roche, 1978), or in combination with PGF (Roche, 1976b). More recently a new intravaginal device (CIDR, controlled internal drug release device) containing progesterone has been developed to treat cycling and non-cycling animals (Macmillan and Day, 1987; Macmillan et al., 1990b).

Synchronization systems can successfully condense most oestrous events into

a 3 to 5 days period (Roche and Ireland, 1984), but there is a considerable variation between animals within this typical period.

Several trials have described plasma progesterone concentrations (PPC) in cycling and ovariectomized animals treated with a progesterone intravaginal device. PRID was used by Roche and Gosling (1977), Munro and Moore (1985, 1986), Munro (1987), and Robinson et al. (1989). CIDR was used by Munro (1987), Macmillan et al. (1990c), and Peterson and Henderson (1990). However, the importance of progesterone treatment and associated PPC at the different stages of the cycle on ovarian follicular status have not been studied. Thus, the specific aims of this study were to characterize the interactions involving follicles in the ovaries, to profile the changes in plasma progesterone, to measure treatment effects on cycle lengths, and to closely observe the oestrus response in animals treated with CIDR devices at different stages of the cycle.

LITERATURE REVIEW

Ultrasonic Imaging and Reproductive Events in Cattle.

Diagnostic ultrasound instrumentation has been available to the medical community since the early 1970s. The early history of the development of diagnostic ultrasound commenced in 1880 with the discovery of the piezoelectric effect. Sonar equipment was used in the 1940s to demonstrate echoes deep within body tissues. In the 1960s, the contact scanner permitted visualising two level images (black and white). The use of the electronic scan converter in the early 1970s and gray-scale imaging, together with the development of real-time or dynamic imaging in the late 1970s made this powerful technology adaptable for the study of the internal reproductive tract in large domestic animals by using a transrectal probe.

Piezoelectric properties of certain crystals permit the conversion of electric current to ultrasound waves, and the subsequent conversion of the mechanical energy of echoes into electric current. This principle was utilised during World War II when ultrasound waves were used to detect submarines. It was also adopted for the detection of tissue reflectors in which a water-path scanner was used with patients who were observed in a tank. A submerged transducer moved in a circle around the patient. This represented an important advance, because the transducer could be applied directly to the subject without passage of the sound waves and echoes though a water bath. A layer of gel between the skin surface and the transducer served to eliminate air, which would have blocked the passage of the ultrasound waves. These older scanners used a system in which the wide range of echo amplitudes was compressed into two levels (the images were either black or white).

Many amplitudes of echoes were represented by levels of the gray scale. The signals were stored in a screen converter and then displayed on a television monitor. The original converters were replaced finally by digital scan converters, which stored the echo information in a memory similar to that used in personal computers. The development of real-time or dynamic imaging allowed the operator to observe movements as they occurred (e.g., heartbeats, moving fetal limbs).

Several reports in the early 1980s generated excitement over the potential of the technique in evaluating and monitoring reproductive structures and events in mares. Since then, ultrasound scanners have been integrated rapidly into clinical and commercial programmes in the breeding of animals (Pierson et al., 1988a).

Although the form of diagnostic ultrasonography to be described in detail is the B-mode for two-dimensional imaging, two other modes are available for study of soft tissue. The A-mode (amplitude mode) produces a one-dimensional display of echo amplitudes for various depths. It is in widespread use for evaluating the fat and lean portions of meat animals. It has been used for pregnancy diagnosis in ewes. The M-mode (motion mode) form of imaging is an adaptation of the B-

mode which is used for evaluating moving structures of the heart. Doppler ultrasound systems are used to monitor fetal heartbeat and blood flow in large vessels; this system has been used for pregnancy diagnosis in the late fetal stage in large animals by detecting the enlarged principal uterine vessels (Ginther, 1986).

Principles.

Ultrasonography is becoming increasingly important in veterinary medicine. It is harmless, painless and less expensive than other alternative imaging techniques. It uses high frequency sound waves, ranging from 2-10 megahertz (MHz), or 2-10 million cycles per second (audible sound varies from 20-20,000 Hz), to produce images of soft tissues and internal organs in a similar way to sonar being used to map the ocean floor. The sound waves are produced from a vibrating object, the transducer, which is made of a piezo-electric material. The vibrating frequency is determined by the thickness of the transducer, with thinner transducers producing higher frequencies. The wave is produced by applying a high voltage electrical pulse to the crystal for a few microseconds. The transducer receives reflected sound waves. These are converted to a set of rhythmic voltages which are modified within the machine, and ultimately produce an image on a cathode ray tube as dots of light on a black background. The amplitude of the echo determines the brightness of the dots. The image on the screen results from displaying all the echo signals from all the interfaces at the same time. The ultrasound beam must be moved to produce a succession of images. This is done either mechanically with the transducer moving, or electronically, where the transducer is stationary, but the beam is redirected using electronics (Powis, 1986). When the beam is moved in the form of an arc, it is called a sector scan. The rectal transducers used with cows produce a rectangularly shaped image. These are referred to as linear array scans.

Short bursts of ultrasound are emitted into a patient or subject from the transducer. The sound waves travel though the patient or subject at a constant speed until they meet a reflecting surface. A small component of the sound beam is reflected back to the transducer at the reflecting surface. The rest of the sound beam continues, but subsequently may send back echoes at all reflecting surfaces. A reflecting surface is an interface between two tissues with different acoustic impendance. The best images of structures and boundaries usually come from mirror-like reflections. This occurs when the ultrasound beam is perpendicular to the reflecting surface.

Sound is a mechanical wave of compressions and rarefaction within a medium. A sound wave can be compared to a longitudinal wave having a wavelength, frequency and velocity. If the speed of sound in a particular medium is known, then the time it takes for a pulse of sound to be emitted and returned to its source can be measured, and consequently the distance the sound has travelled can be calculated. The propagation velocity for ultrasound is 1540 m/s, with a wavelength of less that 1 mm in typical soft tissues.

Velocity of wave = frequency x wavelength
Distance travelled = velocity x time (Fig. 1.1)

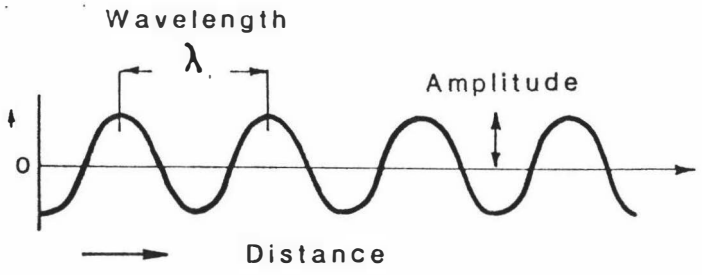


Fig 1.1 Properties of a typical longitudinal wave. Each wave has a characteristic wavelength (λ), frequency, and velocity. The amplitude represent the intensity of the sound (Herring and Bjornton, 1985).

Instead of reporting the actual distance the sound beam has travelled from the transducer to the reflecting surface as a number, the ultrasound scanner converts this information into dots of light on a screen. The amplitude of the echoes received is reported in terms of brightness of these small dots of light on the screen. The position of the dot of light on the screen is proportional to the actual distance travelled. Thus, an image of the tissues and organs traversed can be constructed by the sound beam.

Interaction of the Sound Beam with Tissues.

The characteristics of time determine what proportion of the sound beam can be reflected.

The reflected position is represented on the ultrasound image by shades of gray, extending from black to white. Liquids do not reflect sound waves (i.e. are nonechogenic or anechoic). Therefore, the image of a liquid-containing structure will appear as a black zone on the screen. Dense tissues (bovine cervix) will reflect much of the sound beam (i.e. they are hyperechogenic, or hyperechoic) and will appear as white areas on the screen. Other tissues are seen in various shades of grey, depending upon their echogenicity (or ability to reflect sound waves).

The sound beam is attenuated as it travels though the body until a reflecting tissue is reached.

The principal causes of attenuation are:

a) absorption;

b) reflection; and

c) scattering.

Absorption by the tissues can be as heat. Waves lose energy in the form of heat. This loss of energy limits the depth to which the ultrasound waves can penetrate. A 5.0 MHz transducer penetrates 100-120 mm compared to only 30-50 mm for a 7.5 MHz transducer.

Reflection refers to small portions of the ultrasound beam that strike reflective surfaces and are returned to the transducer. The remainder of the waves are reflected in non-perpendicular directions.

Scattering occurs when small irregular surfaces are encountered and the sound is scattered in all directions in an unpredictable manner.

A sound beam travels at approximately 1540 m/sec in soft tissue. Therefore, the only variable that contributes to the difference in acoustic impedance of one soft tissue to another is its density. This creates an impedance mismatch (reflecting surface) which is relatively small, but a small portion of the beam is reflected back to the transducer. The rest of the beam continues though to the next tissue producing an interface.

A sound beam travels at approximately 3100 m/sec in bone (almost twice as fast as in soft tissue). Thus, a soft tissue/bone interface represents a relatively large impendence mismatch. A high percentage of the beam is reflected back to the transducer, and the rest of the beam is absorbed to create an acoustic shadow.

A sound beam travels at approximately 221 m/sec in air. Thus, a soft tissue/bone interface with air again represents a large impedance mismatch. A strong echo is returned to the transducer, and little of the sound beam traverses the air medium; the bone and air have acted as barriers to the sound waves. Ultrasound technology is most suitable for soft tissues or soft tissue/fluids where the proportion of beam reflected is large enough to be registered on a TV monitor, but not large enough to cause significant attenuation of the sound beam. Locating a scan plane that avoids bowel gases, lung or bony structures is called "finding an acoustic window" (Fig. 1.2).

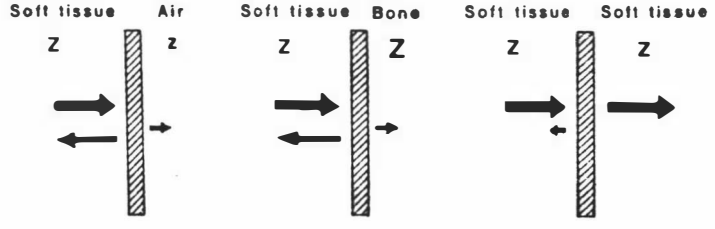


Fig 1.2 The various potential acoustic interfaces encountered by the sound beam. The arrows represent pulses of sound (Herring and Bjornton, 1985).

The resolution of an ultrasound system can be defined as its ability to distinguish closely related structures. It is divided into axial resolution and lateral resolution. Axial resolution is a measure of the ability to show two interfaces when they are closely separated along the axis of the ultrasound beam. It also is determined by the length of the ultrasound pulse generated by the transducer. The smaller the axial resolution, the better the image. The longer the wavelength, the poorer the axial resolution. Therefore, high frequency transducers are preferred for maximum axial resolution. Lateral resolution refers to the ability to distinguish between two echo-forming surfaces lying side by side in parallel with the sound beam. The major contribution to image quality and system resolution is the lateral resolution, which depends on the shape of the ultrasound field, produced by the transducer (Ligtvoet et al., 1989).

The mammalian body is composed of many layers of tissue. A tissue interface occurs wherever tissues of different densities or acoustic impedance (measured of the resistance of the tissue to propagation of the ultrasound waves) are in contact. The reflected wave is directly proportional to the difference in acoustic impendance across the tissue interface. The density and organization of a tissue result in a characteristic ultrasonic pattern (echotexture), which allows the identification of many of the internal organs. The reproductive organs for example, may vary depending on reproductive status. Morphological changes occur in the uterus in response to changes in the pattern of secretion of the ovarian hormones. These morphological changes are reflected in the echotexture of the uterus (Herring and Bjornton, 1985; Rantanen, 1986; Pierson et al., 1988b).

Artifacts may be due to sound beam properties, transducer quality, instrument adjustment or scan techniques. Some artifacts, such as acoustic shadowing and distance enhancement can improve the diagnostic accuracy. Others, such as reverberation and mirror images can cause confusion.

The commonly encountered artifacts are:

a) acoustic shadows which are caused by the disminished transmission of sound due to attenuation and/or reflection of the sound beam at acoustic interfaces such as soft tissue gas or bone (Herring and Bjornton, 1985). For example, forms of shadowing may occur when the path of the sound beam is obstructed by bowel gas, or if there is a lack of intimate contact between the transducer and rectal wall as when faecal material has adhered to the transducer;

b) refraction occurs when the portion of the sound wave which strikes the side of the curved boundary of a structure at less than 90° may refract, causing a shadowing or lack of echo formation beyond the site of refraction. These artifacts are common in images of the ovary, as the beam encounters fluid-filled follicles or the sides of spherical embryonic vesicles (Pierson et al., 1988b);

c) enhanced-though-transmission which is when the sound beam passes though a relatively homogenous medium such as urine, bile or fluid of ovarian follicles or embryonic vesicles. Less attenuation takes place than in the surrounding echogenic areas. When the sound beam strikes the far wall of the structure, the echoes appear brighter than the surrounding structures (Herring and Bjornton, 1985);

d) specular reflection occurs if the portion of the beam which strikes the upper and lower surface of a fluid-filled spherical structure produces a highly echogenic reflection on the ultrasound image of the structure. Specular reflection can be a considerable aid in some instances, e.g. examination of small vesicles (3-6 mm diameter);

e) reverberation produces artifacts which are commonly seen during rectal examination of the reproductive tract, because gas filled segments of the intestine reflect sound waves back to both the transducer and also off the near and far surfaces of the gas filled structure. Due to the lag time of each returning echo, bright echoes are recoded on the image at deeper and deeper levels. There are distinguishing characteristics of reverberations which are: equidistant, gradually diminish in intensity and are parallel to the reflective interface (Pierson et al., 1988b);

f) mirror-image artifacts occur at highly reflective interfaces and are caused by multiple internal reverberations. Returning echoes reach the transducer with a time delay and are registered on the image as being beyond the highly echogenic interface (Herring and Bjornton, 1985).

Equipment.

The resolving power of the equipment is dependant on the frequency of the sound waves. High frequency provides greater detail, and lower frequency provides greater tissue penetration. A larger area is viewed, with a low frequency transducer but with less detail. A smaller area is viewed, with a high frequency transducer but with more detail. High frequency transducers (e.g. 5.0-7.5 mHz) are intended for detailed study of structures close to the transducer and are preferred for intrarectal examinations of the reproductive tract of cattle.

There are two basic types of portable ultrasound equipment. These are:

a) the mechanical sector scanner which provides a small acoustic window and good image quality, with the capability of two dimensional imaging; and,

b) the linear array scanner where single small transducers are fixed sequentially to give a rectangular shaped image. The

main disadvantage is that the large scanheads require large acoustic windows.

The ultrasound imaging is done in real-time. This refers to the continuous upgrading and display of the image on a non-storage monitor. Its big advantage is that movement of the patient becomes less important. Recording of the image is possible using videotapes or multiformat cameras.

Since the ultrasound frequencies do not penetrate air. It is important to have close skin contact with the transducer head. This is achieved in the body scan, by clipping the hair and by using an aqueous coupling gel.

The ultrasonographer is an integral part of each ultrasonic examination at three distinct levels. The most basic level is the eye to hand coordination necessary to locate the organs. Difficulties occasionally may arise when structures such as a loop of bowel or faecal material disrupt the image. Secondly, once the structures of interest are located, the "ultrasonic anatomy" must be evaluated. Finally, the basic principles of diagnostic ultrasound and the biological subject under study must be constantly integrated.

Uses.

Ultrasonography has opened several new lines of research for clinicians and reproductive biologists.

Diagnostic ultrasound may be used to examine virtually any soft tissue in large animals. When first introduced into theriogenology, ultrasound scanners were used primarily for early pregnancy diagnosis and detection of twins in mares and ewes. As a result of recent equipment modifications and evaluations, it now has a more fundamental role in research programmes in reproduction.

Ultrasonography enables the visualization of ovarian and uterine structures for the identification of both physiological and pathological conditions. Also, the applications to the embryo transfer industry include evaluation of the follicular population and ovarian response to superovulation regimes as well as evaluating the reproductive tract of potential recipients, and diagnosing pregnancy and embryonic loss in recipients.

Controlled Breeding in Cattle.

For at least 35 years, direct research contributions have been made towards a general goal of developing effective methods for the control of oestrus in cattle.

A major reason for the development of effective techniques for regulation of the oestrous cycle has been to facilitate the use of Artificial Insemination (AI) in a breeding programme designed to accelerate genetic improvement for increased production efficiency.

Although AI has been widely and routinely used in most dairy industries for

decades, its successful application has been compromised in various ways by the added requirement it imposes on farm staff for accurate oestrus detection.

Succes in the use of AI can be enhanced by implemention a reproductive management system in a dairy herd which takes advantage of techniques available for improving reproductive efficiency in dairy cows. These aspects include semen processing, packaging and storing; semen and sire fertility; detection of oestrus; computerized dairy reproductive herd health records and controlled breeding programmes (Macmillan, 1988b).

Although "synchronization of oestrus" has been routinely used to identify this last area of reproductive management, the term "controlled breeding" better fits the situation, since not all aspects of controlled breeding are directed at obtaining synchronous oestrus and ovulation.

Oestrus detection has been shown to be a significant problem related to AI. It requires time, labour, and skill and can be expensive. It has often been cited as a major factor limiting the widespread use of AI in cattle (Smith, 1986; Macmillan, 1985; Foote, 1975; O'Farrel, 1984), and its economic implications have been frequently reviewed (Oltenacu et al., 1981; Bailie, 1982).

If oestrus detection is not correctly applied, then some of the potential advantages from successfully using synchronization techniques will be reduced, and may be lost (Klingborg, 1987).

There are other factors which may affect detection of oestrus. As herd size increases, more efficient use of labour in dairy herds becomes essential. Any means which allow groups of animals to be brought into oestrus over a short period of time and conceive within a defined postpartum interval offer distinct advantages. However, problems of insemination, travel costs and the practical use of AI in cows and heifers are disadvantages for some dairy farmers.

Oestrous cycle control offers several applications in addition to synchronized breeding programmes. These include management of replacement heifers in beef and dairy herds, breeding control for planned lactations in dairy cows and oestrous synchronization in embryo transfer programmes.

There are several programmes involving the use of hormonal compounds. Their use depends on the requirements of each farm or ranch which may be operating under different management principles and have different economic constraints. They have different realities, requirements and objectives. For these reasons the goal of veterinary involvement in dairy cattle herds must be greater productivity, efficiency and profitability, especially in controlling reproductive cycles. It is necessary to consider at least three major contributory factors:

a) general on-farm herd management;

b) level of nutrition; and,

c) relevance of a herd health programme.

In cyclic animals, control of the timing of ovulation is partly dependent on controlling regression of the corpus luteum (CL). The two main methods of controlling the time of ovulation are:

a) the induction of premature but predictable regression of the cyclic CL with prostanglandin F2 alpha (PGF), or its analogues; and

b) to maintain animals in an artificial luteal phase with exogenously administered progesterone until endogenous regression of the CL has occurred in all animals (Roche and Ireland, 1984).

Oestrus has been synchronized in cattle with progestagens; prostaglandins; progestagen and oestrogen combinations; progestagen and prostaglandin combinations; and progestagen and prostaglandins combined with gonadotrophin releasing hormones (GnRH).

Progestagens administered for 14 to 20 days are effective in synchonizing oestrus. However, fertility at the synchronized oestrus is subnormal. Duration of progestagen treatment can be reduced by combining it with an oestrogen at treatment initiation.

Prostaglandins can be used in double or single injection programmes. Fertility of the oestrus after treatment is similar to or better than that of untreated contemporaries. Oestrus also has been synchronized effectively by combining a 5 to 9-day progestagen treatment with PGF at or near the end of treatment.

Ideally, a synchrony system will be one which can at least maintain normal fertility, produce a high degree of synchrony, and be economically used to control the oestrous cycle before the first insemination, as well as before subsequent inseminations among those animals which return to service (Macmillan, 1988c; Odde, 1990).

Progestagens.

Progestational compounds have been widely used to control the oestrous cycle of cattle. Several compounds were researched in the 1960's as possible synchronization compounds.

One of the more widely used progestagens is melengestrol acetate (MGA) which is administered orally (Zimbelman and Smith, 1966; Rousell et al., 1969). Oestrus and ovulation is suppressed in cattle receiving MGA for 10-18 days. The percentage of MGA-treated females in oestrus in a 6-day period after treatment was similar to the percentage of controls in oestrus in a 20-day period (Zimbelman et al., 1970). The fertility at first service was 14% lower for treated animals than for the controls. Fertility also was reduced after administration of 6-chloro-6-dihydro-17-acetoxy-progesterone (CAP; Hansel et al., 1966), 6-methyl-17-acetoxy-progesterone (MAP; Zimbelman et al., 1966; Hansel et al.,1961), and dihydroxyprogesterone acetophenide (DHPA; Wiltbank et al., 1967).

Progestagen-Oestrogen Combinations.

In general, treatment of cattle with short term progestagen systems (a 7-12 day period) has not been associated with reduced fertility (Roche, 1974a, 1876a), but a luteolytic agent should be used to be effective in synchronizing oestrus. However, some reports showed that short-term treatments sometimes produced poor fertility (Smith et al., 1986; Van Cleeff et al., 1989; Chenault et al., 1990).

Most trials using a subcutaneous ear implant containing 6 mg of norgestomet plus an injection of 5 mg of oestradiol valerate and 3 mg of norgestomet given at the time of implant insertion (Syncho-Mate B) produced a high percentage of cattle showing oestrus soon after treatment. The range of animals showing oestrus within 5 days after treatment was 77 to 100%. However, the fertility at first service ranged between 33 and 68% (Odde, 1990).

Capsules containing oestradiol benzoate which are fixed to the surface of a PRID also were effective for oestrus synchronization in cattle (Roche, 1978; Sprott et al., 1984). The CIDR has also been used successfully with an oestradiol benzoate capsule to synchonise oestrus in heifers using a treatment interval of 12 days (Macmillan et al., 1985, 1988a).

Prostaglandin F2 alpha and its Analogues (PGF).

The luteolytic properties of PGF have been well established in cattle (Lauderdale, 1972; Rowson et al., 1972; Roche, 1974b; Jackson et al., 1979; Dobson et al., 1975; Ireland and Roche, 1982; Moffeo et al., 1983). In general, PGF and its analogues are ineffective in causing luteolysis during metoestrus (Lauderdale, 1972; Rowson et al., 1972; Jackson et al., 1979; Kirocofe et al., 1985).

One method of synchronizing oestrus with PGF alone is to give two injections from 10 to 14 days apart. If cattle are distributed equally across each day of the oestrous cycle, then approximately 70% of the cycling animals should show oestrus after the first injection. These animals, and the remainder of the cycling animals should then be at a stage of the oestrous cycle where they can respond to the second injection. The range of animals showing oestrus over a 5 day period after the two injection system treatment ranged from 11 to 66%. However, the fertility at first service ranged between 44 and 68% (Young, 1989; Odde, 1990). Fertility was similar for cattle that were bred at detected oestrus or at a fixed time 80 h after the second injection (Hafs and Manns, 1975). The interval to oestrus after PGF is shorter for heifers than for cows (King et al., 1982). The response pattern also varies with PGF injection at different stages of the oestrous cycle. (Macmillan, 1978; Refsal and Seguin, 1980; King et al., 1982; Macmillan, 1983; Macmillan et al., 1984; Stevenson et al., 1984; Tanabe and Hann, 1984; Watts and Fuquay, 1985; Momont and Seguin, 1988). Cows initially treated in midcycle have a greater oestrus response, and showed oestrus later than cows injected between days 5 and 9 of the oestrous cycle ([oestrus = day 0], Odde, 1990).

PGF can be used in a single injection programme. This method of using prostaglandin involves detecting oestrus, and inseminating animals for 4 days, then injecting those which have not been inseminated on day 5 and continuing

oestrus detection with inseminating from day 5 though 9. A variation of this method is to inject those which have not been in oestrus 6 to 11 days after their first injection and inseminated at oestrus over the next 4 days after the second injection. Another single injection programme is to inject PGF, and then breed each animal at detected oestrus over 5 days. PGF can also be used in a single injection programme, using bulls fitted with a chinball harness in the pre-mating or pre-treatment period. The animals marked in the first 11-day period are injected once with PGF at 17 days after bull introduction and inseminated on detection 3 and 4 days after treatment. The remaining animals marked in the second period are injected once with PGF 11 days after the first group, with inseminations conducted over the next 4 days.
These methods increase the proportion of animals conceiving over short periods of time after treatment compared with the controls (Lauderdale et al., 1980; Macmillan, 1986).

Watts and Fuquay (1985) and Stevenson et al. (1984) have shown contradictory results in fertility following late and early luteal phase injections. In the first study, the fertility was higher with PGF injected in the late luteal phase. However, the second study reported no differences in fertility. Figueroa et al.(1988) increased the oestrous response by injecting oestradiol benzoate 40-48 h after the injection of PGF. However, pregnancy rates were not improved. Other studies have reported similar results (Peters et al., 1977; Dailey et al., 1983). In another study, lactating dairy cows were injected once or twice at selected intervals with PGF and inseminated at 72 h or 72 h and 96 h post-injection. In a series of 9 trials, the average pregnancy rate to first insemination for over 2000 PGF-treated cows was 69%, compared to 60% in a comparable number of untreated herd mates (Macmillan and Day, 1982). Malmo (1988) reported that the conception rate of cows in the "why wait" group was greater than in the control group of cows which had their first service less than 80 days after calving. This system involves a period of oestrus detection about 10-11 days prior to the planned start of mating [PSM]. Cows in oestrus from day 11 to 6 prior to PSM are injected with PGF at the first day of the PSM. It would be expected to have animals in oestrus in the next 3-6 days. Cows in oestrus from day 6 to 0 prior to PSM are injected with PGF 6 days into the breeding season and inseminated as they are detected in oestrus.

Progestagen-Prostaglandin Combinations.

Several combinations have been tested with progestagens when using a Norgestomet implant for 7 to 9 days with an injection of PGF 1-2 days before, or at implant removal. More than 90% of beef heifers were in oestrus in a 5-day period, and 62% conceived; the control group had a 60% conception rate to first AI (Heersche et al., 1979; Brown et al., 1988). In another study, a group of dairy heifers was treated for 7 days with a PRID and injected with PGF on day 6 (Smith et al., 1984). The same treatment was used by Beal (1983) in lactacting beef cows. In both studies, the fertility to first insemination using timed insemination was greater than in animals treated with two injections of PGF 11 days apart. The fertility of the oestrus after treatment in some cattle may be reduced, even though the progestagen treatment is less than 9 days (Patterson et al., 1989). Several reports using different progestagen treatments have shown that this

reduced fertility was influenced by the stage of the oestrous cycle when the treatment was initiated (Brink and Kiracofe, 1988; Beal et al., 1988; Patterson et al., 1989; Chenault et al., 1990). This reduced fertility was restricted to cattle in which the treatment was initiated during the second half of the oestrous cycle.

Subsequent forms of treatment with progesterone inserted during the metoestrous stage compromised normal luteal function (Woody et al., 1967; Guinter, 1970; Folman et al., 1984; Battista et al., 1984; Macmillan et al., 1989, 1990c).

CIDR is a recently developed intravaginal device to treat cycling and non-cycling animals. Three types of device have been developed for use with different species such as cattle, sheep, goats and deer. The type B is for use with cattle (Fig. 1.3).

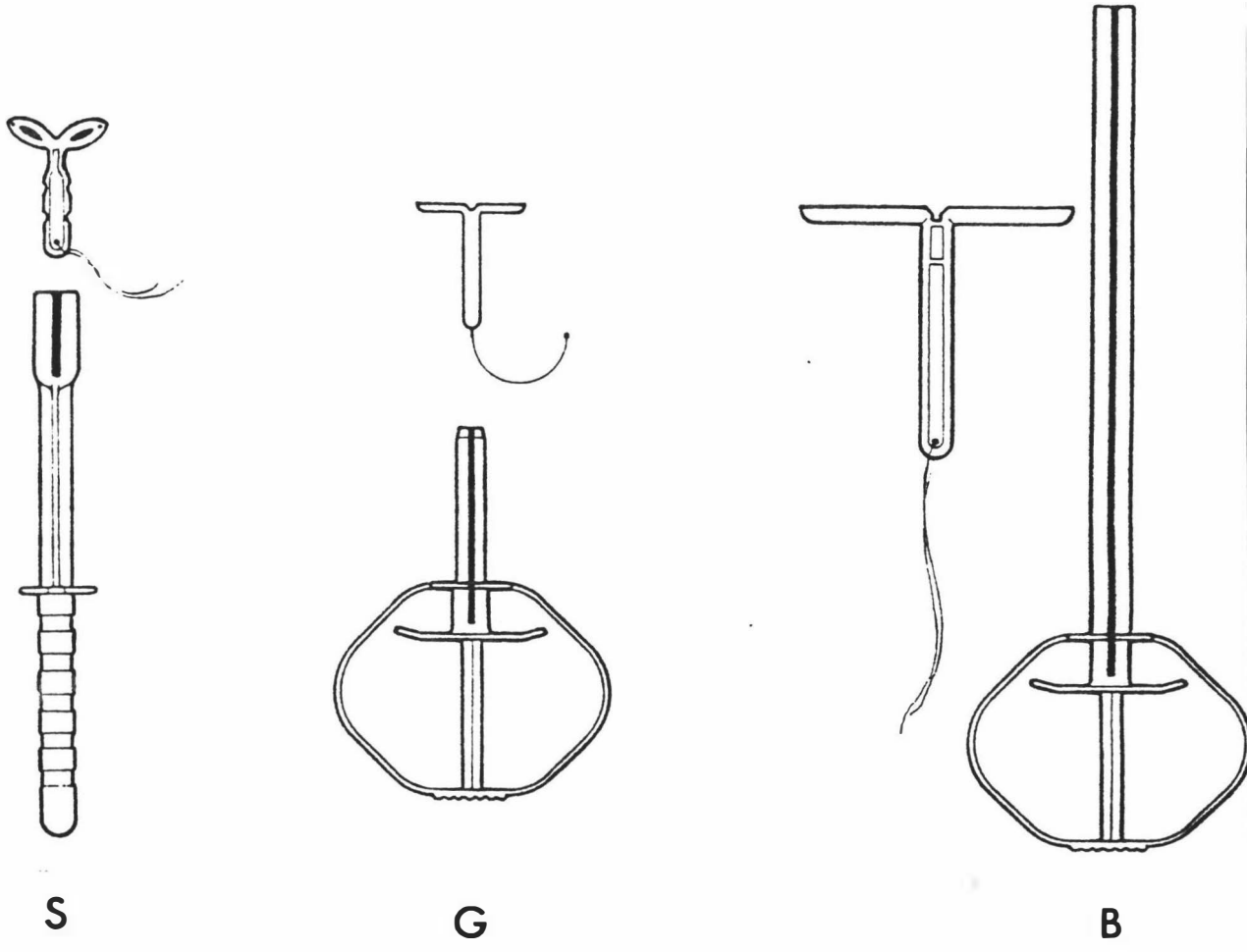


Fig 1.3 **CIDR** Devices/applicators

The mode of action is to release progesterone at a controlled rate into the blood stream of treated animals, thereby inhibiting ovulation. After removal of the device, the level of progesterone in the blood stream falls precipitously, thus initiating behaviourial oestrus and ovulation, as in the normal cycling animal. The CIDR was designed to have a gelatin capsule attached to it to allow an optional short-term treatment such as with oestrogen. In the development of the CIDR,

design and shape changes were made to achieve average plasma progesterone concentrations (PPC) of at least 2 ng/ml for at least 15 days.

Macmillan (1987, see Duirs et al., 1986) reported that PPCs in ovariectomised heifers 12 days after insertion of a re-used CIDR devices were maintained at levels of 2-2.5 ng/ml. This result indicates the potential for re-using CIDR's and maintaining an adequate PPC because of the ability for residual progesterone in the silicone matrix to migrate to the surface and maintain a high PPC after re-insertion.

CIDR-B Indications.

The most commonly recognised indications for CIDR use include:

i) oestrous cycle control in yearling and adult cattle;

ii) improving pregnancy rates following insemination and shortening the interval from first insemination to conception by synchronizing returns to service;

iii) as a contraceptive; and

iv) treating non-cycling cows

i) Oestrous cycle control in adult cattle.

The strategic use of PGF and CIDR-B with lactating dairy cows has the potential to concentrate breeding programmes in seasonal dairy herds and to maintain a 365-day calving interval in year-round herds.

The treatment schedule which was used by Macmillan et al.(1986) was as follows:

All the animals were tailpainted (Macmillan and Curnow, 1977) during the premating period and every oestrus date recorded for eight days. At this time, cows which had been detected in oestrus were re-painted with a second colour. Each of the remaining cows had a CIDR device inserted. CIDR's were removed 9 days later and all cows injected with a luteolytic dose of PGF (5 ml Lutalyse, Upjohn N.Z.). Artificial breeding started one day after CIDR removal and/or PGF had been given. Among the CIDR-treated cows detected in oestrus, 81.5% were inseminated from 48h to 96h after CIDR removal. Longer response intervals were more common among CIDR-treated cows which had no premating heat dates. The overall average pregnancy rate was 56%. Among the PGF group it was 63%, compared with 52% in the CIDR-treated group. Most of the cows which had failed to conceive at first insemination were detected in oestrus and re-inseminated. A 92% submission rate was obtained in 6 days using this CIDR and PGF programme (Macmillan et al., 1986).

ii) Effects of using CIDR-B after first insemination on pregnancy rate and subsequent synchrony.

CIDR devices were used post-insemination in 7 herds, insertion being at periods from 14 to 17 days post-insemination and removal at 21 days post-first insemination.

CIDR removal at 21 days post-insemination caused 67.4% of 186 reinseminated cows to have return intervals of 23 or 24 days.
These cows had a second insemination either 48 or 72h after CIDR removal. By comparison, 63.2% of 172 herdmates used as control animals were re-inseminated over the normal period of 18 to 24 days.
There were fewer returns to service of around 6 weeks in the treated cows (36-50 days; 8.2% for treated cows vs 15.2% for untreated cows). This use of a CIDR device did not alter pregnancy rate to second insemination (Duirs et al., 1986).

In another study, heifers in 4 trials were synchonized using CIDR devices for 9 days and PGF on day 7. Overall, 83.9% of heifers were in oestrus 48-72h after CIDR removal. CIDR devices after AI (new CIDR or 9-day used CIDR for days 1-8 or 2-9 post AI, respectively) reduced conception rate in two trials when re-used CIDR's were used. Treatment groups were 18.6 and 17.2% vs 42 and 52.8% for the control groups, respectively. However, there was a positive effect on fertility in 2 of 3 trials when the devices were re-inserted between days 17-22 post AI. CIDR devices synchronized returns to oestrus significantly in non-pregnant heifers compared with control groups, being 81.3% on day 24 in the treated group vs 12.5 and 2.7% for control groups (Van Cleeff et al., 1989).

A series of trials was completed by Macmillan et al. (1990d), in which CIDR devices were inserted into lactating cows at 4 to 17 days after first insemination. Significant increases in pregnancy rate for individual days occurred when CIDR devices were inserted on days 6 to 8 post-insemination. Over this 3 day period CIDR-treated cows had a pregnancy rate of 79.2%, compared with 65.7% in contemporary controls. No differences in pregnancy rates were found between treatment and control groups when the treatment was initiated on other days of the cycle, or the duration of CIDR insertion was for periods varying from 4 to 12 days.

A Cornell study used PRID for 7 days plus PGF at 24h prior to PRID removal, or PRID + PGF as described plus PRID re-insertion from day 12-19 post-insemination in lactating dairy cows, in order to concentrate oestrus detection for AI into 1 week out of every 3 weeks. Seventy percent (70%) of the treated cows were observed in oestrus within the first post-treatment insemination week. Eighty six percent (86%) of all breeding (first and repeat services) in treated cows occured during the established insemination week.
First service conception rates were lower and services per conception higher in treated cows due to appointment breeding with this treatment (Smith et al., 1987).

iii) CIDR-device use as a contraceptive.

CIDR devices have been used for extended insertion periods to study plasma progesterone concentrations over long intervals. One experiment in intact and ovariectomized heifers showed that CIDR treatment prevented oestrus and ovulation for at least 7 weeks. After this time, there were some ovulations without oestrus, and occasional vaginal perforation. The entire heifers had concentrations of progesterone between 2.5 ng/ml in the second week and 1.1 ng/ml in the seventh week, but the ovariectomized heifers showed concentrations between 2.2 ng/ml and 0.6 ng/ml (second and seventh week respectively). The authors suggested that development of a contraceptive CIDR had considerable market potential but required design changes (Duirs et al., 1986).

Programmes for oestrus synchronization (using prostaglandins, progesterone devices or progestagen and oestrogen treatments) were successfully used in cows with a single fixed-time insemination as an alternative to the tedium and inaccuracies of oestrus detection (Drew, 1984). Although it is important to recognise that this procedure represents a compromise which may sometimes succeed and often fail, methods of synchronization of oestrus are not used routinely in milk production programmes.

Synchronization systems can successfully condense most oestrus events into a 3 to 5 day period (Roche and Ireland, 1984), but there is considerable variation between herds in the pattern of oestrus events within this period. It has been proposed that the length of pro-oestrus is influenced by ovarian follicular status at the start or at the end of treatment (Scaramuzzi et al., 1980; Roche, 1986; Sirois and Fortune, 1988; Momont and Seguin, 1988). Animals treated with prostaglandins in early dioestrus had shorter and much better synchrony between treatment and onset of oestrus than animals treated at midcycle (Macmillan et al.,1984; Watts and Fuquay, 1985; Momont and Seguin, 1988).

Synchronization systems with progesterone or its analogues must suppress plasma gonadotrophin concentrations as well as oestrus to avoid any subsequent reduction in fertility (Roche and Ireland, 1984). This reduced fertility may be associated with altered ovarian folliculogenesis modulated by associated alterations in secretion of LH (Roberson et al., 1989). It could contribute to variation in follicular development among animals at the end of treatment.

Plasma Progesterone Concentrations in Cycling and Ovariectomized (OVX) Animals Treated with a Progesterone Intravaginal Devices.

Several trials have described plasma progesterone concentrations (PPCs) in cycling and ovariectomized animals treated with progesterone intravaginal devices. PRID was used by Roche and Gosling (1977); Munro and Moore (1985, 1986); Munro (1987), and Robinson et al. (1989). CIDR was used by Munro (1987), Macmillan et al. (1990c) and Peterson and Henderson (1990).

Average PPCs 24h after PRID or CIDR insertion in heifers and cows vary considerably and incremental increases appear to be related to variation in metabolic clearance rates (Peterson and Henderson, 1990). It has been shown that this increase is less in cycling than ovariectomized animals. Also, the

increase appears to be less in animals treated with PRID rather than CIDR (Robinson et al., 1989; Macmillan et al., 1990c). Moreover, these changes are influenced by the stage of the cycle at CIDR insertion, for example heifers with CIDR inserted during metoestrus had an average PPC of 5.0 ng/ml by 24 h after insertion, and for heifers inserted in dioestrus on days 9 to 12 of 14.5 ng/ml (Macmillan et al., 1990c).

Average PPCs 24 h after PRID insertion in ovariectomized heifers varied from over 6 to 9 ng/ml (Munro and Moore, 1986; Munro, 1987;), while in ovariectomized cows the average increase was 3.0 ng/ml (Robinson et al., 1989). In ovariectomized heifers treated with CIDR, PPCs increased to 8.7 ng/ml within 6 h of device insertion. Moreover, the average increase in PPCs 24 h after CIDR insertion was 6.7 ng/ml for ovariectomized heifers and 4.9 for cycling heifers (Macmillan et al., 1990c). Finally, cycling animals treated in dioestrus with PRID had an average increase in PPCs of 3.7 ng/ml 24 h after insertion while the animals treated with CIDR in the same period had an increase of 5.5 ng/ml (Robinson et al., 1989; Macmillan et al., 1990c).

OBJECTIVES

a) To follow and characterize the dynamic changes of the follicles in the ovaries at the different stages of the oestrous cycle by ultrasonography during and after CIDR-B insertion in cycling cows;

b) To measure associated changes in plasma progesterone concentrations during these periods (pro-oestrus, metoestrus, early dioestrus, late dioestrus), and in ovariectomized cows;

c) To determine the effect of exogenous progesterone on the oestrous cycle length; and,

d) To characterize the interactions involving follicles in the ovaries, profiles of progesterone and cycle lengths in animals treated with CIDR devices from different stages of the cycle.

MATERIAL AND METHODS

Animals.

The experiment was conducted at the Dairy Cattle Research Unit of Massey University from April to November 1989. Five nonlactating cycling Friesian cows, which weighed 470-550 kg and were aged between 4 and 6 years old, were used for this study.

Animals grazed ryegrass-white clover pastures supplemented with hay. They were in good body condition thoughout the study. The animals were only stalled on those days involving hourly blood sampling. On these occasions, they were outside at 6 hourly intervals to be checked for signs of oestrus and for rest periods.

Oestrus detection consisted of four periods of observation each day, with each observation periods lasting at least 30 minutes. Tail-paint and raddle were also used (Macmillan et al., 1988a) to monitor oestrous activity. Oestrus was defined as that period during which a cow would stand to be ridden by her herdmates. In addition, the animals were considered to be, or have been in oestrus when raddle was removed and easily detectable amounts of paint had been rubbed off.

In calculating oestrous cycle length, account was taken of the time of CIDR removal, and half days were defined (starting at CIDR removal) to distinguish between morning and afternoon onset of oestrus.

Experimental Protocol.

A Controlled Internal Drug Release (Eazi-Breed CIDR^{tm}-B; CHH Plastic Moulding Co., Hamilton, N.Z.) device consisting of a silicone elastomer impregnated with 1.9 g progesterone was used in each treatment sequence. When use of a luteolytic agent was necessary, animals received an intramuscular injection of 500 μg (cloprostenol[1]) as PGF.

CIDR's were inserted for 10 days (in one sub-group, an injection of PGF was given during the CIDR insertion to each cow) during three selected stages of the oestrous cycle.

Initial synchrony before the experiment started was achieved by treating with a CIDR-B device for 9 days, with PGF given 2 days before device removal. At 24 h after device removal, it was considered that the cows were in pro-oestrus and at a stage of the oestrous cycle equivalent to 2 days before an expected oestrus (Day-2). At this time another CIDR was inserted and the trial treatment commenced for the " Pro-oestrous sequence " (PCI group).

---

[1] Estrumate, Pitman Moore NZ Ltd, Upper Hutt.

In the following treatment sequences, each animal had a CIDR device inserted on a pre-determined day for a specified stage of the cycle.

The treatment sequence is presented in Fig. 1.4.

i) Pro-oestrous CIDR insertion group (PCI):

CIDR's were inserted on cycle day -2 (CD-2) of the oestrous cycle (Day of oestrus = CD 0).

ii) Metoestrous CIDR insertion group (MCI):

CIDR's were inserted on CD 3.

iii) Dioestrous groups:

a) sub-group, Early-Dioestrous CIDR insertion group (E-DCI):

CIDR's were inserted on CD 7.

b) sub-group, Late-Dioestrous CIDR insertion group (L-DCI):

CIDR's were inserted on CD 13.

c) sub-group, Late-Dioestrous + PGF during CIDR insertion (L-DCI + PGF):

CIDR's were inserted on CD 13 plus PGF on CD 15 of the oestrous cycle.

The same animals were used in each of the treatment groups, and then as a control group when they were monitored thoughout the entire length of a normal oestrous cycle.

Three of these animals were then ovariectomized and then had a CIDR device for 10 days after a 4 week period for post-operative recovery.

Method of Use of the CIDR-B.

The device used in cattle is registered as the Eazi-breed CIDR-B^{tm}.

The sequence for inserting a CIDR device was as follows:

1. The CIDR-B applicator was dipped into a non-irritant antiseptic solution;

2. A CIDR-B device was inserted into the applicator so that the wings of device were folded and only the tips of the wings protruded from the front of the applicator;

3. The front portion of the applicator was dipped into a veterinary obstetrical lubricant;

4. The tail of the animal was lifted and the lips of the vulva wiped clean (disposable tissue);

5. The loaded applicator was inserted sloping slightly upwards, though the vulva and then forward, without forcing, into the anterior portion of the vagina;

6. After releasing the removal string or nylon filament, the handle of the plunger was firmly depressed to expell the CIDR-B device;

7. When the CIDR-B was correctly located, the front wings opened in the anterior portion of the vagina, with the removal string or nylon filament protruding from the vulva;

8. Adequate depth of insertion was checked by using the string or nylon filament attached to the CIDR-B as an indicator of its position;

9. Step 1 was repeated before the insertion procedure was commenced with another animal;

10. The CIDR-B device was removed by firmly grasping the string or nylon filament and pulling the device from the vagina; and

11. Each used CIDR-B device was buried and burned (Duirs et al., 1986).

Blood Collection and Analysis.

Blood samples were collected during different stages of the oestrous cycle and treatment sequences, to measure plasma progesterone concentrations (PPC) by radioimmunoassay.

Before CIDR-B device insertion. Samples were collected by jugular venepuncture from 2 h before until 0 h (CIDR-B insertion). These samples were collected at 1h intervals.

From CIDR-B device insertion to removal. These samples were collected at 1 h intervals from 0 h to 24 h from indwelling jugular catheters, and then at 24 h interval until device removal, by sampling from the jugular vein.

After CIDR-B device removal. These samples were collected at 30-minutes intervals from CIDR-B removal to +12 h and from +12 to 24 h at 1 h intervals.

From +24 h after device removal, samples were collected twice daily until oestrus by jugular venepuncture.

Each sample (8 ml) was drawn into a heparinized tube (Nipro-New Tube System, Vacuum Blood Collecting System, Nissho Corporation, Osaka, Japan), and then immediately centrifuged at 5 °C for 20 minutes at 3000 xg. The extracted plasma was stored at -20 °C until measured by specific radioimmunoassay (RIAs) for progesterone and LH.

The samples selected for assaying plasma progesterone in each treatment group were those which were taken just before a CIDR device was inserted, 1 h after device insertion, at day 3 after device insertion (only in dioestrus and for sub-groups b and c), at day 10 of treatment just before CIDR device removal, and then 6 h later.

The same sequence of samples from the OVX cows was assayed for progesterone, as well as the samples taken 24 h after device removal.

Samples analyzed for plasma progesterone in normally cyclings cows were taken at day -2 (pro-oestrus), and days 3 (metoestrus), 7 (early dioestrus), and 13 (late dioestrus) of the oestrous cycle.

The assayed samples were at treatment stages known to best reflect PPC patterns associated with device use (Macmillan et al., 1987; Peterson et al., 1990).

Normally cycling (NC) group.

Samples were collected by jugular venepuncture. They were collected once daily from oestrus until day 16 of the oestrous cycle, and then twice daily until the next oestrus.

Ovariectomized (OVX) group.

There was also a period of sampling from 3 ovariectomized cows. Devices were inserted after a 4 week post-operative recovery period, and samples collected using the same procedure described for animals in each of the groups of treated cycling cows.

Blood samples for luteinizing hormone (LH) determination were collected in the late dioestrus stage, sub-group b (L-DCI). Samples were collected at 30 minute intervals from an indwelling jugular catheter, from the onset of oestrus until +18 h after the end of oestrus.

Procedure of Jugular Cannulation.

The standard procedure used in the Department of Animal Science, Massey University (Professor S. McCutcheon, unpublished) was followed.

Materials and equipment.

1. Sterile physiological saline (Travenol Lab. N.Z. Ltd, Auckland).

2. Heparin stock solution at 100 IU/ml [in saline] (Heparin,N.Z., Pharmaceutical Ltd, Palmerston Nth).

3. Oxytetracycline (Pfizer, Pfizer Lab. Ltd, Manukau City).

4. Tuberculin syringes [1 ml] and 14-18G stainless steel needles.

5. Cannula tubing, ID 1.0mm OD 1.50mm polyethylene tube Deural Plastics, N.S.W., Australia).

6. Scalpel blades and handle.

7. Cannula hubs made from 18G hypodermic needles.

8. Plastic basin.

9. 70% ethanol in water.

10. Linen Thead.

11. Scissors.

12. Sleek tape (Smith and Nephew Medical Ltd, England).

13. 5 ml syringes.

14. Halters.

15. Rope.

16. Systenet (International Surgical Netting S.P.A., Italy).

17. Small animal clippers.

18. Extension lead.

19. Isolating transformer (as required).

20. Savlon or similar antiseptic (Savlon, ICI Tasman, Upper Hutt, N.Z.).

21. Plastics buckets.

22. Paper towels.

23. Surgical swabs (Tse Fong Chemical Cotton Co. Ltd, Taiwan).

24. Spray-on anaesthetic, Xylocaine 10% Spray (Astra Pharmaceutical Ltd, N.S.W., Australia).

25. Cannulation needle and sleeve Argyle Medicut "T" 14 gauge (Sherwood Medical Co., St Louis, U.S.A.).

26. Cannula plugs (made from old 2 and 12 ml luer lock syringes).

27. 16G needle for suture.

28. Adhesive bandage (elastoplast).

29. Aureomycin power (Cyanamid of N.Z. Ltd, Papakura, N.Z.).

30. Penicillin [Streptopen](Glaxo N.Z. Ltd, Palmerston Nth).

31. 14G stainless steel needles.

32. 18G stainless steel needles (Nipro, Japan).

33. 10 ml syringes (Monoject, Sherwood Medical, St Louis, U.S.A.).

34. Rectal thermometers.

35. Stainless steel trolley.

B. Procedure.

1. Sterile saline containing 100 IU/ml Heparin and 0.04 ml/100ml oxytetracycline was made and stored under refrigeration;

2. Cannula tubing was cut to a length with a scalpel, allowing sufficient tubing to run from between the shoulders to a point of entry 10-15 cm into the vein;

3. Hubs which were made from 18G hypodermic needles cut to 1.5-2.0 cm length were fitted into cannulae. The outside edge of each needle was carefully rounded on a wetsone (connect to old syringe for easy handling), and the internal surface reamed out with the point of an old needle. The external and internal surface were roughened and tubing cut to trap fibrin clots respectively;

4. The cannulae was straightened out and placed in 70% ethanol to be sterilised;

5. Linen thead was cut into 15 cm lengths;

6. Sleek tape was cut into 5 cm lengths;

7. 5 ml syringes were loaded with heparinised saline;

8. An animal was restrained and the neck shaved with small animal clippers. Systenet was placed on the neck of the animal and pushed down to below the cannulation site;

9. The shaved area was washed with an antiseptic solution (Savlon in warm water), and dried with paper towels;

10. Local anaesthetic was sprayed around the site of cannulation;

11. A cannulation needle and sleeve were removed from their sterile cover, and then the plug was removed from the end of the needle. The needle was replaced in the sleeve and examined to ensure that the

cannula fitted though the sleeve;

12. The jugular was occluded by pressing below the cannulation site with a thumb. Needle/sleeve were inserted though the skin and into the vein;

13. When blood flowed freely though needle, it was removed while holding the sleeve in place. The cannula was inserted though the needle with the saline syringe connected. Before an injection of 2 ml of heparinised saline, it was checked to ensure that blood flowed freely into the syringe;

14. The syringe was removed and replaced with a cannula plug;

15. The cannula was then dried at the wound and wrapped tightly with a sleek tape around it. Tie linen was theaded tightly around the sleek so that it embedded itself into the tape;

16. A 16G stainless steel needle was inserted though the skin at right angles to the jugular and ~ 1 cm above the wound. Linen thread was passed though the needle. The needle was removed and the suture tied off;

17. Aureomycin power or spray-on bacteriostat was squirted around the wound;

18. The cannula was run over the shoulder to behind the neck. The adhesive bandage was wrapped around the neck to cover the wound site and the cannula was held in place. It was passed between two layers of adhesive bandage. When bleeding occurred around the wound, a swab was folded under the bandage to apply pressure;

19. Systenet was pulled up to cover the cannula and connected to the halter. The forelegs were passed though holes in the lower part of the Systenet;

20. The animal was put back into its stall, and then injected intramuscularly with penicillin.

C. Removal of a cannula.

1. The systenet was removed. Then the adhesive bandage behind neck was cut and pulled down to expose the cannulation site.

2. Next, the linen thead was cut and removed from the skin. The cannula was withdrawn gently. Pressure was placed on the wound until bleeding (if any) stopped, and aureomycin powder applied.

3. Each animals intake, behaviour and rectal temperature were monitored for the next 2 days.

4. Cannula hubs and plugs were saved before discarding the remnants of each cannula.

5. Hubs and plugs were washed as soon as possible and sterilized

Ovariectomy.

Ovariectomies were performed on three cows. Food was withheld from each animal for 12 h, and water for 6 h prior to surgery.

Each cow was restrained in a metal crush and sedated with intravenous xylazine (Rompun 2% solution, Bayer N.Z. Ltd, Marine Parade, Petone) injected into the coccygeal vein at a dose rate of 0.04 mg/kg. The skin of the left paralumbar fossa was clipped and the line of the surgical incision shaved. Regional local anaesthesia was induced using an inverted L block technique. The clipped area of skin was aseptically prepared for surgery with alternating solutions of aqueous hibitane and hibitane tincture. Finally, the surgical field was sprayed with iodine and appropriately draped. A 20 cm vertical incision was made though the skin from approximately 5-10 cm ventral to the lumbar transverse processes to 10 cm cranial to the tuber coxae. The abdominal wall was divided with sharp scissors, as was the peritoneum. An ecraseur was introduced with the left hand and closed around the ovarian pedicle of the right ovary. The chain loop was slowly tightened to sever the pedicle, and then the ovary was removed from the abdomen. This procedure was repeated for the left ovary. The abdominal wall was closed in two layers with 5 metric chomic gut using a continuous suture pattern. The skin was closed with 6 metric nylon using a blanket stitch. Streptomycin/penicillin (Stretopen 250/250 injection, Pitman Moore NZ Ltd, Upper Hutt, N.Z.) at dose rate of 15mg/kg was given intramuscularly for 3 days following surgery (Jennings, 1984).

Hormone Assays.

Progesterone. Plasma concentrations of progesterone were determined by the method of Kirkwood et al. (1984). Determinations were made on 500 μl sub-samples from each original plasma sample. They were extracted with 5 ml toluene:hexane (1:2 v/v). The solvent-plasma mixture was re-frozen overnight, and the solvent supernatant then decanted into clean tubes, dried under air and redissolved in 500 μl ethanol. Duplicate 100 μl samples of ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 μl samples of standard ethanolic solutions of progesterone (P-1030:Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone levels of 0.625-40 ng/ml. A mixture containing antiserum (courtesy of Dr J. T. France) at a final dilution of 1:18000 (Tungsubutra & France, 1978); [1,2,6,7-H] progesterone(TRK 413, Amersham, Bucks, U.K.) at 10000 c.p.m./100 μl; phosphate-buffered saline containing 0.02 m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 μl) to each tube and vortexed. After overnight incubation at 4 °C, 600 μl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and then incubated at 4 °C for 10 minutes. Tubes were then

centrifuged at 3000 g for 10 minutes at 4 oC. The supernatant was decanted into scintillation vials, and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Assay sensitivity was 0.07 ng/ml. Intra-assay coefficients of variation (CV) were 14, 11.6 and 15% and inter-assay CV were 9.54, 13 and 20% for plasma pools containing mean progesterone concentrations of 5.9, 10.9 and 2.5 ng/ml, respectively (N= 26).

Luteinizing hormone. Plasma LH levels were measured by double antibody radioummunoassay using assay kit ingredients supplied by NIDDK, NIH, Bethesda, Md., U.S.A.. Immunoreagents, prepared by Dr A.F. Parlow, Torrance, Ca, U.S.A., were: NIADDK-oLH-I-3 for radioiodination; NIADDK-anti-oLH-I antiserum; and NIADDK-oLH-24 for assay standars. Separation of free and bound radioiodinated oLH was achieved with donkey anti-rabbit IGG serum (IDS Ltd, Washington, USA). Assay samples were processed in a single assay for which the limit of sensitivity was 0.07 ng/ml and the within-assay coefficient of variation was 7.45% at a mean plasma LH concentrations of 1 ng/ml (n=9).

Ultrasound Examination.

Ovarian follicular populations were examined using a transrectal real time linear array ultrasound (Aloka, Echo Camera, Multicrystal Scanner, model SSD-210-DX, Japan) with a 5.0 megahertz probe.

Presence or absence of corpus luteum (CL) and the size and number of ovarian follicles with antral diameters equal to or greater than 2 mm were recorded. The follicles were classified into three categories described as follow:

Small: <6 mm diameter of clear antral fluid (Class 1).

Medium: from 6 to 9 mm diameter (Class 2).

Large: >9 mm diameter diameter (Class 3).

In the control group, ovaries were examined by ultrasonography on:

CD 0: coinciding with oestrus; CD 2; CD 3; CD 4; CD 6; CD 7; CD 13; and also at CD -2 (2 days before oestrus); and finally CD 0.

In the treatment groups, ovaries were examined by ultrasonography on :

Treatment day (TD) 0 coinciding with CIDR-B insertion and before jugular cannulation; TD 1; TD 3; TD 6; TD 10 before CIDR-B removal and jugular cannulation, and then at post-TD 10 at one day after CIDR-B removal, and on alternate days after 1 day of CIDR-B removal until oestrus. Ovarian examinations were also made at oestrus and CD's 2, 4 and 6 corresponding to days 2, 4 and 6 of the oestrous cycle, respectively.

No chemical methods were used to restrain an animal because none showed signs of chronic discomfort. They were simply held in a race during the scanning. The

method for examining the ovaries by ultrasonography was adopted from the procedures described by other workers (Sirois and Fortune, 1988; Quirk et al., 1986).

The routine established for each examination was as follows:

i) faecal material was removed manually from the rectum before examination;

ii) the transducer probe was inserted into the rectum;

iii) each ovary was separately located, and scanned several times and in more than one plane. When necessary, the image was frozen on the screen and the size of each follicle measured; and

iv) the probe was dipped in antiseptic solution after each examination.

Each ultrasonographic examination was recorded on videotape (Panasonic-VHS-NV-E180SP). The video recorder was a National AG-6200-EN, Matsushita Electric Industrial Co., Ltd Japan. The tape was reviewed on the screen of the scanner, and diagrams of the relative positions of the follicles and their relationship to other ovarian structures were drawn for each ovary. This allowed individual follicles to be identified on successive days. If the image of the follicle being scanned was not spherical, the diameter was estimated by averaging the longest and shortest diameters. All follicles were measured on the screen with a calliper calibrated against the scale provided with the ultrasound unit. All ultrasonographic examinations, and the subsequent reviwing of videotapes were performed by one operator.

Criteria used were:

Dominant Follicle. The largest follicle identified during a given period of time, and with a diameter of > 6 mm.

Maximum Size. The largest diameter measured in a dominant follicle.

Day of Maximum Diameter. The first day at which the maximum diameter was recorded for a dominant follicle for more than one consecutive day.

Validation of ultrasonic measurements of follicular diameter.

Both ovaries (n = 6) in each of three (3) cows were examined first by ultrasound before ovariectomy. The ovaries of each of these animals were recovered within 2 minutes of ovariectomy, and placed in cold saline. Follicles of > 2 mm diameter were counted, and measured with callipers in situ. Follicles of > 4 mm diameter were removed from the ovaries and dissected free of stromal tissue. The diameter was estimated by measuring the average dimensions of the clearly

definable antral fluid volume.

During the following 3 h after dissection, each ovary was examined by ultrasound. The examination was made by firmly holding an ovary in a thermostat and submerging it in a beaker of normal saline at room temperature. The beaker also contained the probe of the ultrasound unit. The ovary was manoeuvred around a fixed probe (Macmillan and Thatcher, 1990).

Statistical Analysis.

Data were analysed using the Panacea Database Management System (PAN Livestock Services Ltd. Department of Agriculture, University of Reading, P.O. Box 236, Reading, Berkshire, England). The total number of follicles, and number of follicles within each class were analysed, including the effects of day of the oestrous cycle and interval from CIDR removal to oestrus, effects of presence or absence of a CL during treatment, and follicular diameter classes (class 1, 2 , 3), and diameter of the largest follicle.

PPCs were analysed, including presence or absence of a CL and ovaries and days of the oestrous cycle at CIDR insertion, and during the treatment period. Animals were grouped for different analyses and tested within groups.

Multiple regression analysis was used to obtain the correlation between the ultrasound diameter and the calliper measurements of follicle diameter.

RESULTS

Animals.

None of the treated animals displayed signs of discomfort due to CIDR treatment or jugular cannulation, and none required veterinary attention during the experiment. No animal was excluded from a treatment period because of effects from a preceding treatment period. In this experiment, 28 CIDR's were inserted. None was lost during the study period.

Pro-oestrous CIDR insertion (PCI) Group.

The average numbers of class 1, class 2 and class 3 follicles at the different stages of the oestrous cycle when CIDR's were inserted are presented in Table 1.1. The number of class 2 follicles declined during the CIDR treatment period (2.5 ± 0.6 vs 1.5 ± 0.3; $P < 0.20$), but numbers of class 1 and 3 follicles were unchanged. The dominant follicle present when the CIDR was inserted in 4 of the 5 cows (-2) was the ovulatory follicle. The fifth cow developed a new ovulatory follicle 1 day after CIDR removal. The average size of the largest follicle at CIDR removal was 15.3 ± 1.3 mm, the post-treatment interval to oestrus was 2 days (± 0), as all cows had a short interval to oestrus after CIDR removal.

There were more class 1 and class 3 follicles during the period of CIDR treatment on the ovulatory ovary (10.8 ± 0.5 vs 12.3 ± 0.7, respectively; $P < 0.10$) and (1.2 ± 0.1 vs 0.2 ± 0.1, respectively; $P < 0.001$). The average number of class 2 follicles was similar (1.8 ± 0.2 vs 1.9 ± 0.2, respectively).

Average plasma progesterone concentrations (PPC) at the time of CIDR insertion (TD 0) and at selected intervals during treatment (TD 0 + 1 h, TD 3, TD 10 and TD 10 +6 h) in cycling and ovariectomized cows are presented in Table 1.2.

The average PPC at CD -2 of the oestrous cycle prior to CIDR insertion (TD 0) for the PCI group was less than the detectable limit of the assay (0.07 ng/ml). Only one hour after device insertion, it was 2.5 ng/ml (± 0.1), representing an average increase of 2.5 ng/ml.

On TD 10, the PPC was 2.3 ng/ml (± 0.1).

By 6 h after CIDR removal, the PPCs were again less than the detectable limit of the assay.

Metoestrous CIDR insertion (MCI) Group.

The number of follicles in each class remained approximately constant thoughout the treatment period (Table 1.1).

The ovulatory follicle was detected in 3 cows on TD 1 (CD 3), and in another on TD 3. The remaining cow developed a new ovulatory follicle 1 day after CIDR removal.

The average size of the largest follicle at CIDR removal was 13.1 ± 1.0 mm, and the average post-treatment interval to oestrus was 3.6 days (± 1.5).

Cows having a short interval to oestrus after CIDR removal (< 3 days) had a lower average number of class 1 follicles on the ovulatory ovary during CIDR treatment than cows having a long interval to oestrus, and also compared with cows having a short or long interval to oestrus on the nonovulatory ovary (15.2 ± 0.1 vs 20.7 ± 0.7, 17.1 ± 0.3, 19 ± 0.1, respectively; $P < 0.05$). There were no significant differences in the average number of class 2 and 3 follicles in cows having a short or long interval to oestrus on the ovulatory or nonovulatory ovaries.

The average PPC at CD 3 at the time of CIDR insertion was 2.3 ng/ml (± 0.1) (Table 1.2).

One hour later (TD 0 + 1 h), it was 2.0 ng/ml (± 1.4).

At TD 10, the average PPC was 5.1 ng/ml (± 1.8).

PPCs at TD 10 + 6 h averaged 3.5 ng/ml (± 1.8) (Table 1.2). At this time the PPCs for 3 animals which had progesterone concentrations less than 1 ng/ml at TD 10 was 0.48, compared to 8.03 ng/ml in the remaining 2 animals.

The average cycle length of cows in this MCI group was 16.6 ± 1.5 days (Table 1.3). However, the average interval from CIDR removal to oestrus for the cows with PPCs < 1 ng/ml at TD 10 was 2.7 ± 0.6 days, compared with 5.0 ± 1.4 days in the 2 cows PPCs with > 1 ng/ml at device removal ($P < 0.10$). The follicular status of the cows with concentrations of progesterone > 1 ng/ml was similar to the cows with progesterone concentrations < 1 ng/ml.

Early-Dioestrous CIDR insertion (E-DCI) Group.

The number of class 1 and 3 follicles was maintained thoughout the CIDR period. However, the number of class 2 follicles had increased by TD 3 (2.2 ± 0.9 vs 4.0 ± 0.6, $P < 0.21$, Table 1.1).

The ovulatory follicle was detected in one cow when the CIDR was inserted (CD 7), and in a second cow on TD 6. The 3 remaining cows developed a new ovulatory follicle after CIDR removal. The average size of the largest follicle at CIDR removal was 10.4 ± 0.3 mm, and the post-treatment interval to oestrus was 7.4 days (± 2.3). Every cow had at least 5.1 days to oestrus after CIDR

removal.

The average number of follicles during CIDR treatment was similar in class 1 (12.3 ± 0.6 vs 11.9 ± 0.8, respectively), and class 2 (1.3 ± 0.2 vs 1.3 ± 0.2, respectively) follicles on the ovulatory and nonovulatory ovaries. However, the average number of class 3 follicles on the ovulatory ovary was higher that on the nonovulatory ovary (1.1 ± 0.1 vs 0.7 ± 0.1, respectively; $P < 0.10$).

The average PPC at TD 0 was 4.8 ng/ml (± 0.1) (Table 1.2). One hour later (TD 0 +1 h) the average was 9.3 ng/ml (± 2.0) equivalent to an increase of 4.5 ng/ml (Table 1.2). The average PPC on TD 10 was 8.5 ng/ml (± 0.1). The average PPC 6 h after device removal (TD 10 +6 h) was 6.7 ng/ml (± 1.1).

The average cycle length in CIDR-treated cows was 24.4 ± 2.3 days (Table 1.3). Although the average interval from CIDR removal to oestrus was 7.4 ± 2.3 days, and the PPCs 6 h after device removal (TD 10 + 6 h) were above 6 ng/ml, 2 out of the 5 animals had no class 2 follicles at CIDR removal.

Late-Dioestrous CIDR insertion (L-DCI) Group.

Similar average numbers of class 1 and 2 follicles were maintained thoughout the treatment period among cows in the L-DCI group (Table 1.1). However, the number of follicles in class 3 had increased slightly by TDs 6 and 10, or from the time of spontaneous luteolysis.

The ovulatory follicle was present as a dominant follicle in 3 cows at the time the CIDR was inserted (CD 13). In another cow, the ovulatory follicle was detected on TD 6, and in the remaining cow a new ovulatory follicle grew 1 day after CIDR removal. The average post-treatment interval to oestrous was 3 days (± 1.7).

L-DCI cows having a short interval to oestrus (< 3 days) after CIDR removal had a lower average number of class 1 follicles during the CIDR treatment on the ovulatory ovary than cows having a long interval to oestrus (13.7 ± 0.7 vs 15.4 ± 0.1, respectively; $P < 0.05$). No difference in the average of class 1 follicles on the nonovulatory ovary was observed between cows having a short or long interval to oestrus (13.7 ± 0.7 vs 13.4 ± 0.7, 13.8 ± 0.9, respectively).

The average PPC at CD 13 when devices were inserted was 8.3 ng/ml (± 1.0) (Table 2). One hour later at TD 0 +1 h it was 10.5 ng/ml (± 1.4). The increase in PPC was 2.2 ng/ml. The average PPC, on TD 3 was 9.9 ng/ml (± 0.1), and on TD 10 it was 3.5 ng/ml (± 0.1). By TD 10 +6 h the average PPC had declined to 2.6 ng/ml (± 1.2).

In the 3 animals with progesterone concentrations <1 ng/ml at TD 10, the average PPC was 0.50 ng/ml, compared with 5.68 ng/ml in the remaining 2 animals.

The average cycle length was 26.0 ± 1.7 days (Table 1.3). The average interval from CIDR removal to oestrus for cows with PPC <1 ng/ml was 2.0 days,

compared with 4.5 ± 2.1 days in cows with progesterone concentrations > 1ng/ml. Also, the cows with concentrations of progesterone > 1 ng/ml had the highest number of class 2 follicles at CIDR removal, compared with cows with progesterone concentrations < 1 ng/ml.

Plasma profiles of LH for 3 cows are presented in Fig. 1.5, 1.6, and 1.7. These cows had the highest concentrations of LH at the first sampling, and then the concentrations declined within 6 to 12 h until the values were less than the detectable limit of the assay.

Late-Dioestrous + PGF during CIDR insertion (L-DCI + PGF) Group.

The number of class 1 and 2 follicles was maintained thoughout the treatment period in cows in the L-DCI + PGF group (Table 1.1). The number of follicles in class 3 was slightly affected by the presence of a CL. The ovulatory follicle was detected in 2 cows when the CIDR was inserted (CD 13). In another 2 cows it was detected on TD 3 or TD 6. In the remaining cow, the ovulatory follicle was detected 1 day after CIDR removal. The average interval to oestrus was 3.0 days (± 1.8).

No differences were observed between cows having a short or long interval to oestrus after CIDR removal on either the ovulatory or the nonovulatory ovary in class 1 or 2 follicles during the CIDR treatment. The difference in the average number of class 3 follicle on the ovulatory ovary was close to statistical significance for cows having a short or long interval to oestrus (1.05 ± 0.1 vs 0.6 ± 0.2, respectively; P < 0.10).

The average PPC at CD 13 before CIDR insertion was 10.3 ng/ml (± 1.3) (Table 1.2). By TD 0 + 1 h, it was 11.9 ng/ml (± 0.1) representing an increase of 1.7 ng/ml. One day after injecting PGF, the average PPC was 4.9 ng/ml (± 0.1)(Table 1.2). The average PPC on TD 10 was 2.0 ng/ml (± 0.1), but only declined to 1.8 ng/ml (± 0.1) by TD 10 + 6 h (Table 1.2).

The average interval from CIDR removal to oestrus for cows with PPCs which averaged 1.8 ng/ml was 1.7 ± 0.6 days, compared with 5 days among cows with plasma progesterone concentrations of 2.2 ng/ml (P < 0.01). No differences were found in follicle classes and ovulatory and nonovulatory ovaries between cows with different intervals to oestrus. The average cycle length in CIDR-treated cows was 25.5 ± 1.9 days (Table 1.3).

Corpus Luteum Effects on Follicular Diameter and Number of Follicles.

In the L-DCI + PGF group, cows were injected with PGF 2 days after CIDR insertion. This injection induced luteolysis of the existing CL and was confirmed by ultrasonography in all cows during the CIDR treatment. However, some remnants of luteal tissue were observed by ultrasonography at the next scanning (TD 6) in all cows.

In the L-DCI group, the number of class 3 follicles was higher in the presence

of a CL, but the difference did not reach quite statistical significance ($P < 0.10$). There was an increase in the number of class 3 follicles at CIDR removal for cows having a CL. However, this increase was not observed in cows which did not have a CL (Table 1.4).

The average diameter of the largest follicle at CIDR removal in cows having a CL was different to the largest follicle at the same time for cows without a CL (12.7 ± 0.8 vs 15.2 ± 0.8 mm respectively, $P < 0.05$, Table 1.5).

The average size of the largest follicle did not change thoughout the CIDR treatment in cows having a CL, neither did it increase among cows in the L-DCI + PGF group until after they were injected with PGF. Then the diameter increased markedly until the day of CIDR removal, (Fig. 1.8).

Average PPCs for cows which had or did not have a CL is presented in Table 1.6. The L-DCI group had higher PPCs than the L-DCI-PGF group from the day following PGF injection (9.9 ± 0.1 vs 4.9 ± 0.1 ng/ml, respectively; $P < 0.01$). The CIDR maintained PPCs above 2ng/ml for the 10-day treatment period in the group without a CL.

Oestrous Cycle and Follicular Status.

The average number of class 1 follicles was similar during the CIDR treatment period among cows at the same stage of the oestrous cycle. In the pro-oestrous group (PCI) there were 20.4 ± 1.1 at CIDR insertion compared to 29.9 ± 2.9 follicles in the metoestrous group (MCI; Table 1.1). At CIDR removal the pro-oestrous treatment group had 24.0 ± 1.4 vs 33.8 ± 2.2 follicles for the metoestrous treatment group.

The E-DCI group had 20.8 ± 1.9 at CIDR insertion compared with 29.4 ± 2.9 follicles in the metoestrous group. At CIDR removal, the early dioestrous group had 24.0 ± 1.2 vs 33.8 ± 2.2 follicles in the metoestrous group (Table 1.1). There were significant differences ($P < 0.05$) between some stages of the oestrous cycle (Table 1.7).

Interactions between Follicle Classes and Interval to Oestrus.

A maximum of 25 post-treatment oestrous events was possible involving the same 5 cows treated 5 times at selected stages of the oestrous cycle, following the treatment sequence as described in Fig. 1.4. There were 60% within the first three days after CIDR removal, and the mean interval was 2.1 ± 0.5 days (Table 1.8).

The average numbers of class 1 (< 6 mm) to 3 (> 9 mm) follicles, for cows having a short (1 to 3 days) or long (4 to 9 days) interval to oestrus from CIDR removal are presented in Table 1.9. The average number of class 2 (6 to 9 mm) follicles present at CIDR removal was different among groups ($P < 0.05$). The number of class 2 follicles was increased with a longer interval to oestrus after CIDR removal (3.0 vs 2.0 class 2 follicles/animal). Numbers of class 1 and 3 follicles were not

significantly different after CIDR removal. Also, there were no significant differences in the total average numbers of follicles for cows having a short or long interval to oestrus (35.8 vs 38.4 follicles/animal, respectively).

Interactions between Follicle Class, Ovary and Interval to Oestrus.

The average numbers of follicles in each class during the CIDR treatment period for the ovulatory and nonovulatory ovary are presented in Table 1.10. There was an interaction between the interval to oestrus, follicle class and ovary in the average number of class 1 and 3 follicles. For class 2 follicles, the average number was similar during the treatment period (0.9 ± 0.1 vs 1.1 ± 0.1 on both ovaries, respectively). Cows having a short interval to oestrus had a higher average number of class 3 follicles on the ovulatory ovary than on the nonovulatory ovary (1.0 ± 0.1 vs 0.7 ± 0.1, respectively; $P < 0.001$), and fewer class 1 follicles on the ovulatory ovary compared with the nonovulatory ovary during CIDR treatment (12.0 ± 0.4 vs 14.2 ± 0.5, respectively; $P < 0.05$). Cows with a short post-treatment interval to oestrus also had fewer class 1 follicles on the ovulatory ovary than cows with a long interval to oestrus (12.0 ± 0.4 vs 14.6 ± 0.6, respectively; $P < 0.01$).

Cows with a long interval to oestrus after CIDR removal had a higher average number of class 3 follicles on the ovulatory ovary than on the nonovulatory ovary (0.9 ± 0.1 vs 0.7 ± 0.1, respectively; $P < 0.05$).

There were no significant differences in the average number of class 1 (14.6 ± 0.6 vs 13.2 ± 0.6, respectively) and 2 (1.2 ± 0.1 vs 1.2 ± 0.1, respectively) follicles on the ovulatory and nonovulatory ovary. Moreover, no differences were found in the total average number of follicles in the various classes either on the ovulatory or the nonovulatory ovary, or for cows with a short or long post-treatment intervals to oestrus after CIDR removal.

The size of the largest follicle at CIDR removal in cows having a short interval to oestrus was similar to that in cows having a long interval to oestrus. The mean diameter and (±SEM) for cows with a short interval to oestrus was 14.5 ± 0.8 mm vs 12.4 ± 0.9 mm ($P = 0.13$; Table 1.11).

The average interval from CIDR insertion to detection of the ovulatory follicle was 2.0 days ± 1.8 for the 14 cows having a short interval to oestrus. Another cow with a short interval to oestrus developed a new follicle during the 10 days of the CIDR treatment period. There were 5 cows cycles where the ovulatory follicle developed during CIDR treatment and they had a long interval to oestrus (3.0 ± 1.8 days). The 5 cases where cows developed the ovulatory follicle after CIDR removal also produced long interval to oestrus.

On the 15 occassions when there was a short intervals to oestrus after CIDR removal, there were fewer follicular waves during the CIDR period than during the 10 occassions when there was a long interval to oestrus (1.7 ± 0.1 vs 2.3 ± 0.3; $P < 0.05$; Table 1.12).

Normally Cycling (NC) Group.

A class 3 follicle was found in one of the ovaries of each of the 5 cows in the NC group at CD -2 (pro-oestrus), as well as a class 2 follicle in 4 of the animals.

An ovulatory class 3 follicle was found as expected at oestrus in each of the cows. At CD 3, a class 3 follicle was found in 3 cows, but all the cows had also at least one class 2 follicle.

All the cows had at least one class 3 follicle at CD 7, and 3 cows also had a class 2 follicle. At CD 13, all the cows had at least one class 2 and class 3 follicle.

Average PPCs on selected days during one oestrous cycle in NC group is presented in Table 1.13. The length of the oestrous cycle in the NC group was 21.4 days (± 2.1; Table 1.3).

Ovariectomized (OVX) Group.

The average PPCs before CIDR insertion in the 3 ovariectomized cows was 0.45 ng/ml (±0.23).

Post CIDR treatment concentrations at 6 h and 24 h after device removal were 0.46 (± 0.23), and 0.31 (± 0.16) ng/ml respectively.

The average PPCs increased to 6.6 ng/ml (± 1.0), at 1 h after CIDR insertion, then declined to 4.2 ng/ml (± 0.7) by TD 3. By TD 10, just before CIDR removal, the average PPCs in these 3 ovx cows was 1.7 ng/ml (± 0.4), (Fig. 1.9).

PPCs in individual animals over the 3 days of sampling during CIDR treatment ranged from 4.6, 7.3 and 7.8 ng/ml at 1 h after CIDR insertion to 3.3, 3.6 to 5.7 ng/ml at TD 3, and they varied from 1.0, 1.6 to 2.3 ng/ml, respectively at CIDR removal.

Individual Animal Variation.

The among animal variation in follicles, cycle length and progesterone profiles was considered during the experiment. None of the animals had consistently more class 1, 2 or 3 follicles, low or high levels of progesterone, or short or long length cycles.

Validation of the Ultrasound Data.

There were 160 follicles identified by ultrasound in the 3 pairs of ovaries, and 157 were correctly located (98.1%). Three small follicles of 2 mm were missed.

The correlation coefficient between the ultrasound diameter, and the calliper measurements of follicle diameter on the video screen was r=0.86, and the

equation describing their relationship was:

Diameter = Diameter calliper 1.259 + 1.054 mm

se = 0.041

DISCUSSION

Oestrous Cycle and Follicular Status.

This experiment involved the study of dynamic changes in the number and size of follicles in the ovaries, as measured by ultrasonography at the different stages of the oestrous cycle after CIDR insertion. However, it has been observed that follicular dynamics during the oestrous cycle of the majority of cows is characterized by the growth of 2 or 3 dominant follicles, producing follicle waves at specific times of the cycle (Ireland and Roche, 1987; Savio et al., 1988; Sirois and Fortune, 1988, 1990).

The average number of class 1 follicles did not differ during the period of CIDR treatment among cows which were at the same stage of the oestrous cycle. However, there was a significant difference between cycle stages, especially during the periovulatory period. In this study, the number of ultrasonically detectable class 1 follicles decreased during the pre-ovulatory period and increased during the post-ovulatory period (Table 1.7). These results are consistent with the finding in a previous report which suggested that the number of antral follicles is not constant thoughout the oestrous cycle (Pierson and Ginther, 1984).

Corpus Luteum Effects on Follicular Diameter and Number of Follicles.

When cows were in dioestrus at device insertion, and then were injected with PGF 2 days later (day + 15 of the oestrous cycle), progesterone concentrations declined significantly within 24 h after injection because the luteolytic action of PGF in cattle. In these animals, the progesterone from the CIDR device maintained levels of nearly 2ng/ml at TD 10 (Table 1.6).

The follicular population in the ovaries was altered by the presence or absence of the CL. The number of large follicles in the ovaries of cows not having a CL at CIDR removal did not increase, but the size of the largest follicle was higher than in cows with a CL. In the former case, the size of the largest follicle increased uniformly thoughout the period of CIDR treatment (Fig. 1.8). The progesterone released by the CIDR appeared to enhance the development and maintenance of a dominant follicle. The number of medium (class) follicles in cows which did not have a CL did not vary during the period of CIDR insertion. This was in contrast to trends observed by Lucy et al. (1990). This could have been due to the short time of exposure to the progesterone from the CIDR, as the CL disappeared only 8 days before the CIDR was removed.

Those cows which had a CL, maintained the same number of medium follicles during treatment, but the number of large follicles increased from the time the CIDR was removed. A previous report related this finding to typical wave-like patterns of follicular growth during the oestrous cycle in the presence of a CL (Lucy et al., 1990).

Follicle Class, Ovary and Interval to Oestrus.

Most cows (60%) came into oestrus within three days of CIDR removal, with an average interval of 2.13 ± 0.51 days (Table 1.8). Cows having a short interval to oestrus had a lower number of medium sized (class 2) follicles on the day of CIDR removal. The numbers of small and large follicles were similar, but there was a tendency in cows with the shortest intervals to oestrus to have fewer class 1 and more class 3 follicles. This tendency was observed also between stages of the oestrous cycle; especially in the pro-oestrous group in which all the cows had short post-treatment intervals to oestrus, and in the early dioestrous group in which all the cows had long intervals to oestrus (Table 1.8).

This result is similar to findings in a previous report (Lucy et al., 1990), in which it was suggested that cows with short intervals to oestrus had an active follicle present on the ovary at the time of CIDR removal, which prevented the growth of smaller follicles. With the removal of the exogenous progesterone, there is a reduced interval to oestrus and ovulation. However, cows having a long interval to oestrus had a greater number of medium follicles, suggesting that follicular dominance was not established at the time of CIDR removal, or that the large follicles present on the ovaries were not physiologically active.

Moreover, cows having shorter intervals to oestrus clearly had a larger number of large follicles and fewer small follicles on the ovulatory ovary, compared with the nonovulatory ovary. These cows had fewer small follicles on the ovulatory ovary than cows with long intervals to oestrus. However, cows having a long interval to oestrus after CIDR removal also had more large follicles on the ovulatory ovary than on the nonovulatory ovary (Table 1.10). These results could be associated with the physiological events involving follicular dominance (Ireland and Roche, 1987).

Although cows having short intervals to oestrus after CIDR removal at different stages of the oestrous cycle had some differences, the tendency confirmed the interaction between interval to oestrus, class of follicle, and CL status of the ovary. The interval to oestrus was longer in animals having more class 2 follicles on the day of CIDR removal, and with a smaller number of class 3 follicles on the ovulatory ovary compared with the nonovulatory ovary.

Most cows which ovulated soon after the CIDR was removed had a large follicle which developed or was maintained from early in the CIDR treatment period. Cows with longer post-treatment intervals to oestrus had more follicular waves during CIDR treatment, and the ovulatory follicle was detected later during CIDR treatment or during the post-CIDR period (Table 1.12). In these latter cows, the side of follicular dominance changed after CIDR removal. This sequence was also observed by Lucy et al. (1990).

Reduced fertility has been reported after some short or long-term treatments using progesterone or potent analogues. Present results show that CIDR-treated cows can ovulate and develop a normal corpus luteum, which is similar to findings in a previous report using a 9 day Norgestomet implant with a luteolytic PGF injection a day before implant removal (Rajamahendran et al., 1989).

Although normal fertility may not be simply a function of the ability of the follicle to ovulate and form a corpus luteum, Lucy et al.(1990) speculated that follicles ovulating after short periods of growth have greater fertility than those which have been statically maintained. The current results do not coincide with those of others who found ovarian follicular cysts after progestagen treatment in cycling animals (Trimberger and Hansel, 1955; Zimbelman, 1963). Present results confirm that follicles develop, persist and are capable of ovulating after 10 days of exposure to progesterone. Although the cows had devices inserted at different stages of the oestrous cycle, most of them (72%) showed similar patterns of follicular dominance. Only 28% of them developed a new follicle after CIDR removal.

With the exception of the early dioestrous group, where the interval from CIDR removal to oestrus was longer and without synchrony, insertion at other stages produced good synchrony within a 3-day period. This does not necessarily imply good fertility.

Several reports show contradictory findings as to whether fertility is normal or decreased after programmes for synchronization of oestrus, using various progestagens either alone or in combination with luteolytic agents (prostaglandin or its analogues and oestrogen) (Hansel et al., 1966; Roche, 1974a, 1974b; Thimonier et al., 1975; Smith et al., 1984; Patterson et al., 1989; Van Cleeff et al., 1990; Chenault et al., 1990). Other reports have postulated possible explanations associated with reduced fertility (Chenault et al., 1990; Odde, 1990), although there is no definitive explanation for the mechanism responsible for reduced fertility following progestagen treatment. The following is offered as a possible explanation: The precision in synchrony increases with treatment duration when using progestagen. However, as Pearce et al., 1990 found, as treatment duration increased, the conception rate decreased from 64% to 37%. Decreased fertility was observed when treatment was initiated late ($\geq$ day 12) in the oestrous cycle (Patterson et al., 1989). Progestagen exposure artificially prolongs dioestrus until administration is ended. It would suggest that metoestrous animals are likely to have the highest fertility because those follicles ovulate after short periods of growth than those which have been statically maintained as in cows undergoing luteolysis, or in pro-oestrus at the time of progestagen treatment. From the result of this study, during treatment a dominant follicle is maintained, suppressing the follicular patterns and resulting in persistent follicles. Therefore, it is logical to think that this situation would produce aged ova.

Plasma Progesterone Profiles.

The daily average PPCs in the 3 ovariectomized CIDR-treated cows varied from 6.6 to 1.7 ng/ml. There was a decline of 2.4 ng/ml over a 3 day period after insertion, and then a decline of 2.2 ng/ml over the next 7 days.

The increased PPCs on the first sampling were less than those obtained within 6 h of CIDR insertion in heifers treated with a new intravaginal device by Macmillan et al. (1990c). Munro (1987), who used CIDR's in ovariectomized Africander cross cows aged between 3 and 6 years, had even higher levels. Munro et al. (1986) also found that PPCs in ovariectomized Hereford heifers were

higher 1 day after PRID insertion than in the present trial with CIDR devices.

Mean concentrations of plasma progesterone prior to treatment and after a treatment period were all less than 0.5 ng/ml and not significantly different (Table 1.2). The concentrations of progesterone on the final day of treatment were also lower in this trial compared with results published by Munro (1987).

These differences may be due to the breed or size of animal, but must also take into account individual animal variation (Peterson and Henderson, 1990). This has been confirmed by Peterson (1990), as each ovariectomized cow had its own unique PPC profile. For example, PPCs (ng/ml; ± SD) on day 11 from CIDR insertion were between 1.9 ± 0.7 and 5.7 ± 2.0 ng/ml (Peterson and Henderson, 1990).

Changes in average PPCs 1 h after CIDR insertion differed between ovariectomized and cycling animals. Also, the change varied in the same animals treated at different stages of their cycles. The average increase from day 0 (before CIDR insertion) to TD 0 + 1 h was 6.2 ng/ml for ovariectomized cows, 2.4 ng/ml with the pro-oestrous insertion group, no difference in the metoestrous group, 4.5 for the early dioestrous group, and 2.2 and 1.6 ng/ml in the L-DCI and L-DCI + PGF groups, respectively (Table 1.2). The incremental increase in PPCs after device insertion was least than 2.5 ng/ml in each group of cycling cows, except for early dioestrous group where it was 4.5 ng/ml. Overall, cycling cows had an average PPC increase of 2.1 ng/ml compared with 6.2 ng/ml in the ovariectomized cows ($P<0.01$).

Although the average increase in the ovariectomized cows (6 ng/ml) was similar to that reported by Macmillan et al. (1990c), this trial had a lower level of increase for progesterone in cycling cows. This is likely due to the wide range of groups involved in the present experiment.

The rate of decline in PPCs was higher during CIDR treatment in ovariectomized cows. However, the same cycling cows in different stages of the oestrous cycle did have different rates of decline in PPCs, depending on the stage of the oestrous cycle at device insertion.

The average PPCs in CIDR treated cows before device insertion were similar to the concentrations of progesterone from untreated NC cows for the same day of the oestrous cycle; CIDR-treated cows had concentrations of progesterone averaging < 0.1 on TD -2, 2.3 on TD 3, 4.8 on TD 7 and 8.3 and 10.3 ng/ml on TD's 13 of the cycle compared with 0.07 on CD -2, 1.8 on CD 3, 4.6 on CD 7 and 7.4 ng/ml on CD 13 of the cycle for untreated normally cycling cows (Table 1.14).

CIDR insertion during metoestrus appeared to compromise normal luteal function. Although an increase in PPC was not observed 1 h after device insertion, the insertion of the device still prevented a decline in average PPCs over the treatment period. NC cows increased average PPCs from 1.8 ng/ml at CD 3 to 7.4 ng/ml on CD 13. In MCI, the average PPC on TD 10 (and its equivalent in the control group on CD 13) was 5.0 vs 7.4 ng/ml, respectively ($P> 0.10$). PPCs increased by 3.1 ng/ml over the treatment period in the MCI group. This differed

to the increase over the same period of time in the NC group which averaged 5.2 ng/ml (P< 0.05; Tables 1.2 and 1.13).

CIDR insertion during early dioestrus or proestrus (E-DCI and PCI) also resulted in a minimal decline in average PPCs for the treatment period, but in the E-DCI group, normal luteal function was not compromised. In this latter group, average PPCs increased from 4.8 ng/ml at CIDR insertion to 9.3 ng/ml 1 h after CIDR insertion with no following decline over the ensuing 10 day period. At CIDR removal, there were 3 out of 5 cows in the MCI group , which had PPCs of around 2 ng/ml. This was similar to the averages at TD 10 in ovariectomized, pro-oestrous and dioestrous PGF treated groups (Table 15). This suggests that premature luteolysis occurred during treatment in these 3 animals.

Average PPCs in both of the dioestrous groups (L-DCI and L-DCI + PGF) increased similarly by 2.1 and 1.7 ng/ml within 1 h of device insertion. In the dioestrous group without PGF, the PPCs then declined steadilly over a 10 day-period (Table 1.2).

In general, the progesterone released from the CIDR during the luteal phase had an additive effect on the PPCs of the cows and did not compromise endogenous progesterone production. A similar result was reported by Macmillan et al.(1990c).

Plasma LH Profiles.

The samples obtained for the LH assays should have been collected from the time of CIDR removal rather than from the commencement of oestrus. The delay in the commencement of intensive sampling meant that the mean concentration of LH in plasma was higher due to the initiation of the periovulatory surge which occurred near the onset of behavioral oestrus. These data showed the highest concentrations of LH were at the first sampling when the animals were observed in oestrus. This could help to study the associations of levels of progesterone, and its effects on the reproductive endocrine system in cattle.

Progesterone and Oestrous Cycle Length.

The average cycle length was significantly reduced (P< 0.01) in the MCI group which had CIDR's inserted on CD 3 (16.6 ± 1.5 vs 21.4 ± 2.1 days; Table 1.3). Short cycles have been reported in experiments in which heifers and cows have had a CIDR or PRID inserted during metoestrus, with or without an injection of PGF (Woody et al., 1967; Ginther, 1970; Folman et al., 1984; Battista et al., 1984; Macmillan et al., 1989). In animals that are treated with progesterone during metoestrus, the patterns of follicular waves may not alter, but it appears likely that the progesterone potentiates the premature release of PGF from the uterus by the first follicular wave (Ottobre et al., 1980). The average cycle length for groups which were CIDR-treated during dioestrous (E-DCI; L-DCI and L-DCI + PGF), did not differ between groups, but the L-DCI and L-DCI + PGF groups were significantly different from the untreated control group. CIDR-treated cows had cycle lengths averaging 24.4 ± 2.3, 26.0 ± 1.7, 25.5 ± 1.9 days

respectively, compared with 21.4 ± 2.1 days for the control group (Table 1.3).

The results of this study suggest that the interval from the end of treatment to oestrus is influenced by the follicular population both at the day of device insertion, and also at the time of device removal. Excluding the PCI group, the synchrony in oestrus among cows treated with a CIDR device was not precise. In the 10 intervals to oestrus >3 days (Table 1.8), most were from CIDR-treated cows in early dioestrus; the follicles in cows with a CL, increased the number of medium follicles by TD 3. During the last 7 days this number was maintained, but the large follicles increased in number from the time the CIDR device was removed. In animals without a CL, the number of large and medium follicles did not increase, but the size of the largest follicle was greater than in cows with a CL. Moreover, the size of the largest follicle increased throughout the period of CIDR treatment. Another study has shown that the oestrous response for treated cows with 10 day-CIDR + PGF at removal were more concentrated into 42 to 93 h, while cows treated with PGF 10 days apart were in oestrus in 30 to 107 h (Dick, 1990). This lack of precision may indicate that the cows were not at a similar stage of the follicular wave at CIDR removal even in the presence or absence of a CL, or at the time of treatment with PGF.

Validation of the Ultrasound Data.

The correlation coefficient (r=0.86) between the ultrasound diameter and the calliper measurements of follicle diameter was similar to the correlation between the follicle volume calculated from the diameter measured by ultrasound and the aspirated volume, which was reported by Macmillan and Thatcher (1990a). Savio et al. (1988) also reported a similar correlation for ultrasound and calliper measurements of follicle diameter. Finally, Spicer and Echternkamp (1986) cited studies which reported similar results for the correlation between fluid volume and calliper measurements of follicle diameter.

CONCLUSIONS

This study showed that inserting a CIDR device into cows at different stages of the oestrous cycle alters the average increase and rates of decline in PPCs depending on the stage of the oestrous cycle at device insertion, and/or the type of animal (cycling or ovariectomized). The follicular population in the ovaries was also altered directly or indirectly by the progesterone released from the CIDR, which appeared to enhance the development and maintenance of a dominant follicle, depending in part on the presence or absence of a CL. Therefore, there were significant interactions between treatments initiated at different stages of the cycle, and the interval to oestrus, number of follicles in each class and CL status of the ovary.

These interactions produced variation in the post-treatment interval to oestrus, and thus affected precision in synchrony. If synchrony is to be improved then follicle wave patterns will also need to be synchronized. This should allow normal fertility to be obtained because the present way of obtaining precise synchrony is by suppressing the follicle wave patterns resulting in persistent follicles which in some animals may produce reduced fertility. Moreover, to ensure a precise synchrony, it must also take into account that the most common problem in breeding management in dairy herds has been and will continue to be : oestrus detection. Simple techniques involving the use of tailpaint (applied when treatment is initiated), and an aerosol raddle (applied at the end of a synchronization treatment) have reduced the occurrence of this problem in seasonal dairy herds.

Fig. 1.4 **Timetable of experimental protocol**

Pre-synchronization with CIDR-B + PGF

Pro-oestrous group

(CD -2)	1	3	6	10	
CIDR IN				CIDR OUT	Oestrus

Late Dioestrous group

(CD 13)	1	3	6	10	
CIDR IN				CIDR OUT	Oestrus

Late Dioestrous + PGF group

(CD 13)	1	3	6	10	
CIDR IN				CIDR OUT	Oestrus

Early Dioestrous group

(CD 7)	1	3	6	10	
CIDR IN				CIDR OUT	Oestrus

Normally Cycling Control group (NC)

Oestrus	2	4	6		Oestrus

Metoestrous group

(CD 3)	1	3	6	10	
CIDR IN				CIDR OUT	Oestrus

Then,
a) Validation of Ultrasound Data.
b) Period of Sampling with OVX Cows.

( ) = Day of the oestrous cycle for CIDR-B insertion.
Oestrus = Day 0 of the cycle.
OVX = Ovariectomized cows

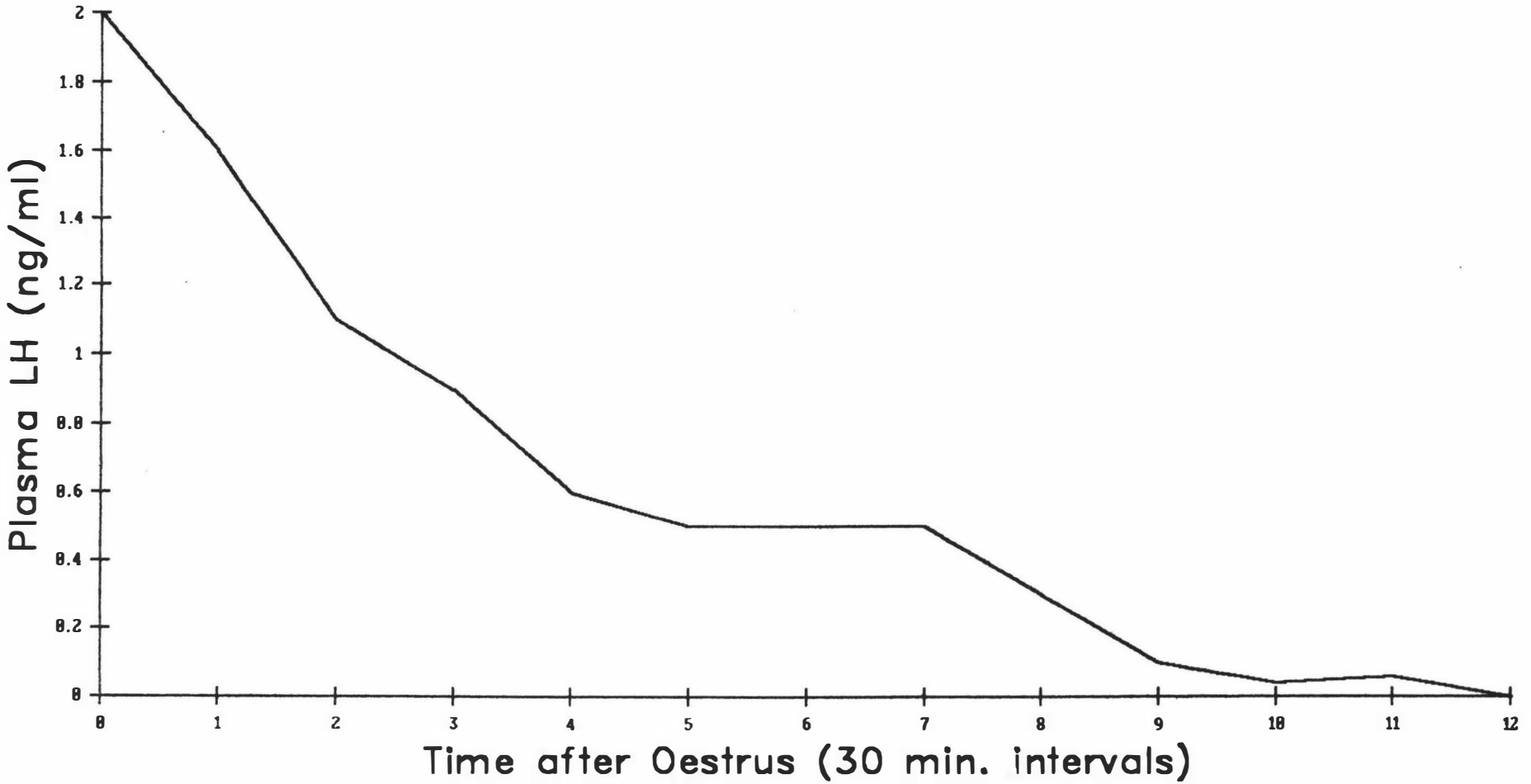


Fig. 1.5 **Plasma LH concentrations in cow A from oestrus until + 6 h after oestrus**

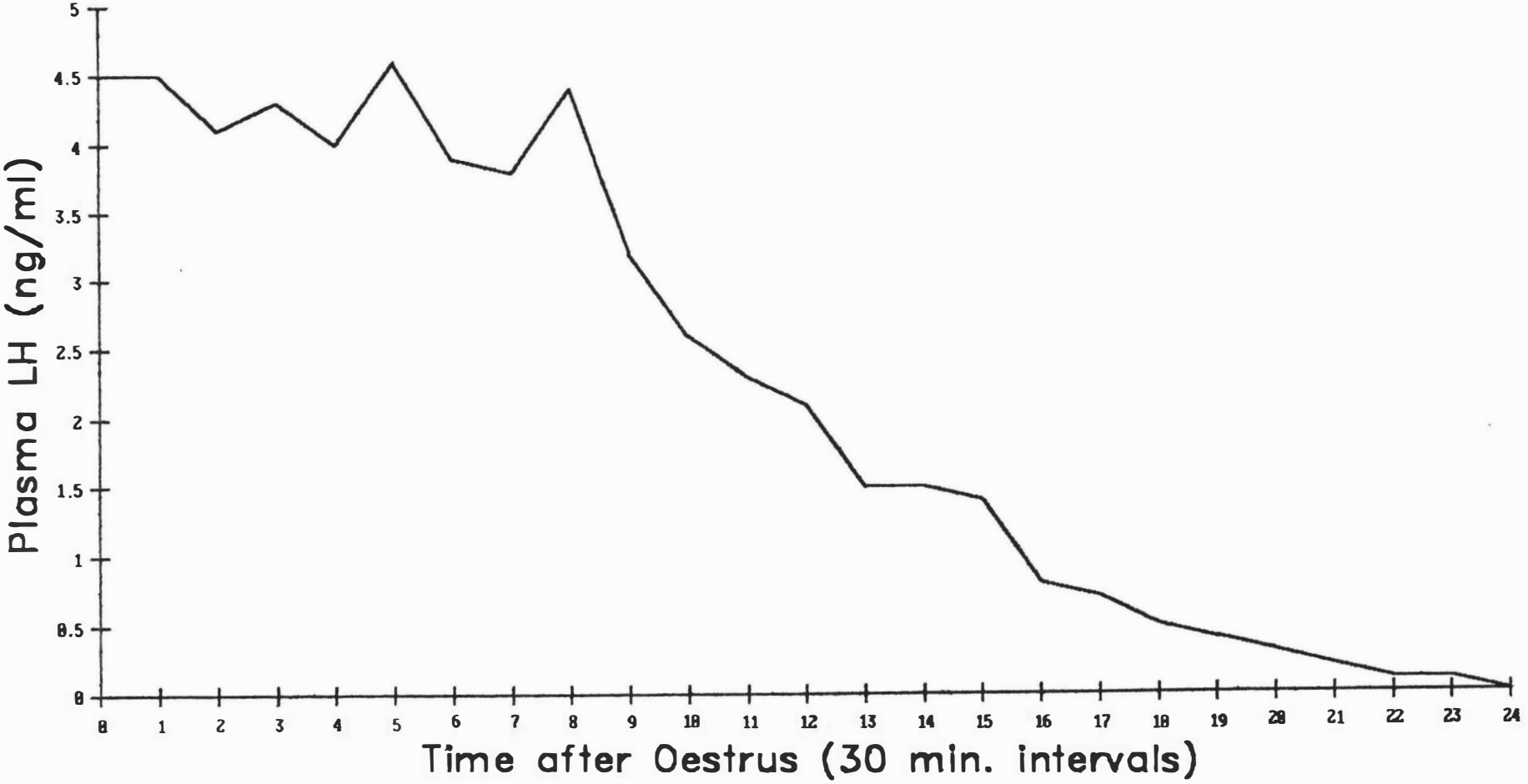


Fig. 1.6 **Plasma LH concentrations in cow B from oestrus until + 6 h after oestrus**

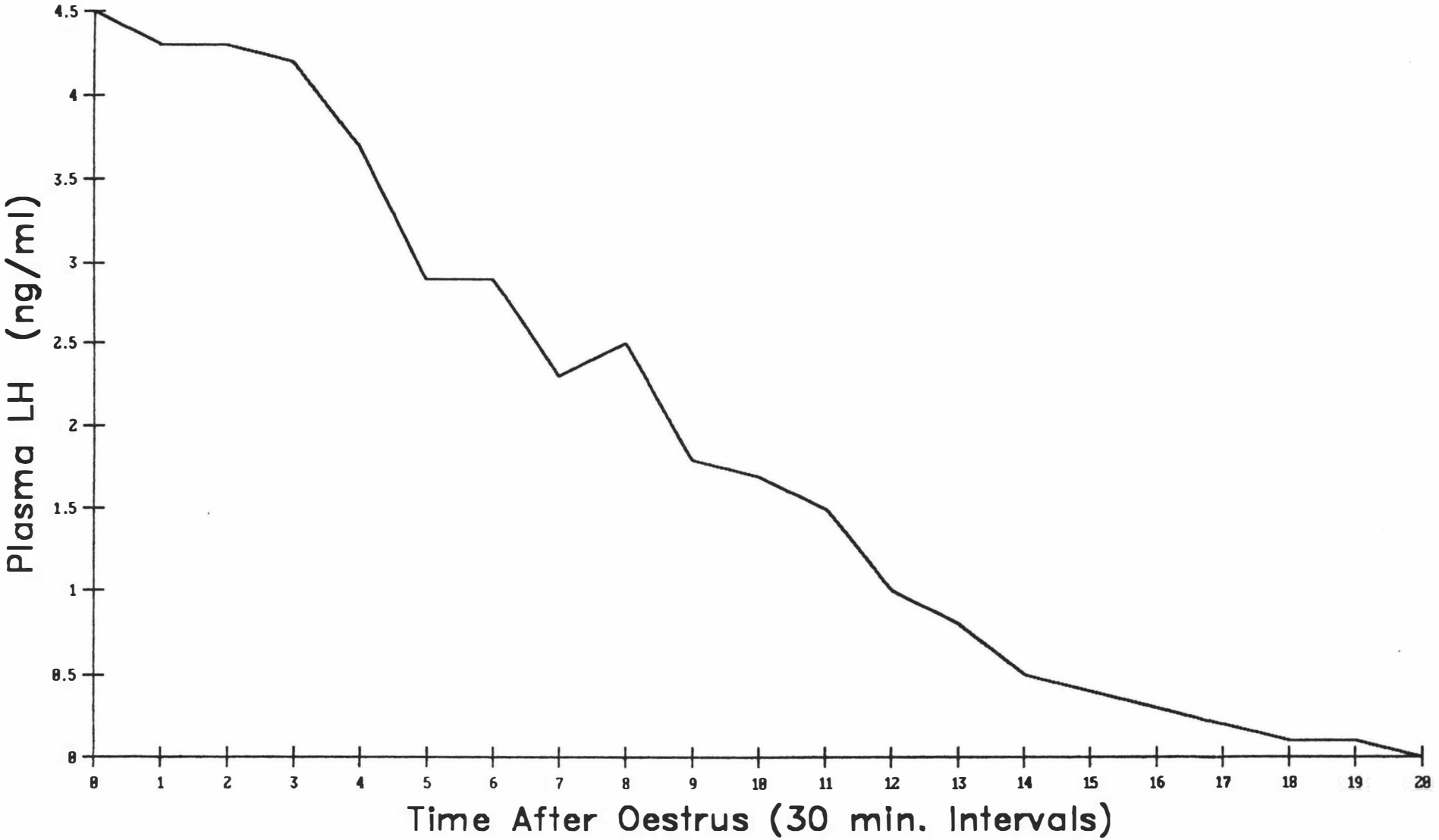


Fig. 1.7 **Plasma LH concentrations in cow C from oestrus until + 6 h after oestrus**

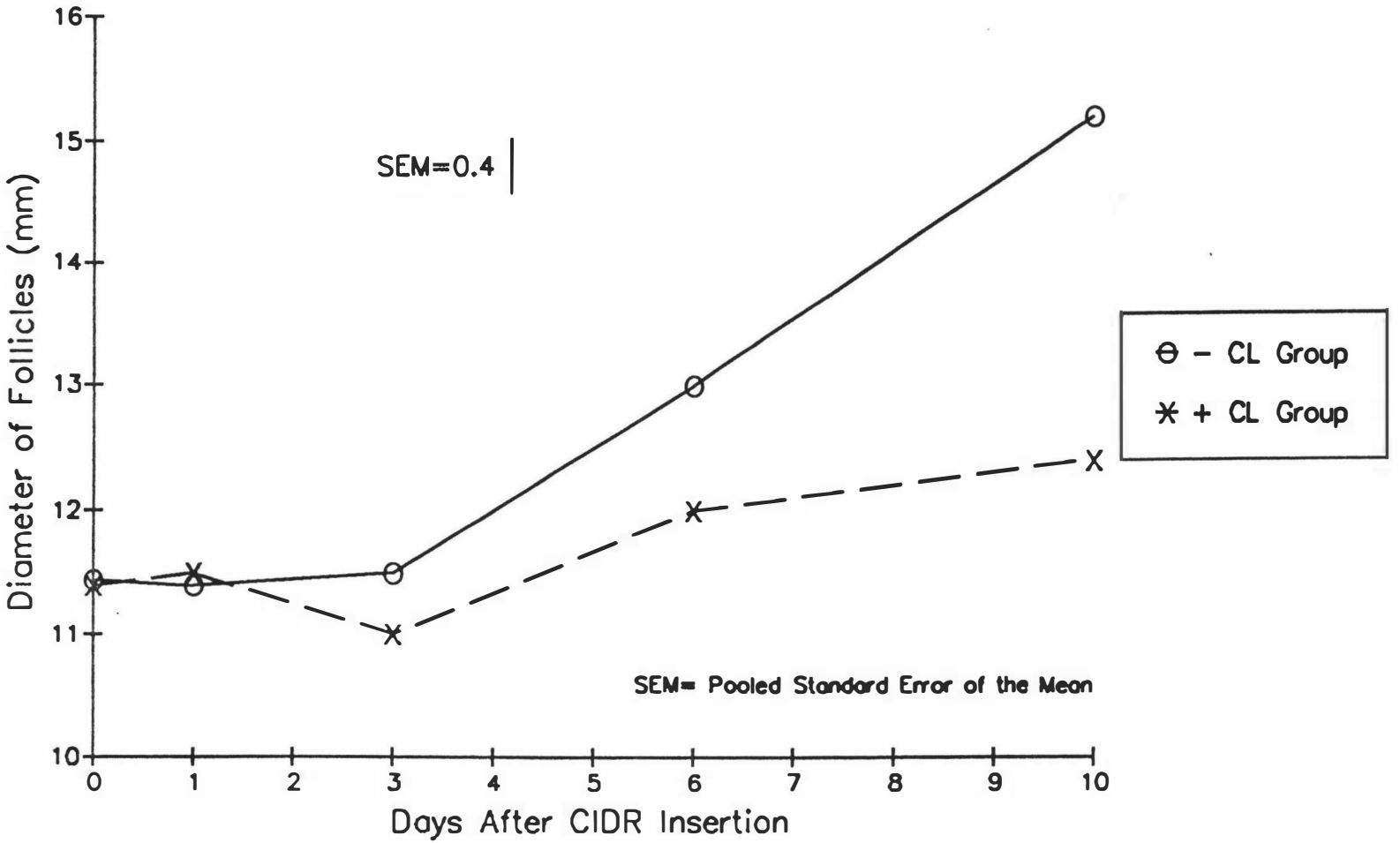


Fig. 1.8 **Average diameter of largest follicle in cows with or without a corpus luteum (CL).**

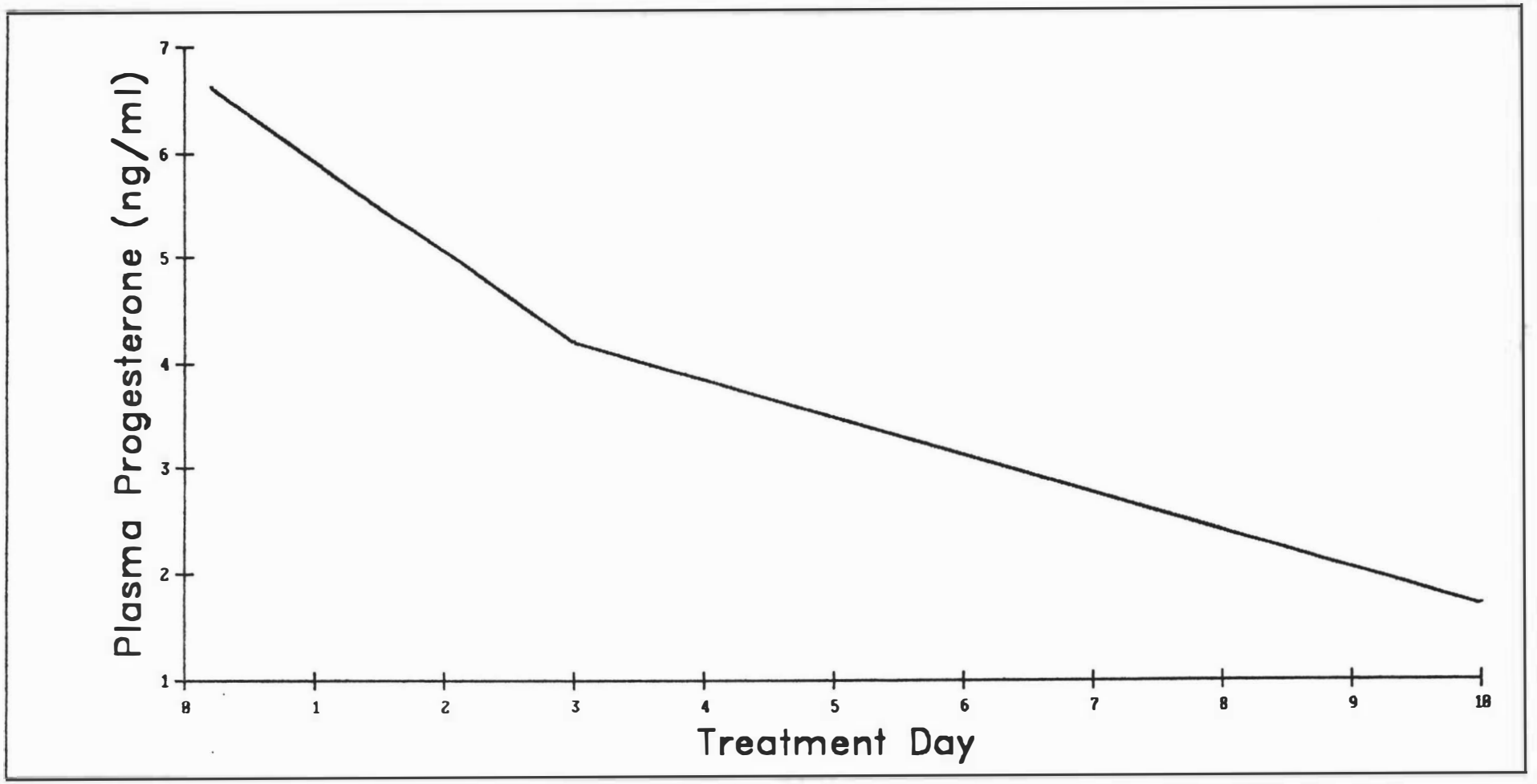


Fig. 1.9. **Average plasma progesterone concentrations in ovariectomized cows treated with CIDR-B for ten days.**

Table 1.1 **Average number of follicles in each class (class 1, <6 mm; class 2, 6-9 mm and class 3, >9 mm) for cows at different stages of the oestrous cycle during the CIDR-B treatment period.**

Day	Class	PCI Mean (SE)	MCI Mean (SE)	E-DCI Mean (SE)	L-DCI Mean (SE)	L-DCI + PGF Mean (SE)
0	1	20.4 (1.1)	29.9 (2.9)	20.8 (1.9)	24.8 (1.1)	26.6 (1.2)
	2	2.5 (0.6)	3.4 (0.4)	2.2 (0.9)	1.8 (0.6)	1.8 (0.4)
	3	1.2 (0.2)	2.0 (0.3)	2.0 (0.4)	1.6 (0.2)	1.2 (0.2)
1	1		31.8 (2.8)	21.6 (1.0)	26.8 (1.1)	28.0 (1.2)
	2		3.4 (0.8)	2.2 (0.2)	2.0 (0.5)	1.8 (0.4)
	3		1.6 (0.2)	1.6 (0.2)	1.6 (0.2)	1.4 (0.2)
3	1	24.4 (1.5)	30.1 (2.3)	23.4 (1.3)	27.2 (2.0)	29.0 (1.5)
	2	2.0 (0.5)	2.2 (0.3)	4.4 (0.5)	1.8 (0.4)	2.5 (0.5)
	3	1.2 (0.2)	2.2 (0.3)	1.6 (0.4)	1.6 (0.2)	1.8 (0.4)
6	1		29.8 (2.2)	23.4 (2.4)	25.0 (0.8)	27.7 (1.1)
	2		2.5 (0.2)	3.5 (1.0)	3.4 (0.8)	3.3 (0.3)
	3		1.4 (0.2)	1.8 (0.6)	2.4 (0.4)	1.7 (0.2)
10	1	24.0 (1.4)	33.8 (2.2)	24.0 (1.2)	27.8 (1.9)	26.0 (1.4)
	2	1.5 (0.3)	3.0 (0.3)	4.0 (0.6)	1.8 (0.4)	2.0 (0.5)
	3	1.4 (0.4)	1.8 (0.2)	1.4 (0.2)	2.4 (0.4)	1.4 (0.2)

PCI = Pro-oestrus; MCI = Metoestrus; E-DCI = Early Dioestrus; L-DCI = Late Dioestrus; L-DCI + PGF = Late Dioestrus + PGF

Table 1.2 **Average plasma progesterone concentrations (PPC[Mean (SEM); ng/ml]) at the time of CIDR insertion (TD 0) and at selected intervals during treatment (TD 0 + 1 h, TD 3, TD 10) and TD 10 + 6 h) in ovariectomized (OVX) and cycling cows.**

Groups	Day of Cycle	TD 0	TD 0 +1 h	TD 3	TD 10	TD 10 +6 h	Number of cows
OVX	-	0.4 (0.2)	6.6 (1.0)	4.2 (0.7)	1.7 (0.4)	0.4 (0.2)	3
PCI	-2	<0.1	2.5 (0.01)	-	2.3 (0.1)	<0.1 (<0.1)	5
MCI	3	2.3 (0.1)	2.0 (0.01)	-	5.0 (1.8)	3.5 (1.8)	5
E-DCI	7	4.8 (0.1)	9.3 (2.0)	-	8.5 (0.1)	6.7 (1.1)	5
L-DCI	13	8.3 (1.0)	10.5 (1.4)	9.9 (0.1)	3.5 (0.1)	2.6 (1.2)	5
L-DCI + PGF	13	10.3 (1.3)	11.9 (0.1)	4.9 (0.1)	2.0 (0.1)	1.8 (0.1)	5

a= Day of the oestrous cycle at CIDR insertion (oestrus=day 0)
b= Injected with 500 mcg 2 days after CIDR insertion

Table 1.3 **Oestrus cycle length in cows treated with a CIDR-B for 10 days from device insertion on CD's 3, 7, or 13, and in untreated controls.**

Group		N	Cycle Length (Days)
Control	(1)[b]	5	21.4 ± 2.1
Metoestrus (CD 3)[a]	(2)[b]	5	16.6 ± 1.5
Early Dioestrus (CD 7)[a]	(3)[b]	5	24.4 ± 2.3
Late Dioestrus (CD 13)[a]	(4)[b]	5	26.0 ± 1.7
Late Dioestrus + PGF (CD 13)[a]	(5)[b]	5	25.5 ± 1.9

(1) vs (2)*; (2) vs (3 + 4 + 5)**; (1) vs (4 + 5)*

a = Day of CIDR-B insertion
b = Probability * $P < 0.01$; ** $P < 0.001$

Table 1.4 **Average difference between class 3 follicles at day of CIDR-B insertion and during the CIDR-B treatment in cows with or without a corpus luteum (CL).**

Day	N	L-DCI +CL	L-DCI + PGF -CL
		Mean (SEM)	Mean (SEM)
0	5	1.6 (0.2)	1.4 (0.2)
1	5	1.6 (0.2)	1.4 (0.2)
3	5	1.6 (0.2)	1.8 (0.4)
6	5	2.4 (0.4)	1.7 (0.2)
10[a]	5	2.4 (0.4)	1.4 (0.2)

a= Day + CL > - CL; P< 0.10

Table 1.5 **Average size of the largest follicle at CIDR-B removal in cows with or without a corpus luteum (CL).**

Day	N	L-DCI +CL	L-DCI + PGF -CL
		Mean (SEM)	Mean (SEM)
10[a]	5	12.7 (0.8)	15.2 (0.8)

a= Day + CL < - CL; P< 0.05

Table 1.6 **Plasma progesterone (ng/ml) during the 10 days of CIDR-B treatment in cows with or without a Corpus Luteum (CL).**

Day of CIDR-B Insertion		L-DCI +CL	L-DCI + PGF -CL	N
		Mean (SEM)	Mean (SEM)	
0		8.3 (1.0)	10.3 (1.3)	5
1		10.5 (1.4)	11.9 (0.1)	5
2	PGF			
3[a]		9.9 (0.1)	4.9 (0.1)	5
10		3.5 (0.9)	2.0 (0.1)	5

a = Day + CL > - CL; $P < 0.05$

Table 1.7 **The average number of class 1 (< 6 mm) for cows at different days of the periovulatory period.**

	Periovulatory Period			
	CD -2	Oestrus	CD 3[a]	CD 7
	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
Follicles class 1	20.4 (1.1)	23.7 (1.2)	29.9 (2.9)	20.8 (1.9)

a = $P < 0.05$
CD = Cycle day

Table 1.8 **Interval to oestrus after CIDR-B removal in cows.**

Interval to Oestrus (days)	Number of Cows	Percentage of Cows (%)	Cows with +CL	Cows without -CL
1	1	4.0	-	1
2	11	44.0	3	8
3	3	12.0	1	2
4	2	8.0	2	-
5	2	8.0	-	2
6	3	12.0	3	-
9	3	12.0	3	-
Total	25	100	12	13

Table 1.9 **Average number of class 1 (<6 mm), class 2 (6-9 mm) and class 3 (> 9 mm) follicles at CIDR-B removal in cows with short (1-3 days) or long (4-9 days) post-removal intervals to oestrus.**

		Class 1	*Class 2*[a]	*Class 3*
Interval to Oestrus (days)	N	Mean (SEM)	Mean (SEM)	Mean (SEM)
1-3	15	32.0 (1.9)	2.0 (0.3)	1.8 (0.2)
4-9	10	34.0 (2.5)	3.0 (0.4)	1.4 (0.2)

a = P <0.05

Table 1.10 **Average number of ovarian follicles in classes 1, 2 and 3 during the CIDR-B treatment for ovulatory and nonovulatory ovary for cows with short[a] (1-3) or long[b] (4-9) post-treatment intervals to oestrus after CIDR-B removal.**

		Ovulatory Ovary		Nonovulatory Ovary	
		Short[a]	*Long[b]*	*Short[a]*	*Long[b]*
Class	1	12.0 (0.4)	14.6 (0.6)	14.2 (0.5)	13.2 (0.6)
Class	2	0.9 (0.1)	1.2 (0.1)	1.1 (0.1)	1.2 (0.1)
Class	3	1.0 (0.1)	0.9 (0.1)	0.7 (0.1)	0.7 (0.1)

Values = Mean (± SEM)

Table 1.11 **Size of largest follicle in cows with differing post-treatment intervals from CIDR-B removal to oestrus.**

Interval to Oestrus	Size of the follicle Mean (SEM)
1-3 days	14.6[a] (0.8)
4-9 days	12.4 (0.9)

a= Interval groups differ; P= 0.13

Table 1.12 **Average number of follicular waves leading to the ovulatory follicle during CIDR-B treatment for cows with different interval to oestrus after CIDR-B removal.**

Interval to Oestrus (Days)	N	Number of waves Mean (SEM)[a]
1-3	15	1.7 (0.1)
4-9	10	2.3 (0.3)

a= Interval groups differ; $P < 0.05$

Table 1.13 **Average plasma progesterone concentrations (PPC) on selected days during one oestrous cycle in control cows.**

		PPC Mean (SEM; ng/ml)			
Group	N	CD -2[a]	CD 3	CD 7	CD 13
Control	5	0.07 (0.01)	1.8 (0.1)	4.6 (0.1)	7.4 (1.0)

a = Day of Cycle (oestrus = day 0)

Table 1.14 **Average plasma progesterone concentrations (PPC[mean (SEM);ng/ml]) at stated time of CIDR-B insertion in different treatments groups and on selected days (CD) during one oestrous cycle in normally cycling (NC) group.**

Group	No of Cows	CD -2[a]	CD 3	CD 7	CD 13
NC	5	0.07 (0.01)	1.8 (0.1)	4.6 (0.1)	7.4 (1.0)
PCI[b]	5	<0.1			
MCI[c]	5		2.3 (0.1)		
E-DCI[d]	5			4.8 (0.1)	
LDCI[e]	5				8.3 (1.0)
LDCI + PGF[f]	5				10.2 (1.3)

a = Day of cycle (oestrus = day 0)
b = Pro-oestrus
c = Metoestrus
d = Early-dioestrus
e = Late dioestrus
f = Late dioestrus + prostaglandin

Table 1.15 **Average plasma progesterone concentrations (PPC[Mean (SEM); ng/ml]) at the time of CIDR-B removal (TD 10) in ovariectomized (OVX) and cycling cows.**

Groups	Number of Cows	Day of the Cycle[a]	TD 10
OVX	3	-	1.7 (0.4)
PCI[c]	5	-2	2.3 (0.1)
MCI[d]	3	3	2.1 (0.1)
L-DCI + PGF[b]	5	13	2.0 (0.1)

a = Day of the oestrous cycle at CIDR-B insertion (oestrus = day 0)
b = injected with 500 mcg 2 days after CIDR-B insertion
c = Pro-oestrus
d = Metoestrus

REFERENCES

BAILIE, J. H. (1982). Management and economic effects of different levels of estrus detection in the dairy herd. **Vet.** Rec. 110: 218-221.

BATTISTA, P. J., REXROAD, C. E. and WILLIAMS, W. F. (1984). Effects of progesterone administered to dairy heifers on sensitivity of corpora lutea tp PGF 2∝ and on plasma LH concentration. Theriogenology 22: 47-58.

BEAL, W. E. (1983). A note on synchronization of oestrus in post-partum cows with prostaglandin F 2∝ and a progesterone releasing device. Anim. Prod. 37: 305-308.

BEAL, W. E., CHENAULT, J. R., DAY, M. L. and CORAH, L. R. (1988). Variation in conception rates following synchronization of estrus with melengestrol acetate anf prostaglandin F 2∝. **J. Anim. Sci.** 66: 599-602.

BRINK, J. T. and KIRACOFE, G. H. (1988). Effect of estrous cycle stage at Syncro-mate B treatment on conception and time to estrus in cattle. Theriogenology 29: 512-518.

BROWN, L. N., ODDE, K. G., KING, M. E., LEFEVER, D. G. and NEUBAUER, C. J. (1988). Comparison of MGA-PGF2 ∝ to Synchro-Mate B for oestrus synchronization in beef heifers. Theriogenology 30: 1-8.

CHENAULT, J. R., McALLISTER, J. F. and KASSON, C. W. (1990). Synchronization of estrus with melengestrol acetate and prostaglandin F 2∝ in beef and dairy heifers. **J. Anim. Sci.** 68: 296-303.

DAILEY, R. A., JAMES, R. E., INSKEEP, E. K. and WASHBURN, S. P. (1983). Synchronization of estrus in dairy heifers with prostaglandin F 2∝ with or without estradiol benzoate. **J. Dairy Sci.** 66: 881-886.

DICK, A. R. (1990). The use of tailpaint and an aerosol raddle to monitor oestrous behaviour of animals after different synchrony treatments. In **Studies on the use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 2: 79-126.

DREW, B. (1984). Benefits, application and problems of oestrous synchronization techniques. "Dairy Cow Fertility" edit. R. G. Eddy and M. J. Ducker, **Brit. Vet. Assoc.** Edit. Services, London 130-131.

DUIRS, G. F., MACMILLAN, K. L., RHODES, A. P., BARNES, D. R. and TAUFA, V. K. (1986). CIDR: Concepts in Breeding Management. **Proc. Dairy cattle of the N.Z. Vet. Assoc.** 127-136.

FIGUEROA, M. R., FUQUAY, J . W. and SHIPLEY, S. K. (1988). Synchronization of estrus in early diestral dairy heifers with prostaglandin F 2∝ and estradiol benzoate. **Theriogenology** 30: 1093-1097.

FOLMAN, Y., KAIM, M, HERZ, Z. and ROSEMBERG, M. (1984). Reproductive management of dairy cattle based on synchronization of estrous cycles. **J Dairy Sci.** 67: 153-160.

FOOTE, R. H. (1975). Estrus detection and estrus detection aids. **J. Dairy Sci.** 58: 248-256.

GINTHER, O. J. (1970). Effects of progesterone on length of estrous cycle in cattle. **Am. J. Vet. Res.** 31: 493-496.

GINTHER, O. J. (1986). Ultrasonic imaging and reproductive events in the mare.

HAFS, H. D. and MANNS, J. G. (1975). Onset of oestrus and fertility of dairy heifers and suckled beef cows treated with prostaglandin F 2∝. **Anim. Prod.** 21: 13-20.

HANSEL, W., MALVEN, P. V. and BLACK, D. L. (1961). Estrous cycle regulation in the bovine. **J. Anim. Sci.** 20: 621-625.

HANSEL, W., DONALDSON, L. E., WAGNER, W. C. and BRUNNER, M. A. (1966). A comparison of oestrus cycle synchronization methods in beef cattle under feedlot conditions. **J. Anim. Sci** 25: 497-503.

HEERSCHE, G. Jr., KIRACOFE, R. C., DeBENEDETTIS. S. W. and McKEE, R. M. (1979). Synchronization of estrus in beef heifers with a norgestomet implant and prostaglandin F2 ∝. **Theriogenology** 11: 197-203.

HERRING D. S. and BJORNTON, G. (1985). Physics, facts, and artifacts of diagnostic ultrasound. **Veterinary Clinics of North America: Small Animal Practice** 15: 1107-1122.

IRELAND, J. J. and ROCHE, J. F. (1982). Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormoes steroids in follicular fluid and gonadotrophin receptors. **Endocrinology** 111: 2077-2086.

IRELAND, J. J. and ROCHE, J. F. (1987). Hypothesis regarding development of dominant follicles during a bovine estrous cycle. **"Follicular Growth and Ovulation Rate in Farm Animals"** eds. J. F. Roche and D. O"Callanghan, Martinus Nijhoff, Publishers, The hague. 1-18.

JACKSON, P. S., JONHSON, C. T., FURR, B. J. and BEATTIE, J. F. (1979). Influence of stage of oestrus cycle on time of oestrus following cloprostenol treatment in the bovine. **Theriogenology** 12: 153-167.

KING, M. E., KIRACOFE, J. S., STEVENSON, J. S. and SCHALLES, R. R. (1982). Effect of stage of the oestrous cycle on interval to estrus after PGF 2∝ in

beef cattle. Theriogenology 18: 191-200.

KIRACOFE, G. H., KEAY, L. E. and ODDE, K. G. (1985). Synchronization of estrus in cyclic beef heifers with the prostaglandin analog alfaprostol. Theriogenology 24: 737-745.

KIRKWOOD, R. N., LAPWOOD, K. R., SMITH, W. C. and ANDERSON, I. L. (1984). Plasma concentrations of LH, prolactin, oestradiol-17B and progesterone in sows weaned after lactation for 10 or 35 days. J. Reprod. Fert. 70: 95-102.

KLINGBORG, D. J. (1987). Normal reproductive parameters in large California-style dairies. Veterinary clinics of North America 3: 483-499.

LAUDERDALE, J. W. (1972). Effects of PGF 2∝ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35: 246 (abstr.).

LAUDERDALE, J. W., McALLISTER J. F., MOODY, E. L. and KRATZER, D. D. (1980). Pregnancy rate in beef cattle injected once with PGF 2∝. J. Anim. Sci. 51 (suppl. 1): 296 (abstr.).

LIGTVOET, C. M., BOM, N. and GUSSENHOVEN, W. J. (1989). Technical principles of ultrasound. Diagnostic Ultrasound and Animal Reproduction 1-9. M.M. Taverne and A. H. Willemse (eds.) Kluwer Academic Publishers.

LUCY, M. C., THATCHER, W. W. and MACMILLAN, K. L. (1990). Ultrasonic identification of follicular populations and return to estrus in early postpartum dairy cows given intravaginal progesterone for 15 days. Theriogenology in press.

MACMILLAN K. L. and CURNOW, R. J. (1977). Tailpainting: a simple form of oestrus detection in new Zealand dairy herds. N.Z. Exper. Agric. 5: 357-361.

MACMILLAN K. L. (1978). Oestrus synchronization with a prostaglandin analogue. III. Special aspects of synchronization. N.Z. Vet J. 26: 104-108.

MACMILLAN, K. L. and DAY A. M. (1982). Prostaglandin F 2∝ - A fertility drug in dairy cattle?. Theriogenology 18: 245-253.

MACMILLAN, K. L. (1983). Prostaglandin responses in dairy herd breeding programmes. N.Z. Vet J. 31: 110-113.

MACMILLAN, K. L. and HENDERSON, H. V. (1984). Analysis of the variation in the interval from an injection of prostaglandin F 2∝ to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. Anim. Reprod. Sci. 6: 245-254.

MACMILLAN, K. L. (1985). Reproductive efficiency in dairy cattle. Proc. Inter.

Conf. on Vet. Prev. Med. and Anim. Prod., Aust. Vet. J. 41-48.

MACMILLAN, K. L. (1986). Condensed breeding programmes. Seminar **New Zealand Dairy Board Livestock Improvement Division. Flock House** 17-21 February.

MACMILLAN, K. L. and DAY, A. M. (1987). Treating the non-cycling cow. **Proc. Ruakura Farmers' Conf.** 39:65-68.

MACMILLAN, K. L., TAUFA, V. K., BARNES, D. R., DAY, A. M. and HENRY, R. (1988a). Detecting oestrus in synchronized heifers using tailpaint and aerosol raddle. **Theriogenolgy** 30: 1099-1114.

MACMILLAN, K. L. (1988b). Maximizing the use of AI in cattle. **Proc. Int. Cong. on Anim. Rep. and AI** 2: 265-275.

MACMILLAN, K. L. (1988c). Trends in breeding management in New Zealand dairy herds. Non-infectious factors affecting reproductive performance of dairy herds. **Dairy Cattle Reproduction Research Workshop.** Werribee, Nov. 15-17.

MACMILLAN, K. L., TAUFA, V. K., DAY, A. M. and BARNES, D. R. (1989). Some effects of administering progesterone per vaginum during metoestrus on oestrous cycle length in heifers. **Proc Australian Society for Reproductive Biology.** 25-27 September, Monash University. 105.

MACMILLAN, K. L. and THATCHER, W. W. (1990a). Effects of an analog of gonadotrophin releasing hormone on ovarian follicles in cattle. **Theriogenology** in press.

MACMILLAN, K. L., DAY, A. M. and TAUFA, V. K. (1990b). Anoestrum update. **Proc. Ruakura Farmers Conf.** 42: 107-114.

MACMILLAN, K. L., TAUFA, V. K., BARNES, D. R. and DAY, A. M. (1990c). Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. **Anim. Reprod. Sci.** submitted.

MACMILLAN, K. L., TAUFA, V. K., DAY, A. M. and PETERSON, A. J. (1990d). Effects of supplemental progesterone on pregnancy rates in cattle. **Third International Ruminant Reproduction Symposium.** March, Nice, France.

MALMO, J. (1988). A review of reproductive management practices currently being used or researchered in seasonally calving Australian dairy herds. Non-infectious factors affecting reproductive performance of dairy herds. **Dairy Cattle Reproduction Research Workshop.** Werribee, Nov. 15-17.

MOFFEO, G., BALLABIO, R., OLGIATI, V. and GUIDOBONO, F. (1983). Induction of estrus in cows by a new analogue of PGF 2∝ (alfaprostol). **Prostaglandins** 25: 541-548.

MOMONT, H. W. and SEGUIN, B. E. (1988). Interval to oestrus after

prostaglandin treatment of dairy heifers in dependent of rate of luteolysis. **Proc. Int. Cong on Anim. Rep. and AI.** 2: 448-449.

MUNRO, R. K. and MOORE, N. W. (1985). Effects of progesterone, oestradiol benzoate and cloprostenol on luteal function in the heifer. **J. Reprod. Fert.** 73: 353-359.

MUNRO, R. K. and MOORE, N. W. (1986). Plasma concentrations of progesterone in ovariectomized and prepuberal heifers following intravaginal and intramuscular administration of progesterone. **Anim. Reprod. Sci.** 11: 81-89.

MUNRO, R. K. (1987). Concentrations of plasma progesterone in cows after treatment with 3 types of progesterone pessaries. **Australian Veterinarian Journal** 64: 385-386.

ODDE, K. G. (1990). A review of synchronization of estrus in postpartum cattle. **J. Anim. Sci.** 68: 817-830.

O'FARREL, K. J. (1984). Oestrous behaviour, problems of detection and relevance of cycle lengths. **"Dairy Cow Fertility"** eds. R. G. Eddy and M. J. Tucker, Brit. Vet. Assoc. Edit. Services, London 47-59.

OLTENACU, P. A., ROUNSAVILLE, T. R., MILLIGAN, R. A. AND FOOTE, R. H. (1981). Systems analysis for designing reproductive management programs to increase production and profit in dairy herds. **J. Dairy Sci.** 64: 2096-2104.

OTTOBRE, J. S., LEWIS, G. S., THAYNE, W. V. and INSKEEP, E. K. (1980). Mechanism by which progesterone shortens the estrous cycle of the ewe. **Biology of Reproduction** 23: 1046-1053.

PATTERSON, D. J., CORAH, L. R., KIRACOFE, G. H., STEVENSON, J. S. and BRETHOUR, J. R. (1989). Conception rate in Bos Taurus and Bos Indicus crossbred heifers after postweaning energy manipulation and synchronization of estrus with melengestrol acetate and fenprostalene. **J. Anim. Sci.** 67: 1138-1147.

PEARCE, M. G., MACMILLAN K. L., TAUFA, V. K. and DAY A. M. (1990). Systems for synchronising oestrus in dairy heifers treated with a CIDR-B intravaginal device with or without prostaglandin F 2α or oestradiol. **Proc. 5th AAAP Taipei Vol. 3.**

PETERS, J. B., WELCH, J. A., LAUDERDALE, J. W. and INSKEEP, E. K. (1977). Synchronization of estrus in beef cattle with PGF 2α and estradiol benzoate. **J Anim. Sci.** 45: 230-235.

PETERSON, A. J. and HENDERSON, H. V. (1990). Plasma progesterone concentrations in ovariectomized dairy cows treated with a CIDR-B breeding device. **Third International Ruminant Reproduction Symposium.** Mach 25-28, Nice, France.

PIERSON, R. A. and GINTHER, O. J. (1984). Ultrasonography of the bovine ovary. **Theriogenology** 21: 495-504.

PIERSON, R. A. and GINTHER, O. J. (1987). Follicular population during the estrous cycle in heifers. I. The influence of day. **Anim. Reprod. Sci.** 14: 165-176.

PIERSON, R. A., KASTELIC, J. P. and GINTHER, O. J. (1988a). Basic principles and techniques for transrectal ultrasonography in cattle and horses. **Theriogenology** 29: 3-20.

PIERSON, R. A. and GINTHER, O. J. (1988b). Ultrasonic imaging of the ovaries and uterus in cattle. **Theriogenology** 29: 21-37.

POWIS, R. L. (1986). Ultrasound science for the veterinarian. **Veterinary Clinics of North America: Equine Practice** 2: 3-27.

QUIRK, S. M., HICKEY, G. J. and FORTUNE, J. E. (1986). Growth and regression of ovarian follicles during the follicular phase of the oestrous cycle in heifers undergoing spontaneous and PGF-2 alpha-induced luteolysis. **J. Reprod.** Fert. 77: 211-219.

RAJAMAHENDRAN, R., EIDE, A., ROBINSON, J. TAYLOR, C. and WALTON, J. S. (1989). Effect of norgestomet on follicular dynamics, corpus luteum growth, progesterone, LH. estrus and ovulation in cycling heifers. **J. Anim. Sci.** 67: (suppl. 1): 383.

RANTANEN, N. W. (1986). General considerations of ultrasound examinations. **Veterinary Clinics of North America: Equine Practice** 2: 29-32.

REFSAL, K. R. and SEGUIN, B. E. (1980). Effect of stage of dioestrus and number of cloprostenol (ICI 80.996) injections on intervals to estrus, LH peak, and ovulation in hifers. **Theriogenology** 14: 37-48.

ROBERSON, M. R., WOLFE, M. W., STUMPF, T. T., KITTOK, R. J. and KINDER, J. E. (1989). Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. **Biology of Reproduction** 41: 997-1003.

ROBINSON, N. C., LESLIE, K. E. and WALTON, J. S. (1989). Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. **J. Dairy Sci.** 72: 202-207.

ROCHE, J. F. (1974a). Effect of short term progesterone treatment on oestrus response and fertility in heifers. **J. Reprod. Fertil.** 40: 433-440.

ROCHE J. F. (1974b). Synchronization of oestrus and fertility following artificial insemination in heifers given prostaglandin F 2α. **J. Reprod. Fertil.** 37: 135-138.

ROCHE, J. F. (1976a). Calving rate of cows following insemination after a 12-

day treatment with silastic coils impregnated with progesterone. **J. Anim. Sci.** 43: 164-169.

ROCHE, J. F. (1976b). Synchronization of oestrus in cattle. **World Review Animal Production.** 12: 79-88.

ROCHE, J. F. and GOSLING, J. P. (1977). Control of estrus and progesterone levels in heifers given intravaginal progesterone coils and injections of progesterone and estrogen. **J. Anim. Sci.** 44: 1026-1029.

ROCHE, J. F. (1978). Control of estrus in cattle using progesterone coils. **Anim. Reprod. Sci.** 1: 145-154.

ROCHE, J. F. and IRELAND, J. J. (1984). Manipulation of ovulation in cattle. **Proc. 10th Inter. Cong. on Anim. Reprod. and A.I.** 4: (4) 9-17.

ROUSELL, J. D. and BEATTY, J .F. (1969). Effects of melengestrol acetate on synchronization of estrus subsequent fertility and milk constituents of lactating dairy cows. **J. Dairy Sci.** 52: 2020-2023.

ROWSON, L. E., TERVIT, A. R. and BRAND, A. (1972). The use of prostaglandin for synchronization of oestrus in cattle. **J. Reprod. Fertil.** 29: 145-154.

SAVIO, J. D., KEENAN, L. BOLAND, M. P. AND ROCHE, J. (1988). Patterns of growth of dominant follicles during the oestrous cycle of heifers. **J. Reprod. Fert.** 83: 663-441.

SIROIS, J. and FORTUNE J. E. (1988). Ovarian follicular dynamics during the oestrous 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. **Biology of Reproduction** in press.

SCARAMUZZI, R. J., TURNBULL, K. E. and NANCARROW, C. D. (1980). Growth of graafian follicles in cows following luteolysis induced by the prostaglandin F 2α analogue, Cloprostenol. **Aust. J. Biol. Sci.** 33: 63-69.

SMITH, R. D., POMERANTZ, A. J., BEAL, W. E., McCANN, J. P., PILBEAM, T. E. and HANSEL, W. (1984). Insemination of Holstein heifers at a present time after estrous cycle synchronization using progesterone and prostaglandin. **J. Anim. Sci.** 58: 792-800.

SMITH, R. D. (1986). Estrus detection. **Current Therapy in Theriogenology** ed. D. Morrow. W. B. Saunders Company.

SMITH, R. D., HANSEL, W. and PILBEAM, T. E. (1986). PRID plus PGF 2α-a programmed approach to managing reproduction in lactating dairy cows. **J. Dairy Sci.** (abstr.) 69: (suppl. 1) 93.

SPICER, L. J. and ECHTERNKAMP, S. E. (1986). Ovarian follicular growth, function and turnover in cattle: a review. **J. Anim. Sci.** 62: 428-451.

SPROTT, L. R., WILTBANK, J. N., SONGSTER, W. N. and WEBEL, S. (1984). Estrus and ovulation in beef cows following use of progesterone-releasing devices, progesterone and oestradiol valerate. **Theriogenology** 21: 349-356.

STEVENSON, J. S., SCHMIDT, M. K. and CALL, E. P. (1984). Stage of estrus cycle, time of insemination and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin F 2∝. **J. Dairy Sci.** 67: 1798-1805.

TANABE, T. Y. and HANN, R. C. (1984). Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin F 2∝ I. Influence of stage of cycle at treatment. **J. Anim. Sci.** 58: 805-811.

THIMONIER, J., CHUPIN, D. and PELOT, J. (1975). Synchronization of oestrus in heifers and cyclic cows with progestagens and prostaglandins analogues alone or in combination. **Ann. Biol. Anim. Biochim. Biophys.** 15: 437-449.

TRIMBERGER, G. W. and HANSEL, W. (1955). Conception rate and ovarian function following estrus controlled by progesterone injections into dairy cattle. **J. Anim. Sci.** 14: 224-232.

TUNGSUBUTRA, V. and FRANCE, J. T. (1978). Serial changes in plasma levels of progesterone, unconjugated oestradiol and unconjugated oestriol in normal pregnancy. **Aust. N. Z. Jl. Obstet. Gynaec.** 18: 97-103.

VAN CLEEFF, J., MACMILLAN, K. L., THATCHER, W. W. and LUCY, M. C. (1989). Estrous synchronization and fertility in heifers treated with CIDR before and after insemination. **J. Anim. Sci.** 67: (suppl. 1): 383.

WISHART, D. F. and YOUNG, I. M. (1974). Artificial insemination of progestin (SC21009)-treated cattle at predetermined times. **Vet. Rec.** 95: 503-508.

WATTS, T. L. and FUQUAY, J. W. (1985). Response and fertility of dairy heifers following injection with prostaglandin F 2∝ during early, middle, and late diestrus. **Theriogenology** 23: 655-661.

WILTBANK, J. N., SHUMWAY, R. P., PARKER, W. R. and ZIMMERMAN, D. R. (1967). Duration of estrus, time of ovulation and fertilization rate in beef heifers synchronized with dihydroxyprogesterone acetophenide. **J. Anim. Sci.** 26: 764-767.

WILTBANK, J. N. and GONZALEZ-PADILLA, E. (1975). Synchronization and induction of estrus in heifers with a progestagen and estrogen. **Ann. Biol. Anim. Biochim. Biophys.** 15: 255-262.

WOODY, C. O., FIRST, N. L. and POPE, A. L. (1967). Effect of exogenous progesterone on estrous cycle length. **J. Anim. Sci.** 26: 139-141.

YOUNG, I. M. (1989). Dinoprost 14-day oestrus synchronization schedule for dairy cows. **Veterinary Record.** 124: 587-588.

ZIMBELMAN, R. G. (1963). Determination of minimum effective dose of 6a-methyl-17a-acetyoxyprogesterone for control of the estrual cycle of cattle. **J. Anim. Sci.** 22: 1051-1058.

ZIMBELMAN, R.G. and SMITH, L.W. (1966). Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. **J. Reprod. Fertil.** 11: 185-191.

ZIMBELMAN, R. G., LAUDERDALE, J. W., SOKOLOWSKI, J. H. and SCHALK, T. G. (1970). Safety and pharmacologic evaluations of melengestrol acetate in cattle and other animals: A review. . **Am. Vet. Med. Assoc.** 157: 1528.

CHAPTER 2

THE USE OF TAILPAINT AND AN AEROSOL RADDLE TO MONITOR OESTROUS BEHAVIOUR OF ANIMALS AFTER DIFFERENT SYNCHRONY TREATMENTS

ABSTRACT

The aim of this study was to evaluate the effectiveness of tailpainting combined with the use of an aerosol raddle technique in detecting oestrus in synchronized animals. Other objectives were to compare the patterns of onset of oestrus with different synchrony treatments, to measure the duration of oestrus, and to monitor oestrous behaviour in some animals forming sexually active groups (SAGs).

The study used groups of cycling maiden heifers and cycling and non-cycling cows. Tailpainting always followed the injection of a prostaglandin (PGF), or insertion of a controlled internal drug release device (CIDR). The paint strip on each animal was sprayed with an aerosol raddle at the end of a synchronization treatment. Those heifers or cows which had lost raddle at each checking time after the end of treatment were paint-scored before the original tail-painted skin region was re-sprayed with a raddle of a different colour than that used at the end of the synchronization treatment.

Each paint strip was scored at selected post-treatment intervals. This score was based on the estimated proportional loss of the paint. Oestrus was defined as that period during which an animal would stand to be ridden by its mates. Also, the animals were considered to be or have been in oestrus when raddle was removed and varied amounts of paint were rubbed off. They were also observed visually, and details recorded of activity associated with oestrus such as mounting and standing-to-be-mounted, and whether an animal was part of a sexually active group.

The tailpainting and raddle technique combined with scoring, and re-raddling gave a precise correlation with visual oestrus detection, and with patterns in the onset of the oestrus in groups of heifers and cows synchronized with different treatments. Although some heifers had initial scores which were equal to or less than 3 (30% to 50% of tailpaint removed) when they were visually classified as riding, in all cases the score was reduced to 0 (>90% of tailpaint removed) or 1 (70% to 90% of tailpaint removed) within 8 h of the onset of oestrus. When heifers and cows were first observed in oestrus they had scores between 0 and 2 (50% to 70% of tailpaint removed).

A practical problem peculiar to heifers in a group which has been synchronized is the difficulty of accurately identifying the individuals in oestrus. This problem was largely solved by use of this technique. Three of the six cows which were not seen in oestrus, but which were eventually scored and inseminated on the basis of their paint scores became pregnant to first insemination.

The average interval from the end of treatment to onset of oestrus in heifers was similar among treatment groups, and in cows compared with heifers. However, the response patterns for treated cows showed a delay of about 2 to 6 h, compared with heifers. In general the data from this study indicated that the

average interval to oestrus was concentrated between 30 and 120 h after using different treatments for oestrus synchronization. There was a tendency for most of the heifers to be in oestrus within 48 h post-treatment in heifers (Trial 1) treated with CIDR for 15 days. A similar proportion of heifers were in oestrus in the PGF group, but heifers treated with a CIDR for 10 days with PGF injected at device removal (10-day CIDR + PGF) had a spread in synchrony with more heifers in oestrus by 72 h post-treatment. The response patterns for cows (Trial 2) treated with 10-day CIDR + PGF were concentrated into 2 periods at from 42 to 54 and 81 to 93 h after device removal. However, cows treated with two PGF injections 10 days apart were not well synchronized.

The mean duration of oestrus in heifers and cows was not affected by synchrony treatment, but was longer with cows than with heifers.

The SAG formed by cows when at pasture was mainly composed of animals in oestrus, pro-oestrus, and metoestrus with only a few cows in dioestrus within this active group. The number of cows in dioestrus which were involved was less during night periods than in daylight hours. During the night periods, the sexual activity of the dioestrous cows only involved sniffing of the vulva, chin rubbing, and walking behind cows in oestrus, but not active mounting. However, during daylight, cows in dioestrus would also mount oestrous herd mates to become riders.

The SAG with heifers was also composed for few animals in dioestrus. They showed similar mounting activities to oestrous group members. Each SAG was composed of more animals than SAGs with cows per group, and had greater mobility than dioestrous cows about the paddock. Moreover, the SAG was less stable and broke up to reform after short periods of time.

INTRODUCTION

Inefficient oestrus detection has been identified as a major factor contributing to calving intervals in excess of 365 days in year-round herds with a consequent reduction in the annual returns and profit margins (Esslemont, 1974; Lauderdale, 1974). For dairy farmers, the successful implementation of a breeding programme using artificial insemination depends to a large extent on accuracy of oestrus detection. It is also necessary to achieve a high submission rate within a compact breeding period in seasonal herds (O'Farrel, 1984b). The consequences of this requirement and the importance of maintaining an optimum calving interval have been well documented (Esslemont, 1974; Foote, 1975; Stevenson and Britt, 1977). The economic implications related to oestrus detection also have been reported (Oltenacu et al., 1981; Bailie, 1982). The nature of the problem has been described from a veterinary view point by Zemjanis et al. (1969), and Moller (1978).

Methods of oestrus detection have been reviewed or compared (Donaldson, 1968a; Foote, 1975; Stevenson and Britt, 1977). The intensive studies of Williamson et al. (1972b), Hurnik et al. (1975) and Esslemont and Bryant (1976), in which groups of animals were observed continuously throughout 24 h periods, clearly indicated that because behavioural interactions change continuously, results based on occasional observations (Kilgour et al., 1977) or where animals are yarded (Donaldson et al., 1968a) can produce variations in terms of number of times an oestrous animals is mounted or the duration of oestrus. In general, silent oestrus is an infrequent occurrence, although the duration and intensity of oestrus is variable, because behavioural interactions are continuously changing (Esslemont and Bryant, 1976; O'Farrel, 1984a).

The formation of a sexually active group (SAG) is an important behavioural phenomenon in oestrus detection (Williamson et al., 1972a; Kilgour et al., 1977). A SAG will mostly comprise animals in oestrus, pro-oestrus, or metoestrus, with dioestrous animals mounting less frequently (Mylrea and Beilharz, 1964). The formation and stability of a SAG with cows under pasture conditions is important for the effective use of aids in oestrus detection. The most effective aids are associated with mounting activity by cows within a SAG, or the interest of a teaser (bull or cow) fitted with a chin-ball harness (Kilgour et al., 1977; O'Farrel, 1984b). Another report showed that heat mount detectors were the most effective aid in oestrus detection. The comparison between the methods of oestrus detection showed that the percentage of cows detected in oestrus by these devices, team observations 24 h per day, herdsmen checking daily, and two dairyman checking at milking were 98, 89, 56 and 56% respectively. Heat mount detectors may be lost when a large SAG is formed, without animals being observed to be mounted (Williamson et al., 1972a). A similar situation can be expected with oestrous synchronization.

An alternative as an aid to detect oestrus in cows under pasture conditions with seasonal calving patterns is the tailpaint system (Macmillan and Curnow, 1977a; Williamson, 1980). This system has been evaluated for detecting oestrus among

dairy cows which had been injected twice with PGF at 10 day intervals to synchronize oestrus (Macmillan et al., 1977b). A recent estimate is that some 80% of New Zealand herd owners using artificial breeding also use this system, and the effects of herd size on non-return rate have been reduced from 11% in 1969 to 4 in 1982, mainly because of a reduction in the incidence of errors of detection of oestrus in larger herds which almost invariably used tailpainting (Macmillan, 1988a). Its use therefore, has increased the average conception rates of many herds. It has also been successfully used by O'Farrel (1984a) in Ireland. A paste has been used for oestrus detection in beef cows following oestrus synchronization (Elmore et al., 1986). It has also been successfully used with dairy and suckled beef cows (Kerr and McCaughey, 1984; Gould, 1985). Another study reported that tailpaint was less reliable when used with heifers, and when milk progesterone profiles were used to judge when ovulation took place; with 28% of cows which were first observed in oestrus and ovulated, the paint had not been removed, and in 26% of cows where tail paint was removed there was no observed oestrus (Ducker et al., 1983). However, Ball et al. (1983) reported that 14 pregnancies followed 17 inseminations based only on tailpaint score. Macmillan et al. (1988b) reported that detecting oestrus using tailpaint and aerosol raddle increased the reliability of the technique, especially in groups of synchronized heifers. It allowed comparisons of patterns on the onset of synchronized oestrus in large and small groups of heifers. However, an accurate assessment of aspects of cow behaviour which were related to the effectiveness of tailpainting and it use for oestrus detection in commercial herds have not been reported. Thus, the aim of this study was to evaluate the effectiveness of tailpainting combined with the use of an aerosol raddle technique in detecting oestrus in synchronized animals. Other objectives were to compare the patterns of onset of oestrus with different synchrony treatments, and the duration of oestrus, together with some observations of oestrus behaviour in animals forming SAGs.

LITERATURE REVIEW

Oestrous Synchronization in Cattle.

A major reason for the development of effective techniques for the regulation of the oestrous cycle has been to facilitate the use of artificial insemination (AI) in breeding programmes designed to accelerate genetic improvement for increased production efficiency.

Oestrous cycle control has several applications, in addition to synchronized breeding programmes. These include management of replacement heifers in beef and dairy herds, breeding control for controlled parturition in dairy cows, and oestrous synchronization in embryo transfer programmes.

There are several programmes involving the use of hormonal compounds. Their use depends on the requirements of each farm which may be operating under different management principles, and have different economic constraints. They have different realities, requirements, and objectives.

In cyclic animals, control of the timing of oestrus is partly dependent on controlling the time of regression of the corpus luteum (CL). The two main methods for controlling the time of oestrus are:

a) the induction of a premature but predictable luteolysis of the cyclic CL with PGF, or its analogues; or,

b) maintaining animals in an artificial luteal phase with exogenously administered progesterone until spontaneous regression of the CL has occurred in all animals (Roche and Ireland, 1984).

Oestrus has been synchronized in cattle with progestagens, prostaglandins, progestagen and oestrogen combinations, progestagen and prostaglandin combinations, and progestagen and prostaglandins combined with gonadotrophin releasing hormone (GnRH).

Prostaglandin F2 alpha and its Analogues (PGF).

The luteolytic properties of PGF have been well established in cattle (Lauderdale, 1972; Ireland and Roche, 1982). However, there is a refractory period at the beginning of the oestrous cycle when the CL is not susceptible to luteolysis by a single injection of PGF.

One method of synchronizing oestrus with PGF alone is to give two injections at a 10 to 14-day interval. The range of animals showing oestrus over a 5-day period after these two injection systems varied from 11% to 68%, and the fertility at first service ranged from 44% to 68% (Young, 1989; Odde, 1990).

The post-treatment interval to oestrus after PGF is shorter for heifers than for cows (King et al., 1982). The response pattern also varies with PGF injection at different stages of the oestrous cycle (Macmillan, 1983; Macmillan and Henderson, 1984; Watts and Fuquay, 1985). Cows initially treated in midcycle have a greater oestrous response, and showed oestrus later than cows injected between days 5 and 9 of the oestrous cycle (oestrus = day 0).

PGF synchrony systems can also be used with a single injection. One method involves detecting oestrus and inseminating animals for 5 days, then giving a PGF injection to those which have not been inseminated by this fifth day, and continuing oestrus detection with inseminating from day 5 through to day 9 of the breeding programme. A variation of this method is to give a second PGF injection only to those animals which have not been in oestrus 6 to 11 days after their first injection, and then to inseminate at oestrus over the next 4 days. Another single injection programme is to inject PGF and breed at detected oestrus for 5 days. These methods increase the proportion of animals conceiving over short periods of time after treatment compared with the controls (Lauderdale et al., 1980; Watts et al., 1985; Macmillan, 1986).

An increased oestrous response by injecting oestradiol benzoate 40-48 h after the injection of PGF has been reported (Peter et al., 1977; Dailey et al., 1983; Figueroa et al.,1988). However, these studies did not report an increase in pregnancy rate.

Contradictory results in fertility following late and early luteal phase injections have been reported by Stevenson et al. (1984) and Watts and Fuquay. (1985). In the second study, the fertility was higher with PGF injected in the late luteal phase. However, the first study reported no differences in fertility. Another study reported that in lactating dairy cows, the average pregnancy rate to first insemination for over 2000 PGF-treated cows was 69%, compared with 60% in a comparable number of untreated herd mates (Macmillan and Day, 1982).

Progestagens.

Progestagens administered for 14 to 20 days are effective in synchronizing oestrus. The duration of progestagen treatment can be reduced by combining it with PGF at or near the end of treatment, or with an oestrogen at the beginning of treatment.

The CIDR is a recently developed intravaginal device to treat cycling and non-cycling animals. The type B device is for cattle.

CIDR-B Indications.

The most commonly recognised indications for CIDR use include:

i) oestrous cycle control in yearling and adult cattle;

ii) improving pregnancy rates following insemination and

shortening the interval from first insemination to conception by synchronizing returns to service;

iii) as a contraceptive; and

iv) treating non-cycling cows.

Synchronization of Heifers for Artificial Insemination using CIDR-B.

A comprehensive series of experiments has been completed to evaluate the potential of the CIDR-B device for synchronizing oestrus in heifers. Some of these experimental series are summarized in the following sections:

1. First Experimental Series.

In extensive trials conducted from Ruakura, CIDR devices were used with 15-month old heifers under two treatment regime's. These were:

i) A 7-day CIDR insertion with PGF at CIDR removal was used with 184 maiden heifers in 4 commercial herds each with from 20 to 77 animals; (CIDR + PGF);

ii) A 12-day CIDR insertion with 10 mg of oestradiol benzoate (OB) injected at CIDR insertion was used in another 4 commercial herds each with from 32 to 118 heifers; (CIDR + OB).

Tailpaint was applied to each animal when a device was inserted and the synchronization treatment programme commenced. A drying aerosol raddle of a contrasting colour was sprayed over the tailpaint strip when the CIDR was removed. Heifers were then checked at selected post-treatment intervals, and their tailpaint scored according to the criteria described by Macmillan et al. (1988b). In those trials in which animals were inseminated, heifers which had lost some raddle by 48 h post-treatment were inseminated, the paint scored and the tailpaint strip re-raddled with a second colour. This checking process was repeated at 72, 96 and 120 h; but only those animals which had scores further reduced by >2 units were re-inseminated. The heifers were inseminated with thawed deep frozen semen. Each heifer not detected in oestrus by 96 h or 120 h post-treatment had its reproductive tract palpated per rectum to determine the cause of treatment failing to produce an oestrus. A double PGF treatment group was also included in one comparison.

A total of 97.8% of the heifers treated with CIDR + PGF were detected in oestrus and inseminated within 120 h of the end of treatment.
The insemination times after treatment with CIDR + OB showed that 94.7% of heifers were inseminated from 48 to 96 h, and only 0.4% were inseminated at 120 h. There were 2.8% of heifers which were not inseminated.
In total (CIDR + PGF and CIDR + OB), 96.1% were inseminated from 48 to 96 h, with 0.4% of heifers inseminated at 120 h, and only 3.4% of heifers not

inseminated.
The CIDR + PGF treatment had an average of 50% of inseminations at 48 h and 40% at 72 h, compared to 80% of heifers inseminated at 48 h and 15% at 72 h with the CIDR + OB treatment (Macmillan et al., 1988).

The insemination time in the 2 x PGF (injections at a 12 days interval) group had 82% of heifers inseminated from 48 to 96 h, another 3% of heifers were inseminated by 120 h, and 15% of them were not inseminated. The average pregnancy rate in heifers following CIDR + PGF was 61.9% with a herd range of 57% to 70%. With the CIDR + OB treatment, it was 62.9% ranging from 56% to 65%.

2. Second Experimental Series.

This was a series of experiments designed to study the use of a specifically formulated oil based enamel paint for use in oestrus detection with synchronized oestrus regimes in heifers.

Heifers were treated with systems to synchronize oestrus including:

i) 2 injections of PGF 12 days apart;

ii) inserting a CIDR for 7, 10, or 12 days (7-day CIDR, 10-day CIDR or 12-day CIDR) with an injection of OB (10 mg) at CIDR insertion or PGF at CIDR removal; and

iii) The Syncromate-B (SMB) system with implant removal after 10 or 12 days.

The distributions of intervals to oestrus and insemination differed between treatments within trials and also between groups of heifers within a treatment (Table 2.1).

Trial	Treatment	No. Herds	No. Heifers	% in oestrus by 48-54 h	% not insem'd by 120 h
1	12-Day CIDR + OB		78	71	5
	7-Day CIDR + PGF	1	99	54	5
	2 x PGF		102	21	14
2	12-Day CIDR + OB	4	184	79	5
	7-Day CIDR + PGF	6	365	49	2
3	7-Day CPRO + PGF	1	122	76	12
	7-Day CPRO + PGF	1	126	64	7
4	12-Day CIDR + OB	1	40	88	2
	12-Day SMB		40	95	2
	10-Day CIDR + OB	1	36	41	0
	10-Day SMB		35	60	0
	TOTAL		1227	60	5

Only 5.5% of the 1227 heifers included in the 4 trials were not detected in oestrus by 120 h from the end of treatment.
Among this total of undetected heifers, only 0.8% of the 1227 had ovulated. This represented a probable detection failure of the technique. On average, 93,8% of the heifers included in the trials were inseminated after being detected in oestrus. Only 10.6% were re-inseminated on consecutive days (usually at 48 and 72 h).
The pregnancy rate in Trial 1 including all treated heifers was 59.1%, compared with 62.7% in Trial 2.
In Trial 3, the pregnancy rate was only 41.8% because the CIDR prototype (CPRO) did not maintain adequate concentrations of plasma progesterone if PGF was injected at device insertion, compared with 51.6% in the group with CPRO + PGF at removal.
Animals in Trial 4 were not inseminated (Macmillan et al., 1988b). In conclusion: the combination of tailpaint, raddling, tailpaint scoring and re-raddling is a simple sequence which can be effectively used to detect oestrus among synchronized heifers.

3. Third Experimental Series.

This experiment was conducted to evaluate the onset of oestrus and fertility in heifers synchronized with a CIDR device for 15 days.

Heifers, aged 17 to 21 months each had a CIDR device inserted into the vagina. They were injected with a luteolytic dose of PGF 5 days later (to terminate any endogenous progesterone production from a functional corpus luteum). The CIDR's were removed after a treatment period of 15 days.

Heifers were used for either one or two 15-day treatment periods (the second group of heifers had a second CIDR inserted when detected in oestrus at 48 h after removal of the first CIDR).

None of the heifers was in standing oestrus within 28 h of initial CIDR removal. Only 4% had been mounted by 30 h, but 77% were in oestrus at 48 h. In total, 94% of animals were in oestrus from 30 to 72 h after CIDR removal. After the second treatment period, 20% of the heifers were in oestrus at 48 h, another 60% at 72 h and the remaining 20% by 96 h. The overall pregnancy rate was 55.3%, but only one of the 4 heifers with a prolonged post-treatment interval to oestrus conceived. The results of this experiment showed that the interval to standing oestrus was at least 30 h after the end of this 15-day treatment period. Most animals which did not have a functional CL at device removal were detected in oestrus by 48 h post-treatment (Macmillan et al., 1988c). However, when the device was inserted at oestrus or during metoestrus, then more animals are likely to be precisely synchronized by 72 h.

Detection of Oestrus.

Accurate oestrus detection is a key to efficient reproduction. The inability of farmers to detect oestrus in dairy cattle is being widely recognized as one of the major factors limiting reproductive efficiency, and therefore the efficiency of milk production (Foote, 1974; Williamson et al., 1972a).

Inefficient oestrus detection has been found to be a major factor contributing to calving intervals in excess of 365 days with a consequent reduction in the annual returns and profit margins (Esslemont, 1974; Lauderdale, 1974). However, an improvement in rates of oestrus detection is of little value unless there is an associated reduction in the interval from calving conception (Williamson et al., 1975). Other reports have shown that extended calving intervals were a result of problems with oestrus detection rather than because of anoestrous cows (Williamson et al., 1972a; Macmillan, 1975).

For the dairy farmers using AI, the successful implementation of a breeding programme depends to a large extent on accuracy of oestrus detection to achieve a high submission rate within a compact breeding period in seasonal herds, or to maintain an optimum calving interval in year-round herds (O'Farrel, 1984b). The consequences of this problem and the importance of maintaining an optimum calving interval have been well documented (Esslemont, 1974; Foote, 1975, Stevenson and Britt, 1977). The economic implications related to oestrus detection also have been reported (Oltenacu et al., 1981; Bailie, 1982).

The best criterion that a cow is in oestrus is that it stands to be ridden by its herdmates or by a herd sire. However, using this criterion alone can result in a small number of cows being wrongly diagnosed, and a larger number of others not being detected. Mounting othes cows in oestrus, and ruffling and abrasion of the rump have been shown to be useful secondary signs in diagnosis, but neither symptom could be considered completely reliable (Williamson et al., 1972b).

Not all cycling animals show the same behavioural signs of oestrus. The frequency of mounting behaviour in dairy cattle varies with the stage of the oestrous cycle, type of animal, status, and number of animals simultaneously in oestrus. Oestrous cows tend to congregate together forming SAGs, which are restless and move throughout the herd, with others joining or leaving them so that intense riding activity is maintained within the group. On many farms, especially in large herds, there can be major difficulties in implementing more effective oestrus detection, and consequently many oestrous periods may be missed (Macmillan, 1985). Under these circumstances, tailpainting has been shown to be an effective aid (Macmillan and Curnow, 1977a; Williamson, 1980)

The Duration of Oestrus.

While Esslemont and Bryant (1976) reported that the average duration of oestrus was 14.9 ± 4.7 hours, O'Farrel (1984a) found it was only 9.2 ± 5.7 hours. This latter study also found that the duration of the oestrus was not affected by herd size, and 23.3% of oestrous periods were 4 hours or less in duration. Moreover, pregnancy rates were not affected significantly by duration of oestrus.

Other factors which may affect the duration of the oestrus are:

i) an interruption in standing behaviour by breaks when the cow was not mounted for a several hours before standing again (O'Farrel, 1984a). Another study reported that some cows which were clearly in oestrus in the paddock showed little or no activity in the milking yard (Williamson et al., 1972b); and,

ii) different types of management and whether animals are at pasture or housed during observation periods showed that mounting activity was more common during darkness when management activities were minimal (King et al., 1975; O'Farrel. 1980a). In contrast, another report showed that cows at pasture had more mounting activity during the day. This study also reported that the riding activity within the SAG was inhibited by the immediate presence of bulls, by feeding supplements, or by inclement weather (Kilgour et al., 1977).

Frequency and Time of Observation.

Three observations periods each day for 30 minutes generally can give a detection rate of around 70%, whereas five observations can give over 90% (Donaldson, 1968a; Esslemont and Bryant, 1976; O'Farrel, 1984a). From these studies, it was clear that frequent and timely oestrus observations must be made during the early morning and late evening to achieve high detection rates.

Methods of Oestrus Detection.

Visual observation is the most common method used in detecting oestrus in most dairy herds.

The value of the various aids to oestrus detection can be assessed by the following criteria: The method should be easy to use and applicable under at least some of the wide range of conditions on commercial farms, effective, accurate, inexpensive for practical application, and should result in satisfactory conception rates.

In an attempt to reduce the labour of oestrus detection, and improve its efficiency a wide variety of oestrus detection aids have been developed.

There are three different approaches to the problem:

i) to measure the hormonal changes related to oestrus, and ovulation by assaying progesterone (or other hormones) in plasma or milk;

ii) to detect changes closely associated with oestrus such as cervical mucus, body temperature, pheromones, or non-sexual activities; and

iii) detection of standing and mounting behaviour other than by visual observation by using a heat mount detector, prepared bulls, cows or steers, television cameras, or tailpaint (Boyd, 1984).

Hormonal Changes.

At present, only progesterone has been assayed as an aid for oestrus detection. Progesterone production from the CL in cycling cows falls 2 to 4 days before ovulation. It is not the complete answer to detecting oestrus and ovulation (Boyd, 1984). Milk is the most suitable fluid for frequent sampling, and although a radioimmunoassay (RIA) has been widely used, an enzyme-linked immunoassay (ELISA) test which can be carried out under " on farm conditions " means that results can be obtained rapidly to avoid the shipping, and processing, and reduce response times. Moreover, the ELISA test is more accessible with low cost and can be made with innocuous reagents (Booth and Hollandsworth, 1976; Blake and Gould, 1984).

Foulkees et al. (1982) have reported the successful use of milk progesterone assay and timed insemination after the decline in milk progesterone concentration.

Changes Associated with Oestrus.

i) Electrical resistance of the vaginal mucus.

Resistance can be measured in the living animal by using a suitable probe which incorporates electrodes to which a current is applied before measuring the electrical resistance between the electrodes. The hormonal changes at oestrus produce the characteristic oestrous mucus which has a reduced electrical resistance. Boyd (1984) described other circumstances which may also cause lowered electrical resistance such as mucus produced in cases of ovarian cysts and with purulent material in cows with endometritis or vaginitis. Cows inseminated on the basis of change in the electrical resistance of vaginal mucus had a similar conception rate (measured by rectal palpation diagnosis), to cows inseminated on observed oestrus (Foote et al., 1979). More detailed information has been reported by Aizinbud et al.(1984). However, the cost of the equipment and its application to on farm conditions need to be considered in more detail. Each cow entering the breeding season or mating period needs to be inspected daily by intravaginal examination with the probe for about 11 days. This can

create a management problem. Moreover, the potential problems of hygiene and trauma to the cow would be a consideration.

ii) Body temperature.

Oestrus appears to be associated with a very small rise (0.1 °C) in body temperature. However, the increase of body temperature also can be caused by systemic or local inflammatory reactions.

Body temperature can be measured either in the rectum or in the milk. Ball et al.(1978) reported a formula for assessing the significance of the recorded temperature changes, but this study did not report pregnancy rates obtained with inseminations based on temperature rise.

iii) Oestrous odour.

Kiddy and Mitchel (1981) have reported that dogs can be trained to detect the specific odour produced by cows in oestrus.

iv) Other behaviour.

Cows in oestrus exhibit non-sexual behavioural changes such as bellowing and restlessness. Attempts to measure restlessness by using electronic pedometers have been successful (Vasquez et al., 1984). These results indicated that a significant increase in walking activity occurred for 18 h preceding oestrus and ovulation.

Detection of Standing Oestrus other than by Visual Observation.

i) Television cameras.

Hurnick et al.(1975) used television cameras to improve oestrus detection efficiency and to study oestrous behaviour in housed cattle. The data indicated that the time distribution of mounts showed evidence of a circadian rhythm with the highest frequency occurring during the nocturnal period. The total number of mounts and mounting/mounted ratios per oestrus varied among individuals and were affected by oestrous synchronization. The number of mounts per cow increased from an average 11.2 with one cow in oestrus to 52.6 with three cows in oestrus at the same time. The sequence of post-partum oestrus periods was associated with a gradual increase both in mounted and mounting activities and mounting/mounted ratios. Also, on average, more mounted cows were in oestrus than mounting ones. The length of mounting between cows in synchronized oestrus lasted longer than between non-synchronized cows with only one oestrous partner. Detected duration of oestrus was 7.5, 7.8 and 10.1 h if there were one, two and three cows in oestrus together. Behavioural signs of onset of oestrus differed among animals and revealed a dependency on social factors. Finally, oestrous stage caused changes in the proportional composition of daily activities i.e. a high increase of walking time at the cost of both resting time and time spent at the feeder.

ii) Vasectomized bulls or testosterone treated teaser cows or steers with chin-ball harness.

These animals has been shown to be very useful for identifying cows in oestrus. They require the same management as entire bulls, and in large herds several teasers are required for maximum efficiency. O'Farrel (1984b) reported that a considerable variation in efficiency was found between teasers cows which had been treated with testosterone to stimulate mounting behaviour. R. Morris (personal communication) found that teaser bulls with chin-ball harnesses tended to favour certain cows among those in oestrus in a large herd, and therefore did not necessarely perform any better than visual methods.

In order to determine the effectiveness of steers and heifers treated with oestrogen and testosterone in the detection of oestrus in cattle, two experiments were carried out in Australia (Sawyer and Fulkerson, 1981). There was no effect of age at castration of steers on development of male behaviour. Steers and heifers treated with oestradiol benzoate were superior at detection of oestrus in cattle than animals treated with testosterone or those receiving no hormone. These oestrogen-treated animals generally detected heifers in oestrus in less than 3 minutes after introduction and mounted these animals between 20 and 30 times in one hour. This response was consistent throughout the duration of the experiment. Moreover, the rate and degree of development of male behaviour in steers was tested in response to a weekly subcutaneous injections of 0, 2, 4, 8 or 16 mg oestradiol benzoate per 250 kg body weight, 250 mg testosterone or 150 mg dihydrotestosterone for a period of 15 weeks. Steers treated with oestradiol benzoate proved to be more successful than untreated or testosterone-treated steers at consistently detecting and mounting oestrous heifers. The best response was obtained from steers treated with 8 mg/250 kg body weight.

In another experiment Fulkerson et al. (1983) assessed the accuracy and efficiency of oestrus detection using behavioural observations only in a large commercial dairy herd of 120 cows, or in combination with hormone-treated steers or tailpaint. The oestrus detection rate was 50, 88 and 80%, respectively, with no significant difference in conception rate between methods following artificial insemination. Progesterone analysis confirmed oestrus in all cows detected, except for 5 cows detected with steers and 4 with tailpaint. All except 2 of these animals conceived at that oestrus, the accuracy of the 3 methods was considered to be the same. In a study to determine the effect of various factors on the reproductive performance of approximately 1700 cows in 12 seasonally calving dairy herds in North Western Tasmania, the same author (1984) found that the presence of the male did not improve oestrus detection or non-return rate of cows in the early post-partum period.

"Other Methods".

Alternative methods of oestrus detection were evaluated by Johnson and Reneau (1989) for dairy cows in tie stall barns. Oestrus was detected by visual observation during exercise or by a system which combined a test of progesterone in milk, a probe to measure conductivity of cervical mucus, and a selective injection of PGF. These data showed that the combined system was at

least as effective as the visual observation.

Although the duration and intensity of oestrous behaviour is variable, and behavioural interactions change continuously, silent oestrus was an infrequent occurrence (Esslemont and Bryant, 1976; O'Farrel, 1984a).

The importance of the formation of a SAG is a significant behaviourial phenomenon in oestrus detection (Williamson et al., 1972b; Kilgour et al., 1977). The formation and stability of a SAG with cows under pasture conditions would seem important to the effective use of aids in oestrus detection. The most effective aids are associated with mounting activity by cows within the group or the interest of a teaser (bull [Donaldson, 1968a], cow [Britt, 1976], or steer [Sawyer and Fulkerson, 1981]) fitted with a chin-ball harness. Previous reports showed that heat mount detectors were the most effective aid in oestrus detection. The comparison between the methods of oestrus detection showed that the percentage of cows detected in oestrus by these devices, team observation 24 h per day, herdsmen checking daily, and two dairymen checking at milking were 98, 89, 56 and 56% respectively (Williamson et al., 1972a).

An alternative as an aid to detect oestrus in cows under pasture conditions with seasonal calving patterns is the tailpaint system (Macmillan and Curnow, 1977a; Williamson, 1980). Tailpainting has increased submission rates, reduced the incidence of short return-to-service intervals and increased non-return rates. It has also reduced the difference in non-return rates between small and large herds (Macmillan, 1988a).

Oestrus Detection Rate.

As the accuracy and efficiency of oestrus detection has a direct effect on conception rates it is important to be able to determine its contribution to overall herd reproductive performance.

Efficiency in detection may be estimated by calculating the mean inter-oestrous interval. An interval of 21 days indicates 100%, and 42 days a 50% detection rate (O'Farrel, 1984a).

It is well known that adequate records and their reliable analysis and interpretation are very important to effective reproductive management. However, there is wide variation in calculation methods for reproductive performance indices. In order to make comparisons between different methods and a correct interpretation, Fetrow et al. (1990) have proposed standards for the calculation of indices used to evaluate dairy reproduction. One of the indices is to measure the completeness of oestrus detection. This is the percentage of possible oestrous events which are detected. It provides an estimate of the percentage of detection. The indices can be calculated by accounting for all inseminations and reported oestrous events in eligible cows for a defined period, and the oestrous cycle days in the period in these cows is divided by 21.

Another method is to calculate the proportion of all returns which are normal (18-24 days), short (less 18 days), or long (more than 24 days). Macmillan

(1970) showed that about 17% of all return to service intervals after first insemination were less than 18 days. Many of these short intervals were errors in detection. Another study conducted by Macmillan and Watson (1971) found that the proportion of normal returns fell from 67% for herds of 20 to 50 cows to 52% for herds over 200 cows. As this occurred, the proportion of short returns increased from 12 to 29%. This was suggestive of errors in oestrus detection increasing with the size of the herd. However, some of these short cycles were identified when using milk progesterone assay results as an intermediate phase between post-partum anoestrus and normal cycling. Therefore, short cycles were more prevalent in herds with a high incidence of anoestrus. Even when these short cycles were excluded, the incidence of short returns still exceeded 12% of all intervals (Macmillan, 1988a).

The use of vasectomized bulls was recommended to assist oestrus detection in large herds in New Zealand, but tailpainting is now an accepted procedure in an efficient breeding programme. Some 80% of herd owners using artificial breeding also use this system, and the herd size effects on non-return rate have been reduced from 11% in 1969 to 4% in 1982, mainly because of a reduction in the incidence of errors of detection of oestrus in larger herds (Macmillan, 1988a).

OBJECTIVES

a) To evaluate the effectiveness of tailpainting combined with the use of an aerosol raddle technique in detecting oestrus in synchronized dairy cattle;

b) To compare the patterns of onset of oestrus with different synchrony treatments;

c) To estimate the duration of oestrus; and,

d) To observe the behaviour of some animals forming sexually active groups.

MATERIAL AND METHODS

Groups of cycling maiden heifers and cycling and non-cycling mature cows were used to evaluate 2 or 3 different brightly coloured enamel paints as tail-paint combined with contrasting colours of rapidly drying aerosol raddles among synchronized animals and untreated herdmates.

Animals.

All the maiden heifers were 14 to 16 months of age and located in one herd (Massey N° 4 Herd) when examined rectally to determine ovarian activity. All the mature cows (Massey N° 4 Herd) were examined rectally to determine the progress of uterine involution and the nature of ovarian structures.

All groups of dairy heifers and cows were grazing ryegrass-white clover pastures when the study was performed. Stock density varied from 50 to 57 animals per hectare, and animals could interact freely for most of the time.

Oestrus was defined as that period during which an animal would stand to be ridden by its herd mates. The animals were considered to be or have been in oestrus when raddle was removed and varied amounts of paint rubbed off.

Heifers and mature cows were observed visually and details recorded of activity associated with oestrus, such as mounting and standing-to-be-mounted. These activities were subdivided into the following classifications: "riding", "mounted and stood well", "mounted but refused by moving away", and "stood when mounted?". Also, the observer noted if an animal was part of a SAG. Heifers and mature cows were also checked for tail-paint and raddle removal due to mounting activity.

In some heifers, blood samples were taken during oestrus for subsequent assaying for progesterone. Milk samples were taken from some cows for the same purpose.

Observations of Oestrus and Tailpainting and Raddle Scoring.

This method for detecting oestrus in synchronized heifers was developed by Macmillan et al.(1988b), and has been described as tail-paint and raddle scoring.

Heifers were checked for 30 minutes at selected post-treatment intervals (from 0 h after the end of a synchronization treatment until 144 h, at 4 h intervals), and their tail-paint scored according to the following criteria:

Raddle not removed; no paint removed, 5+ not yet in oestrus.

Raddle removed; < 10% paint removed,	5 just in oestrus.
Raddle removed; 10% to 30% paint removed,	4 in oestrus.
Raddle removed; 30% to 50% paint removed,	3 in oestrus.
Raddle removed; 50% to 70% paint removed,	2 in oestrus.
Raddle removed; 70% to 90% paint removed,	1 in oestrus.
Raddle removed; > 90% paint removed,	0 in oestrus.

Cows were checked during dayligth hours for 30 minutes at 8 h intervals (time 0 h coincided with the end of a synchronization treatment) until 168 h. Their tail-paint scored at 12 h intervals from 0 h until 192 h.

Scores were recorded at each checking time to obtain an indication of the occurrence of further occasional riding (score change of 1 or 2) or intensive riding (score change > 2). In Trial 2, those cows which had scores further reduced by > 2 units from one milking to the next were re-inseminated.

Those heifers or mature cows which had lost raddle were paint-scored before the original tail-painted skin region was sprayed with a raddle of a different colour than that used at the end of the synchronization treatment. This system allowed for cumulative progress of the synchronization response to be monitored visually at any time.

The heifers which were not seen in oestrus within 144 h post-treatment were examined per rectum to diagnose the reason for lack of behavioural oestrus, including detection failure (which was when a heifer had ovulated without being detected in oestrus).

The cows which were not seen in oestrus within 192 h post-treatment were also examined per rectum to identify developing or ovulated follicles.

The post-treatment interval to oestrus was defined as the period from the end of treatment until an animal was seen to be mounted by herdmates, when the raddle was removed and a score could be given. The time for end of oestrus was when an animal was last mounted, the raddle was not removed, and the next score given was unchanged from that at the previous checking time.

Tailpainting.

Each animal was tail-painted when a synchronization treatment program commenced. This involved applying a strip of red, blue or green tail-paint (Coopers Animal Health, N.Z.) approximately 20 cm long and 5 cm wide running posteriorly from about the first coccygeal vertebra (Macmillan and Curnow, 1977a).

The paint was applied with a 5 cm brush after loose hair or dirt had been brushed off, to achieve an average of 70 to 80 applications per litre.

Tail-painting always followed injection (PGF), or insertion (CIDR-B) to ensure every animal in a group was treated, and to allow untreated animals to be identified if groups became mixed.

Raddling.

The paint strip on each animal was sprayed with a rapidly drying aerosol raddle of a contrasting colour ("Spray Line Stock Marker" Donaghys Industries Limited, N.Z.), at the end of a synchronization treatment.

Experimental Protocol.

Trial 1.

Cycling Friesian heifers from within a herd of 118 animals were randomly allocated to three treatment groups (n = 10 each), and one control group (rest of the herd).

Animals in each treatment group received one of three different synchronization treatments which were applied so that each treatment was completed at the same time on the same date. This allowed patterns of onset of oestrus to be compared within groups.

These treatments included:

1) Inserting a CIDR-B for 15 days ([15-day CIDR] Eazi-Breed CIDR^{tm}-B; CHH Plastic Moulding Co., Hamilton, N.Z.);

2) Inserting a CIDR-B for 10 days and administering prostaglandin F 2α (PGF, 2 ml, i.m. of Estrumate, Coopers, N.Z.) at device removal (10-day CIDR + PGF);

3) PGF (two injections) 10 days apart (2xPGF); and

4) A control group which did not receive any treatment and comprised all of the heifers remaining in the herd.

Trial 2.

Groups of Friesian cows from within a herd of 270 animals were randomly allocated to three treatment groups (n = 10 each), and one control group (rest of the herd).

Each treatment group received a different synchronization treatment applied so that each treatment ended at the same time.

These treatments included:

1) Anoestrous cows (10-day CIDR + PMSG) which each had a CIDR inserted for 10 days from at least at 50 days post-partum. 400 I.U. of Serum Gonadotrophin of Pregnant Mares (PMSG, Folligon, Intervet, Chemavet Division, N.Z.) was injected, when the device was removed.

   Cycling cows were divided in three sub-groups (2, 3 and 4).

They each received one of the following treatments described below:

2) A CIDR was inserted for 10 days from about 50 days post-partum. Prostaglandin F 2α (PGF, Estrumate, 2 ml, i.m., Coopers, N.Z.) was injected at device removal (10-day CIDR + PGF);

3) Two intramuscular injections of PGF 10 days apart (2xPGF); and

4) The control group in which animals did not receive any treatment.

Blood Collection.

Blood samples (10 ml) were taken from a coccygeal venipuncture, and into heparinized tubes (Nipro-New Tube System, Vacuum Blood Collecting System, Nissho Corporation, Osaka, Japan). The sample was temporarily stored in ice until centrifuged (within 2 h of collection) at 3000 xg for 20 minutes. The plasma was collected and stored at -20 °C until progesterone was measured using a specific radioimmunoassay.

Milk samples (5 ml) were taken from a healthy quarter and collected in a clean plastic tube, then stored at -20 °C until measured by a specific radioimmunoassay for progesterone using the method of Dobson et al. (1975) which had previously been validated by Lapwood (Moller et al., 1986). The first 3-5 squirts of milk from the teat were discarded.

Samples were also taken to measure progesterone concentrations when the animals were in oestrus.

Hormone Assay.

Plasma concentrations of progesterone were measured by the method of Kirwood et al. (1984).

Plasma progesterone. Determinations were made on 500 μl sub-samples from each original plasma sample. They were extracted with 5 ml toluene:hexane (1:2 v/v). The solvent-plasma mixture was frozen overnight, and the solvent

supernatant then decanted into clean tubes, dried under air and redissolved in 500 µl ethanol. Duplicate 100 µl samples of the ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 µl samples of standard ethanolic solutions of progesterone (P-1030:Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone levels of 0.625-40 ng/ml. A mixture containing antiserum (courtesy of Dr J. T. France) at a final dilution of 1:18000 (Tungsubutra & France, 1978); [1,2,6,7-H] progesterone(TRK 413, Amersham, Bucks, U.K.) at 10000 c.p.m./100 µl; phosphate-buffeted saline containing 0.02 m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 µl) to each tube and vortexed. After overnight incubation at 4 C, 600 µl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and then incubated at 4 °C for 10 minutes. Tubes were then centrifuged at 3000 g for 10 minutes at 4 °C. The supernatant was decanted into scintillation vials and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

All samples were processed in a single assay for which the limit of sensitivity for progesterone was 0.06 ng/ml, and the intra-assay coefficient of variation was 10.5%.

Milk progesterone. Progesterone concentrations were measured by direct radioimmunoassay, without extraction using the method of Dobson et al. (1975) which had previously been validated in this faculty by Lapwood (Moller et al., 1986).

Duplicate 20 µl aliquot of standard progesterone solutions (range 0-80 ng/ml) were evaporated to dryness under air, then 20 µl ovariectomized cow's milk was added to each standard tube. After dispensing 20 µl unknown milk samples into assay tubes, 600 µl buffer containing rabbit anti-progesterone serum (1:3000; courtesy of Dr J.L. France) and tritiated progesterone (8000 c.p.m.; Radiochemical Centre, Amerham, U.K.) was added to all tubes, mixed, then incubated overnight at 4 °C. After addition of 600 µl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia. U.S.A.) suspension in buffer, tubes were vortexed incubated for 10 minutes at 4 °C. The supernatant was decanted into scintillation vials and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Mean assay sensitivity was 0.40 ng/ml. The samples were run in one assay; and the intra-assay coefficient of variation was 11.6%.

Statistical Analysis.

Data were analyzed using The Panacea database management program (PAN Livestock Services Ltd. Department of Agriculture, University of Reading, P.O. Box 236, Reading, Berkshire, England).

Differences in mean concentrations of progesterone, and average intervals from the end of treatment to onset of oestrus and average duration of oestrus among groups were evaluated by analyses of variance. The proportional distributions of cows and heifers in oestrus at different periods post-treatment

were analyzed by chi-square.

RESULTS

Trial 1.

Observations of oestrus and initial tailpaint raddles scores for each individual animal.

The distribution of animals with low tailpaint and raddle scores after first being detected in oestrus was similar for all four groups (Table 2.2). In group 1 (15-day CIDR), all the heifers detected in oestrus had scores between 0 and 2. There were 2 animals which were first classified as "stood when mounted?", but subsequently were classified as "mounted and stood well" 3 and 6 h later, and were scored 0.

In group 2 (10-day CIDR + PGF), all heifers were detected in oestrus, and had initial scores between 0 and 2. There were 2 heifers which had initial scores of 1 when classified as "stood when mounted?". One of them was observed in oestrus 1 hr later, but now with score 0. The remaining heifer was again observed in oestrus one day later which was 24 h after first being scored 1.

In group 3 (2 x PGF) only 9 of the 10 heifers was observed in oestrus. These 9 animals had scores between 0 and 2. There were 2 heifers which were scored 2 when they were classified as "stood when mounted?". However, when one of them was seen in oestrus 4 h later, it was scored 1. The remaining heifer was not in standing oestrus until 8 h later, and the score was 1.

In the control group (spontaneous oestrus), 8 out of the 10 heifers detected in oestrus had scores between 0 and 1. The 2 remaining heifers each had an initial score of 3 at oestrus detection, but when next checked 4 h later the score was reduced to 0.

Patterns of onset of oestrus.

The average interval from the end of treatment to onset of oestrus was not significantly different among the three treatment groups.

Group 1 was 50.5 ± 7.1 h, vs 66.0 ± 6.5 and 56.4 ± 8.1 h for groups 2 and 3 respectively (Table 2.3). The different distributions of heifers in oestrus at different post-treatment periods was not significantly different (Figure 2.1).

The distribution of oestrus onset in heifers in group 1 (15-day CIDR) was:
7 of 10 were first detected in oestrus between 30 and 55 h post-treatment. The 3 remaining heifers were first detected in oestrus between 56-68, 69-81, and 95-107 h post-treatment, respectively.

In group 2 (10-day CIDR + PGF): there were 3 heifers first seen in oestrus between 30 and 55 h post-treatment. Another 4 were first seen in oestrus between 56-68 h, and the 3 remaining heifers between 82 and 107 h post-

treatment.

In group 3 (2 x PGF): 7 of the 10 heifers were first in oestrus between 30-55 h post-treatment. Another two heifers were first in oestrus from 95-107 h post-treatment. The remaining heifer was not detected in oestrus until 8 days after the second injection of PGF had been given.

Duration of the oestrus.

The duration of oestrus was similar among the treatment groups and contemporary controls (the first 10 heifers in oestrus within the period of 30-107 h post-treatment for treated heifers were taken as control oestrus).

In group 1 it was 12.2 ± 0.9 h, compared to 12.8 ± 1.5 h, 13.0 ± 1.3 h, and 13.3 ± 1.1 h in groups 2 and 3 and the control group respectively (Table 2.4).

Sexually active group and its composition.

There were 57 heifers in oestrus over a 6 day post-treatment period, but only 15 dioestrous heifers were involved in at least one SAG during this time.

Four out of these 15 heifers were in a SAG during the night (observations made between 19.00 and 05.00). They showed no obvious riding activity, just vulva sniffing, chin rubbing, and walking behind the heifers in oestrus. The remaining 11 dioestrous heifers were involved in a SAG during the day, and they were mounting animals in oestrus, but were never mounted themselves.

Tailpaint and raddle and mounting activities.

In order to more closely record the sequence of raddle and paint loss among heifers in oestrus, 3 SAGs were observed continuously for a period of 8 h. During this time all the heifers in a group were recorded for the incidence of mounting and other activities, as well as for removal of tailpaint and raddle.

Each observed SAG included 2 to 4 heifers in standing oestrus, with another 3 to 4 heifers as riders, making from 5 to 8 animals in total. When there were up to 8 to 10 heifers in the SAG, it usually split after a short period of time into two groups.

Close observation of these heifers in oestrus showed that after 3 to 5 mounts, more than 70% of the raddle (score 0 and 1) was completely removed. However, the paint needed between 4 and 8 attempted mounting sequences for at least 70% to be rubbed off.

Trial 2.

Observations of oestrus and initial tailpaint scores for each individual animal.

The distribution of animals with low tailpaint and raddle scores after being

detected in oestrus was similar for groups of cows (Table 2.5). In group 1 (10-day CIDR + PMSG; n = 10) only five cows were detected in oestrus. These cows had initial scores which were all between 0 and 2. The 5 cows which were not detected displaying signs of oestrus had scores between 4 and 5.

In group 2 (10-day CIDR + PGF; n = 10), 9 of the 10 cows detected in oestrus had scores between 0 and 2. However, 1 of these cows was scored 1 when it was seen riding one day before oestrus.

In group 3 (2 x PGF; n = 9), 3 of 7 cows were insemimated before being detected in oestrus. The other cows were detected in oestrus and the scores at oestrus were between 0 and 2.

In the control group (spontaneous oestrus; n = 10), all the cows detected in oestrus had scores between 0 and 2.

Patterns of onset of oestrus.

The average interval from the end of treatment to onset of oestrus was similar among the three treatment groups. It was 68.4 ± 23.4 h vs 70.5 ± 9.3 and 59.0 ± 9.8 h for Groups 1, 2 and 3 respectively (Table 2.6). The different proportions of cows in oestrus and inseminated at different periods after treatment among the treated groups were not significantly different (Figure 2.2).

The oestrous response for cows in Group 1 (10-day CIDR + PMSG) was: 4 cows were first in oestrus between 43 and 55 h post-treatment. The remaining cow was in oestrus between 160 and 172 h. The 5 cows which did not display behavioural signs of oestrus included 2 animals which had a corpus luteum identified by ultrasonography at scanning. In the remaining 3 animals, no response to treatment was observed.

In Group 2 (10-day CIDR + PGF): 4 cows were first in oestrus between 43 and 55 h, another 4 cows between 82 and 94 h, and the ninth cow between 108 and 120 h. Only 1 cow did not respond to treatment, even though a small CL was identified at scanning.

In Group 3 (2 x PGF): 2 cows were inseminated between 30 and 42 h even though they had not been seen in oestrus; 1 cow was first in oestrus between 56 and 68 h, another between 82 and 94 h. The third cow which was inseminated before being detected in oestrus was between 95 and 107 h. The other 2 cows were first in oestrus between 69 and 81 h. There were two cows which did not not respond to the second injection of PGF. In both cases, a large luteinized (18 and 20 mm) follicle was identified by ultrasonography at scanning at the 8th day post-treatment.

Duration of the oestrus.

The duration of the oestrus was similar among the treatment groups and contemporary control (the first 10 cows in oestrus within the period of 30-172 h post-treatment for treated cows were taken as control).

In group 1 it was 20.4 ± 2.1 h, compared to 17.8 ± 0.9 h, 17.0 ± 1.2 h, and 17.7 ± 1.4 h in groups 2 and 3 and the control group respectively (Table 2.7).

Sexually active group and its composition.

There were 93 cows in oestrus and inseminated over a 7 day post-treatment observation period, but only 19 dioestrous cows were in at least one SAG. Eight out of these 19 cows were in a SAG during the night (observations made at 21.00 and 04.00). These cows were only sniffing vulvas, and walking behind other cows which were in oestrus. The remaining dioestrous cows were involved in a SAG during the day, and activity included mounting of cows in oestrus.

Tailpaint and raddle and mounting activities.

Two SAGs were observed continuously for a period of 8 h. All cows were recorded for incidence of mounting and other activities, as well as for removal of tailpaint and raddle.

Each SAG usually included 1 cow in standing oestrus with another 2 cows as riders, making 3 in each SAG. Close observation of these cows in oestrus, showed that more than 70% of the paint (score 0 and 1) was rubbed off after 8 to 9 mounting attempts, compared with 6 or 7 for raddle removal.

Progesterone profiles.

Plasma progesterone concentrations measured by RIA of cycling heifers which were compared with tailpaint and raddle scoring at the time of detection of oestrus are presented in Table 2.8.

Concentrations measured by RIA in milk from 6 cows which were not detected in oestrus were between 0.2 and 2.1 ng/ml. The three cows in the 2XPGF group were 1.8, 2.1 and 0.5 ng/ml, while the remaining 3 cows in the control group were 0.2, 0.3 and 0.3 ng/ml.

DISCUSSION

Tailpainting and Raddle Scoring.

The tailpainting and raddle technique combined with scoring and re-raddling gave a precise correlation of visual oestrus detection because those animals in oestrus were identified and the number recorded before or as well as checking the paint and raddle, with patterns in the onset of the oestrus in groups of heifers and cows synchronized with different treatments.

The monitoring sequence using this technique was well correlated with oestrus detection by visual observation (Table 2.2). Some heifers had initial scores which were less than or equal to 3 when they were classified as "riding" and/or "stood when mounted?". However, in all cases the score was reduced to 0 or 1 within 8 h. This need not be a limitation when relating this result to a practical management situation in a commercial herd, where oestrus detection and scoring may only be done once or 2 times each day. Bearing in mind that the tailpaint and raddle system allows correct identification of those heifers suitable for correct detection of oestrus, it is worth noting another practical problem peculiar to synchronized heifers which was also solved by use of this technique. Namely, there are difficulties in accurately identifying the individual animals in oestrus in a group which has been synchronized, because many heifers are simultaneously in oestrus. Confusion occurs with the effects of the number of SAGs and the varied intensity of interactions involving mounting and/or standing-to-be-mounted.

In cows, there was also a good correlation between this technique and visual oestrus detection. Six cows were not detected in oestrus. However, when they were eventually scored and inseminated based on paint and raddle removed, 4 out of 6 became pregnant from the first artificial insemination; 3 of these cows were in the 2xPGF group; the remaining 3 cows were in the control group. The concentrations of milk progesterone from these treated cows showed that 2 cows had levels above 1 ng/ml. The result coincided with the score given which was 4 and 5. Moreover, these cows were not pregnant at this insemination. The remaining treated cow had score 1 and was pregnant at this insemination.
The concentrations of progesterone in milk for the 3 control cows not detected in oestrus showed that in all cases the values were very low, confirming the data from the score given which was 0, 2 and 1, and they became pregnant from the first insemination. There were no differences between concentrations of progesterone in plasma from samples collected from heifers at oestrus, and tailpaint and raddle scores ranging 0 to 3 (Table 2.8).

A previous report mentioned that a high proportion of cows were not detected in oestrus after treatment with prostaglandins (Roche, 1976). The same result occurred in this study. One explanation may be that the incidence of prolonged oestrus after the treatment with PGF and its analogues could be more common than after progesterone.

The data from this study indicated that in synchronized animals, there is a precise

correlation of visual oestrus detection and tailpainting and raddle technique. It therefore suggests that the use of this technique and visual observation could be successfully used in the correct identification of animals in oestrus.

Although the system of tailpaint and raddle is suitable for use with synchronized animals, it may be possibly less suitable for use this with animals which have not been synchronized or in situations with a lower degree of oestrous activity as with one cow in oestrus in a whole herd. However, in the trial 2 of this study, the SAGs were normally composed of 2 or 3 cows at the same time. It suggests that the tailpaint and raddle appears to be suitable even if a small SAG forms, but if no SAG forms, then it may be less reliable.

Patterns of Onset of Oestrus.

In heifers, the average interval from the end of treatment to onset of oestrus was similar among the treatment groups (Table 2.3), and the proportions of heifers in oestrus after different treatments did not differ significantly. However, in heifers treated with a CIDR device for 15 days, there was a tendency for most of the heifers to be in oestrus in less than 48 h post-treatment. The same finding was reported in a previous study (Macmillan et al., 1988c). Unexpectly, a similar proportion of heifers were in oestrus less than 48 h after treatment in the PGF group (Figure 2.1).

Heifers treated with a CIDR for 10 days + PGF, had a spread in synchrony, with most heifers in oestrus by 72 h post treatment. The response pattern for this group was close to the pattern in a previous study using a 7-day CIDR + PGF (Macmillan et al., 1988b).

In Trial 2, when the same treatments (10-day CIDR + PGF and 2 x PGF) were used in cows for oestrus synchronization, the average interval from the end of treatment to onset of oestrus did not differ to that for heifers. However, the response patterns for treated cows showed a delay of about 2 to 6 h, compared with heifers (Tables 2.3 and 2.6). The response patterns for cows treated with 10-day CIDR + PGF were more concentrated into two periods of from 42 to 54 and 81 to 93 h. However, cows treated with PGF 10 days apart were not well synchronized (Figure 2.2).

In general, the data from this study indicated that the average interval to oestrus was concentrated between 30 and 120 h after using different treatments for oestrus synchronization. This finding is in agreement with Roche and Ireland. (1984), who concluded that synchronization systems can successfully condense most oestrus events into a 3 to 5 day-period. Although there is no definitive explanation for this variation between treatments, especially when PGF is used, the following is offered as a possible explanation:

No currently available treatment programme can consistently produce perfect synchrony. The factors which influence this variation are: the stage of the follicle wave at the time of treatment (Macmillan and Henderson, 1984), and the length of the pro-oestrus after treatment. This may be longer in cows than in heifers. There was a slower growth rate of follicles in post-partum cows, suggesting

different patterns of follicular development (Savio et al., 1990). This could partially explain the wider spread in the onset of oestrus in cows treated with PGF in Trial 2.

Duration of the Oestrus.

The mean duration of oestrus in heifers was similar among the groups in this study (Table 2.4). A similar finding was reported in a previous study using continuous observation (Donaldson et al., 1968b). The mean duration of oestrus in cows was longer, but not significantly different among the groups in this study (Table 2.7). A similar result was reported by Ayalon and Wiss (1970), and Esslemont and Bryant (1976) using observations every 8 h and continuously, respectively. However, variation in the duration of the oestrus for heifers and cows of different breeds has been reported by O'Farrel (1984a), ranging from 9.2 ± 5.7 to 20.3 ± 8.4 hours. In this latter study, the duration of oestrus was very variable. This may be due to the frequency of observations, season of the year or presence of a bull (Wishart, 1972). The implications of the other components or factors associated with oestrous behaviour which could affect the duration of oestrus in cattle cannot be ignored.

Sexually Active Group and its Composition.

It is well known that cows in oestrus tend to congregate in an easily identifiable group (Williamson et al., 1972b). These SAGs are formed under spontaneous and synchronized oestrus situations. In this study, the characteristics of the SAGs were similar to a previous report (Kilgour et al., 1977). However, some differences were found between heifers and cows.

The SAG formed by cows when at pasture was mainly composed of cows in oestrus, pro-oestrus, and metoestrus. A few cows in dioestrus tended to be associated within this easily identifiable group. The numbers of cows in dioestrus which were involved was less during night periods than daylight hours. During the night periods of involvement by these dioestrous cows, the sexual activity was only sniffing of the vulva, chin rubbing, and walking behind cows in oestrus, but not actively mounting cows. During daylight hours, however, cows in dioestrus tended to become riders as well.

Although the SAGs formed by heifers also included a few animals in dioestrus, these animals showed similar mounting activities to oestrus group members. However, the SAGs were composed of more heifers per group, and had great mobility about the paddock. These SAGs were less stable and broke up to reform after short periods of time. Sometimes there was so much social excitement among heifers in large SAGs, it prevented the precise identification of heifers in oestrus by visual observation.

The paint and raddle was rubbed off after fewer attempts involving mounting in heifers than cows. This appears to be the result of different skeletal configuration associated with the degree of fat over the back bone, hips, and tail-head. Heifers has a smother skeletal conformation than the lactating cows.

CONCLUSIONS

Tailpainting combined with the use of an aerosol raddle, and scoring was effectively used in detecting oestrus in synchronized animals. This technique allowed a precise correlation with oestrus detection by visual observation, and a successful comparison of patterns in the onset of oestrus in groups of heifers and cows synchronized with different treatments. Moreover, by using this technique it was possible to monitor patterns of oestrus response within short periods of time after treatment, duration of oestrus between groups, and behaviourial interactions of animals forming SAGs.

The practical application of tailpainting and raddle scores can mean that when there is confusion arising from the size or the number of SAGs, and the varied intensity of interactions associated with mounting and/or standing-to-be-mounted in groups of heifers which have been synchronized, animals in oestrus may still be accurately identified with this technique. Moreover, the inseminator can be sure that oestrus animals are being inseminated, and the practitioners can obtain precise information on treatment efficacy with different classes of cattle.

The data from this experiment indicated that after using different treatments for oestrous synchronization, the occurrence of oestrus was concentrated between 30 and 120 h. In the future, treatment programmes should focus on the study of follicle wave regulation to reduce the variation in the post-treatment interval to oestrus without loss in fertility.

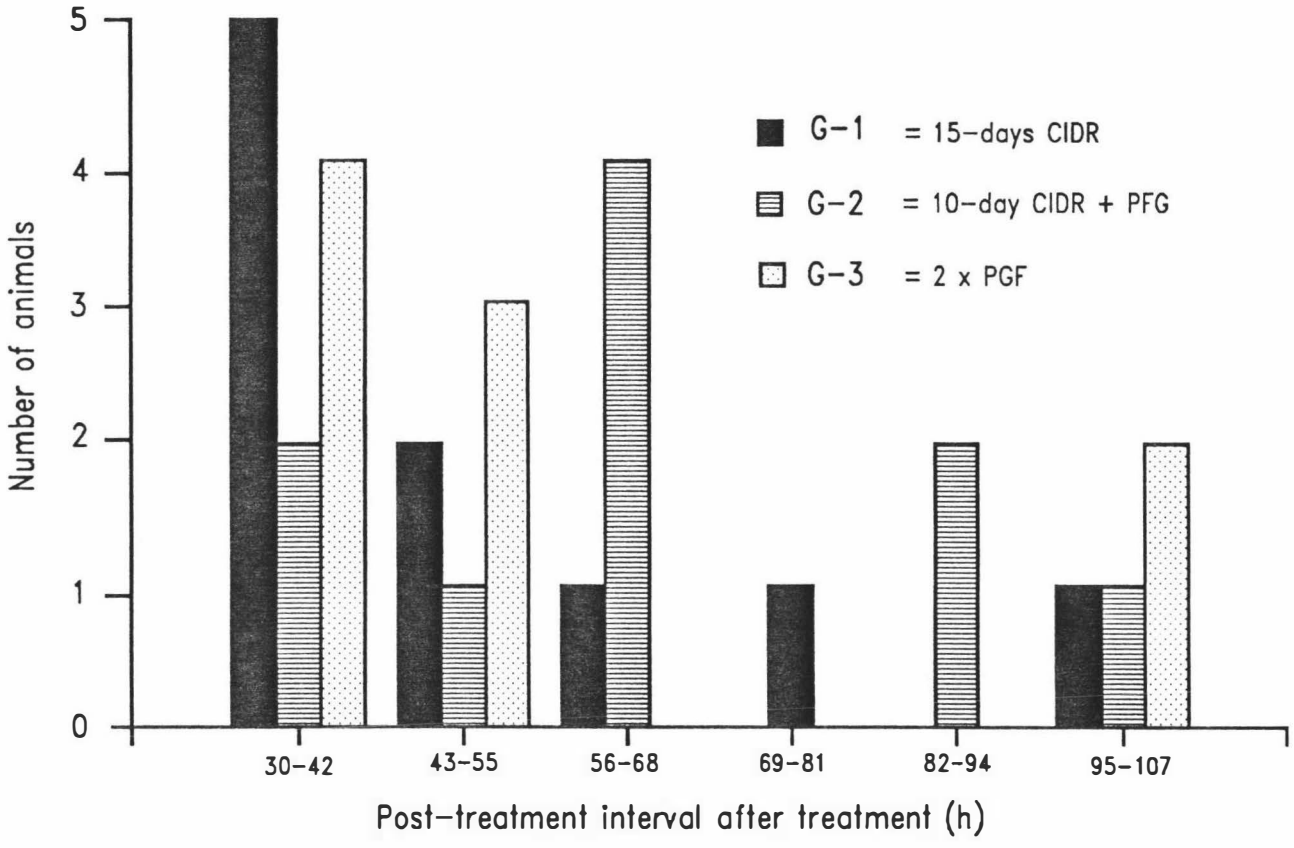


Figure 2.1 Distribution intervals to onset of oestrus in groups of heifers which received different synchrony treatments

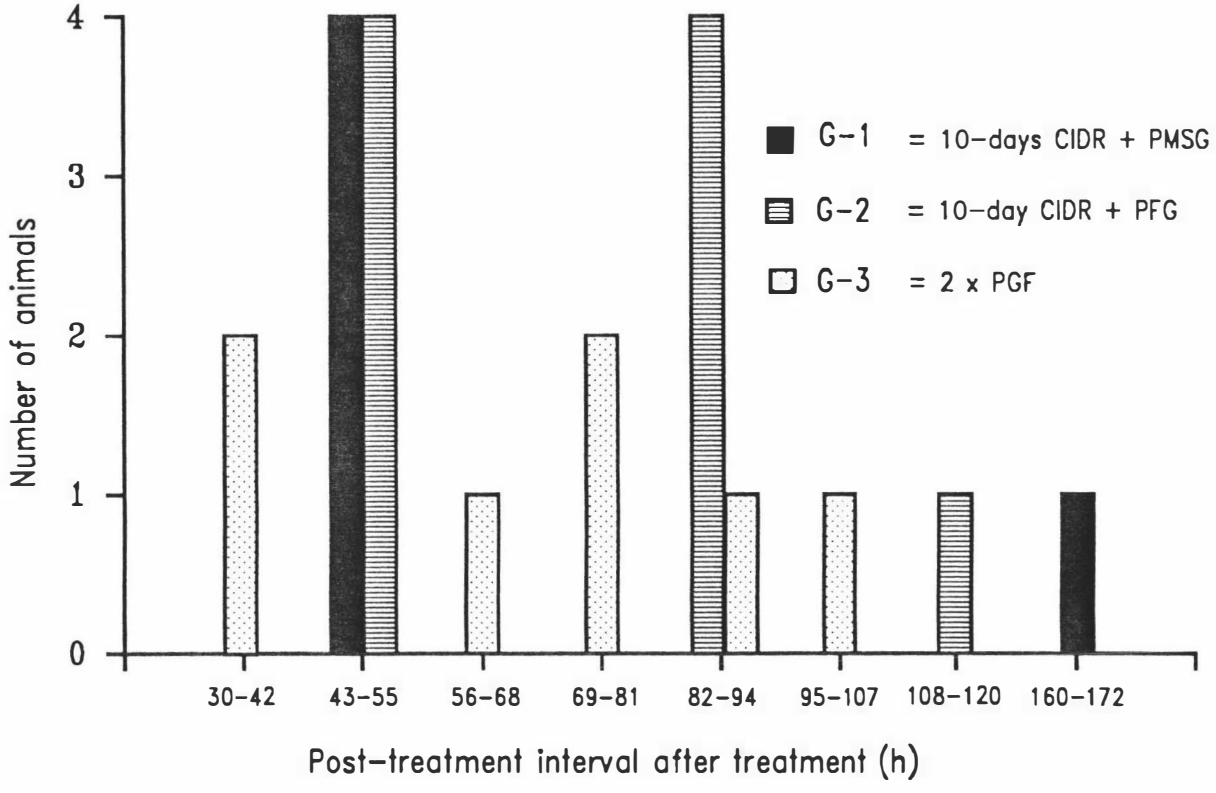


Figure 2.2 Distribution intervals to onset of oestrus and/or first insemination in groups of cows which received different synchrony treatments

Table 2.1 **Tailpaint and raddle scores at the time of initial oestrus detection among heifers included in Trial 1.**

	Number of heifers with tailpaint and raddle scores of					
Treatment	0	1	2	3	4-5	Not in Oestrus
15-Day CIDR	7	1	2			0
10-Day CIDR + PGF	3	6	1			0
2 x PGF	2	5	2			1
Control	5	2	1	2		0

Table 2.3 **Average post-treatment interval to onset of oestrus in groups of heifers which received three different treatments to synchronize oestrus (Trial 1).**

Treatment	Interval to oestrus (Hour) Mean (SEM)
15-Day CIDR	50.5 (7.1)
10-Day CIDR + PGF	66.0 (6.5)
2 x PGF	56.4 (8.1)

Table 2.4 **Average duration of behavioural oestrus in each group of synchronized and control heifers.**

Treatment	Duration of Oestrus (Hour) Mean (SEM)
15-Day CIDR	12.2 (0.9)
10-Day CIDR + PGF	12.8 (1.5)
2 x PGF	13.0 (1.3)
Control	13.3 (1.1)

Table 2.5 **Tailpaint and raddle scores at the time of initial oestrus detection among cows in Trial 2.**

	Number of cows with tailpaint and raddle scores of					
Treatment	0	1	2	3	4-5	Not in Oestrus
10-Day CIDR + PMSG	4	-	1			5
10-Day CIDR + PGF	4	3	2			1
2 x PGF	3	2	2			2
Control	6	2	2			0

Table 2.6 **Average post-treatment interval to onset of oestrus in groups of cows which received three different treatments to synchronize Oestrus (Trial 2).**

Treatment	Interval to Oestrus (Hour) Mean (SEM)
10-Day CIDR + PMSG	68.4 (23.4)
10-Day CIDR + PGF	70.5 (9.3)
2 x PGF	59.0 (9.8)

Table 2.7 **Average duration of behavioural oestrus in each group of synchronized and control cows.**

Treatment	Duration of Oestrus (Hour) Mean (SEM)
10-Day CIDR + PMSG	20.4 (2.1)
10-Day CIDR + PGF	17.8 (0.9)
2 x PGF	17.0 (1.2)
Control	17.7 (1.4)

Table 2.8 **Comparison between plasma progesterone concentrations (PPC, Mean (SEM); ng/ml) by radioimmunoassay and tailpaint and raddle scoring at the time of detection of oestrus in cycling heifers.**

Tailpaint and Raddle scores	Number of samples	PPC
0	5	0.5 (0.1)
1	8	0.4 (0.1)
2	6	0.3 (0.1)
3	2	0.6 (0.2)

REFERENCES

AIZINBUD, E., LEHRER, A. R., FISCHLER, H., TADMOR, A., SCHINDLER, D. and SCHINDLER, H. (1984). Impedometric changes in the vaginal tissue of cows in relation to reproductive events. The **Reproduction Potential of Cattle and Sheep.** Rehovot (Israel) 21-23 February Ed. INRA Publ.

AYALON, N. and WEISS, Y. The influence of a tease bull on oestrus detection. (1970). **Refuah Vet.** 27: 22-25.

BAILIE, J. H. (1982). Management and economic effects of different levels of estrus detection in the dairy herd. **Vet Rec.** 110: 218-221.

BALL, P. J. H., MORANT, S. V. and CANT, E. J. (1978). Measurement of milk temperature as an aid to oestrus detection in dairy cattle. **J. Agric. Sci. Camb.** 91: 593-597.

BALL, P. J. H., COWPE, J. E. D. and HARKER, D. B. (1983). Evaluation of tail paste as an oestrus detection aid using serial progesterone analysis. **Vet. Rec.** 112: 147-149.

BLAKE, C. and GOULD, B. J. (1984). Use of enzymes in immunoassay techniques. A review. **Analyst.** 109: 533-548.

BRITT, J. H. (1976). Testosterone induction of male-like sexual behaviour in cows for use in oestrus detection. **A.I. Digest.** 24: 14-15.

BOOTH, J. M. and HOLLANDSWORTH, R. J. (1976). The establishment and operation of a central laboratory for pregnancy testing in cows. **Brit. Vet. J.** 132: 518-528.

BOYD, H. W. (1984). Aids in oestrus detection: A review. In: **"Dairy Cow Fertility"** eds. R G. Eddy and M. J. Tucker **Proc. Brit. Vet. Assoc. and Brit. Soc. of Anim. Prod.** 60-67.

DAILEY, R. A., JAMES, R. E., INSKEEP, E. K. and WASHBURN, S. P. (1983). Synchronization of estrus in dairy heifers with prostaglandin F2 α with or without estradiol benzoate. **J. Dairy Sci.** 66: 881-886.

DOBSON, H., MIDMER, S. E. and FITZPATRICK, R. J. (1975). Relationship between progesterone concentrations in milk and plasma during the bovine oestrous cycle. **Vet. Rec.** 96: 222-223.

DONALDSON, L. E. (1968a). The efficiency of several methods for detecting oestrus in cattle. **Aust. Vet. J.** 44: 496-498.

DONALDSON, L. E., LITTLE, D. A. and HANSEL, W. (1968b). The duration of

estrus and the time of ovulation in cattle of three breed types with and without synchronization of oestrus with a progestogen. **Aust. Vet. J.** 44: 364-366.

DUCKER, M. J., HAGGETT, A., BLOOMFIELD, G. A. and MORANT, S. V. (1983). An evaluation of tailpaint as an aid or alternative to oestrus detection. **Anim. Prod.** 37: 221-227.

ELMORE, R. G., ADERIBIGDE, A. A. and GARVERICK, H. A. (1986). The use of heat detection aids in estrus synchronization programs. Theriogenology. 26: 239-244.

ESSLEMONT, R. J. (1974). Economic and husbandry aspects of the manifestation and detection of oestrus in cows. I. Economic aspects. Adas Q. **Rev.** 15: 83-95.

ESSLEMONT, R. J. and BRYANT, M. J. (1976). Oestrous behaviour in a herd of dairy cows. **Vet. Rec.** 99: 472-475.

FETROW, J., McCLARY, D., HARMAN, R., BUTCHER, K., WEAVER, L., STUDER, E., EHRLICH, J., ETHERINGTON, W., GUTERBOCK, W., KLINGBORG, D. RENEAU, J. and WILLIAMSON, N. B. (1990). Calculating selected reproductive indices: recommendations of the american association of bovine practitioners. **J. Dairy Sci.** 73: 78-90.

FIGUEROA, M. R., FUQUAY, J. W. and SHIPLEY, S. K. (1988). Synchronization of estrus in early diestral dairy heifers with prostaglandin F2 α and estradiol benzoate. **Theriogenology.** 30: 1093-1097.

FOOTE, R. H. (1975). Estrus detection and estrus detection aids. **J. Dairy Sci.** 58: 248-256.

FOOTE, R. H., OLTENACU, E. A. B., MELLINGER, J., SCOTT, N. R. and MARSHALL, R. A. (1979). **J. Dairy Sci.** 62: 69-73.

FOULKES, J. A., COOKSON, A. D. and SAUER, M. J. (1982). AI in cattle based on daily microtitre plate enzymeimmunoassay of progesterone in whole milk. **Br. Vet. J.** 138: 515-521.

FULKERSON, W. J., SAWYER, G. J. and CROTHERS, I. (1983). The accuracy of several aids in detecting oestrus in dairy cattle. **Appl. Anim. Ethol.** 10: 199-208.

FULKERSON, W. J. (1984). Reproduction in dairy cattle: effect of age, cow condition, production level, calving-to-first-service interval and the male. **Anim. Reprod. Sci.** 7: 305-314.

GOULD, C. M. (1985). A simple procedure for investigating submission rates in dairy practice. **Proc. of Inter. Conf. on Vet. Prev. Med. and Anim. Prod.** Aust. Vet. J., Melbourne, Australia. 103-105.

HURNIK, J. F., KING, G. J. and ROBERTSON, H. A. (1975). Estrous and related behaviour in post-partum Holstein cows. **Appl. Anim. Ethol.** 2: 55-68.

IRELAND, J. J. and ROCHE, J. F. (1982). Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones sterids in follicular fluid and gonadotrophin receptors. **Endocrinology.** 111: 2077-2086.

JOHNSON, D. G. and RENEAU, J. K. (1989). Alternative methods of estrus detection for dairy cows in tie stall barns. **J. Dairy Sci.** (suppl. 1): 447 (abstr.).

KERR, O. M. and McCAUGHEY, W. J. (1984). Tailpainting techniques as an aid to oestrus detection in cattle. **Vet Rec.** 114: 605-607.

KIDDY, C. A. and MITCHELL, D. S. (1981). Estrus-related odors in cows: Time of occurrence. **J. Dairy Sci.** 64: 267-271.

KILGOUR, R., SKARSHOLT, B. H., SMITH J. F., BREMMER, K. J. and MORRISON, M. C. L. (1977). Observations on the behaviour and factors influencing the sexually active group in cattle. **Proc. N. Z. Soc. Anim. Prod.** 37: 128-135.

KING, M. E., KIRACOFE, J. S., STEVENSON, J. S. and SCHALLES, R. R. (1982). Effect of stage of the oestrous cycle on interval to estrus after PGF2 α in beef cattle. **Theriogenology.** 18: 191-200.

KIRWOOD, R. N., LAPWOOD, K. R., SMITH, W. C. and ANDERSON, I. L. (1984). Plasma concentrations of LH, prolactin, oestradiol-17B and progesterone in sows weaned after lactation for 10 or 35 days. **J. Reprod. Fert.** 70: 95-102.

LAUDERDALE, J. W. (1972). Effects of PGF2 α on pregnancy and estrous cycle of cattle. **J. Anim. Sci.** 35: 246 (abstr.).

LAUDERDALE, J. W. (1974). Estrus detection and synchronization of dairy cattle in large herds. **J. Dairy Sci.** 57: 348-354.

LAUDERDALE, J. W., McALLISTER, J. L., MOODY, E. L. and KRATZER, D. D. (1980). Pregnancy rate in beef cattle injected once with PGF2 α. **J. Anim Sci.** 51: (suppl. 1): 296.

MACMILLAN, K. L. (1970). Return intervals to first insemination and conception rates to second insemination in New Zealand dairy cattle. **N. Z. J. Agric. Res.** 13: 771-777.

MACMILLAN, K. L. and WATSON, J. D. (1971). Short estrous cycles in New Zealand dairy cattle. **J. Dairy Sci.** 54: 1526-1529.

MACMILLAN, K. L. and CURNOW, R. J. (1977). Tailpainting: a simple form of oestrus detection in New Zealand dairy herds. **N. Z. J. Exp. Agric.** 5: 357-

361.

MACMILLAN, K. L., CURNOW, R. J. and MORRIS, G. R. (1977). Oestrus synchronization with a prostaglandin analogue. I. Systems in lactating dairy cattle. **N. Z. Vet. J.** 25: 366-372.

MACMILLAN, K. L. and DAY, A. M. (1982). Prostaglandin F2 ∝-A fertility drug in dairy cattle. **Theriogenology.** 18: 245-253.

MACMILLAN, K. L. (1983). Prostaglandin responses in dairy herd breeding programmes. **N. Z. Vet. J.** 31: 110-113.

MACMILLAN, K. L. and HENDERSON, H. V. (1984). Analysis of the variation in the interval from an injection of prostaglandin F2 ∝ to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. **Anim Reprod. Sci.** 6: 245-255.

MACMILLAN, K. L. (1985). Detection of oestrus in dairy cows. Refresher Course for Veterinarians. **Dairy Cattle Production.** 6-10 May. University of Sydney, Australia.

MACMILLAN, K. L. (1986). Condensed breeding programmes. Seminar **New Zealand Dairy Board Livestock Improvement Division.** Flock House, 17-21 February.

MACMILLAN, K. L. (1988a). Trends in brreding management in New Zealand dairy herds. **Non-infectious Factors Affecting Reproductive Performance of Dairy Herds.** Dairy Cattle Reproduction Research Workshop, Nov. 15-17. Australia.

MACMILLAN, K. L., TAUFA, V. K., BARNES, A. M. and HENRY, R. (1988b). Detecting estrus in synchronized heifers using tailpaint and an aerosol raddle. **Theriogenology.** 30: 1099-1114.

MACMILLAN K. L., TAUFA, V. K. and DAY, A. M. (1988c). Onset of oestrus and fertility in heifers synchronized with progesterone from a CIDR-Type B for fifteen days. **Proc. 11th Int. Congr. on Anim. Reprod. and AI.** Dublin-Ireland 444-447.

MOLLER, K. (1978). PAHAPS-Planned animal health and production service on New Zealand dairy farms. **Veterinary Services Council.** 18-30. Wellington.

MOLLER, K., LAPWOOD, K. R. and MARCHANT, R. M. (1986). Prolonged service intervals in cattle. **N. Z. Vet. J.** 34: 128-132.

MYLREA, P. J. and BEILHARZ, R. G. (1964). The manifestation and detection of oestrus in heifers. **Anim. Behaviour.** 12: 25-30.

ODDE, K. G. (1990). A review of synchronization of estrus in postpartum cattle. **J. Anim. Sci.** 68: 817-830.

O'FARREL, K. J. (1984a). Oestrous behaviour, problems of detection and relevance of cycle lengths. In: **"Dairy Cow Fertility"** eds. R. G. Eddy and M. J. Tucker, Brit. Vet. Assoc. Edit. Services, London 47-59.

O'FARREL, K. J. (1984a). The control of dairy herd fertility. **Part II: Animal Health and Machine Milking, paper 10.** Moorepark 25th Anniversary Publication.

OLTENACU, P. A., ROUNSAVILLE, T. R., MILLIGAN, R. A. and FOOTE, R. H. (1981). Systems analysis for designing reporductive management programs to increase production and profit in dairy herds. **J. Dairy Sci.** 64: 2096-2104.

PETER, J. B., WELCH, J. A., LAUDERDALE, J. W. and INSKEEP, E. K. (1977). Synchronization of estrus in beef cattle with PGF2 α and estradiol benzoate. **J. Anim. Sci.** 45: 230-235.

ROCHE, J. F. (1976). Fertility in cows after treatment with a prostaglandin analogue with or without progesterone. **J. Reprod. Fertil.** 46: 341-345.

ROCHE, J. F. and IRELAND, J. J. (1984). Manipulation of ovulation in cattle. **Proc. 10th Inter. Cong. on Anim. Reprod. and AI.** 4: (4):9-17.

SAVIO, J. D., BOLAND, M. P. and ROCHE, J. F. (1990). Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. **J. Reprod. Fertil.** 88: 581-591.

SAWYER, G. J. and FULKERSON, W. J. (1981). The effectiveness of steers and heifers treated with oestrogen or testosterone to detect oestrus in cattle. **Anim. Reprod. Sci.** 3: 259-269.

STEVENSON, J. S. and BRITT, J. H. (1977). Detection of estrus by three methods. **J. Dairy Sci.** 60: 1994-1998.

STEVENSON, J. S., SCHMIDT, M. K. and CALL, E. P. (1984). Stage of estrus cycle, time of insemination and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin F2 α. **J. Dairy Sci.** 67: 1798-1805.

TUNGSUBUTRA, V. and FRANCE, J. T. (1978). Serial changes in plasma levels of progesterone, unconjugated oestradiol and unconjugated oestriol in normal pregnancy. **Aust. N. Z. Jl. Obstet. Gynaec.** 18: 97-103.

VASQUEZ, L., WHITMORE, H., RODRIAN, J., PUCKETT, H., SPAHR, S., McCOY, G., LODGE, R. and OTT, R. (1984). Use of electronic pedometers to measure hourly activity before and after conception in dairy cattle. **Proc. 10th Int. Congr. on An. Reprod. and AI,** Urbana, Illinois, USA. p. 298.

WATTS, T. L. and FUQUAY, J. W. (1985). Response and fertility of dairy heifers following injection with prostaglandin F2 α during early, middle and late diestrus. **Theriogenology.** 23: 655-661.

WILLIAMSON, N. B., MORRIS, R. S., BLOOD, D. C. and CANNON, C. M. (1972a). A study of oestrous behaviour and oestrus detection mehtods in a large commercial dairy herd. I. The relative efficiency of methods of oestrus detection. **Vet. Rec.** 91: 50-58.

WILLIAMSON, N. B., MORRIS, R. S., BLOOD, D. C., CANNON, C. M. and WRIGHT, P. J. (1972b). A study of oestrous behaviour and oestrus detection methods in a large commercial dairy herd. II. Oestrus signs and behaviour patterns. **Vet Rec.** 91: 58-62.

WILLIAMSON, N. B. (1975). The use of decision analysis to evaluate the economic effects of heat mount detectors in two dairy herds. **Aust. Vet. J.** 51: 114-121.

WILLIAMSON, N. B. (1980). Tailpainting as an aid to oestrus detectionin cattle. **Aust. Vet. J.** 56: 98-100.

WISHART, D. F. (1972). Observations on the oestrous cycle of the Friesian heifer. **Vet. Rec.** 90: 595-597.

YOUNG, I. M. (1989). Dinaprost 14-day oestrus synchronization schedule for dairy cows. **Vet. Rec.** 124: 587-588.

ZEMJANIS, R. (1969). Anestrus, the practitioners dilemma. **Vet. Scope.** 14: 15-21.

CHAPTER 3

CONTROLLED BREEDING MANAGEMENT THROUGH THE STRATEGIC USE OF THE CIDR-B INTRAVAGINAL DEVICE FOR COWS IN HERDS WITH A DAILY MILK QUOTA

ABSTRACT

This study was designed to evaluate a management system for such dairy herds which would reduce the time commitment for oestrus detection through control of the oestrous cycle, and to minimize the variation in returns to oestrus after a second synchrony treatment. The oestrous responses to different dose rates of prostaglandins (PGF) combined with CIDR devices in cycling animals, and the possible effects of treatment with progesterone on fertility and plasma progesterone concentrations (PPC) in cattle were also evaluated.

Systems for oestrous synchronization have focused on treatment preceding first insemination. However, oestrus detection rates are commonly lower among inseminated animals which return to service than among the same animals preceding first insemination. Control of the oestrous cycle, in order to facilitate detection of oestrus and minimize the variation in return to service intervals was evaluated in this study in which the basic programme was: i) all cows which had calved at least 35 days and were identified to start the programme were examined; each cow had a CIDR inserted and was tail-painted; ii) the CIDR's were removed 10 days later, and cycling cows were injected with either the recommended luteolytic dose of Estrumate or a half dose. The non-cycling cows received an injection of PMSG (400 IU); iii) all cows had raddle applied over the paint at device removal; iv) cows were inseminated on detected oestrus over the first period of artificial insemination (AI); v) a used CIDR was re-inserted into each cow on day 17-19 post-insemination, and tail-paint re-applied; vi) the re-used CIDR's were removed 6 days after insertion and the paint strips were re-raddled. The second period of AI on oestrus detection commenced and was completed within two days.

In a herd with a year-round milking system, this programme meant that a new group of cows would enter the breeding schedule on every fourth wednesday. This time coincided with CIDR re-insertion for cows from the previous month. Insemination of the control cows commenced at the first oestrus from 45 days post-partum.

The mean interval from Planned Start of Mating to first insemination was shorter in all treated groups in the three year-round calving herds (PSM; day 0 for treatment and control groups was taken as the day when a new CIDR device was inserted into each cow in a new treatment group). This difference was also seen in cycling early calvers from the autumn seasonal herd. The mean interval from PSM to conception in year-round milking herds was significantly different between groups. Treated cows had fewer days open than controls, ranging from a difference of 5.3, 6.3 or 14.8 days between treated cows among the 3 individual herds with "12 month" calving programmes. In this measure no difference was found between groups in seasonal milking herds. Conception rate to first insemination differed statistically between groups, but not for second inseminations. As a consequence, the mean number of services per conception also differed significantly between groups.

Only 8.5% of cows were diagnosed as non-cycling in the year round herds, compared with 12.7% in the seasonal herd. The proportion of cows inseminated after treatment was 53% in the year-round herds and 71% in the seasonal herd. The percentage of cows detected in oestrus varied from 40% to 63%. The mean interval from PSM to conception and the mean number of services per conception were significantly lower among the treated cows.

The two periods of AI were concentrated into 5-day synchrony periods in the treated groups. Oestrous responses in the four farms averaged 76% in the treatment group, and differed significantly from the control group (63%; $P < 0.001$). There were 67% of the treated cows observed in oestrus and inseminated within the first period of AI (3 days), with the highest proportion (32%) of these inseminations at 48 h after device removal. The return to oestrus was synchronized in 85% of non-pregnant cows on days 22 to 25 after first insemination.

The average responses to the two doses of PGF at CIDR removal was 72% for half dose vs 68% for full dose.

Plasma progesterone concentrations maintained by the used CIDR at device removal was about 2 ng/ml in both groups of non-pregnant cows (CIDR/PGF and CIDR/PMSG). This declined to 0.3 ± 0.1 ng/ml the morning after the re-used CIDR was removed. Progesterone concentrations of pregnant cows in both treated groups were similar, when the re-used CIDR was removed and the day after (8.7 ± 0.4 and 9.3 ± 1.1 vs 7.1 ± 0.4 and 8.4 ± 0.7, respectively).

The CIDR treatment together with an injection of PGF or PMSG significantly reduced the PSM to first AI, but this management advantage involving systematic control of the oestrous cycle was partly lost because of the lower fertility, and because of mistakes relating to the interpretation of the tailpainting system which were frequently made by the owners.

INTRODUCTION

Dairy farms in New Zealand can be broadly classified into two types; namely seasonal supply herds or "town milk herds". Town milk herds operate an a " quota " system which requires that a designated quantity amount of milk must be produced every day. This milk is paid for at a higher rate than that received by the seasonal producer (Fielden et al., 1980). To achieve this relatively constant output, cows are inseminated to calve throughout the year, particularly in the late summer, autumn, and winter months. This calving pattern is not associated with maximum " in situ " pasture utilization. These farms must feed more crop, hay and silage than seasonal supply farms (Brooks and Holmes, 1988). Therefore, the costs for town supply production are higher.

Reproductive performance in dairy herds has a major influence on the efficiency of milk production because it influences the synchrony between feed supply and demand, as well as the percentage of each year during which a cow is producing milk. It also influences the rate of genetic progress and culling rates. All these factors directly or indirectly affect profit. The calving interval depends on the calving to conception interval. One frequently reads that a treatment programme may either reduce the interval from calving to first insemination, or increase the pregnancy rate at that insemination. But, many of these apparently beneficial effects on individual components of reproductive performance fail to significantly reduce the mean interval from calving to conception. The solution to this critical problem relates to the development of either detection techniques or synchronization techniques which focus on returns to service (Macmillan, 1988). Ideally, a synchrony system will be one which can at least maintain normal fertility, produce a high degree of synchrony, and be economically used to control the oestrous cycle before the first insemination, as well as before subsequent inseminations among those animals which return to service (Macmillan, 1988b; Odde, 1990).

Inefficient oestrus detection has been found to be a major factor contributing to calving intervals in excess of 365 days in all the recognised dairying industries in the world with a consequent reduction in production and profit. Moreover, in herds with daily milk quotas, oestrus detection must be carried out throughout the year. This is a time-consuming tak, and it is difficult to maintain the enthusiasm of the farm staff. Hence reproductive performance in such herds frequently suffers in comparison with seasonal calving herds (R. Morris, personal communication). For the dairy farmers using AI, the successful implementation of the breeding programme depends to a large extent in accuracy of oestrus detection. Accurate oestrus detection in necessary to achieve a high submission rate, a compact breeding period in seasonal herds, and optimum calving intervals in year-round herds (O'Farrel, 1984).

The introduction of tailpainting for efficient breeding management in New Zealand has reduced differences in non-returns rates to first insemination associated with herd size, mainly because of a reduction in the incidence of detection errors in large herds. Moreover, the improved oestrus detection has contributed to an

increase in non-returns rate (Macmillan and Curnow, 1977; Macmillan, 1988b).

Synchronization of oestrus in dairy herd reproductive programmes has been used to test the effectiveness of two progestagen systems for the control of oestrus in normal and anoestrous cows, and to synchronize oestrus during each of seventeen 3-week breeding periods (Britt et al., 1972). In order to concentrate oestrus detection and insemination into short periods of time, lactating dairy cows were treated with a progesterone-releasing intravaginal device (PRID) for 7-9 days combined with prostaglandin F 2 α ([PGF]; Folman et al., 1984; Smith et al., 1986). The use of single or double injection system with PGF in a controlled breeding programme has also been reported (Britt, 1977; King at al., 1983; Seguin et al., 1983; Sudweeks and Randel, 1985; Belschner, 1986).

The most common procedures used in New Zealand to condense the breeding programme in dairy herds involved a single injection of PGF given to cows that were in oestrus 7 to 16 days before treatment. This system was originally called the " Why Wait System " (Macmillan, 1986a). The breeding programme can be further concentrated into short periods by using a controlled internal drug release device (CIDR) in some cows and PGF in others (Macmillan, 1986a). CIDR's were used after insemination in another trial, with device insertion from 14 to 17 days after artificial insemination (AI), and removal at 21 days after first insemination (Macmillan, 1986a). CIDR's have also been used for 9 days with PGF on day 7 to synchronize oestrus for first insemination, and to synchronize returns to oestrus of non-pregnant animals (Van Cleeff et al., 1989). Dairy herd reproductive management systems using reproductive tract examination at 3 week interval, with or without concurrent synchronization of oestrus with PGF was evaluated between treated herds, visited and unvisited control herds (Washburn and Dailey, 1987).

PGF had been administered to regulate the bovine oestrous cycle by intrauterine (Shelton, 1973; Louis et al., 1974; Moore, 1975), intramuscular (Lauderdale et al., 1974; Roche, 1974; Hafs et al., 1975), intravaginal (Louis et al., 1973), or intravulvosubmucous routes (Ono et al., 1982). Several management systems have been investigated involving 1 or 2 injections of PGF at various dose rates, with and without the detection of oestrus (Lauderdale, 1972; Lauderdale et al., 1974; Donaldson, 1980; Donaldson et al., 1982).

A functional corpus luteum (CL) must be maintained for up to approximately 215 days of gestation before the placenta and/or adrenal is able to produce adequate amounts of progesterone for the maintenance of pregnancy (Sachs, 1984). As a consequence, insufficient circulating progesterone has been associated with infertility in cattle (Garverick and Smith, 1986). The findings of previous studies of direct progesterone supplementation in cows and their effects on increasing the pregnancy rates through increased embryonic survival rates suggest a tendency for progesterone supplementation to increase pregnancy rates of cows. This is particularly noticeable in repeat breeder or low fertility cattle. Sreenan and Diskin (1983) had shown no effect on pregnancy rate in cows. However, others researchers found a positive effect on pregnancy rate (Wiltbank et al., 1956; Johnson et al., 1958; Marcus and Ayalon, 1981; Shemesh et al., 1981; Folman et al., 1983; Diskin and Sreenan, 1986; Macmillan and Taufa, 1987b; Robinson et al., 1989; Macmillan et al., 1990a). In this last study, the beneficial effects of

progesterone supplementation were specific to the period from 6 to 8 days after insemination.

The specific aims of this study were to evaluate a management system for dairy herds involving control of the oestrous cycle, to reduce the time required for detection of oestrus and to consequently improve oestrus detection rate, by reducing the variation in return to service intervals. Other aims were to evaluate oestrous responses to different dose rates of prostaglandins combined with CIDR devices in cycling animals, and to determine the possible effects of treatment with progesterone on fertility and plasma progesterone concentrations in cattle.

LITERATURE REVIEW

Dairy farms in New Zealand can be broadly classified into two types.

a) Seasonal Supply Herds.

b) "Town milk" herds.

Town milk herds produce a year round supply of fresh liquid milk, cream, and cultured food products (Baldwing, 1989). The herd owners operate under a " quota " system which requires that a designated volume of milk must be produced every day. This milk is paid for at higher rate than that received by the seasonal producer (Fielden et al., 1980). To achieve this relatively constant output, cows are inseminated to calve throughout the year, particularly in the late summer, autumn and winter months. This calving pattern is not associated with maximum "in situ" pasture utilization. These farms must feed more crop, hay and silage than seasonal supply farms (Brooks and Holmes, 1988). Therefore, the costs for town supply production are higher.

Nutritional and environmental factors which contribute to reduced reproductive efficiency may be more significant in these herds (Fielden et al., 1980). In addition, a reduced emphasis on breeding management is common in herds where cows are calving throughout the year, and breeding efficiency is not easily monitored. The detection of oestrus is not restricted to a limited intensive period as in seasonal herds, and few cows are in oestrus simultaneously. Moreover, a common reason for the prolonged calving interval in these herds is failure to detect returns to service. The cows which fail to conceive to a first or second insemination can continue to be milked and have long lactations. This situation increases production per cow per lactation; but cows are less efficient in late lactation (Macmillan, 1985a).

Reproductive performance in dairy herds has a major influence on the efficiency of milk production because it influences the synchrony between feed supply and demand, as well as the percentage of each year during which a cow is producing milk. It also influences the rate of genetic progress and culling rates. All these factors directly or indirectly affect profitability.

The calving interval (this specific term is defined as the period between consecutive calvings) depends on the calving to conception interval. One frequently reads that a treatment programme may either reduce the interval from calving to first insemination, or increase the pregnancy rate at that insemination. But, many of these apparently beneficial effects on individual components of reproductive performance fail to significantly reduce the mean interval from calving to conception. The solution to this critical problem relates to the development of either detection techniques or synchronization techniques which focus on returns to service (Macmillan, 1988c).

The term "synchronization of oestrus" has been routinely used to refer to

manipulation of the oestrous cycle in the interests of reproductive management. The term controlled breeding better fits the situation, since not all aspects of controlled breeding are directed at obtaining synchronous oestrus and ovulation.

Studies of Synchronization of Oestrus in Dairy Herd Reproductive Programmes.

Britt et al.(1972) designed experiments to test the effectiveness of medroxyprogesterone acetate (MAP) and melengestrol acetate (MAG) for the control of oestrus in normal and anoestrous dairy cows, and to synchronize oestrus in dairy cows during each of seventeen 3-week breeding periods. The progestagens reduced the variation among herds in the interval from calving to first service and average days open. They concluded that this system for controlling oestrus could be beneficial as part of a total system to obtain a high reproductive rate in large herds.

High yielding dairy cows were allocated to a control group, or to one of two treatment groups for synchronization of oestrus in a large trial by Folman et al. (1984). The treatments were: i) inserting a progesterone releasing intravaginal device (PRID) for 7 days with 500 μg of a prostaglandin F2 α (PGF) analogue injected 1 day before PRID removal; or ii) by administration of 500 μg of PGF followed 13 days later by a PRID inserted for 9 days. Following each oestrus synchronization regime, cows were inseminated during a fixed 6 day-period. A second PRID was inserted for 9 days into one-half of the cows of each treatment group 12 days following the fixed time insemination. Pregnancy rates at 25 days following the fixed time insemination and at 100 days after calving were greater in treatment groups than control groups. This indicated that the synchronization treatment, in which observations for oestrus detection and inseminations were made only during 6 days out of each 3 weeks, significantly increased the percentage of cows pregnant within 100 days of calving.

King et al.(1983) indicated that a controlled breeding programme could have application in dairy herds, but should be used with caution. They found that the percentage of dairy cows which were inseminated and became pregnant during 42 days of the programmed breeding periods was not different from animals which have been routinely observed for oestrus and inseminated when detected, compared with animals inseminated at oestrus following a single injection of PGF, or inseminated at a fixed time after two PGF treatment 11 days apart. However, in the control group, some animals were not inseminated. Their inclusion significantly reduced the overall pregnancy rate.

Another experiment involved 3 herds in a reproductive management system (Seguin et al., 1983), by combining selective use of PGF with insemination based on oestrus detection. Cows or heifers assigned to the experimental system were inseminated and become pregnant sooner than controls. The pregnancy rates in treated animals for first insemination were higher than in controls. Distributions of oestrous activity by day of the week of inseminations were significantly affected by the experimental management system. Therefore, it was concluded that the routine use of PGF in cycling cows, at weekly visits can improve reproductive performance and reduce the cost of

labour in oestrus detection.

The use of a PRID in combination with PGF for concentrating oestrus detection and artificial insemination (AI) activities into 1 week out of every 3 was also studied in lactating dairy cows by Smith et al. (1986). Oestrus detection in treated cows was increased, interval to first AI was reduced, conception rate at first AI was reduced and services per conception was higher than in control cows. Days open and percentage of cows pregnant before 120 days postpartum did not differ.

Oestrus detection efficiency and conception rate at first insemination were compared in treated and control dairy cows, where animals with a corpus luteum (CL) were either injected with PGF or left untreated on a random basis (Sudweeks and Randel, 1985). Oestrus detection was significantly enhanced in the treatment group, but conception rate at first AI was not affected by treatment. However, the final pregnancy rate was significantly greater in the treated group.

Controlled internal drug release devices (CIDR) were used for 9 days with PGF on day 7 in 4 trials to synchronize oestrus, to determine the effect of exogenous progesterone during week one post-insemination on conception rate, or to synchronize returns to oestrus of non-pregnant animals (Van Cleeff et al., 1989). In summary, 83.9% of heifers were in oestrus between 48-72 h after CIDR removal. CIDR's may have had a positive effect on fertility between days 17-22, and could be used to synchronize the interval of returns to service in non-pregnant heifers.

The most common procedure used in New Zealand to condense the breeding programme in dairy herds has involved a single injection of PGF with cows that were in oestrus 7 to 16 days before treatment (Macmillan, 1986a). A single group or several groups of cows can be injected once as each group of cow reaches the seventh day of the cycle. This system was originally called the "Why Wait System". It was used so that cows with premating heats in the 2 weeks before the start of artificial breeding which would otherwise have been inseminated in the second and third week of the breeding performance were inseminated in the first 12 days of the programme.

Breeding programmes can be concentrated even further into short periods by using CIDR's in some cows and PGF in others. In one trial, all cows were initially tailpainted (Macmillan, 1986a), 21 days before the start of artificial breeding. Every three days, those cows which had been bulling were repainted with the original paint colour. Nine days before the start of AI, those cows which had been bulling in the previous 9 days were repainted with a different colour. The remaining cows had a CIDR device inserted for 7 days. Two days before the start of AI, CIDR's were removed and the repainted cows were injected with PGF. These regimes meant that 60% of the herd conceived during the first 6 days of the AI programme. Among the CIDR treated cows detected in oestrus, 81.5% were inseminated from 48 to 96 h after CIDR removal. No significant difference in pregnancy rate was found, being 63% in PGF treated cows versus 52% in CIDR treated cows. A submission rate of 92% was obtained in 6 days of artificial breeding.

CIDR's were used after insemination in another trial in 7 herds, with insertion being at periods from 14 to 17 days after AI, and removal at 21 days after first insemination (Macmillan et al., 1986). CIDR removal at 21 days after insemination caused 62.4% of 186 re-inseminated cows to have a return interval of 23 or 24 days. These cows had a second insemination between 48 and 72 h after CIDR removal, whereas 63.2% in the control group of animals were re-inseminated over the normal period of 18 to 24 days. There were fewer return-to-service intervals of around 6 weeks in the treated group compared with controls (35-50 days; 8.2% vs 15.2%, respectively). This form of CIDR treatment did not alter conception rates to second insemination for treated and control cows (67.4% vs 63.2%, respectively. The trial showed that the CIDR device as a post-insemination treatment can substantially reduce variation in return to service intervals.

One seasonal herd with low fertility indices was selected to test a regimen including synchronization of oestrus and fixed time insemination using PGF (11 days apart) together with pregnancy diagnosis using a progesterone assay in milk, in samples collected 23-24 days after insemination (Eddy, 1983). The interval from calving to conception and the culling rate were reduced, and the conception rate was improved.

PGF products (Cloprostenol and Lutalyse) were used in a clinical trial consisting of biweekly herd health visits to six dairy herds. The objective was to reduce time for oestrus detection (Belschner, 1986). All cows were palpated and PGF injection was given if a CL was present. The results of the programme were compared with historical data from the preceding 12-month period. A reduction in days open and services per conception, and increased conception rate at first AI were obtained. In addition the culling rate was reduced.

A model for using PGF treatment in dairy herds was described by Britt (1977). The model utilized a scheme of grouping all cows which calved in a 3 week period and treating them as a single management unit. Cows failing to conceive at the timed AI will usually return to oestrus about 3 weeks later.

A dairy herd reproductive management system using reproductive tract examinations at 3 week intervals, with or without concurrent synchronization of oestrus with PGF was evaluated. Cows in treated herds, were injected when they were more than 40 days postpartum. Also, PGF was given to cows after 82 days postpartum if they were not observed in oestrus in control visited herds. This management system was evaluated for a period of 6 months in treated herds, visited control herds and unvisited control herds (Washburn and Dailey, 1987). Routine synchronization of oestrus did not enhance herd reproductive efficiency compared with no synchronization of oestrus in the visited herds.

Management Systems for Synchronization of Oestrus in Cattle utilizing PGF at Various Dose Rates.

Since 1972, when it seemed that the luteolytic property of PGF could be used to synchronize oestrus and ovulation in various species including cattle, several management systems have been investigated involving 1 or 2 injections of PGF at various dose levels, or different routes of administration. PGF has been administered to regulate the bovine oestrous cycle by intrauterine (Shelton, 1973; Tervit et al., 1973; Louis et al., 1974; Moore, 1975), intramuscular (Lauderdale et al., 1974; Roche, 1974; Hafs et al., 1975), intravaginal (Louis et al., 1973), or intravulvosubmucous routes (Ono et al., 1982).

In one of these studies, cows with a palpated CL were subjected to an intravulvosubmucous injection of PGF at 3 different doses (Ono et al., 1982). Oestrous response and subsequent fertility were compared for different doses. No difference was found in the proportions of cows showing oestrus within 3 days after each treatment, and the pregnancy rates 60-90 days post insemination were similar among the various doses of PGF. In a second study, heifers and dairy cows were subjected to an intravulvosubmucous injection of PGF at ½ and ¼ of the dose usually given by intramuscular injection. Two injections of PGF were administered 11 days apart to the animals not detected in oestrus after a single injection. The difference in the mean interval from injection to the onset of the oestrus between groups was not significant.

Lauderdale (1972) and Lauderdale et al. (1974) have described management systems which involved 2 injections of PGF at an 11 day interval with cycling cattle. Inseminations were made after detection of oestrus or with inseminations at approximately 80 h after the second injection. The dose range was 0, 5, 15, 25, or 35 mg of PGF per injection. There was no difference in oestrous response to the 15, 25, or 35 mg doses of PGF.

A synchronization system used by Donaldson (1980) in Australia was a 10 day management system, using twice-daily oestrus detection and an injection of PGF on day 5 of the breeding programme in cows which were not detected in oestrus, and were not inseminated during the first 5 days. There were no differences between dose rates of PGF (8, 15, 20 mg) in initiating an oestrous response with this system.

In a later trial, 2 dose rates of PGF (12.5-mg, 25-mg) and 2 management systems for the synchronization of oestrus in cattle were compared in 3 dairy and beef heifer herds (Donaldson et al., 1982). No difference was found in oestrous response between herds or breeds, or in the oestrous response produced by the two doses of PGF. The 10 day, 1 injection management system produced a 21% better oestrus response than that produced by the 2 injection system (11 days apart). The interval from injection to oestrus after 1 PGF injection was shorter than after 2 PGF injections.

These reports show that selective use of a reduced dose of PGF (½ of the dose) could be effective for the regression of the CL and be more economical.

Effect of Treatment with Progesterone on Fertility.

Normal luteal function is essential for establishment and maintenance of pregnancy in cattle (Estergreen et al., 1967). A functional CL must be maintained for up to approximately 215 days of gestation before the adrenal and/or placenta is able to produce adequate amounts of progesterone for the maintenance of pregnancy (Sachs, 1984). As a consequence, insufficient circulating progesterone has been associated with infertility in cattle (Garverick and Smith, 1986). Premature luteolysis during gestation usually results in abortion, except in species in which the placenta becomes the major source of progesterone (Auletta et al., 1988). Systemic progesterone concentrations early after insemination may (Hansel et al., 1978; Lukaszewska and Hansel, 1980) or may not (Echternkamp and Maurer, 1983) be related to pregnancy maintenance. In addition, progesterone concentration during the pre-breeding oestrous cycle may be involved in pregnancy maintenance (Folman et al., 1973; Fonseca et al., 1983).

The increase of progesterone has been reported to be similar between days 4 and 8 of the oestrous cycles in cycling animals, and inseminated cows which were subsequently confirmed pregnant or which returned to service (Lamming et al., 1989). Shemesh et al. (1968) also reported similar plasma progesterone concentrations during days 10 to 18 after ovulation in cycling and inseminated cows whether the latter were pregnant or not. In contrast, other reports maintained that pregnant animals have higher concentrations of progesterone than cycling animals, and that the increase of progesterone started on day 14 of the oestrous cycle and continued until day 18 of the cycle (Hansel, 1981), or started as early as day 9 of the cycle (Henricks et al., 1972).

Because this infertility contributes to reproductive losses in dairy cattle reproduction (Diskin and Sreenan, 1986), several attempts have been made to improve pregnancy rates with the use of progesterone directly, such as in the form of oral administration, injections, implants, sponges, and controlled releasing devices. Others have used indirect methods (or luteal stimulation) where certain antiluteolytic substances have been administered to increase or maintain luteal function. Both direct and indirect treatments have been carried out at varying times following insemination in order to increase conception rates; most of the treatments have been given at the time of initiation or during the luteal phase of the oestrous cycle. Some of the treatments have also been given at or immediately after insemination.

Indirect supplementation of progesterone can be given in the form of substances such as human chorionic gonadotrophin (HCG), and gonadotrophin releasing hormone (GnRH), which cause luteal stimulation and/or result in accessory CL formation. These treatments have sometimes been shown to increase peripheral progesterone concentrations during the luteal phase of the oestrous cycle in cow. In some situations these treatments have tended to result in an improvements of the female reproductive performance. Treatment with HCG can be luteotrophic in the bovine and have therefore been used to stimulate luteal function in the early post-insemination period (Holness et al., 1982), or when HCG is administered during the early or mid-luteal phases as a luteotrophic substance. Therefore, the effect of the HCG treatment influences pregnancy rate,

either directly or indirectly through an increased production of progesterone. This treatment has also been used at around the time of luteolysis to supplement the action of a possible inadequate luteotrophic or antiluteolytic signal from the developing embryo (Christie et al., 1979). Treatment with HCG in cattle sometimes produce small improvements in conception rates (Holness et al., 1982; Greve and Lehn-Jensen, 1982; Santos-Valadez, 1982; Sreenan and Diskin, 1983; Helmer and Britt, 1986; Lewis et al., 1990).

The administration of GnRH is another method that has been used to increase conception rates possibly, by reducing early embryonic mortality. Treatment has been given at the time of insemination and also post-insemination. It has also been used to increase fertility in cows (Lee et al., 1983; Macmillan and Taufa, 1983; Aboul-Ela and El-Keraby, 1986; Macmillan et al., 1986b; Phatak et al., 1986). However, latest reports do not support the use of GnRH 15 days after AI (Lewis et al., 1990), by altering either timing of AI or hormone injection relative to the onset of oestrus (Mee et al., 1990) as method to enhance conception rates in dairy cattle. The exact mode of action that GnRH has in increasing fertility is not known. It may possibly produce dose-related increases in serum concentrations of LH in cattle (Fernandes et al., 1978; Macmillan et al., 1985d). The action of GnRH when administered during the mid-luteal phase of the oestrous cycle after insemination is to stimulate the function of the CL either directly or indirectly (Macmillan et al., 1985c; McMillan et al., 1986). GnRH has been shown to increase the length of the oestrous cycle through prolonging the lifespan of the CL, and progesterone production. This increases the probability that maternal recognition of the presence of a developing embryo will occur (Macmillan et al., 1986b). The injection of GnRH during dioestrus in cows has also been shown to influence CL and progesterone synthesis, but the precise action of the drug has not been defined (Macmillan et al., 1985c). Low-dose GnRH treatment may produce an effect which modifies the induced luteolysis (Macmillan et al., 1985d). Alternatively, the GnRH treatment may produce an LH release which has a luteoprotective effect (McMillan et al., 1986).

The findings of previous studies of direct progesterone supplementation in cows and its effects on increasing the pregnancy rates through increased embryonic survival rates suggests a tendency for progesterone supplementation to increase pregnancy rates of cows. This is particularly noticeable in repeat breeder or low fertility cattle. Sreenan and Diskin (1983) had shown no effect on pregnancy rate in cows. However, others researchers found a positive effect on pregnancy rate (Wiltbank et al., 1956; Johnson et al., 1958; Marcus and Ayalon, 1981; Shemesh et al., 1981; Folman et al., 1983; Diskin and Sreenan, 1986; Macmillan and Taufa, 1987b; Robinson et al., 1989; Macmillan et al., 1990a). In this last study, the beneficial effects of progesterone supplementation were specific to the period from 6 to 8 days after insemination.

Anoestrum.

Postpartum anoestrum and oestrus detection efficiency are the major factors affecting rates at which cows in seasonal dairy herds are submitted for artificial insemination (Macmillan and Watson, 1973). Improved oestrus detection through the use of tailpainting has emphasised the impact of anoestrum on a seasonal herd's breeding programmes, as for example in New Zealand (Macmillan, 1988b). Non-cycling cows with no post-partum ovarian activity have extended calving intervals in year-round herds. Therefore, it affects production per cow per year and production per lactation. This condition is the major factor contributing to differences in submission rates (SR) in seasonally calving dairy herds. SR is defined as the percentage of cows in a herd presented for insemination during a period of time in the AI programme. Therefore, a successful treatment for prolonged postpartum anoestrum would be a major breakthrough in the reproductive management of these animals because high SR's are required to maintain concentrated calving patterns (Jubb et al., 1989).

A wide range of treatments have been used with these anoestrous cows in an attempt to induce oestrus and ovulation. They include injections of vitamins and minerals, utero-ovarian massage and hormonal treatments with oestrogen or GnRH (Macmillan and Day, 1987a). Some recommendations have included the adjustment of calving date to suit pasture growth patterns, early mating of maiden heifers, better feeding of younger cows during the postpartum period, and inducing cows to calve close to the start of the calving season (Macmillan and Day, 1987a). The association of stress with high production levels in early lactation, combined with reduced appetite and loss in body condition contribute to a period after calving when there is no normal ovarian activity producing oestrus and ovulation. Moreover, this condition can be increased by underfeeding both before and after calving or by diseases (Macmillan and Day, 1987a).

One early report showed that treatment with a progestagen sponge for 7 days, and 1000 IU of PMSG at sponge removal produced a 4-week pregnancy rate of 38% (Fielden et al., 1976). The initial trial which used a CIDR device for 7 days with PMSG at removal, demonstrated an increased incidence of oestrus within 7 days of CIDR removal. It was 85% in treated cows vs 22% for control cows being detected and inseminated within 14 days after initial diagnosis. The fertility of the post-treatment oestrus was normal (Macmillan and Day, 1987a).

A second trial compared the use of a CIDR device for 7 or 10 days, using the same dose of PMSG at CIDR removal. Results showed that the oestrous response and pregnancy rates were similar in both treated groups, with 77% and 59% in 7 day CIDR group vs 75% and 37% in the 10 day CIDR group. The conception rate in untreated cows was 70% (Macmillan et al., 1990).

This trial also demonstrated that anoestrous cows which failed to respond to the CIDR/PMSG treatment should be re-examined about 14 days after CIDR removal, and then differentially treated either with PGF (if they had ovulated), or re-treated with a new or previously used CIDR plus PMSG at device removal.

Oestrus Detection.

Inefficient oestrus detection has been found to be a major factor contributing to calving intervals in excess of 365 days in all the recognised dairying industries in the world, with a consequent reduction in production and profit. For the dairy farmers using AI, successful implementation of the breeding programme depends to a large extent in accuracy of oestrus detection. Accurate oestrus detection is necessary to achieve a high SR, a compact breeding period in seasonal herds and to maintain an optimum calving interval distribution in year-round herds (O'Farrel, 1984). The consequences of inefficient oestrus detection and the importance of optimum calving intervals have been well documented (Esslemont, 1974; Foote, 1975; Stevenson and Britt, 1977). The importance of having a high SR, which is in turn dependent on thorough and accurate detection of oestrus has been reported for a single, seasonally concentrated calving period (Macmillan, 1988b).

The economic implications related to oestrus detection have also been reported (Oltenacu et al., 1981; Bailie, 1982). Williamson et al.(1972) showed how extended calving intervals may be the result of problems with oestrus detection rather than because of anoestrus cows. Inter-service intervals greater than 38 days are common, even in herds with reproductive management programmes (Washburn and Daily, 1987). However, in one study it was concluded that the herd health management programme significantly improved the recorded occurrence of oestrus (N. Williamson, personal communication).

It is essential that herdsmen should be familiar with signs of oestrus, so that they can accurately select cows which are in oestrus. It is defined as that period during which a cow will stand to be ridden by her mates or by a herd sire. Mounting behaviour of dairy cattle varies with the stage of the oestrous cycle, type of animal, status and number of animals simultaneously in oestrus. These oestrous cows tend to congregate together, forming sexually active groups which are restless and move throughout the herd, with other cows joining or leaving them (the riding activity increases within the group). On many farms, especially in large herds, there are major difficulties in implementing more effective oestrus detection, and as a result many oestrus periods are missed (Macmillan, 1985b).

Failure to improve the rate of detection of oestrus is the most significant factor frustrating improvements in reproductive efficiency. For this reason, the challenge is to produce a simple inexpensive detection method to attract the attention of stockman towards animals most likely to be in oestrus. This system should allow animals to be distinguished (visually if possible) from those which are pregnant, have recently calved, or have recently been inseminated (Macmillan, 1988c).

The introduction of tailpainting for efficient breeding management in New Zealand has reduced differences in non-return rates to first insemination associated with herd size, mainly because of a reduction in the incidence of detection errors in large herds. Moreover, the improved oestrus detection has contributed to an increase in non-return rates (Macmillan and Curnow, 1977; Macmillan, 1988b).

OBJECTIVES

The objectives of the proposed experiment were:

a) To evaluate a management system for dairy herds involving control of the oestrous cycle, to reduce the time commitment required for detection of oestrus and consequently improve oestrus detection rate, and to reduce the variation in return to service intervals.

b) To evaluate oestrous responses to different dose rates of prostaglandins combined with CIDR devices in cycling animals, and the possible effects of treatment with progesterone on fertility and plasma progesterone concentrations in cattle.

MATERIALS AND METHODS

The study was carried out in four commercial (4) town-supply dairy herds in the Manawatu area of New Zealand. It involved the use of controlled internal drug release devices (Eazi-Breed CIDR^{tm}-B, Carter Holt Harvey Plastic Products, Hamilton. N.Z.). Each device consisted of a silicone elastomer impregnated with 1.9 g progesterone. This was combined with the strategic use of PGF (Estrumate, Coopers, Upper Hutt. N.Z.) and Pregnant Mare Serum Gonadotrophin (PMSG, Folligon, Intervet Chemavet Distributors Ltd, Auckland. N.Z.).

Experimental Design.

The following protocol was used with the treatment group in each herd:

Day 0: Wednesday, All cows which had calved at least 35 days and were identified to start the programme were examined by an experienced veterinarian before being randomly divided into three sub-groups (defined below), based on calving date. Each cow had a CIDR device inserted and was tail-painted.

Day 10: Saturday, CIDR's were removed. The cycling cows (ovaries of good size and with palpable structures) were injected with either the recommended luteolytic dose of Estrumate (500 μg i.m. Estrumate, group TF) or half the dose of PGF (250 μg i.m. Estrumate, group TH). Raddle was applied over the tailpaint. The non-cycling cows (ovaries were small, hard and without any palpable structures) received PMSG dose (400 I.U. i.m. Folligon), plus raddle over the paint strip.

Day 12-14: Monday-Wednesday. Cows were inseminated on detected oestrus. It was expected that 80-90% of cows would be inseminated during these 3 days.

Day 28: Wednesday. A used/washed/stored CIDR was re-inserted into each cow, and tailpaint re-applied.

Day 33: Tuesday. CIDR's were removed and the paint strips were also re-raddled.

Day 35-36: Thursday-Friday. The second period of artificial insemination on oestrus detection commenced and was completed within two days.

In a herd with a year-round milking system, this programme meant that a new group of cows would enter the breeding schedule on every fourth Wednesday. This time coincided with CIDR re-insertion for cows from the previous month (Figure 3.1).

This regimen started on three farms in the same month; the fourth farm

started at a different time because it had cows calving only for a restricted period each autumn and spring. The study period in each farm was 10 months, with a half of the cows being in the treatment group.

Experimental Procedure.

This regimen was designed to improve oestrus detection, by reducing time spent on this task, by producing less variation in the post-treatment interval to oestrus, by stimulating a fertile oestrus in anoestrous cattle, and by developing a method by which most of the cows in the herd would conceive within a defined interval postpartum. Reproductive and production records were collected for this period of time.

In this treatment regime, all the cows entered the study when they were between 45 and 72 days postpartum, and at a fixed date in a 4-weekly cycle. The treatment started when treated cows were examined at 36 ± 7 days postpartum. Control cows were also examined rectally to determine the progress of uterine involution and the nature of ovarian structures.

Throughout the experiment, treatment and control cows were observed for signs of oestrous behaviour twice daily at approximately 12 h intervals, with each observation period lasting at least 20-30 minutes in conjunction with tailpainting (Macmillan et al., 1988a). Observation periods did not coincide with feeding and milking. Paint and raddle of different colours were used to identify individual groups of cows.

Although oestrus was defined as that period during which a cow stood to be ridden by herd mates or by a herd sire, they were also considered to be or have been in oestrus when the paint and raddle were rubbed off (observation periods during milking). All cows with loss of paint and raddle, or displaying oestrus were inseminated.

Following detection of oestrus, both treatment and control groups were inseminated in accordance with normal farm practice (ie. artificial insemination normally on the morning after first detection).

Diagnosis of pregnancy was by rectal palpation of the uterus at about 6 weeks following the insemination date.

Control Group.

The cows were managed and inseminated following to standard procedures used in each herd. Insemination of the control cows commenced at the first oestrus from 45 days postpartum.

Cows in the control group which were not observed in oestrus by day 120 postpartum and cows that failed to conceive from previous inseminations were examined, and then treated appropriately.

Progesterone Determination.

Three blood samples were collected for progesterone assay. Ten (10) ml of blood was collected by coccygeal venipuncture into heparinized tubes (NIPRO-New Tube System, Vacuum Blood Collecting System, Nissho Corporation, Osaka Japan). Samples were centrifugated within 3 h at 3000xg for 20 minutes, and plasma collected and stored at -20 °C for subsequent assaying for progesterone.

The summary of the blood sampling regime is as follows:

i) sample 1 was taken when a new CIDR was removed;

ii) sample 2 was taken when the re-used CIDR was removed; and

iii) sample 3 was taken at the morning after the re-used CIDR was removed.

This strategic sampling sequence was used to check the variation in progesterone concentrations at critical stages during the treatment programme.

Hormone Assay.

Plasma concentrations of progesterone were measured by the method of Kirwood et al.(1984).

Determinations were made on 500 µl sub-samples from each original plasma sample. They were extracted with 5 ml toluene:hexane (1:2 v/v). The solvent-plasma was frozen overnight, and the solvent supernatant then decanted into clean tubes, dried under air and redissolved in 500 µl ethanol. Duplicate 100 µl samples of ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 µl samples of standard ethanolic solutions of progesterone (P-1030: Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone level; of 0-20 ng/ml. A mixture containing antiserum (courtesy of Dr J. T. France, National Women's Hospital, Auckland, New Zealand) at a final dilution of 1:1800 (Tungsubutra & France, 1978); [1,2,6,7-H] progesterone (TRK 413, Amersham, Bucks, U.K.) at 8000 c.p.m./100 µl; phosphate-buffered saline containing 0.02m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 µl) to each tube and vortexed. After overnight incubation at 4 °C, 600 µl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and, then incubated at 4 °C for 10 minutes. Tubes were then centrifugated at 3000xg for 10 minutes at 4 °C. The supernatant was decanted into scintillation vials and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Assay sensitivity was 0.08 ng/ml. Intra-assay CV's were 16%, 12.1, and 10.5, and inter-assay CV's were 13.7%, 8.9, and 11% for plasma pools containing mean progesterone concentrations of 2.5, 5.8, and 10.9 ng/ml,

respectively (N = 12).

Statistical Analysis.

Data were analyzed using the Panacea database management program (PAN Livestock Services Ltd. Department of Agriculture, University of Reading, P.O. Box 236, Reading, Berkshire, England).

Variation in the degree of synchronization of oestrus among groups was evaluated by Chi-squared analysis. Oestrous responses to treatment were evaluated and grouped by percentage of cows inseminated each day during 1-2-3 days after device removal (the first 3-day period after synchronization), cows inseminated during the first and second oestrus after synchronization (3 day-period and 2 day-period, respectively), and the differences due to stage postpartum at treatment (early, $\leq$ 60 days versus late, $>$ 60 days). Chi-squared analysis were used as well as to evaluate the variation in conception rates (%) of control and treatment groups. Conception rates at first and second inseminations, services per conception and pregnancy rates were also analyzed.

Differences in measures of reproductive efficiency between the control and treatment groups were evaluated by analysis of variance including intervals (days) from Planned Start of Mating (PSM) to first AI, PSM to conception, and interval between first AI and conception. These analyses were done using groups means rather than individual cow data. The first day of Planned Start of Mating (PSM) for treatment and control groups, was taken as the day when a new CIDR device was inserted into each cow in a new treatment group.

Differences in progesterone concentrations between the groups were evaluated by analyses of variance.

RESULTS

Herd Reproductive Efficiency.

1. Farm A:

Measures of reproductive efficiency are presented in Table 3.1.

The mean interval from PSM to first AI was 10.7 days less among cows in the treatment group (27.9 vs 38.6 days ; $P< 0.01$; Table 3.1), but the difference in PSM to conception was only 6.3 days because the interval from first AI to conception was 4.5 days longer ($P> 0.10$) in this group (26.6 vs 22.1).

The longer interval from first AI to conception in the treatment group was associated with lower conception rates to first and second inseminations (49% vs 58%; and 62% vs 74%; $P> 0.10$), and consequently more services per conception (1.9 vs 1.7; $P> 0.10$; Table 3.1).

Similar patterns occurred among the cycling cows in this herd (Table 3.1), except that the interval from PSM to first insemination was 11.1 days less in the treated group and this difference was significant ($P< 0.01$).

2. Farm B:

Measures of reproductive efficiency are presented in Table 3.2.

The mean interval from PSM to first AI was 11 days less among cows in the treatment group (24.8 vs 35.8 days; $P< 0.01$; Table 3.2), but the difference in PSM to conception was only 5.3 days because the interval from first AI to conception was 4.3 days longer ($P> 0.10$) in this group (13.6 vs 9.3).

The longer interval from first AI to conception in the treatment group was associated with lower conception rates to first and second inseminations (58% vs 78%; $P< 0.05$, and 52% vs 73%; $P> 0.10$), and consequently more services per conception (1.6 vs 1.4; $P> 0.10$; Table 3.2).

Similar patterns occurred among the cycling cows in this herd (Table 3.2), except that the interval from PSM to first insemination was 12.4 days less in the treated group and this difference was significant ($P< 0.01$), the conception rate to first insemination was 60% in the treatment group vs 80% in the control group ($P< 0.05$), and the number of services per conception differed significantly (1.67 vs 1.3; $P< 0.10$; Table 3.2).

3. Farm C:

Measures of reproductive efficiency are presented in Table 3.3.

The mean interval from PSM to first AI was 11.6 days less among cows

in the treatment group (27.1 vs 38.7 days; P< 0.01; Table 3.3). There was a significant difference in PSM to conception (62.1 vs 76.9 days; P< 0.10). The interval from first AI to conception was only 1.2 days shorter (P> 0.10) in this group (31.7 vs 32.9).

The treatment group had lower conception rates to first and second inseminations (43% vs 50%, and 60% vs 61%; P> 0.10), and consequently a higher figure for services per conception (2.1 vs 1.9; P> 0.10; Table 3.3).

Similar patterns occurred among the cycling cows in this herd (Table 3.3). The reduction in interval from PSM to first insemination of 12.9 days was also significant at a higher level of probability (P< 0.01).

4. Farm D:

4. 1. Measures of reproductive efficiency in the autumn-calving herd are presented in Table 3.4.

The mean interval from PSM to first AI was 2.8 days less but not statistically significant in the treatment group (9.1 vs 11.9 days; P> 0.10). However, the interval from PSM to conception was 8 days longer (P> 0.10) in the treatment group (29.8 vs 21.8 days). The mean intervals PSM to first AI and to conception were similar for early and late calvers (Table 3.4).

Early and late calvers had similar conception rates to first inseminations (44% and 67% vs 54% and 60%; P> 0.10). Similar patterns occurred among early and late calvers with the second inseminations (Table 3.4).

Similar patterns occurred among the cycling cows in this herd (Table 3.4), except for early calvers where the interval from PSM to first insemination of 7.4 days was significant at a higher level of probability (P< 0.01).

4. 2. Measures of reproductive efficiency in the spring-calving herd are presented in Table 3.5.

The mean interval from PSM to first AI was 9.4 days less among cows in the treatment group (21.1 vs 30.6 days; P< 0.10). The interval from PSM to conception was 3.4 shorter (P< 0.10) in the treatment group (35.6 vs 39 days). The mean interval PSM to first AI differed significantly in early calvers between groups (17.5 vs 28.5 days; P< 0.01; Table 3.5). However, the mean interval from PSM to conception in early calvers was similar between groups. No differences were found in these parameters between groups among late calvers.

Conception rate to first insemination differed significantly between groups. Treated cows were 43%, compared with 63% for cows in the control group (P< 0.10). However, the conception rate to second insemination was 43% in treated cows vs 18% in control cows (P< 0.01; Table 3.5).

Similar patterns occurred among the cycling cows in this herd (Table 3.5), except for early calvers where the interval from PSM to first insemination was 10.2 days less in the treated group and this difference was significant (P< 0.01).

Herd reproductive efficiency in " Town-milk " herds.

Measures of Reproductive efficiency are presented in Table 3.6.

The mean interval from PSM to first AI was 11.1 days less for cows in the treatment group (26.6 vs 37.7 days; P< 0.01; Table 3.6). Also, the interval from PSM to conception differed in both groups (P< 0.10), being 54.7 days in treatment group vs 62.7 days in control group. The interval from first AI to conception was 3 days longer in treated cows (P> 0.10) in this group (28 vs 25).

The longer interval from first AI to conception in the treatment group was associated with lower conception rates to first and second inseminations (50% vs 62%; P< 0.01, and 59% vs 67%; P> 0.10), and consequently more services per conception (1.9 vs 1.7; P< 0.05; Table 3.6).

The final pregnancy rate was similar in both groups (92% vs 92.1% for treatment vs control).

Similar patterns occurred among the cycling cows in the whole group (Table 3.6), except that the conception rate to first insemination and number of services per conception were significant at a higher level of probability (P< 0.001), and the interval from PSM to conception was no longer significant (54.8 vs 61.2 days).

The final pregnancy rate in treated cows was 91% compared with 93% in control cows.

Anoestrum in Lactating Dairy Herds.

Town supply herds.

Measures of reproduction efficiency in non-cycling cows are presented in Table 3.7.

The difference between the treatment and control groups in the mean interval PSM to first AI was 10 days (36 vs 46.7 days; P> 0.10). The mean interval from PSM to conception differed between groups to a mild extent (P< 0.10). For the treatment group it was 54.2 vs 77 days in the control group. The mean interval from first AI to conception in the treatment and control groups was 12 days (18.2 vs 30.2 days; P> 0.10).

Conception rates to first and second inseminations were similar between treatment and control groups (64% and 71% vs 60% and 50%, respectively). The mean number of services per conception in treated cows (1.65) was significantly less than in control cows (2.25; P< 0.10). The overall pregnancy rate was 94 % in treated cows compared with 80 % in the control cows (P< 0.01).

There were 51 (8.5%) animals from among 435 dairy cows, which were

diagnosed as non-cycling. Only 53 % of the CIDR treated anoestrous cows were mated after treatment, although the percentage detected in oestrus varied from one herd to another. The range between farms was from 40 to 63 %. Another 24% of the treated cows ovulated, but were not seen in oestrus. These cows were inseminated within 5 days of re-used CIDR removal. The variation in response patterns, both between and within herds was great. On average, 18 % of the non-cycling cows were 4 and 5 years old, 15 % 3 and 4 years old, and 13 % between 2 and 3 years old. The rest of non-cycling animals (54%) were from 5 to 10 years old.

Autumn-Spring calving herd.

Measures of reproductive efficiency in non-cycling cows are presented in Table 3.8.

The mean interval from PSM to first AI was 4.1 days longer among cows in the treatment group (25.1 vs 21 days; $P > 0.10$), but the difference in PSM to conception was 57.8 days longer in the treated cows (90.4 vs 32.6 days; $P < 0.05$; Table 3.8). Conception rates to first and second inseminations were lower in treatment group (21% and 35% vs 43% and 42%; $P > 0.10$), and they consequently had more services per conception (3.3 vs 1.6; $P < 0.05$; Table 3.8)

Of 173 cows, 11 cows (12.7 %) were diagnosed as non-cycling. There were 71 % of the CIDR treated anoestrous cows inseminated after treatment.

Oestrous Response after Synchronization by using CIDR-B, PGF and PSMG.

When the oestrous response was grouped for percentages of cows responding to the first and second periods of artificial insemination, without regard to each individual cow being in the treatment or control groups, the period of artificial insemination for control groups started when the new CIDR device was inserted, and finished with the second period of artificial insemination in the treatment groups while those cows in the treatment groups responding outside of the 2 periods (5-day period) of artificial insemination were considered out of synchrony.

The percentage (%) of cows in oestrus and inseminated in the treatment and control groups during the insemination periods are presented in Table 3.9. Percentage total (%) of cows in oestrus and inseminated in the treatment groups during the first 3-day period of AI are presented in Table 3.10.

1. Farm A:

The standard treatment programme was run 9 times in the 10 month-period. Treated cows ($n = 53$) had greater synchrony than control cows ($n = 49$), ($P < 0.05$), as the percentage of cows in oestrus and inseminated for 1 or 2 services in the 5 day-period was 79% versus 59%, respectively. The degree of synchronization of oestrus among treatment groups was between 100 and 57%. However, the degree of synchronization of oestrus among control groups was between 100 and 20 %.

Overall, 60% of cycling and non-cycling cows were in oestrus and inseminated between 48-96 h after CIDR removal, with 26% for the first day, 17% for the second day and 17% for the third day of this period.

Comparable figures for cycling cows were 65% in oestrus and inseminated between 48-96 h after CIDR removal, with 28%, 19% and 17% on the first, second and third days, respectively. All 53 cows in treatment group were inseminated, whereas 2 of 49 cows in the control group were not inseminated

2. Farm B:

The standard treatment programme was also run 9 times in the 10 month-period. Treated cows (n = 69) had greater synchrony than control cows (n = 55), ($P < 0.05$), as the percentage of cows in oestrus and inseminated for 1 or 2 services in the 5 day-period was 71% versus 56%, respectively. The degree of synchronization of oestrus among treatment groups was between 100 and 45 %. However, the degree of synchronization of oestrus among control groups was between 100 and 18%.

Overall, 59% of cycling and non-cycling cows were in oestrus and inseminated between 48-96 h after CIDR removal, with 36% for the first day, 9% for the second day, and 14% for the third day of the period.

Comparable figures for cycling cows were 63% in oestrus and inseminated between 48-96 h after CIDR removal; and 35%, 10% and 17% on the first, second and third days, respectively.

Whereas 2 of 69 cows in the treatment group were not inseminated, 3 of 55 cows in the control group were not.

3. Farm C:

The standard treatment programme was run 9 times in a 10 month-period. Treated cows (n = 114) had greater synchrony than control cows (n = 93), ($P < 0.06$), as the percentage of cows inseminated for 1 or 2 services in a 5 day-period was 70% versus 54%, respectively. The degree of synchronization of oestrus among treatment groups was between 100 and 40 %. However, the degree of synchronization of oestrus among control groups was between 85 and 33%.

Overall, 55% of cycling and non-cycling cows were in oestrus and inseminated between 48-96 h after CIDR removal, with 22% for the first day, 25% for the second day and 7% for the third day of the period.

Comparable figures for cycling cows were 65 % in oestrus and inseminated 48-96 h after CIDR removal; and 25%, 29% and 10% on the first, second and third days, respectively.

Of 114 cows in the treatment group, only 1 cow was not inseminated compared with 3 of cows in the control group.

4. Farm D:

4.1. The Autumn-calving season:

The percentage of early calvers which were treated (n = 28), and which were inseminated for 1 or 2 services in the 5 day-period was 85% vs 95% for the control (n = 27) cows, while treated later calvers (8) had 89% vs 80% for the control (7) cows (P> 0.10).

4.2. The Spring-calving season:

Early calvers in the treatment (n = 42) group had greater synchrony than those in the control (n = 44) group (83% vs 63%; P< 0.05). However, in late calvers the difference was not statistically significant (66% vs 55%; P> 0.10).

The mean for the total treatment group among the autumn calvers was 87%. It was a similar total in the control group (87%). However, the treatment group in spring calvers achieved 75% synchrony compared with 59% in the control group (P< 0.05).

In autumn calvers, 94% of cycling and non-cycling cows were in oestrus and inseminated 48-96 h after CIDR removal, with 45% on the first day, 27% for the second day and 21% for the third day of the period. There were 93% of cycling cows in oestrus and inseminated 48-96 h after CIDR removal, with 45%, 26% and 22% on the first, second and third days, respectively.

In spring calvers, 63% of cycling and non-cycling cows were in oestrus and inseminated 48-96 h after CIDR removal, with 32% for the first day, 16% for the second day and 14% for the third day of the period.

There were 68% of cycling cows in oestrus and inseminated 48-96 h after CIDR removal, with 34%, 19% and 14% on the first, second and third days, respectively.

All cows in the treatment and control groups were inseminated.

General Oestrous Response after Synchronization.

The oestrous response for the treatment groups in the four farms (n = 324) averaged 76% as compared with 63% in the control groups (n = 284; P< 0.001; Table 3.9). Between 48 and 96 h after CIDR removal, 67% were inseminated, with 32%, 19% and 21% on the first, second and third days of the period, respectively.

There were 71% of cycling cows in oestrus and inseminated between 48-96 h after CIDR removal, comprising 33%, 21% and 16% on the first, second and third days, respectively.

Within the treated groups, the postpartum interval at treatment did not

affect the number of cows observed in oestrus after the 2 periods of artificial inseminations.

Oestrous Response and use of Full and Half Dose of Prostaglandins at CIDR-B Removal.

Oestrous responses to the two PGF doses were evaluated on individual cows rather than within each herd. Oestrous responses were grouped by the percentage responding within the 3 days of the first period of artificial insemination. Therefore, those cows responding after the first period of artificial insemination were considered to be out of synchrony.

The effects of dose rates of PGF at CIDR removal on synchrony and fertility in cows are presented in Table 3.11.

A 2 ml (500 μg) and 1 ml 250 μg) dose of PGF as Estrumate at CIDR removal did not affect the number of cows observed in oestrus during the first period of artificial insemination (3 days; 68% vs 72%).

The postpartum interval at treatment did not affect the proportions of cows in oestrus. Conception rates for first inseminations were similar (44% versus 48%).

Effect of Treatment with Progesterone on Pregnancy Rate.

Within each treatment group, all cows had a used CIDR device re-inserted by day 17-19 post insemination. The control cows inseminated over the second period of AI were used as contemporary control cows.

Conception rate from second inseminations was not statistically different for treatment and control groups (n = 84, 76, respectively; 50% vs 58%; Table 3.12).

Plasma Progesterone.

Progesterone levels of non-pregnant and pregnant cows treated with CIDR/PGF and CIDR/PMSG are presented in Table 3.13.

Plasma progesterone concentrations in non-pregnant cows (n = 50) which had previously received CIDR/PGF was 3.1 ng/ml at new CIDR removal, 2.3 ng/ml at re-used CIDR removal and 0.3 ng/ml one day after the re-used CIDR had been removed. However, progesterone concentrations of pregnant cows (n= 50) which had received the same treatment was 4.1 ng/ml at new CIDR removal, 8.7 ng/ml when the re-used CIDR was removed and 7.1 ng/ml one day after the re-used CIDR had been removed. The progesterone levels differed significantly between both pregnant and non-pregnant cows at the last two sampling ($P < 0.01$), but not at the first sampling.

Plasma progesterone concentrations of non-pregnant cows (n = 26) which

had received CIDR/PMSG was 2.8 ng/ml at new CIDR removal, 2.6 ng/ml at re-used CIDR removal and 0.3 ng/ml one day after the re-used CIDR had been removed. However, progesterone concentrations of pregnant cows (n = 33) which had also received the same treatment was 5.0 ng/ml at new CIDR removal, 9.3 ng/ml at re-used CIDR removal and 8.4 ng/ml one day after the re-used CIDR had been removed. Progesterone levels differed significantly between both pregnant and non-pregnant cows at the last two samplings ($P < 0.01$), but not at the first sampling.

Levels of progesterone for non-pregnant and pregnant cows which had received CIDR + PGF or CIDR + PMSG were similar. Similar levels were obtained for pregnant cows with different treatments.

DISCUSSION

The main objective of this study was to evaluate a management system involving systematic control of the oestrous cycle, in order to facilitate detection of oestrus and minimize the variation in returns to oestrus after treatment.

The strategic use of CIDR plus PGF or PMSG in lactating dairy cows has the potential to concentrate breeding programmes in seasonal dairy herds and to maintain a 365-day calving interval in year-round herds.

Herd Reproductive Efficiency.

The mean interval from PSM to first insemination was significantly reduced among the treated cows in year-round milking herds (Table 3.6). This difference was seen also in the seasonal milking herd (spring-calving cows; Table 3.5). However, this parameter was only statistically different in cycling early calvers from the Autumn herd (Table 3.4). It is important to emphasise that in herd D during spring, there was a staffing problem which affected oestrus detection efficiency. For this reason, there were differences in the proportions of cows inseminated between seasons.

It was clear that the interval PSM to first AI was shorter in all treated groups. The variation among treated groups within each herd can be explained by several factors:

Oestrus detection rates appeared to be affected by management. Undoubtedly there were differences between year-round and seasonal herds. The importance of the formation and stability of the sexually active groups in the expression of oestrus is well known and, this group activity also influences the effectiveness of aids to oestrus detection (Kilgour et al., 1977). This was seen in herd D with treated cows in the autumn compared with the other three year-round farms; but in these town milk herds, the effect of the treatment was associated with an increase in the detection rate when it is common to find low oestrus detection rates especially during winter months. Another factor was the length of the synchrony period which was necessarily longer to allow the control group to be inseminated during the treatment period. This was seen in farm D between the treatment and control group in the autumn herd (Table 3.9), but in year-round herds, although the percentage of treated cows inseminated during the 5-day period was almost similar, treatment groups had higher percentages ranging from 15%, 16% and 20% between herds compared with control groups (Table 3.9). The seasonal herd also had differences between treatment groups in the different periods of the year.

The mean interval from PSM to conception in year round milking herds was significantly different between groups (Table 3.6). Treated cows had fewer days open than controls, ranging from 5.3, 6.3 to 14.8 days between treated cows in individual herds (Table 3.1, 3.2 and 3.3). No difference was found between

groups in the seasonal milking herd (Table 3.4 and 3.5).

Even though the PSM to first AI was reduced in almost all treated cows, the effect on the interval to conception was less significant because the lower conception rate to first AI increased the interval from first AI to conception. This lower conception rate at first AI was a significantly limiting problem. Although the CIDR treatment together with an injection of PGF or PMSG reduced the PSM to first AI, this management advantage was partly lost because of lower fertility. Moreover, it total increased expense and labour related to the procedure of CIDR insertion, without increasing the pregnancy rate.

The variation among herds was due mainly to decreased fertility at the first AI, and secondly to the overall reproductive efficiency between herds.

Conception rate to first insemination differed statistically between groups, but not for second inseminations. As a consequence, the mean number of services per conception also differed significantly between groups. Although in one year-round herd (herd B) there was a remarkably high conception rate to first AI in the control group, the final results from this study did not change. In fact, the general tendency achieved with this treatment showed that the PSM to first AI was reduced, but the effect on the interval to conception was less significant because the lower conception rate to first AI increased the interval from first AI to conception.

This high conception rate in the herd B means that the aspects which are considered important in AI are of a good standard and quality, and the cows are fertile. Therefore, it is logical to think that the problem in these herds was related to the percentage of cows in a herd presented for insemination during a period of time in the AI programme. One interesting observation in this study was that the percentage of cows detected in oestrus and inseminated throughout the experiment in year-round herds was quite variable, but especially in late winter when the percentage of cows inseminated was low compared with other periods of this study. Moreover, the lowest percentage of cows inseminated during that period was the farm which had the lowest overall reproductive efficiency (Figure 3.2). This finding can be explained as follows:

In early lactation, cows experience energy stress of milk production which coincides with a reduced appetite, and substantial loss in body condition. The mean pasture cover and mean cow condition score were reported to be decreased slightly over winter periods in town milk herds (Baldwin, 1989). Therefore, pasture production during winter months is affected and the farms during this time are not associated with maximum "in situ" pasture utilization in which Stewart (1988) estimated wastage of in situ pasture for June-August milking cows at 25%, 40% and 55% for light, medium and heavy soils respectively. For this reason, possibly at any stage or for a period of time during early lactation some cows have experienced degrees of nutritional stress depressing the external signs of oestrus (N. Williamson, personal communication). This situation can be increased by underfeeding; a previous study reported that undernutrition depressed the occurrence of oestrus by 31% (Juneja and Arora, 1989). However, the problems of detection involving cow, human or environmental factors that could affect sexual behaviour in dairy cows cannot be ignored.

The fertility of the oestrus after treatment in cattle may be reduced even though the progestagen treatment is less than 9 days (Patterson et al., 1989). Many reports using different progestagen treatments showed that this reduced fertility was influenced by the day of the oestrous cycle at treatment initiation (Beal et al., 1988; Van Cleeff et al., 1989; Patterson et al., 1989; Chenault et al., 1990). Therefore, the reduction in fertility is not due simply to the presence of progesterone, but also to an interaction of progesterone present in the latter stages of the cycle (Chenault et al., 1990). There is no explanation for the mechanism responsible for reduced fertility following progestagen treatment. The following facts may be contributing to this reduced fertility: The precision in synchrony is increased with an increase in the duration of the treatment when a progestagen is used. However, as treatment interval is increased, the fertility is decreased from 64% to 37% (Pearce et al., 1990). Reduced fertility at first AI has also been related to an extended inter-oestrus interval (Brink and Kiracofe, 1988), and a reduced fertility was observed when treatment was initiated late ($\geq$ day 12) in the oestrous cycle (Patterson et al., 1989). Moreover, a delayed cleavage rate of fertilized ova was demonstrated when a long term progestagen treatment was used (Wishart and Young, 1974). In cycling animals, control of the timing of ovulation is partly dependent on controlling the time of regression of the CL (Roche and Ireland, 1984). Treatment with a progestagen artificially extends dioestrus until administration is ended. It would suggest that metoestrous animals are likely to have the highest fertility, because those follicles ovulate after shorter periods of growth than in those which have been statically maintained cows undergoing luteolysis or in pro-oestrus at the time of progestagen treatment. From the result of another study (Dick, 1990), a dominant follicle is maintained during treatment, suppressing the follicular patterns. This result in persistent follicles, and a short post-treatment interval to oestrus. Moreover, these data showed that short intervals to oestrus were associated with lower fertility. Therefore, this situation of reduced fertility would be the consequence of aged ova.

Anoestrus in Lactating Dairy Herds.

Only 8.5% of cows were diagnosed as non-cycling in the year-round herds, compared with 12.7% in the seasonal herd. The proportion of cows inseminated after treatment was 53% in year-round herds and 71% in the seasonal herd. The percentage of cows detected in oestrus after CIDR/PMSG treatment varied from 40% to 63%. There were only 24% of treated cows which had ovulated but were not seen in oestrus. This suggests that use of AI by appointment is not a reliable recommendation with this form of treatment. This emphasises the desirability of re-examining all non-responders and then differentially treating cows with PGF or a CIDR + PMSG at this time.

Although the mean interval from PSM to conception, and the mean number of services per conception were significantly lower in treated cows, in general the reproductive performance of treatment groups in the year-round milking herds was better than in control groups (Table 3.7). This shows that treatment was preferable to no treatment in these problem cows.

Contrasting results were obtained with a small number of non-cycling cows

treated with progesterone and PMSG in the seasonal herd (Table 3.8). Although this herd had less satisfactory response patterns, because it is known that the response to treatment is quite variable. This may be possibly because the condition of anoestrus is essentially nutritional anoestrus, rather than simply postpartum or lactational anoestrus (Macmillan et al., 1990b). However, results of a recent trial reported that the treatment programme achieved a successful reduction in the average date at which conception occurred, and the non-pregnant rate in treated cows (Macmillan et al., unpublished). For this reason, the treatment of this major form of infertility in New Zealand dairy cows is always recommended.

Oestrous Response after Synchronization.

The two periods of artificial insemination were concentrated into 5-day synchrony periods in the treated groups. Oestrous responses in the four farms averaged 76% in treatment groups, and differed significantly from the control groups (63%; $P< 0.001$; Table 3.9). There were 67% of the treated cows observed in oestrus and inseminated within the first period of AI (3 days). The highest proportion of inseminations during the period were at 48 h, involving 32% for the whole treated group (Table 3.10). Although the degree of synchronization of oestrus was higher compared with the control groups, there was wide variation among groups within and between-herds throughout the study period.

Previous reports for cycling cows and heifers treated with CIDR and PGF have shown higher proportions of animals are in oestrus and inseminated. For example 81.5%) of cows were inseminated from 48 to 96 h after CIDR removal (Macmillan et al., 1987), and 83,9% of heifers were in oestrus between 48 and 72 h after CIDR removal (Van Cleeff et al., 1989) and 97% of heifers were in oestrus between 48 and 96 h after CIDR removal (Macmillan, 1988b). The results of the current study reveal that the proportion of cows in oestrus and inseminated was low compared with the other studies and the expectation that 80% to 90% of all first and second inseminations would be done on Monday to Friday of every fourth week was not reached. The oestrous response of 71% was similar to findings in a previous report which showed that 70% of cows treated with PRID for 7 days and PGF given at removal were in oestrus within the first week post-treatment (Smith et al., 1986).

If the oestrous synchrony response had been greater than 90%, there would have been a greater reduction in PSM to first AI interval because more cows had been detected in oestrus and inseminated. If these cycling cows would have had a normal pregnancy rate at first AI, the effect on the calving to conception interval would have been significantly reduced as well as reducing the interval from first AI to conception and the number of services per conception. This treatment can be expected to be more successful in herds with a high oestrus detection rate. But even in herds with a good oestrus synchrony response (i.e. autumn calving, herd D), the effect of a lower conception rate at first AI will be an important limiting problem, especially in seasonal herds because the PSM to first AI interval in autumn calving cows was significantly reduced while the PSM to conception was similar (Table 3.4). Moreover, if achieving a compact breeding period in seasonal herds, or maintaing an optimum calving interval in

year-round herds is one of the aims, then the selective use of CIDR with PGF or PMSG in cows not inseminated by days 82 post-partum would be beneficial.

In this study, the degree of oestrus synchronization was less than expected due to failure to detect oestrus. This problem can be seen clearly in farm D, in which the difference in oestrous response was 12% between the autumn and spring herd (Table 3.9). It is important to emphasise that the average synchrony patterns were different from previous trials, and within a herd from one treatment group to another (Table 3.10). It could have been possible that herd owners made more detection mistakes with treated cows and had them inseminated at the wrong time because the percentage of cows inseminated at 48 h seems to be low. It may be possible that some cows inseminated at 72 h should have been inseminated at 48 h because the percentage of animals inseminated at 48, 72, and 96 h after treatment within and between-herds was very variable. On the other hand, there was a reasonable number of cows not seen in oestrus after the first synchrony treatment which were seen in oestrus after the re-treatment. In most of these cows a CL was found when a rectal palpation was made before re-insertion of the used CIDR. It could explain partially that the owners were missing synchronized cows. In addition, there is a possibility that cows in late winter show oestrus less clearly as seen in Figure 3.2, and that tailpaint and raddle on winter coats which are long are less successful than in spring when hair coats are short. Even though many cows had ovulated, the paint and the aerosol were not rubbed off. Under all of these circumstances, mistakes relating to the interpretation of the tailpainting system were frequently made by the owners. Few cows were in oestrus outside the insemination periods. Asynchronous responses were mainly observed in non-cycling cows having longer responses to the treatment with CIDR/PMSG.

In relation to the interval to onset of oestrus, it is expected that most cycling animals which did not have a functional CL at device removal would be detected in oestrus by 48 h post-treatment. In this study the lower fertility was associated with cows being inseminated by 48 h in which the difference in fertility at the first AI was 7% less compared with cows being inseminated by 72-96 h. It may reinforce the concept that the lower fertility was due to CIDR treatment effects were because follicle persistence produced a short post-treatment interval to oestrus (Dick, 1990).

In this study, CIDR's inserted for 6 days by day 17-19 post-insemination were associated with decreased conception rate at first insemination. However, this may not have been a CIDR treatment effect. The conception rate at second insemination was similar to the control group (Table 3.12). Moreover, the conception rate to this insemination in all the farms was high compared with that of the first insemination (Table 3.4, 3.5 and 3.6).

Progesterone treatment after insemination had no effect on conception rates. A similar finding was reported by Munro and Bertram (1990), who showed that the fertility of treated and control cows inseminated at the second AI was not significantly different. This is in contrast with a number of reports which have indicated that the strategic use of CIDR or PRID as a post-insemination treatment can increase the chance of conception to the preceding insemination (Shemesh et al., 1981; Robinson et al., 1989). These findings could explain why the

administration of exogenous progesterone had no effect on fertility when given to groups of cows having normal levels of fertility (Diskin and Sreenan, 1986). However, Macmillan et al. (1990a) also remarked on the relevance of timing of device insertion to increase fertility in relation to the oestrous cycle in treated cows with progesterone after insemination.

CIDR's can also be used to synchronize returns to oestrus in non-pregnant animals. Eighty five per cent (85%) of the cows in this study were in oestrus on days 22-25. The results of the current study suggest that although supplementation with exogenous progesterone post-insemination had no effect on fertility at the second AI, it does reinforce the recommendation that this management system involving device re-insertion should be included in breeding programmes to synchronize returns to service. In Van Cleeff's study (1989), 81.3% of non-pregnant heifers were in oestrus on day 24 after treatment. Oestrus detection in animals synchronized with this regimen was more successful than in control cows. Other reports also have demonstrated positive effects on oestrus detection of synchronization of oestrus using PGF (Sudweeks and Randel, 1985; Washburn and Dailey, 1987). Although the proportion of cows in oestrus and inseminated, differed among groups within and between-herds, it was clear that the oestrus detection was improved significantly in all of the farms (Table 3.9). The variation among herds can be explained partly by the organization of oestrus detection and of the beneficial effects of having a single, seasonally concentrated calving period.

Oestrous Response and Use of Full and Half Dose of PGF at CIDR-B Removal.

Previous reports compared several doses of PGF alone in different management systems for synchronization of oestrus in dairy and beef herds. There were no differences with dose rates in oestrus response, but there were differences between management systems, ranging from 42.8 to 64.5% (Lauderdale, 1972; Lauderdale et al., 1974; Donaldson, 1980; Donaldson et al., 1982).

Responses to two different doses of PGF at CIDR removal on oestrus and fertility were studied. Treated cows injected with the full dose of PGF had a 68% oestrous response within 48 to 96 h, which was similar (72%) in treated cows injected with the half dose of PGF (Table 3.11). This result shows that the selective use of a reduced dose of PGF could be effective for the regression of the CL, and consequently be more economical.

Plasma Progesterone.

Progesterone concentrations in non-pregnant and pregnant cows at first sampling given CIDR/PGF in cycling cows and CIDR/PMSG in non-cycling cows were similar (Table 3.13).

Plasma progesterone concentrations maintained by the used CIDR at device removal was above 2 ng/ml in both groups of non-pregnant cows (Table 3.13). This suggests that luteolysis had occurred during the 6 days after the insertion of the used CIDR. The concentrations of progesterone were maintained by the

progesterone released from the used CIDR. Similar concentrations of progesterone were found in cycling cows without a CL, and in ovariectomized cows before the new CIDR was removed 10 days after insertion (Dick, 1990). This shows that progesterone from the used CIDR was sufficient to maintain plasma progesterone to concentrations similar to that produced by a new device. As expected, the progesterone concentrations of non-pregnant cows in both treated groups at third sampling was very low (0.3 ng/ml; Table 3.13). Progesterone concentrations of pregnant cows in both treated groups at second and third sampling were similar. This suggests that the pregnancy was established when the treatment with the used CIDR was initiated in late dioestrus.

CONCLUSIONS

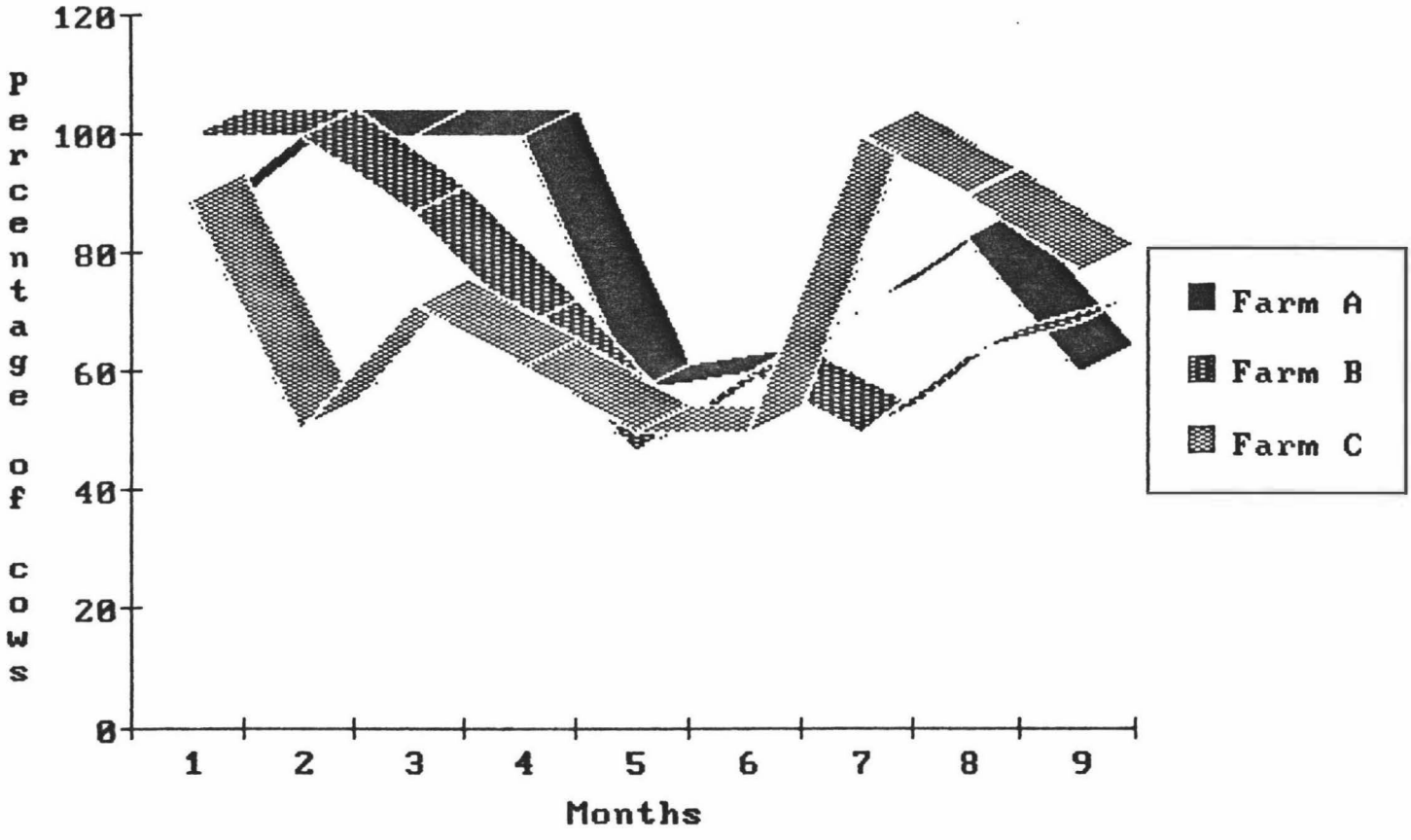
The results of this study demonstrated that CIDR treatment together with an injection of PGF or PMSG significantly reduced the interval from PSM to first AI, but this management advantage involving the systematic control of oestrous cycle was partly lost because of lower fertility at first AI, so that the effect on the interval to conception was less significant. It therefore: i) increased the interval from first AI to conception; ii) increased the number of services per conception, and iii) reduced the benefits of synchrony, because of failure to detect oestrus. Thus the proportion of cows in oestrus and inseminated at the 1st and 2nd synchrony periods was less than expected. In addition, the synchrony patterns were so different within a herd from one treatment group to another. This was possibly due to: i) the tailpaint system which was not sufficiently sensitive in cows during which may show oestrus less clearly in winter than in any other season; ii) the variation in diagnostic value of tailpaint and raddle on the different coats and the different skeletal conformations of various animals, associated with the degree of fat over the back, bone, hips, and tail-head. Therefore, the effects of these interactions produced a wide variation in the interval to onset of detected oestrus after treatment among groups within and between-herds, and lower fertility in some cows. Thus, it affected precision in synchrony and fertility at the synchronized oestrus.

If the oestrous cycle response by using this management had been greater than 90% with a normal pregnancy rate, then there would have been a greater reduction in the calving to first AI and calving to conception intervals. In order to achieve this objective it is necessary to achieve high synchrony and normal fertility. The areas to study in cycling cows are: i) research on the control of the oestrous cycle should be focused on regulating follicle wave patterns, reducing the duration of treatments programmes when a progestagen is used to obtain high pregnancy rates; ii) increasing fertility and minimizing the variation in return to oestrus after treatment by using CIDR devices after insemination. Moreover, it is important to recognize that the most common problem in breeding management in dairy herds is oestrus detection. The tailpainting system may be less satisfactory in herds with continuous calving patterns, possibly because it was not sufficiently sensitive, especially with animals during winter months. It shows a particular potential for further studies between animals which may not display oestrus, and the associations of the tailpaint and raddle technique in animals with different coats and skeletical conformation. The areas to study in non-cycling cows are: i) reduce the incidence of anoestrus; ii) identify factors which affect the effectiveness of the treatment response for this condition through herd management, including concentrating the calving pattern through greater use of controlled breeding programmes to condense conception patterns, and hence to increase the interval from the next calving to the start of AI period.

Figure 3.1 **Basic programme of experimental protocol.**

Day		Task
0	Wednesday	Insert CIDR, Tailpaint
10	Saturday (am)	Remove CIDR, Raddle over Paint
12-14	Monday-Wednesday	1st Insemination on Detection
28	Wednesday *	Re-insert Used/Washed/Stored CIDR, Tailpaint
34	Tuesday (am)	Remove and Discard CIDR, Raddle
36-37	Thursday-Friday	2nd Insemination on Detection.

* In herds with year-round milking, a new group of cows enters the breeding schedule on every fourth Wednesday.



1 = April; 5 = August; 9 = December

Figure 3.2 Percentage of cows in oestrus and inseminated in treatment groups throughout the study period in Year-round herds.

Table 3.1 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and interval from 1st AI to conception (days) in treatment and control groups in Farm A (Year-round herd).**

	All Animals		Cycling Animals	
Group	Treatment (53)	Control (49)	Treatment (45)	Control (47)
PSM to 1st Insemination	27.9[a] 3.7	38.6 5.0	25.2[a] 3.8	36.3 4.9
PSM to Conception	54.5 5.8	60.8 5.9	55.5 6.4	59.5 6.0
Interval 1st AI to Conception	26.6 5.3	22.1 4.8	23.1 5.0	30.1 6.1
Conception Rate 1st Insemination	49	58	43	56
Conception Rate 2nd Insemination	62	74	62	74
Services per Conception	1.9	1.7	2	1.7

Probability a = $P < 0.01$

( ) Number of cows in parentheses.

Table 3.2 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and interval from 1st AI to conception (days) in treatment and control groups in Farm B (Year-round herd).**

	All Animals		Cycling Animals	
Group	Treatment (69)	Control (55)	Treatment (59)	Control (47)
PSM to 1st Insemination	24.8[a] 2.7	35.8 4.0	23.0[a] 2.7	35.4 4.5
PSM to Conception	44.2 4.6	49.5 5.6	40.4 4.4	46.0 4.9
Interval 1st AI to Conception	19.3 3.9	13.6 4.7	17.4 3.5	10.6 3.3
Conception Rate 1st Insemination	58[a]	78	60[a]	80
Conception Rate 2nd Insemination	52	73	50	78
Services per Conception	1.6	1.4	1.6	1.3[b]

Probability a = $P < 0.01$, b = $P < 0.10$

( ) Number of cows in parentheses.

Table 3.3 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and intervals from 1st AI to conception (days) in treatment and control groups in Farm C (Year-round herd).**

	All Animals		Cycling Animals	
Group	Treatment (114)	Control (93)	Treatment (104)	Control (80)
PSM to 1st Insemination	27.1[a] 2.8	38.7 4.2	25.4[a] 3.0	38.3 4.5
PSM to Conception	62.1[b] 4.6	76.9 8.7	63 5.1	75.5 9.2
Interval 1st AI to Conception	31.1 4.0	32.7 6.5	37.5 4.8	37.1 8.1
Conception Rate 1st Insemination	43	50	38	52
Conception Rate 2nd Insemination	60	61	58	63
Services per Conception	2.1	1.9	2.3	1.8

Probability a= P< 0.01, b= P< 0.10
( ) Number of cows in parentheses.

Table 3.4 **Conception rates (%) and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI) and PSM to conception (days) in treatment and control groups in Farm D (Seasonal-herd, Autumn-calving).**

		All Animals		Cycling Animals	
Group		Treatment (36)	Control (34)	Treatment (33)	Control (30)
PSM to 1st Insemination	EC (50)	6.7 2.1	10.7 1.4	3.4[a] 0.8	10.8 1.5
	LC (20)	17.6 0.7	14.8 4.5	17.7 0.8	12.5 6.0
PSM to Conception	EC	46.8 12.8	25.8 7.2	24.2 9.0	27.2 7.8
	LC	60.6 8.7	27.8 7.1	44.5 9.0	23.0 7.9
Conception Rate 1st AI	EC	44	54	60	59
	LC	67	60	67	71
Conception Rate 2nd AI	EC	20	33	15	31
	LC	22	40	22	29

Probability a = $P < 0.01$; ( ) Number of cows in parentheses.
EC = Early Calvers; LC = Late Calvers.

Table 3.5 **Conception rates (%) and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI) and PSM to conception (days) in treatment and control groups in Farm D (Seasonal-herd, Spring-calving).**

		All Animals		Cycling Animals	
Group		Treatment (51)	Control (53)	Treatment (48)	Control (52)
PSM to 1st Insemination	EC (86)	17.5[a] 2.5	28.5 3.4	18.3[a] 2.7	28.5 2.9
	LC (18)	38.4 7.2	40.2 6.5	21.5 8.0	39.1 8.4
PSM to Conception	EC	32.1 3.3	37.3 3.8	31.6 3.1	37.3 3.8
	LC	51.0 6.5	46.5 7.2	36.7 10.7	45.5 9.1
Conception Rate 1st AI	EC	43	63[c]	49	62
	LC	44	67	50	58
Conception Rate 2nd AI	EC	43[b]	18	38	19
	LC	44	22	50	25

Probability a = P< 0.01 b = P< 0.05 c = P< 0.10; ( ) Number of cows in parentheses. EC = Early calvers; LC = Late calvers.

Table 3.6 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and intervals from 1st AI to conception (days) in treatment and control groups in Year-round herds.**

	All Animals		Cycling Animals	
Group	Treatment (236)	Control (197)	Treatment (200)	Control (182)
PSM to 1st Insemination	26.6[a] 1.7	37.7 2.5	24.7[a] 1.8	36.7 2.6
PSM to Conception	54.7[c] 2.9	62.7 4.1	54.8 3.2	61.2 4.3
Interval 1st AI to Conception	28.0 2.6	25.0 3.6	30.0 2.9	24.4 3.6
Conception Rate (%) 1st AI	49	62[a]	46	62[a]
Conception Rate (%) 2nd AI	59	67	57	65
Services per Conception	1.9	1.7[b]	1.9	1.6[a]

Probability a = P< 0.01 b= P< 0.05 c = P< 0.10
( ) Number of cows in parentheses.

Table 3.7 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and intervals from 1st AI to conception (days) in non-cycling cows in Year-round Herds.**

Group	Treatment (36)	Control (15)
PSM to 1st Insemination	36.0 4.8	46.7 7.2
PSM to Conception	54.2[a] 7.0	77.0 14.4
Interval 1st AI to Conception	18.2 6.1	30.2 14.8
Conception Rate (%) 1st Insemination	64	60
Conception Rate (%) 2nd Insemination	71	50
Services per Conception	1.6[a]	2.2

Probability a= $P < 0.10$
( ) Number of cows in parentheses.

Table 3.8 **Conception rates (%), services per conception and least squares means (SEM) of planned start of mating (PSM) to first insemination (AI), PSM to conception and intervals from 1st AI to conception (days) in non-cycling cows in Seasonal herd.**

Group	Treatment (6)	Control (5)
PSM to 1st Insemination	25.1 3.3	21.0 2.2
PSM to Conception	90.4 16.1	32.6[a] 5.9
Interval 1st AI to Conception	39.1 12.4	28.1 7.5
Conception Rate (%) 1st Insemination	21	43
Conception Rate (%) 2nd Insemination	35	42
Services per Conception	2.9	1.5[a]

Probability a= $P < 0.05$
( ) Number of cows in parentheses.

Table 3.9 **Percentage (%) of cows in oestrus and inseminated in treatment group during the 5-Day period and in control cows during the 38-Day period.**

Farms	N° of Animals	Type of Farm	Treatment Group (%)	Control Group(%)
A	102	Year-round	79[c]	59
B	124	Year-round	71[b]	56
C	207	Year-round	70[c]	54
D	70	Seasonal (Autumn)	87	87
	103	Seasonal (Spring)	75[c]	59
TOTAL	608	-	76[a]	63

Probability a = P< 0.001 b = P< 0.06 c = P< 0.05

Table 3.10 **Percentage (%) of cows in oestrus and inseminated in treatment group during the first period of artificial insemination (3-day period).**

	Cycling-Non-cycling cows % Insem'd at:				Cycling cows % Insem'd at:			
Farms	Total	48 h	72 h	96 h	Total	48 h	72 h	96 h
A	60	26	17	17	65	28	19	17
B	59	36	9	14	63	35	10	17
C	55	22	25	7	65	25	29	10
D[a]	94	45	27	21	93	45	26	22
D[b]	63	32	16	14	68	34	19	14
TOTAL	67	32	19	21	71	33	21	16

a = Autumn-calving Herd b = Spring-calving Herd

Table 3.11 **Effect of dose rates of prostaglandins (PGF) at CIDR-B removal on oestrus and fertility in cows.**

Dose of PGF	N° of Cows	Oestrous Response (%)	Conception Rate at 1st AI (%)
500 mcg	154	68	44
250 mcg	138	72	48

Table 3.12 **Effect of treatment with progesterone on conception rate at second artificial insemination (AI) in treatment and control groups.**

Group	Number of Cows	Conception Rate at 2nd AI (%)
Treatment	84	50
Control	76	58

Table 3.13 **Least squares means (SEM) of plasma progesterone concentrations (ng/ml) in non-pregnant (NP) and pregnant (P) cows treated with CIDR-B, and prostaglandins (PGF) or pregnant mare serum gonadotrophin (PMSG).**

	CIDR-B/PGF		CIDR-B/PMSG	
Treatment	NP (50)	P (50)	NP (26)	P (33)
Sample 1[b]	3.1 0.4	4.1 0.5	2.8 0.4	5.0 1.8
2[c]	2.3[a] 0.3	8.7 0.4	2.6[a] 0.4	9.3 1.1
3[d]	0.3[a] 0.1	7.1 0.4	0.3[a] 0.1	8.4 0.7

Probability a = $P < 0.01$
b = sample taken when a new CIDR was removed
c = sample taken when the re-used CIDR was removed
d = sample taken at the morning after the re-used CIDR was removed
( ) Number of cows in parentheses.

REFERENCES

ABOUL-ELA, M. B. and EL-KERABY, F. E. (1986). The effect of treatment with a GnRH analogue on postpartum reproductive performance in Friesian cows. **Anim. Reprod. Sci.** 12: 99-107.

AULETTA, F. J. and FLINT, A. P. F. (1988). Mechanism controlling corpus luteum function in sheep, cows, nonhuman primates, and women especially in relation to the time of luteolysis. **Endocr. Rev.** 9: 88.

BAILIE, J. H. (1982). Management and economic affects of different levels of estrus detection in the dairy herd. **Vet. Rec.** 110: 218-221.

BALDWIN, G. W. (1989). A study of winter milk production and a comparison of town milk and seasonal supply dairy farms in the Manawatu. **Thesis, Master of Agricultural Science.** Animal Science Department, Massey University.

BEAL, W. E., CHENAULT, J. R., DAY, M. L. and CORAH, L. R. (1988). Variation in conception rates following synchronization of estrus with melengestrol acetate and prostaglandin F 2α. **J. Anim. sci.** 66: 599.

BELSCHNER, A. (1986). A breeding program for dairy cattle. **Agri-Practice** 7: 7-12.

BRINK, J. T. and KIRACOFE, G. H. (1988). Effect of estrous cycle stage at Syncro-mate B treatment on conception and time to estrus in cattle. **Theriogenology.** 29: 513-518.

BRITT, J. H., HUERTAS VEGA, E. and ULBERG, L. C. (1972). Managing reproduction in dairy cattle. I. Progestogens for control of estrus in dairy cows. **J. Dairy Sci.** 55: 598-605

BRITT, J. H. (1977). Strategies for managing reproduction and controlling health problems in groups of cows. **J. Dairy Sci.** 60: 1345-1353.

BROOKES, I. M. and HOLMES, C. W. (1988). The assessment of pasture utilization on dairy farms. **Proc. of the New Zealand Grassland Association** 49: 123.

CHENAULT, J. R., McALLISTER, J. F. and KASSON, C. W. (1990). Synchronization of estrus with melengestrol acetate and prostaglandin F 2α in beef and dairy heifers. **J. Anim. Sci.** 68: 296-303.

CHRISTIE, W. B., NEWCOMBE, R. and ROWSON, L. E. A. (1979). Embryo survival in heifers after transfer of an egg to the uterine horn contralateral to the CL and the effect of treatments with progesterone and hCG on

pregnancy rate. **J. Reprod. Fertil.** 56: 701-706.

DICK, A. R. (1990). Ovarian follicular population, oestrous response, plasma progesterone concentrations and length of cycles in cows treated with an intravaginal device at different stages of the oestrous cycle. In **Studies on the use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 1: 2-71.

DISKIN, M. G. and SREENAN, J. M. (1986). Progesterone and embryo survival in the cow. In: **Embryo Mortality in Farm Animals**. eds. J. M. Sreenan and M. G. Diskin. Martinus Nijhoff, Boston. 142-158.

DONALDSON, L. E. (1980). The development and marketing of estrus synchronization in cattle in Australia using prostaglandins. **Theriogenology**. 14: 391-401.

DONALDOSON, L. E., GLAPHIN, S. P. and GREEN, G. A. (1982). Comparison of two dose rates and two management ssystems for synchronization of estrus in cattle. **Am. J. Vet. Res.** 43: 1873-1975.

ECHTERNKAMP, S. E. and MAURER, R. R. (1983). Conception, embryonic development and corpus luteum function in beef cattle open for two consecutive breeding seasons. **Theriogenology** 20: 627-637.

EDDY, R. G. (1983). The use of prostaglandin analogue cloprostenol and the milk progesterone test to control breeding policy in one dairy herd. **Br. Vet. J.** 139: 104-108.

ESSLEMONT, R. J. (1974). Economic and husbandry aspects of the manifestation and detection of oestrus in cows. I. Economic aspects. **ADAS Q. Rev.** 12: 175-184.

ESTERGREEN, V. L., FROST, O. L., GOMES, W. R., ERB, R. E. and BULLARD, J. F. (1967). Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. **J. Dairy Sci.** 50: 1293-1295.

FERNANDES, L. C., THATCHER, W. W., WILCOX, C. L. and CALL, E. P. (1978). LH release in response to GnRH during the postpartum period of dairy cows. **J. Anim. Sci.** 46: 443-448.

FIELDEN, E. D., HARRIS, R. E., MACMILLAN, K. L. and SHRESTHA, S. L. (1980). Some aspects of reproductive performance in selected town-supply dairy herds. **N.Z. Vet. J.** 28: 131-142.

FOLMAN, Y., ROSINBERG, M., HERZ, Z. and DAVIDSON, M. (1973). The relationship between plasma progesterone concentration and conception in post-partum dairy cows maintained on two levels of nutrition. **J. Reprod. Fertil.** 34: 267.

FOLMAN, Y, McPHEE, S. R., CUMMING, I. A., DAVIS, I. F. and CHAMLEY, W.

A. (1983). Conception rates in cows after various synchronization techniques using progestrone releasing intravaginal devices. **Aust. Vet. J.** 60: 44-47.

FOLMAN, Y., KAIM, M. HERZ, Z and ROSEMBERG, M. (1984). Reproductive management of dairy cattle based on synchronization of estrous cycles. **J. Dairy Sci.** 67: 153-160.

FONSECA,F. A., BRITT, J. H., McDANIELS, B. T., WILK, J. C. and RAKES, A. H. (1983). Reproductive traits of Holstein and Jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rates, and days open. **J. Dairy Sci.** 66: 1128-1147.

FOOTE, R. H. (1975). Estrus detection and estrus detection aids. **J. Dairy Sci.** 58: 248-256.

GARVERICK, H. A. and SMITH M. F. (1986). Mechanism associated with subnormal luteal function. **J. Anim. Sci.** 62: (suppl. 2): 92 (abstr.).

GREVE, T and LEHN-JENSEN, H. (1982). The effect of hCG administration on pregnancy rate following non-surgical transfer of viable bovine embryos. **Theriogenology.** 17: 91.

HAFS, H. D., MANNS, J.G. and DREW, B. (1975). Onset of estrus after prostaglandin F 2α in cattle. **Vet. Rec.** 96: 134-135.

HANSEL, W., LUKASZESWKA, J. and BEAL, W. (1978). Maintenance of the bovine corpus luteum of early pregnancy. **Biol. Reprod.** I: (suppl. 18): 27 (abstr.).

HANSEL, W. (1981). Plasma hormone concentrations associated with early embryo mortality in heifers. **J. Reprod. Fertil.** 30: (suppl.): 231-239.

HELMER, S. D. and BRITT, J. H. (1986). Fertility of dairy cattle treated with human chorionic gonadotropin (hCG) to stimulate progesterone secretion. **Theriogenology.** 26: 683-695.

HENRICKS,D. M., DICKEY, J. F., HILL, J. R, and JOHNSTON, W. E. (1972). Plasma estrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. **Endocrinology.** 90: 1336-1342.

HOLNESS, D. H., McCABE, C. T. and SPROWSON, G. W. (1982). Observations on the use of hCG during the post-insemination period on conception rates in synchronized beef cows with sub-optimum reproductive performance. **Theriogenology** 17: 133-140.

JOHNSON, K. R., ROSS, R. H. and FOURT, D. L. (1958). Effect of progesterone administration on reproductive efficiency. **J. Anim. Sci.** 17: 386-390.

JUBB, T. F., BRIGHTLING, P., MALMO, J., LARCOMBE, M. T., ANDERSON, G.

A. and HIDES, S. J. (1989). Evaluation of a regimen using a progesterone releasing intravaginal device (CIDR) and PMSG as a treatment for post partum anoestrus in dairy cattle. **Aust. Vet. J.** 66: 334-336.

JUNEJA, S. C. and ARORA, S. P. (1989). Occurrence of oestrus depressed by undernutrition in crossbred cows. **J. Anim. Sci.** 67: (suppl. 1): 384.

KILGOUR, R, SKARSSHOLT, B. H., SMITH, J. F., BREMNER, K. L. and MORRISON, M. C. L. (1977). Observations on the behaviour and factors influencing the sexually active group in cattle. **Proc. N. Z. Soc. Anim. Prod.** 37: 128-135.

KING, G. L., BURNSIDE, E. B. and CURTIS, R. A. (1983). Controlled breeding of dairy cows with cloprostenol. **Can. Vet. J.** 24: 105-107.

KIRWOOD, R. N., LAPWOOD, K. R., SMITH, W. C. and ANDERSON, I. L. (1984). Plasma concentrations of LH, prolactin, oestradiol-17B and progesterone in sows weaned after lactation for 10 or 35 days. **J. Reprod. Fertil.** 70: 95-102.

LAMMING, G. E., DARWASH, A. O. and BACK, H. L. (1989). Corpus luteum function in dairy cows and embryo mortality. **J. Reprod. Fertil.** 37: (suppl.): 245-252.

LAUDERDALE, J. W. (1972). Effects of PGF 2α on pregnancy and estrous cycle of cattle. **J. Anim. Sci.** 35: 246 (abstr.).

LAUDERDALE, J. W., SEGUIN, B. E., STELLFLUG, J. N., CHENAULT, J. R., THATCHER,W. W., VINCENT, C. K. and LOYANCANO, A. F. (1974). Fertility of cattle following PGF 2α injection. **J. Anim.Sci.** 38: 964-967.

LEE, C. N., MAURICE, E., AX, R. L., PENNINGTON, J. A., HOFFMAN, W. F. and BROWN, M. D. (1983). Efficacy of gonadotrophin-releasing hormone adminietered at the time of artificial insemination of heifers and postpartum and repeat breeder dairy cows. **Am. J. Vet. Res.** 44: 2160-2163.

LEWIS,G. S., CALDWELL, D. W., REXROAD, C. E. Jr., DOWLEN, H. H. and OWEN, J. R. (1990). Effects of gonadotropin-releasing hormone and human chorionic gonadotropin on pregnancy rate in dairy cattle. **J. Dairy Sci.** 73: 66-72.

LOUIS, T. M., HAFS, H. D. and SEQUIN, B. E. (1973). Progesterone, LH, estrus and ovulation after prostaglandin F 2α in heifers. **Proc. Soc. Exp. Biol. Med.** 143: 152-155.

LOUIS, T. M., HAFS, H. D. and MORROW, D. A. (1974) Intrauterine administrations of prostaglandin F2 α in cows. Progesterone, Estrogen, LH, and ovulation. **J. Anim. Sci.** 38: 347-353.

LUKASZEWSKA, J. and HANSEL, W. (1980). Corpus luteum maintenance during

early pregnancy in the cow. **J. Reprod. Fertil.** 59: 485-493.

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 a herd reported in calf to AB. **N. Z. Exper. Agric.** 1: 309-314.

MACMILLAN, K. L. and CURNOW, R. J. (1977). Tailpainting: a simple form of oestrus detection in New Zealand dairy herds. **N.Z. J. Exper. Agric.** 5: 357-361.

MACMILLAN, K. L. and TAUFA, V. K. (1983). Increasing pregnancy rates in New Zealand dairy cattle. **Proc. N. Z. Soc. Anim. Prod.** 43: 53-57

MACMILLAN, K. L. (1985a). Production related effects of calving patterns and calving intervals. Proc. Dairy Cattle Production. 6-10 May, Refresher Course for Veterinarians, University of Sydney, Australia.

MACMILLAN, K. L. (1985b). Detection of oestrus in dairy cows. **Proc. Dairy Cattle Production.** 6-10 May, Refresher Course for Veterinarians, University of Sydney. Australia.

MACMILLAN, K. L., DAY, A. M., TAUFA, V. K., GIBB, M. and PEARCE, M. G. (1985c). Effects of an agonist of gonadotrophin releasing hormone in cattle. I. Hormone concentrations and oestrus cycle length. **Anim. Reprod. Sci.** 8: 203-212.

MACMILLAN, K. L., DAY, A. M., TAUFA, V. K., PETERSON, A. J. and PEARCE, M. G. (1985d). Effects of an agonist of gonadotrophin releasing hormone in cattle. II. Interactions with injected prostaglandin F 2α and unilateral ovariectomy. **Anim. Reprod Sci.** 8: 213-223.

MACMILLAN, K. L. (1986a). Condensed breeding programmes. Seminar **New Zealand Dairy Board Livestock Improvement Division. Flock House.** 17-21 February.

MACMILLAN K. L., TAUFA, V. K. and DAY, A. M. (1986a). Effects of an agonist of gonadotropin releasing hormone (Buserelin) in cattle. III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. **Anim. Reprod. Sci.** 11: 1-10.

MACMILLAN, K. L. and DAY, A. M. (1987a). Treating the non-cycling cow. **Proc. Ruakura Farmer's Conf.** 39: 65-68.

MACMILLAN, K. L. and TAUFA, V. K. (1987b). Effects of using bovine CIDRs after first insemination on pregnancy rate and subsequent synchrony. **Proc. 4th AAAP Animal Science Congress.** 224.

MACMILLAN, K. L., TAUFA, V. K., BARNES, D. R., DAY, A. M. and HENRY, R. (1988a). Detecting oestrus in synchronized heifers using tailpaint and aerosol raddle. **Theriogenology.** 30: 1099-1114.

MACMILLAN, K. L. (1988b). Trends in breeding management in New Zealand dairy herds. Non-infectious factors affecting reproductive performance of dairy herds. **Dairy Cattle Reproduction Research Workshop** Werribee, Nov. 15-17.

MACMILLAN, K. L. (1988c). Maximizing the use of AI in cattle. **Proc. Int. Cong. on Anim. Rep. and AI.** 2: 265-275.

MACMILLAN, K. L., TAUFA, V. K., DAY, A. M. and PETERSON, A. J. (1990a). Effects of supplemental progesterone on pregnancy rates in cattle. **Third International Ruminant Reproduction Symposium.** March, Nice, France.

MACMILLAN, K. L., DAY, A. M. and TAUFA, V. K. (1990b). Anoestrum update. **Proc. Ruakura Farms Conf.** 42: 107-114.

MARCUS, S. and AYALON, N. (1981). Improving conception rate in dairy cattle using progestin-impregnated intravaginal sponges. **Refuah Vet.** 38: 55-56.

McMILLAN, W. H., KNIGHT, T. W. and MACMILLAN, K. L. (1986). Effects of gonadotrophin releasing hormone (Buserelin) on sheep fertility. **Proc. N. Z. Soc. Anim. Prod.** 46: 161-163.

MEE, M. O., STEVENSON, J. S., SCOBY, R. K. and FOLMAN, Y. (1990). Influence of gonadotropin-releasing hormone and timing of insemination relative to estrus on pregnancy rates of dairy cattle at first service. **J. Dairy Sci.** 73: 1500-1507.

MOORE, N. W. (1975). The use of prostaglandin F 2α given by either intrauterine infusion or by intramuscular injection for the control of estrus and ovulation in cattle. **Ann. Biol. Anim. Biochim. Biophys.** 15: 451-460.

MUNRO, R. K. and BERTRAM, J. (1990). Progesterone administered after insemination did not affect the fertility of cattle following a controlled breeding program. **Australian Journal of Experimental Agriculture.** 30: 179-181.

ODDE, K. G. (1990). A review of synchronization of estrus in postpartum cattle. **J. Anim. Sci.** 68: 817-830.

O'FARREL, K. J. (1984). Oestrous behaviour, problems of detection and relevance of cycle lengths **"Dairy Cow Fertility"** eds. R. G. Eddy and M. J. Tucker, Vet. Assoc. Edit. Services, London 47-59.

OLTENACU, P. A., ROUNSAVILLE, T. R., ,ILLIGAN, R. A. and FOOTE, R. H. (1981). Systems analysis for designing reproductive management programs to increase production and profit in dairy herds. **J. Dairy Sci.** 64: 2096-2104.

ONO, H., FUKUI, Y. TERAWAKI, Y., OHBOSHI, K. and YAMAZAKI, D. (1982). An intravulvosubmucous injection of prostaglandin F2 α in onoestrous cows. **Anim. Reprod. Sci.** 5: 1-5.

PATTERSON, D. L., CORAH, L. R., KIRACOFE, G. H., STEVENSON, J. S. and BRETHOUR, J. R. (1989). Conception rate in Bos Taurus and Bos Indicus crossbred heifers after postweaning energy manipulation and synchronization of estrus with melengestrol acetate and fenprostalene. **J. Anim. Sci.** 67: 1138-1147.

PEARCE, M. G., MACMILLAN, K. L., TAUFA, V. K. and DAY, A. M. (1990). Systems for synchronizing oestrus in dairy heifers treated with a CIDR-B intravaginal device with or without prostaglandin F 2∝ or oestradiol. **Proc. 5th AAAP Animal Science Congress Taipei** Vol. 3.

PHATAK, A. P., WHITMORE, H. L. and BROWN, M. D. (1986). Effect of gonadotrophin releasing hormone on conception rate in repeat-breeder dairy cows. **Theriogenology**. 26: 605-609.

ROCHE, J. F. (1974). Synchronization of estrus and fertility following artificial insemination in heifers given prostaglandin F 2∝. **J Reprod. Fertil.** 37: 135-138.

ROCHE, J. F and IRELAND J.J. (1984). Manipulation of ovulation in cattle. **Proc. 10th Inter. Cong. on Anim. Reprod. and AI.** 4: 9-17.

ROBINSON, N. C., LESLIE, K. E. and WALTON, J. S. (1989). Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. **J. Dairy Sci.** 72: 202-207.

SACHS, M (1984). The control of parturition in the bovine. In: **The reproductive Potential of cattle and Sheep.** eds. R. Ortavant and H. Schindler. INRA, Paris. 327-337.

SANTOS-VALADEZ, S. de los, SEIDEL, G. E. Jr. and ELSDEN, R. P. (1982). Effect of HCG on pregnancy rates in bovine embryo transfre recipients. **Theriogenology.** 17: 85.

SEGUIN, B. E., TATE, D. J. and OTTERBY, D. E. (1983). Use of cloprostenol in a reproductive management system for dairy cattle. **J. Am. Vet. Med. Assoc.** 183: 533.

SHELTON, J. N. (1973). Prostaglandin F 2∝ for synchronization of estrus in beef cattle. **Aust. Vet. J.** 49: 442-444.

SHEMESH, M., AYALON, N. and LINDER, H. R. (1968). Early effect of conceptus on plasma progesterone levels in the cows. **J. Reprod. Fertil.** 15: 161164.

SHEMESH, M., AYALON, N., MARCUS, S., DANIELLI, Y. SHORE, L. and LAVI, S. (1981). Improvement of early pregnancy diagnosis based on milk progesterone by the use of progestin-impregnated vaginal sponges. **Theriogenology** 15: 459-462.

SMITH, R. D., HANSEL, W. and PILBEAM, T. E. (1986). PRID plus PGF 2∝- a

programmed approach to managing reproduction in lactating dairy cows. **J. Dairy Sci.** (abst.) 69: (suppl. 1) 93.

SREENAN, J. M. and DISKIN, M. G. (1983). early embryonic mortality in the cow: its relationship with progesterone concentration. **Vet. Rec.** 112: 517-521.

STEVENSON, J. S. and BRITT, J. H. (1977). Detection of estrus by three methods. **J. Dairy Sci.** 60: 1994-1998.

STEWART, J. D. (1988). Some economic aspects of Autumn calving. **Proc. of the 5 th Seminar of the Dairy Cattle Society of the new Zealand Society of the New Zealand Vet, Assoc.** pp 39-47.

SUDWEEKS, E. M. and RANDEL, R. D. (1985). The effect of using Estrumate on estrus detection and conception rates in lactating dairy cows. **J. Anim. Sci.** 61: (suppl. 1): 35 (abstr.).

TUNGSUBUTRA, V. and FRANCE, J. T. (1978). Serial changes in plasma levels of progesterone, unconjugated oestradiol, and unconjugate oestriol in normal pregnancy. **Aust. N. Z. Jl.** Obstet. Gynaec. 18: 97-103.

VAN CLEEFF, J., MACMILLAN, K. L., THATCHER, W. W. and LUCY, M. C. (1989). Estrous synchronization and fertility in heifers treated with CIDR before and after insemination. **J. Anim. Sci.** 67: (suppl. 1): 383.

WASHBURN, S. P. and DAILEY, R. A. (1987). Dairy Herd Reproductive management programs with or without synchronization of estrus. **J. Dairy Sci.** 70: 1920-1926.

WILLIAMSON, N. B., MORRIS, R. S., BLOOD, D. C., CANNON, D. C., CHRISTINE, M. and WRIGHT, P. J. (1972). A study of oestrus behaviour and oestrus detection methods in a large commercial dairy herd. II. Oestrous signs and behaviour patterns. **Vet. Rec.** 91: 58-62.

WISHART, D. F. and YOUNG, I. M. (1974). Artificial insemination of progestin (SC21009)-treated cattle at predetermined times. **Vet. Rec.** 95: 503-508

WILTBANK, J. N., HAWK, H. W., KIDDER, H. E., BLACK, W. G., ULBERG, L. C. and CASIDA, L. E. (1956). Effect of progesterone therapy on embryo survival in cows of lowered fertility. **J. Dairy Sci.** 39: 456-461.

CHAPTER 4

THE USE OF MILK PROGESTERONE TESTS TO IDENTIFY NON-PREGNANT ANIMALS AND CONSEQUENTLY IMPROVE THE OESTRUS DETECTION IN A MANAGEMENT SYSTEM FOR DAIRY HERDS INVOLVING THE USE OF CIDR DEVICES

ABSTRACT

This study was designed to evaluate the effectiveness of using a milk progesterone test to identify non-pregnant animals and consequently improve oestrus detection rates in a management system for dairy herds involving systematic control of the oestrous cycle. The ideal synchrony system will be one which can at least maintain normal fertility, produce a high degree of synchrony and be economically used to control the oestrous cycle for first insemination, as well as for subsequent inseminations among those animals which return to service. The controlled breeding programme used in this study involved cows which had calved at least 35 days post-partum and were selected for the programme. They were examined and randomly allocated to treatment or control groups. Each treated cow had a CIDR inserted for 10 days and was tailpainted. At device removal, an injection of prostaglandin F2 α (PGF) or pregnant mare serum gonadotrophin (PMSG) was given depending on whether animals were cycling or non-cycling, respectively. They also had raddle applied over the paint at device removal. Cows were inseminated on detected oestrus over the first synchronization period. The used CIDR was re-inserted for 6 days from 14-16 days after original removal and each cow was tailpainted again. At removal of the used device, the paint strips were also re-raddled. The second period of AI on oestrus detection commenced and was completed within 2 days. In a herd with a year-round milking system, this programme meant that a new group of cows would enter the breeding schedule on every fourth Wednesday.

Milk and blood samples were collected for progesterone assay at the morning milking 24 h after the re-inserted CIDR was removed. Plasma progesterone concentrations were measured using a radioimmunoassay (RIA) technique, and each milk sample was measured by RIA and a progesterone ELISA assay kit. Each farmer was informed on the same day as samples were taken which cows could be expected in oestrus over the next 2 days.

The average percentage of non-pregnant cows inseminated during the second period of AI was 63%, and varied from 44% to 77.2% among individual herds.

97.6% of pregnant cows had milk progesterone concentrations between 3 and 39.9 ng/ml, and 99.5% of the non-pregnant animals were <3 ng/ml. In non-pregnant cows, the average concentration of progesterone was 0.2 ± 0.1 ng/ml one day after the re-used CIDR had been removed. In pregnant cows the average was 9.7 ± 0.7 ng/ml ($P < 0.001$).

These data showed that in general, the identification of non-pregnant animals did not improve the percentage of animals inseminated over the second period of AI. Therefore, providing information on pregnancy status did not lead to high detection rates. The incidence of false positive diagnoses (0.6%) and embryonic death (2.4%) were remarkably low.

INTRODUCTION

It is recognized that the most common problem in breeding management in dairy herds has been and will continue to be oestrus detection (Macmillan, 1988). Traditional methods of oestrus detection are time consuming, requiring at least two daily observation periods of 30 minutes (Elmore, 1987). Since the introduction of simple techniques like tailpainting (Macmillan and Curnow, 1977), there has been a reduction of the occurrence of this problem in seasonal dairy herds in New Zealand. Controlled breeding programmes should have the potential to reduce this problem in all dairy herds. However, control must extend beyond the first insemination (Macmillan, 1988).

Good reproductive management requires accurate pregnancy diagnosis as soon as possible after insemination to identify both non-pregnant and pregnant animals (Melrose, 1979). Measuring progesterone concentrations in either milk or blood provides an effective way of monitoring ovarian activity in cattle as well as detecting pregnancy (Melrose, 1976).

Several reports have demonstrated the usefulness of measuring milk progesterone for monitoring reproductive status. These include:

i) the early diagnosis of non-pregnant and pregnant animals (Cox et al., 1978);

ii) confirmation and prediction of oestrus (Reimers et al., 1985);

iii) identification of the resumption of post-partum ovarian activity (Fagan and Roche, 1986);

iv) monitoring reproductive components associated with ovarian activity and embryo mortality (Booth, 1980; Moller et al., 1986); and,

v) integration of hormone assays into fertility programmes (Lamming and Bulman, 1976; Foote et al., 1979; Benmrad and Stevenson, 1986).

Since the development of enzyme immunoassay techniques (ELISA), new and faster methods of measuring progesterone are available commercially. These tests have been compared with a previously validated radioimmunoassay ([RIA]; Nebel et al., 1989). ELISA tests have been used to select non-lactating beef cows for prostaglandin treatment in an oestrus synchronization programme (Meyers et al., 1988). A breeding programme involving the incorporation of a rapid progesterone assay and prostaglandin F2 α (PGF) treatments was used to detect and solve the problem of oestrus detection (Elmore, 1987). It was also used to determine the reproductive status of cows on days 21-24 after insemination (Wimpy et al., 1986; Worsfold et al., 1987; Nebel et al., 1987), to

confirm oestrus (Van de Wiel et al., 1982; Foulkes et al., 1982), and to monitor reproductive activity in cows at various stages of the oestrous cycle (Stanley et al., 1986). The use of a rapid progesterone assay in embryo transfer programmes has been also reported (Allen and Foote, 1988; Foote, 1988). However, the potential uses of the controlled internal drug release device (CIDR) for controlling return to service intervals, combined with milk progesterone estimation to improve oestrus detection and detect non-pregnant animals, has not been reported. The aim of this study was to evaluate the effectiveness of using milk progesterone test to identify non-pregnant animals and consequently improve oestrus detection rates and to reduce the average return to service intervals in a management system for dairy herds, involving systematic control of the oestrous cycle.

LITERATURE REVIEW

Measuring progesterone concentrations in either milk or blood provides an effective way of monitoring ovarian activity in cattle. The changes in concentration of progesterone in cow's milk have been shown to be an indicator of the cow's reproductive status, and can be used in reproductive management.

The uses of a progesterone test to monitor reproductive status include:

a) early diagnosis of non-pregnant and pregnant animals;

b) confirmation and prediction of oestrus;

c) identification of the resumption of post-partum ovarian activity;

d) monitoring reproductive components associated with ovarian activity and
embryo mortality; and

e) integration of hormone assays into fertility control programmes.

Improved reproductive efficiency depends on being able to inseminate cows at the correct time, and to know as soon as possible that they are pregnant. Precise monitoring should allow fertility problems to be detected before treatment or culling (Melrose, 1979).

Early Detection of Non-pregnant and Pregnant Animals.

It is frequently suggested that good reproductive management requires accurate pregnancy diagnosis as soon as possible after insemination, to identify both pregnant and non-pregnant animals without having the diagnosis confounded by subsequent embryonic mortality. One possibility to achieve this objective involves the use of a system based on progesterone assay results from a single milk sample at 20-26 days after insemination. The negative results (non-pregnant) were between 93.9 and 100% accurate, whereas the accuracy of the positive results ([Pregnant] 67.2-95.2%) was complicated by the incidence of embryonic mortality, and by the initial insemination of a cow during dioestrus instead of at true oestrus (Melrose, 1979; Foote et al., 1979; Van de Wiel et al., 1982; Davies, 1983; Chang and Estergreen, 1983; Reimers et al., 1985; Moller, et al., 1986; Worsfold et al., 1987).

Later pregnancy testing also was carried out when each cow was potentially 42-45 days pregnant. For this system, it was necessary to take two or three milk samples at fixed days (Davies, 1983). Other researchers have reported that identification of non-pregnant animals by rectal palpation at 60 days was significantly more accurate than progesterone determination at 20 days post-insemination (Roche and Prendiville, 1978). In that study, veterinarians predicted

pregnancy by rectal palpation, with 92.5% accuracy.

Davies (1983) also remarked that in herds with a large number of negative results for pregnancy, the positive identification of pregnant cows was liable to be less accurate. This was due to the poor fertility of these herds.

Confirmation and Prediction of Oestrus.

Accurate detection of oestrus is essential for successful use of artificial insemination (AI). The verification of progesterone concentrations in milk from cows with questionable signs of oestrus is an important use of milk progesterone assays.

Low milk progesterone concentration alone is not a positive indicator of oestrus. However, a high milk progesterone concentration confirms that a cow is not in oestrus. Reimers et al. (1985) have shown that the proportion of cows in dioestrus when inseminated varied from 0 to 60% among different herds.

Artificial insemination after a decline in milk progesterone concentration has been used by Foulkes et al. (1982). Cows in the treatment group were bred 2 days after detecting the fall in milk progesterone concentration accompanying luteolysis of the corpus luteum, whereas cows used as controls were observed for oestrus and then inseminated during the same period. There were 97.5% of cows in the progesterone assay group inseminated during the study-period of 30 days, compared with 70.7% of the cows in the control group. At 24 days after insemination, a similar proportion of those animals inseminated in both groups had high progesterone (64% vs 67%).

In a study designed to predict oestrus using an ELISA test (Eddy and Clark, 1987), milk samples from 2 herds were collected and analyzed for progesterone concentration between 16 and 24, or 17 and 23 days after insemination. A heat detector was applied following a fall in progesterone concentration, and cows were inseminated either after an observed oestrus or after the heat detector device was triggered. The proportions of 18-24 day inter-service intervals were increased by 39% and 24% in the two herds. Moreover, among cows with low concentrations of progesterone on day 24, 66.4% were low on day 19 and 94% low on day 22 after insemination. Also, 82% of cows had low progesterone concentrations for more than 48 h before they were inseminated, and only 66% of the inseminations were made between 3 and 4 days after initial progesterone testing. This indicated that because progesterone concentrations remained low for more than 48 h after luteolysis, AI by appointment was considered unacceptable because of the possibility of reduced pregnancy. Sampling on days 18, 20, 22 and 24 after insemination could allow oestrus to be predicted with a high degree of accuracy, but with an increased cost.

Eddy (1985, see Quinlan, 1987) attempted to evaluate the effect of alternate day milk testing for progesterone on oestrus detection rates following the first insemination. As a result of the use of progesterone assays in this study, the accuracy of oestrus detection increased and hence the percentage of 2-17 days inter-service intervals declined from 20.9% to 7.6%. Moreover, the

percentage of 18-24 day inter-service intervals increased from 24.7% to 53.3%, and number of inter-service intervals greater than 35 days was reduced from 40.5% to 18.8%.

Another study using assays of milk progesterone showed that only 53% of first post-partum ovulations resulting in increased milk progesterone concentrations were associated with an oestrus which was detected by a herdsman. However, the milk progesterone profiles showed that over 80% of cattle had actually ovulated within 60 days post-partum (Ball, 1980). In this study, cycling cows which were at least 50 days post-partum, but not observed in oestrus, samples were assessed using RIA, and injected with PGF if they had a progesterone concentrations indicative of luteal activity. The improved detection of oestrus using the progesterone assay and subsequent treatment of such cattle with PGF reduced the mean interval from calving to conception compared with the control group of herdmates (96.8 vs 105.7 days, respectively). Non-cycling cows at 35 days post-partum, which were confirmed as non-cyclic by milk progesterone assay, were treated with a PRID (progesterone releasing intravaginal device) which was inserted for 10 days. The mean interval from calving to conception was reduced from 94.4 days (in controls) to 80 days in treated cows.

Another experiment involved a herd having poor oestrus detection and a pregnancy rate to first service about 40%. The milk progesterone records starting at 50 days post-partum revealed that 98% of cows were cycling. It was found that signs of oestrus were missed, and that many cows apparently were reported for insemination on the basis of incorrect interpretation of signs of oestrus. When the animals were inseminated on oestrus detection which coincided with low progesterone values, the pregnancy rate was over 60% (Foote et al., 1979).

In order to solve the problem of oestrus detection, a breeding programme involving the incorporation of a rapid progesterone assay and PGF treatments was reported by Elmore (1987). Milk samples were collected from all normal cows known to be more than 60 days post-partum. Each cow with a high concentration of progesterone was injected with PGF and inseminated 72 and 86 h after injection, regardless of signs of oestrus. The cows with low concentrations of progesterone were re-sampled and re-tested 7 days later. Those cows with continually low progesterone concentrations were examined to determine the cause of anoestrus, and those with high progesterone concentrations were injected with PGF and inseminated 72 and 96 h later. The diagnosis of pregnancy was done twice; first by progesterone in milk with a sample taken on day 21 after insemination, and then by rectal palpation. Any non-pregnant cow was put back into the breeding programme. The pregnancy rates were similar to prior pregnancy rates in the herd (40%). Although this programme did not improve the poor pregnancy rate commonly experienced during the hot months, the benefit of the programme was to save labour.

Ruiz et al. (1989) used modelling and simulation to evaluate the effect of using an on-farm milk progesterone test for early detection of non-pregnancy and for prevention of insemination errors. For early detection of non-pregnancy, three testing schemes were compared against a control. The test extended the interval from calving to first insemination by 3 days, decreased the interval from calving

to conception by 4 days, and reduced the replacement rate by 2%. Also, the net return per cow per year was increased. For preventing insemination errors, two error rates in oestrus detection were compared. The test resulted in 0.16 fewer services per conception, but increased the interval from calving to conception by 3 days. The net return per cow per year was less than cows in the control group. Although the effect of the test was greater in herds with problems of oestrus detection, it was found to be unprofitable under the assumed cost and returns.

Identification of Resumption of Ovarian Activity after Calving.

Acyclicity in non-pregnant animals may be an indication of post-partum anoestrus, ovarian follicular cysts or uterine disturbances. Early diagnosis of any of these disorders is essential if a calving interval of 365 days is to be maintained. Because progesterone values vary according to the stage of the cycle, more than one sample is required for diagnosis. In order to study the accuracy of 2 progesterone samples in identifying cycling dairy cows, Heinonen et al. (1988) found that in cows which had started to cycle by 50 days post-partum, samples should be taken at intervals of 8 days. However, in cows which have started to cycle by 60 days post-partum, the lowest percentage of false positive results occurred when samples were taken at intervals of 10 days.

Fagan and Roche (1986) monitored the post-partum reproductive activity of 463 dairy cows, based on progesterone assay and rectal palpation. The mean interval from calving to first progesterone rise was 30.6 ± 0.9 days. Based on progesterone concentrations in milk, 31% of cows were non-cycling by 30 days and 7% by day 50 post-partum. There were 719 ovulations confirmed in all, of which 38% were associated with observed oestrus; 10% of observations of oestrus were found not to be associated with ovulation, and short cycles occurred in 4% of cows.

Monitoring of Reproductive Components associated with Ovarian Disorders and Embryo Mortality.

It is difficult to distinguish between follicular and luteal cyst by rectal palpation. Once the presence of an ovarian cyst has been determined by rectal palpation, differentiation can be made on the basis of progesterone concentrations in blood or milk.

Milk samples were taken from cows with supposed cystic ovarian disease. A correlation made between the diagnosis by rectal palpation from the veterinarian at the time of examination, and that made as a result of the progesterone assay showed that the veterinarian was correct in the diagnosis of follicular cysts at 80% of examinations, but only 52% were correct for the diagnosis of luteal cysts (Booth, 1980). In contrast, Leslie and Bosu (1983) found that rectal palpation accurately determined the presence of luteal cysts as confirmed by progesterone in plasma, but palpation was much less accurate for the diagnosis of follicular cysts.

In another study, milk progesterone assessed by ELISA was used to make

a differential diagnosis of follicular and luteal cysts and cystic corpora lutea, because it was not possible to accurately differentiate between these structures by rectal palpation (Nakao et al., 1983).

Several studies have used milk progesterone profiles to monitor possible embryonic loss in cattle. It occurred in 10.5% of cattle between 25 and 60 days after insemination, with the majority of losses occurring between 30 and 45 days after insemination (Kummerfeld et al., 1978; Ball, 1978).

Foote et al., (1979) had estimated a 22.7% loss between 28 and 75 days after insemination. This clearly over estimated the embryo mortality because many cows in oestrus before 28 days were missed according to normal cyclic patterns in milk progesterone. The embryo mortality by milk progesterone taking into account cycles longer than 27 days after insemination, was 7.2%.

A New Zealand trial was based on progesterone measured by RIA in milk in samples which were collected on the day of insemination and 7, 23 and 30 days later from 2274 cows in 14 herds (Moller et al., 1986). Two additional samples were collected from cows which returned to service more than 35 days after the first AI, one on the day of the return, and another 7 days later. Late returns after the 35th day occurred in 8.6% of the cows, and the estimated incidence of losses of concepta was 4.8%. These results also indicated that of these apparently late-returning cows, 55.9% suffered a loss of the conceptus, 22.1% had not been observed but had been in oestrus around 21 days after AI, 11.8% had a late return, 2.6% were in pro-oestrus or dioestrus at insemination, and 2.1% went into anoestrus after an oestrous insemination.

The systematic use of milk progesterone assays has produced lower estimates of embryonic mortality than estimates based in return to service intervals. In Moller's study, the average prevalence at late returns after the 35th day was 8.6% and the estimated prevalence of embryonic mortality was 4.8%. Other studies have shown higher incidences of late returns which ranged from 12.5% to 20.3% for returns greater than 25 days after insemination (Wijeratne, 1973; Kummerfeld et al., 1978; Kidder, et al., 1984). However, Kummerfeld et al. (1978) reported only 7.2% of long returns were related to embryonic mortality. A prevalence of 7.2% based on RIA would have been estimated at 22.7% if a delayed return to oestrus had been the method employed.

Integration of Hormone Assays into Fertility Control Programmes.

Assaying progesterone in milk can be useful in evaluating responses to endocrine treatment, and to interpret experiments and field trials designed to study the reproductive endocrinology of cows and to improve their reproductive efficiency.

A herd health programme in 10 herds was used in a study of the value of milk progesterone in detection of oestrus, early detection of non-pregnancy and for diagnosis of problem cows (Foote et al., 1979). Milk samples were taken three times weekly between 35 and 60 days post-partum in normal animals, three times over a 28-day period in cows with reproductive problems, on the day of each insemination and at 21 and 23 days after insemination. The predicted

pregnancy and non-pregnancy rates in comparison with diagnosis by rectal palpation were 98% accuracy for non-pregnant and 80% for pregnant animals, respectively. Also, 82% of the cows had initiated and completed oestrous cycles by 60 days post-partum. The remaining 18% had persistent high or low progesterone profiles. These profiles were associated with pyometra and with follicular cysts.

In one seasonal herd, a regime including synchronization of oestrus with fixed time insemination and pregnancy diagnosis by progesterone assay in milk on days 23 and 24 after insemination, was conducted in the U.K (Eddy, 1983). Calving to conception interval and culling rate were reduced, and the conception rate was improved.

Samples collected twice-weekly for milk progesterone assay have been successfully used for fertility control by identifying non-pregnant animals at 21 days after insemination, or cows inseminated at the wrong stage of the cycle, and also for differential diagnosis of ovarian cysts, pyometra and problems associated with control of oestrus. Lamming and Bulman (1976) found that twice a week sampling of milk for progesterone assay provided a more rational basis for differentiating problems of silent oestrus, anoestrus and persistent corpus luteum. Bulman and Lamming (1978) used a progesterone assay to study the onset of ovarian activity post-partum, the relationship between progesterone concentrations and conception rate, and profiles of progesterone in repeat breeder animals.

Milk progesterone testing 10 days after injection of gonadotrophin releasing hormone (GnRH) was used to monitor effective luteinization of follicular cysts, and showed that those cows with follicular cyst as defined by ELISA of milk progesterone, had a 70% response to treatment. Therefore, progesterone assays could be used to determine if re-treatment was needed (Nakao et al., 1983).

Response to treatment with PGF is dependent upon the presence of a functional corpus luteum. Milk progesterone testing before and 3 days after administration of PGF accurately assessed the success rate of the treatment (Benmrad and Stevenson, 1986).

The use of a rapid progesterone assay in embryo transfer programmes has been reported (Foote, 1988). Three milk samples were collected from each of 5 cows. The first was collected to determine if each cow selected to become an embryo donor had a functional corpus luteum. Cows with low progesterone concentrations at this time could be discarded from the programme. This saved time and reduced cost of drugs and semen. The second sample was taken 24 h after injecting PGF to ensure that luteolysis of the corpus luteum occurred in cows with non-visible signs of oestrus but with low progesterone concentrations so that they could then be inseminated. The third sample was collected after the donors were inseminated and just before the embryos were flushed and recovered. This sampling was to predict the ovarian response and possible number of embryos which could be collected.

Assay Methods of Measuring Progesterone.

The first routine analyses were conducted using RIA methods.

RIA's for this purpose used tritiated progesterone as a label (Heap et al., 1973), but an alternative RIA using progesterone labelled with ^{125}I was then proposed (Allen et al., 1980). Both of these methods gave accurate and reliable results, but they were relatively expensive and slow as they required specialised and expensive equipment and qualified technical support. They suffered from the problems associated with the use of radioisotopes, e.g.short shelf life of label (^{125}I), cost of scintillation and radioactive waste disposal (^{3}H,^{14}C), and a restriction of use to institutions which had approval and facilities to handle radioisotopes (Arnstadt and Cleere, 1981). Also, shipping, processing and response time produced variations in the notification of results from 2 to 10 days (Bishop et al., 1976). Such problems have prompted the search for alternative labels for use in immunoassays.

Enzymes have received the greatest attention because enzyme markers offer the possibility of achieving assays with practicability, sensitivity, and reproducibility similar to those of RIA (Wisdom, 1976; Voller and Bedwell, 1978). New and faster methods of measuring progesterone such as ELISA assays and a latex agglutination assay are now commercially available. Development of these methods has removed many of the constraints associated with RIA use because of their general accessibility, low cost and the innocuous nature of reagents employed. Moreover, their use in on-farm testing can eliminate shipping and processing, and reduce response time (Bishop et al., 1976), without reducing accuracy (Nebel et al., 1987). Many of these tests can be performed on the farm and results are available within minutes. Their are designed to identify high or low progesterone concentrations, rather than numeric values of concentrations. The evaluation of the result is based on either a colour change or an agglutination reaction which is compared with known standards. The type of reaction is determined by the conjugate-substrate system which is employed.

Correlation coefficients of ELISA results with those from a previously validated RIA where both methods were performed in the laboratory have been from 0.93 to 0.9 respectively (Chars and Estergreen, 1983). However, where the ELISA was performed on-farm and the RIA was performed in the laboratory, the correlation coefficient was only 0.79 (Nebel et al., 1987).

Principles and Characteristics of Milk Progesterone Tests.

1. Enzyme-linked immunoabsorbent assay.

Procedures for performing ELISA milk progesterone assays vary with each type of test. However, the principles of each assay are similar and are based on the same principles as RIA. The difference between ELISA and RIA is related mainly to use of an enzyme rather than a radioactive isotope to label progesterone. The ELISA test utilizes the principles of competitive absorption of progesterone in milk to a specific antibody for progesterone, which is coated onto a plastic tube. When milk is added to the system, progesterone attaches to specific antibodies

at the progesterone binding sites.

A progesterone-enzyme conjugate is added, immediately following milk addition (three conjugates have been tested: alkaline phosphatase, B-galactosidase and horseradish peroxidase). The progesterone-enzyme conjugate attaches to unbound antibody sites. Therefore, the greater the concentration of progesterone, the lower the binding of conjugate to antibody. Following a brief incubation (1-15 minutes), tubes are emptied and rinsed with water. The next step is the addition of the substrate. The degradation of substrate is inversely proportional to the concentration of progesterone. The final step involves addition of developer (chromogen) which reacts with modified substrate to produce a colour reaction. This reaction can be evaluated after a brief incubation of 1 to 5 minutes, depending upon the specific test being used.

The amount of colour development is inversely proportional to the concentration of progesterone in the milk sample. For example, a sample from a cow with a high concentration of progesterone will produce a slight colour change or remain clear. In contrast, a sample from a cow with a low concentration of progesterone will show a dark or intense colour change.

The classification of the colour reaction is one limitation of ELISA tests. To overcome this problem, specific samples, each of known concentration of progesterone, are analyzed at the same time as the unknown milk samples. Most test kits include two standards for comparison; one for oestrus (low level of progesterone); and one for pregnancy (high level of progesterone). However, other tests utilize only a single known standard for comparison. Using the single standard system, concentrations associated with oestrus result in a darker than standard reaction, whereas concentrations above the standard are associated with the luteal phase or pregnancy.

2. Latex Agglutination Test.

Equal amounts of milk, antibody, and progesterone-coated latex beads are mixed together and applied to a reaction slide. The mixture diffuses across the slide in a narrow channel so that, the latex beads and solutions interact with each other to provide a thin milk film. Milk containing a high concentration of progesterone results in progesterone antibody binding. This prevents agglutination, with the mixture appearing as a smooth milk film in the readout window. In contrast, a low concentration of progesterone allows the progesterone-coated latex beads to agglutinate to varying degrees, resulting in a grainy appearance in the readout window. This test does not use a standard for comparison, because the final indicator is the smooth versus grainy appearance of the milk film (Nebel, 1988).

There are at least 8 commercially available milk progesterone kits throughout the world. In the present experiment, the B.E.S.T. test was used. It is also the only non-ELISA milk progesterone test commercially available in New Zealand. It is based on a reaction between latex beads coated with progesterone molecules and antibodies, which have a specific attraction to progesterone.

The development of milk progesterone kits in other countries has resulted in recommended uses which are more suitable for town supply farms in New

Zealand. Basically, it involves testing for low or no progesterone levels.

The progesterone assay can be used to provide information which a herd owner may use to reduce the wide variability among cows in inter-service intervals. This can be done by identifying those cows which are likely to return to oestrus following the first or any other insemination. Non-pregnant cows return to oestrus 18 to 24 days after insemination. If this oestrus is not detected, then the return to service interval is about 42 days. This assay can be used as follows:

i) to improve conception rates, by avoiding those animals which would otherwise be inseminated when they are not in oestrus;

ii) to identify cows thought to be in anoestrus by the dairyman; and,

iii) to aid in the diagnosis of cystic ovaries, so that the dairyman can identify the affected cow by taking a milk sample from this cow prior to veterinary examination by rectal palpation, to increase the accuracy of veterinary diagnosis. As a result, a more specific treatment may be given to this condition.

In these cases, the calving to first AI interval can be reduced for some cows.

The potential uses of CIDR's for controlling return to service intervals among cows in autumn-calving or town supply farms combined with milk progesterone testing could have a great application for detecting non-pregnant animals and increasing rates of oestrus detection in herds where it is common to find low rates, especially during winter.

OBJECTIVE

a) To evaluate the effectiveness of using a "cow side" milk progesterone test to identify non-pregnant animals and consequently improve oestrus detection in cows previously synchronized in a controlled breeding management system.

MATERIALS AND METHODS

This study was carried out in four commercial (4) town-supply dairy herds in the Manawatu area of New Zealand. It involved the use of a controlled internal drug releasing device (Eazi-Breed CIDR^{tm}-B, Carter Holt Harvey Plastic Products, Hamilton. N.Z.). Each device consisted of a silicone elastomer impregnated with 1.9 g progesterone. This was combined with strategic use of PGF (Estrumate, Coopers, Upper Hutt. N.Z.) and pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet Chemavet Distributors Ltd, Auckland. N.Z.).

Experimental Design.

The ideal synchrony system will be one which can at least maintain normal fertility, produce a high degree of synchrony, and be economically used to control the oestrous cycle before first, as well as before subsequent inseminations among those animals which return to service.

This concept was included in the following protocol which was used with the treatment group of cows in each herd:

Day 0: Wednesday, All cows which had calved at least 35 days and were identified to start the programme were examined by an experienced veterinarian before being randomly divided into three sub-groups (defined below), based on calving date. Each cow had a CIDR device inserted and was tail-painted.

Day 10: Saturday, CIDR's were removed. The cycling cows (ovaries of good size and with palpable structures) were injected with either the recommended luteolytic dose of Estrumate (500 μg i.m. Estrumate, group TF) or half the dose of PGF (250 μg i.m. Estrumate, group TH). Raddle was applied over the tailpaint. The non-cycling cows (ovaries were small, hard and without any palpable structures) received PMSG (400 I.U. i.m. Folligon), plus raddle over the paint strip.

Day 12-14: Monday-Wednesday. Cows were inseminated on detected oestrus. It was expected that 80-90% of cows would be inseminated during these 3 days.

Day 28: Wednesday. A used/washed/stored CIDR was re-inserted into the vagina of each cow, which was tailpainted again.

Day 33: Tuesday. CIDR's were removed and the paint strips were also re-raddled.

Day 35-36: Thursday-Friday. The second period of artificial insemination on oestrus detection commenced, and was completed within two days.

In a herd with a year-round milking system, this programme meant that a

new group of cows would enter the breeding schedule on every fourth Wednesday. This time coincided with CIDR re-insertion for cows from the previous month (Figure 4.1).

This regime started on three farms in the same month. The fourth farm started at a different time because it had cows calving only for a restricted period in autumn and spring. The study period in each farm was 10 months, with half of the cows being in the treatment group.

Experimental Procedure.

This regimen was designed to improve oestrus detection by reducing time spent on this task as a result of reduced variability in the post-treatment interval to oestrus, by stimulating a fertile oestrus in anoestrous cattle, and by developing a method by which most of the cows in the herd would conceive within a defined interval post-partum. Reproductive and production records were collected for that period of time.

In this treatment regimen, all the cows entered the study when they were between 45 and 72 days post-partum, and at a fixed date in a 4-weekly cycle. The treatment started when treated cows were examined at 36 ± 7 days post-partum. Control cows were also examined rectally to determine the progress of uterine involution and the nature of ovarian structures.

Throughout the experiment, treatment and control cows were observed for signs of oestrous behaviour twice daily at approximately 12 h intervals (with each observation period lasting at least 20-30 minutes) in conjunction with tailpainting (Macmillan et al., 1988). Observation periods did not coincide with feeding and milking. Paint and raddle of different colours were used to identify individual groups of cows.

Although oestrus was defined as that period during which a cow stood to be ridden by its herd mates or by a herd sire, they were also considered to be, or have been in oestrus when the paint and raddle were rubbed off (observation periods during milking times). All cows with loss of paint and raddle, or displaying oestrous behaviour, were inseminated.

Following detection of oestrus, both treatment and control groups were inseminated in accordance with normal farm practice (ie. artificial insemination normally on the morning after first detection). Diagnosis of pregnancy was by rectal palpation of the uterus at about 6 weeks following the last insemination date.

Control Group.

These animals were managed and inseminated according to standard procedures used in each herd. Insemination of the control cows commenced at the first oestrus from 45 days post-partum.

Cows in the control group which were not observed in oestrus by day 120 post-partum and cows that failed to conceive from previous inseminations were examined, and then treated as appropriate.

Progesterone Determination.

Milk and blood samples were collected for progesterone assay. These samples were taken at the morning milking 24 h after the re-inserted CIDR device was removed. Ten (10) ml of blood was collected by coccygeal venepuncture into heparinized tubes (NIPRO-New Tube System, Vacuum Blood Collecting System, Nissho Corporation, Osaka Japan). Samples were centrifugated within 3 h at 3000xg for 20 minutes. The plasma was collected and stored at -20 °C until progesterone was measured using a specific radio-immunoassay.

Milk samples (5 ml) of first milk and from any healthy quarter, were collected in a clean plastic bag provided in the supply kit, then refrigerated until tested. The remaining amount of milk was stored at -20 °C until assayed for progesterone by a validated RIA. The first 3-5 squirts from the teat were discarded. The ELISA test was run in the laboratory within 6 h of collection. Each farmer was informed on the same day which cows could be expected in oestrus over the next 2 days.

This strategic sampling sequence was used to check the variation in progesterone concentrations, and to identify non-pregnant and pregnant animals.

The plasma progesterone concentrations was measured using an RIA technique and the milk sample was measured by RIA and a rapid progesterone assay kit (Bovine Estrus Slide Test, B.E.S.T.™ Test; Alfa Laval (NZ) Ltd, P.O. Box 10241, Te Rapa, Hamilton, New Zealand).

Hormone Assay.

Milk progesterone. Progesterone concentrations were measured by direct radioimmunoassay without extraction, using the method of Dobson et al. (1975) which had previously been validated in this laboratory by Lapwood (see Moller et al., 1986). Duplicate 20 µl aliquot of standard progesterone solutions (range 0-80 ng/ml) were evaporated to dryness under air, then 20 µl ovariectomized cow's milk was added to each standard tube. After dispensing 20 µl unknown milk samples into assay tubes, 600 µl buffer containing rabbit anti-progesterone serum (1:3000; courtesy of Dr J.L. France) and tritiated progesterone (8000 c.p.m.; Radiochemical Centre, Amersham, U.K.) was added to all tubes, mixed, then incubated overnight at 4° C. After addition of 600 µl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia. U.S.A.) suspension in buffer, tubes were vortexed incubated for 10 minutes at 4 °C. The supernatant was decanted into scintillation vials, and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Mean assay sensitivity was 0.42 ng/ml. Intra-assay coefficients of variation were 20%, 18.2% and 21% and inter-assay coefficients of variation

were 8%, 6.9% and 13.1% for milk pools containing mean progesterone concentrations of 9.3, 14.8 and 30.3 ng/ml, respectively (n = 16).

The commercial milk progesterone test (B.E.S.T.) was performed according to the instructions provided with the kit.

The B.E.S.T. kit was run using a disposable plastic slide. The milk, antibody and latex were mixed together in the mixing well of the slide. The mixture then travelled from the arrow through the channel in the slide until it appeared at the opposite end in the readout window where the results were visualized and recorded.
When the milk contained less than 5 ng/ml of progesterone, the latex and the antibody linked together into chain-like structures, producing a grainy appearance. This result indicated that a cow was not pregnant. The smooth appearance indicated that a cow was in mid-cycle or pregnant because progesterone concentrations were high.

Plasma progesterone. Plasma concentrations of progesterone were determine by the RIA method of Kirwood et al.(1984).

Determinations were made on 500 μl sub-samples from each original plasma sample. They were extracted with 5 ml toluene:hexane (1:2 v/v). The plasma was frozen overnight, and solvent was then decanted into clean tubes, dried under air and redissolved in 500 μl ethanol. Duplicate 100 μl samples of ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 μl samples of standard ethanolic solutions of progesterone (P-1030: Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone level; of 0.625-40 ng/ml. A mixture containing antiserum (courtesy of Dr J. T. France, National Women's Hospital, Auckland, New Zealand) at a final dilution of 1:1800 (Tungsubutra & France, 1978); [1,2,6,7-H] progesterone (TRK 413, Amersham, Bucks, U.K.) at 8000 c.p.m./100 μl; phosphate-buffeted saline containing 0.02m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 μl) to each tube and vortexed. After overnight incubation at 4 °C, 600 μl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and, then incubated at 4 °C for 10 minutes. Tubes were then centrifugated at 3000xg for 10 minutes at 4 °C. The supernatant was decanted into scintillation vials and 6 ml toluene-triton scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Assay sensitivity was 0.08 ng/ml. Intra-assay coefficients of variation were 12.1%, 10.5% and 16% and inter-assay coefficient of variation were 8.9%, 11% and 13.7% for plasma pools containing mean progesterone concentrations of 2.5, 5.8, and 10.9 ng/ml, respectively (n = 12).

Statistical Analysis.

Data were analyzed using Panacea Database Management (PAN Livestock Services LTD. Department of Agriculture, University of Reading, P.O. Box 236, Reading, Berkshire, England).

Differences in progesterone concentrations between non-pregnant and pregnant cows were evaluated by analysis of variance. Multiple regression analysis was used to obtain the correlation between plasma and milk progesterone.

RESULTS

Each farmer was informed (on the same day as samples were taken) which cows could be expected in oestrus over the next 2 days after CIDR removal. The average percentage of non-pregnant cows inseminated during the second period of AI was 63% and varied between 44% and 77.2% among herds (Table 4.1). Among the 70 cows inseminated, 55% of them were inseminated at 48 h. after device removal, and the remaining 45% of cows at 72 h. The conception rate to second insemination was similar between treatment and control groups (52.3% vs 56.7%, respectively).

Milk progesterone concentrations measured by RIA of non-pregnant and pregnant cows are presented in Table 4.2.

In non-pregnant cows, the average was 0.2 ± 0.1 ng/ml one day after the re-used CIDR had been removed. In pregnant cows it was 9.7 ± 0.7 ng/ml ($P < 0.001$).

Concentrations measured by RIA in milk from 82 pregnant animals were between 1.29 and 39.9 ng/ml; only two samples (2.4%) ranged from 1.2 to 2.9 ng/ml, whereas 97.6% were between 3 and 39.9 ng/ml. The 182 non-pregnant cows had concentrations of progesterone in milk of between 0 and 3.19 ng/ml, 95% ranging between 0 to 1 ng/ml. Only 4.9% were between 1 and 3 ng/ml, and the remaining 0.5% were above 3 ng/ml (Figure 4.2). These data show that 97.6% of pregnant cows were identified and 99.5% were non-pregnant. Therefore, an arbitrary decision was made to designate all cows whose milk contained less than 3 ng/ml as non-pregnant. The sensitivity and specificity (these concepts in this case are defined as the ability of a test to give a positive result when the animal is pregnant, or as the ability to give a negative result when the animal is not pregnant, respectively) for different concentrations of progesterone in milk by RIA, combined with rectal palpation showed that for 2 ng/ml, they were 98-98; for 3 ng/ml, they were 98-99; for 4 ng/ml, they were 94-100, and for 5 ng/ml, they were 82-100, respectively (Table 4.3).

The results of the reverse comparison of milk progesterone concentrations measured by RIA and pregnancy status are presented in Table 4.4. In this study, the classification of cows (made one day after CIDR removal based on milk progesterone concentrations by RIA) to estimate non-pregnant and pregnant status was adopted from a previous study (Reimers et al., 1985). The current study found that 0% ($n=0$), 2.4% ($n=2$), and 97.6% ($n=80$) of cows were pregnant as determined by rectal palpation when concentrations of progesterone were less than 1, 1 to 3, and greater than 3 ng/ml respectively. For non-pregnant animals, the percentages were 95% ($n=172$), 4.9% ($n=9$), and 0.5% ($n=1$) respectively (Table 4.5).

One cow diagnosed non-pregnant at rectal palpation 42 days after AI had a concentration of progesterone in milk of 3.1 ng/ml, but the remaining 181 (99.5%) were < 3 ng/ml. In contrast, 2 cows had concentrations of

progesterone in milk less than 3 ng/ml 1.2 and 2.9 ng/ml, respectively), but they were diagnosed pregnant by rectal palpation 6 weeks after insemination; the remaining 80 pregnant cows (97.6%) were > 3 ng/ml.

The comparison between the BEST milk progesterone test classified as low and high level and concentrations of progesterone by RIA classified as low or high is shown in Table 4.6. The comparison with RIA was 90.3% for low progesterone, and 85.5% for high progesterone (values greater than 3 ng/ml).

The distributions of progesterone concentrations determined by RIA in each category (pregnant and non-pregnant) are shown in Table 4.7. Of the samples classified as having progesterone concentrations ≤ 2 ng/ml, correct classification was made for 92.2%. In the three samples with progesterone concentrations greater than 2 to 4 ng/ml, all the samples were classified as pregnant. Of the samples classified as having progesterone concentrations greater than 4 to 10 ng/ml, a correct classification was made with 80% of cases, and samples having a progesterone concentrations greater than 10 ng/ml, correct classification was made in 92% of cases.

The sensitivity of the B.E.S.T. test kit was 83% and the specificity was 93%. The sensitivity and specificity were determined by the comparison between the B.E.S.T. results and the values from the RIA. However, the corrected sensitivity was 85% and for specificity 95%, which were determined by comparison of the test with the results of rectal palpation. The sensitivity and specificity for different concentrations of progesterone in plasma by RIA, combined with rectal palpation showed that for 1 ng/ml, they were 100-95; for 2 ng/ml, they were 98-98; and for 3 ng/ml, they were 93-100, respectively (Table 4.8). Concentrations measured by RIA in plasma from non-pregnant and pregnant animals are presented in Figure 4.3. In non-pregnant cows the average concentration of progesterone was 0.3 ± 0.1 ng/ml one day after the re-used CIDR was removed, compared with 7.7 ± 0.6 ng/ml for pregnant cows ($P < 0.001$; Table 4.9). The correlation between milk and plasma progesterone was 0.8 and the equation describing their relationship was:

Milk progesterone = 1.112 PLasma progesterone - 0.143 ng/ml

se = ± 0.052

The multiple coefficient of determination is at 0.638 (64%).

DISCUSSION

A milk progesterone test can be used effectively to identify non-pregnancy earlier after breeding than pregnancy diagnosis by rectal palpation and non-return to oestrus. In this study, a breeding programme incorporating ELISA milk progesterone assay with CIDR-B, PGF or PMSG treatment for controlling return to service intervals did not improve the percentage of cows inseminated over the second period of artificial insemination in all the farms. Moreover, the return-to-service intervals of around 36-50 days (6 weeks) were similar for treated cows, compared with control cows (9.9% vs 13.1%, respectively). This lack of improvement is not in agreement with a previous study (Duirs et al., 1986), in which the use of CIDR devices as a post-insemination treatment on days 14-17 with removal on day 21 significantly reduced the incidence of 6 weeks return from 15.2% to 8.2%.

Clearly, the data show that in general the identification of non-pregnant animals did not improve the percentage of animals inseminated over the second period of AI. In some farms (A and B) the oestrus detection rate was increased (Table 4.1), as seen in a previous study (Dick, 1990a), in which treatment groups in those farms showed an average of 76% of animals observed in oestrus and inseminated during the 5-day period, while in the same farms, control groups had an average of 63%. The low percentage of animals observed and inseminated, mainly in Farms C and D was due in part to failure in detecting oestrus. Most animals which were not observed in oestrus had a corpus luteum when they were examined rectally to diagnose the reason for lack of behavioural oestrus. However, the paint and raddle from these cows with low levels of progesterone were not rubbed off. This suggests that the poor percentage of cows observed and inseminated may require further study among cows which may not display oestrus in some of these herds. Alternatively, relying on tailpaint may not be appropriate with cows in these herds.

Identification of non-pregnant animals is an important component of reproductive management. Identifying these animals earlier after insemination could possibly be successfully used in herds with high detection rates. Moreover, the progesterone concentrations on first milk samples taken at the morning milking one day after CIDR removal showed that there was a significantly higher value in pregnant cows than in non-pregnant cows (Table 4.2). These progesterone concentrations in milk are similar to those reported for cows in oestrus in another study using RIA of progesterone in milk (Dick, 1990b) and to those in a previous report with cyclic cows (Pennington et al., 1985; Eddy and Clark, 1987; Nebel et al., 1989).

One of the practical applications for an ELISA progesterone test is for oestrus prediction in dairy cows. Set-time inseminating after a decline in milk progesterone has been reported (Foulkes et al., 1982). A breeding programme involving the use of a rapid progesterone assay and PGF treatment was reported by Elmore (1987), in which each cow with high concentrations of progesterone was injected with PGF and inseminated 72 and 96 h after injection, regardless of

signs of oestrus. Pregnancy rates did not differ from prior pregnancy rates in the herd. However, in a previous study designed to predict oestrus using an ELISA progesterone test, it was found that more than 80% of cows had low progesterone concentrations for more than 48 h before they were inseminated, and only 66% of the inseminations were made on days 3 and 4 after initial progesterone testing (Eddy and Clark 1987). In another study, low levels of progesterone estimated by the test kits which were associated with oestrus, ranged from 6.5 to 9.7 days. Samples collected during early and mid-dioestrus had similar levels compared with RIA (Nebel et al., 1989). This suggests that the prediction of oestrus based on ELISA progesterone test with a single sample cannot be used to determine correct timing of insemination (Eddy and Clark, 1987). It was suggested that the application of milk progesterone ELISA assay which incorporates synchrony treatments may need to be re-evaluated (Nebel et al., 1989).

This concept may be applicable to the first synchrony treatment under this management system with CIDR devices, as CIDRs can be successfully used to synchronize returns to oestrus in non-pregnant animals. Other studies showed that 85% of cows were in oestrus in a 2-day period (Dick, 1990a), and that 81.3% of heifers were in oestrus on day 24 after treatment (Van Cleeff et al., 1989). It suggests that when high synchrony is achieved after an initial treatment before first insemination, as well as at subsequent inseminations among cows which return to service (Macmillan 1988), the kit may have an important role because the interval from first AI to conception can be reduced. The concept of improving breeding management will be particularly useful and important in year-round herds and in any seasonal herds with poor oestrus detection. The possibility for set time inseminating at 48 or 72 h would have to be investigated because only 55% of the cows were seen in oestrus and re-inseminated at 48 h after the re-used CIDR was removed. Recent studies using modelling and simulation showed that the use of an on-farm milk progesterone test for early detection of non-pregnant animals improved reproductive performance in terms of days open, replacement rate, and net return per cow per year (Ruiz et al., 1989), and was economically profitable (Elmore, 1986; Ruiz et al., 1989).

Different limits, due to the influence of techniques of sampling and analysis to separate pregnant and non-pregnant categories have been selected by other authors (Heap et al., 1976; Hoffmann et al., 1974). In this study, the decision to use 3 ng/ml as the upper limit for non-pregnant cows, and the lower limit for pregnant cows showed that 97.6% of the values were above the limit in pregnant cows, and 99.5%% of the values were below the limit for non-pregnant cows when status was confirmed by rectal palpation. The value of 3 ng/ml is similar to findings in a previous report with the same commercial kit (Nebel et al., 1989).

A previous report showed a high percentage of false-positive results in three herds, ranging between 14 and 31%, and the author indicated several common reasons for it (Elmore, 1987). In this study, the incidence of false positive results was very low. Only one pregnant cow was detected as non-pregnant with progesterone concentrations in milk more than 3 ng/ml (0.6%). This can be explained in part because the use of this reproductive management technique, involving the control of the oestrous cycle, produced a reduction in the incidence of ovarian and uterine disturbances, and achieved a good synchrony after the

second treatment. It may also had reduced the incidence of dioestrous first insemination.

Only 2 cows in this study were pregnant with concentrations of progesterone from 1 to 3 ng/ml; but the same concentrations of progesterone were found in 9 cows which were diagnosed as non-pregnant by rectal palpation. Only 1 cow which had a concentration greater than 3 ng/ml was diagnosed as non-pregnant.

In this study, the incidence of embryonic death between the day after CIDR removal and diagnosis by palpation was remarkably low, only 2 cows which were given as pregnant by RIA were found to be non-pregnant by rectal palpation (2.4%). Other New Zealand reports (Macmillan et al., 1977; Moller et al., 1986) have also estimated a lower incidence when it was compared with an overseas report (Wijeratne, 1973) which had accepted the premise that an extended inter-service interval was synonymous with embryonic loss. However, Kummerfeld et al. (1978) remarked that the data from milk progesterone profiles to monitor possible embryonic loss in cattle was more accurate and reliable than that collected from records of inter-service intervals because not all the cows when they return to a second service are re-inseminated. Such a differentiation is possible by taking milk samples. Therefore, an accurate and practical method of identifying pregnancy status is through the immunoassay techniques. From the results of this study, the important point is that the probable loss rate of a pregnancy from milk progesterone sampling to pregnancy testing in these 4 herds was only 1 of 82 cows (1.2%). The values of progesterone in milk and plasma for this cow at one day after CIDR removal were 3.1 and 2.6 ng/ml, respectively, which was classified as being pregnant. Therefore, it could be considered as an embryo mortality.

On the other hand, it is possible that the lower prevalence found in this study occurred because most losses occurred within 3 weeks of insemination. It therefore does not give long intervals (Moller et al., 1986), and does not affect the initiation of the next phase of follicular growth and ovulation (Lamming et al., 1989). The results of this current study support the hypothesis that early losses are more common than later ones (Sreenan and Diskin, 1983; Lamming et al., 1989).

CONCLUSIONS

A milk progesterone test can be used effectively to identify pregnancy status earlier after breeding than pregnancy diagnosis by rectal palpation and non-return to oestrus. However in this study, the breeding programme incorporating ELISA milk progesterone assay and CIDR, PGF, or PMSG treatment for controlling return to service intervals in general did not improve the percentage of cows inseminated over the second period of AI. The low percentage of animals observed and inseminated was due in part to failure to detect oestrus. This observation would require further study among cows which may not display oestrus.

The identification of non-pregnant animals is an important component of reproductive management, possibly this technique could be succesfully used in herds with high detection rates. Also, the test may have an important role because this management system can be successfully used to synchronize returns to oestrus in non-pregnant animals. Moreover, the possibility for set-time inseminations would have to be investigated.

The estimated incidence of embryonic mortality was low (1 of 82 pregnancies, 1.2%). The use of milk and plasma progesterone assays has produced lower and accurate estimates of embryo mortality. However, it should not be confused with failure to detect non-pregnant cows when they were in oestrus.

Figure 4.1 **Basic programme of experimental protocol.**

Day		Task
0	Wednesday	Insert CIDR, Tailpaint
10	Saturday (am)	Remove CIDR, Raddle over Paint
12-14	Monday-Wednesday	1st Insemination on Detection
28	Wednesday *	Re-insert Used/Washed/Stored CIDR, Tailpaint
34	Tuesday (am)	Remove and Discard CIDR, Raddle
36-37	Thursday-Friday	2nd Insemination on Detection.

* In herds with year-round milking, a new group of cows enters the breeding schedule on every fourth Wednesday.

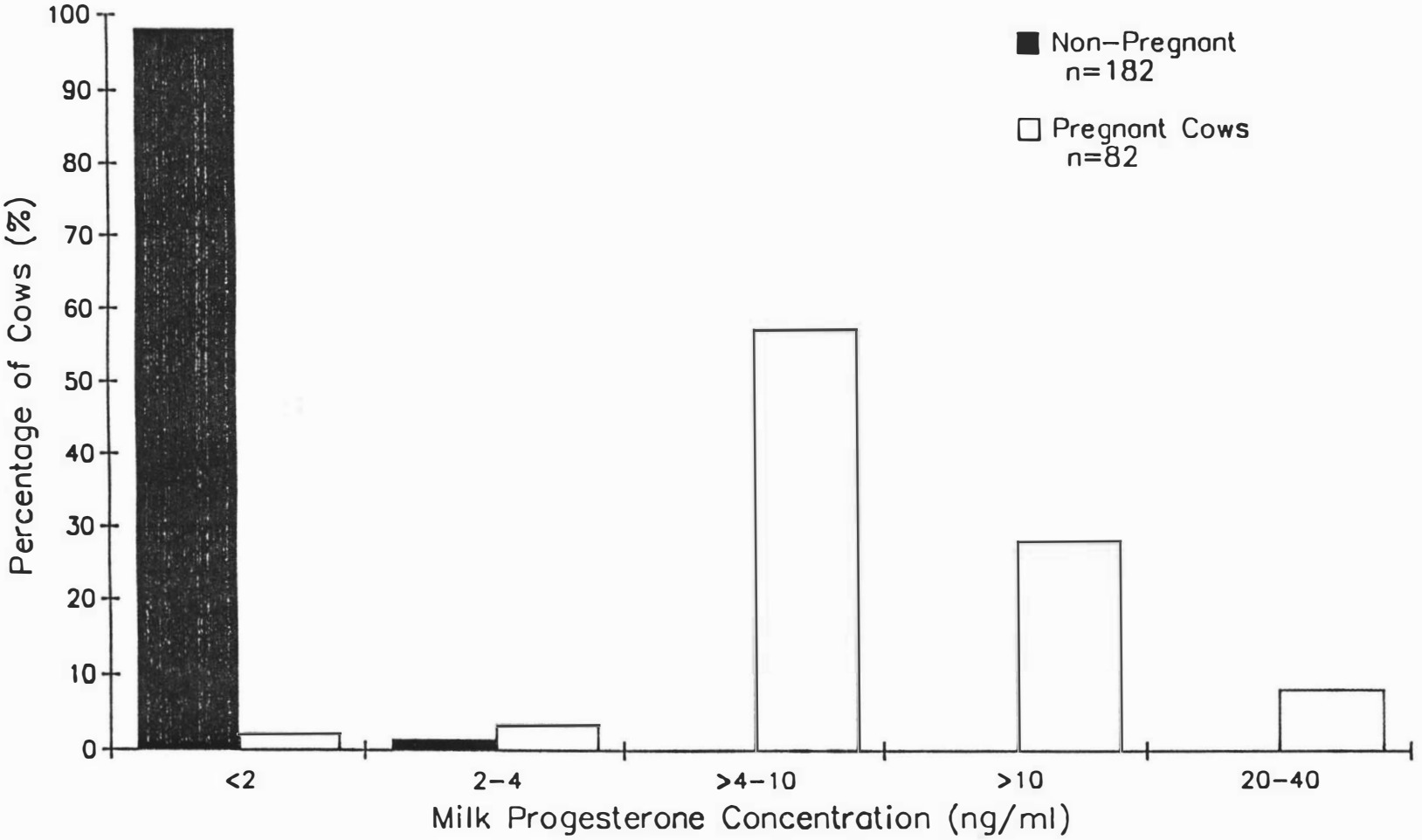


Fig. 4.2 **Non-pregnant and pregnant cows and progesterone in milk by radioimmunoassay (RIA)**

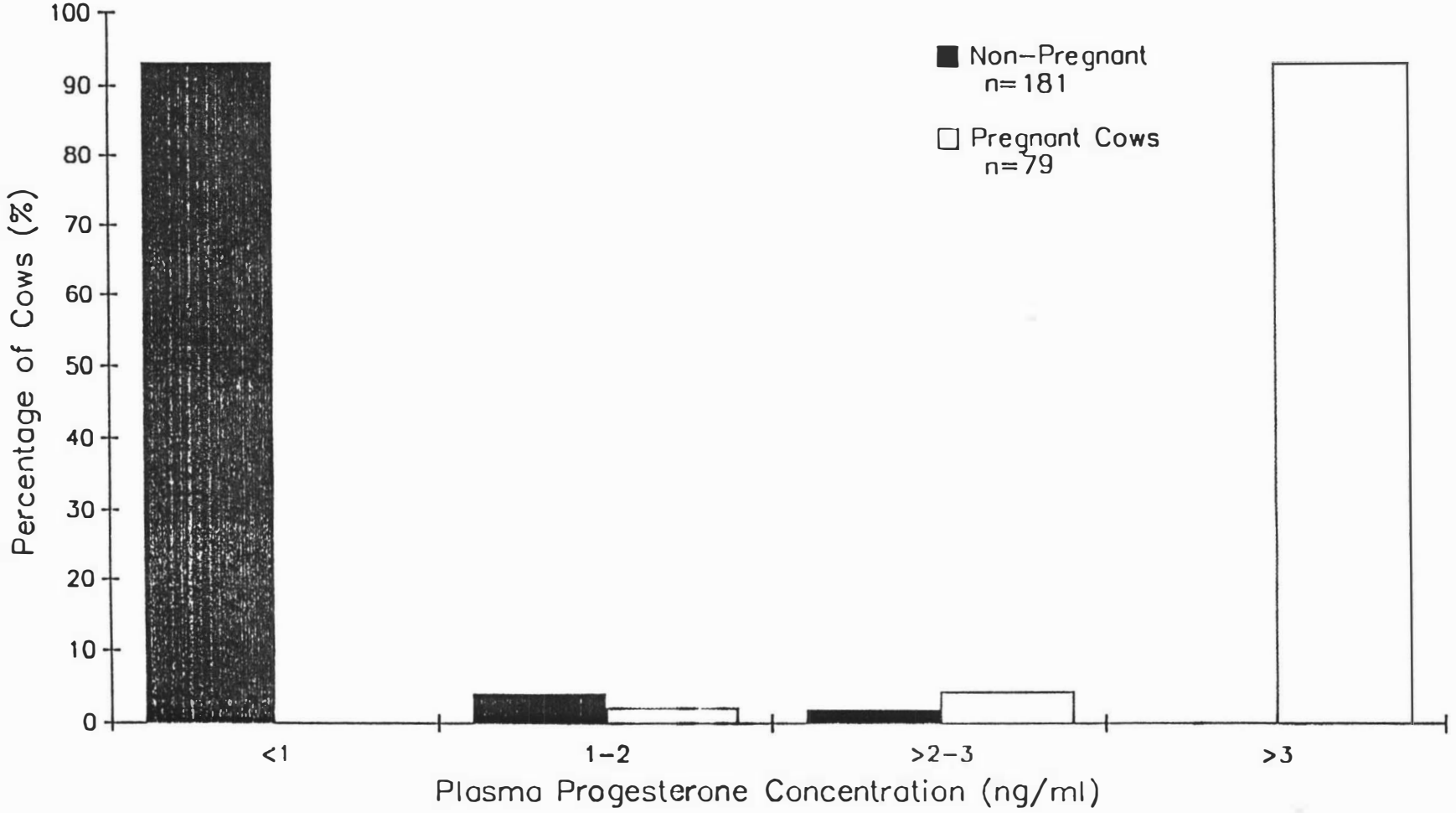


Fig. 4.3 **Non-pregnant and pregnant cows and progesterone in plasma by radioimmunoassay (RIA)**

Table 4.1 **Percentage (%) of cows inseminated over the second period of artificial insemination after being identified as non-pregnant by B.E.S.T. Test Kit.**

Farm	Nº of Non-pregnant Cows	Cows Inseminated	Percentage (%)
A	22	17	77.2 %
B	24	18	75 %
C	50	22	44 %
D	23	13	56.5 %
Total	120	70	63 %

Table 4.2 **Means (SEM) of milk progesterone concentrations (ng/ml) by radioimmunoassay (RIA) of non-pregnant and pregnant cows at one day after CIDR-B removal.**

Status	N° of Animals	Progesterone ng/ml[a]
Non-pregnant	182	0.2 ± 0.1
Pregnant	82	9.7 ± 0.7

Probability a= $P < 0.001$

Table 4.3 **Sensitivity and specificity for different concentrations of progesterone in milk (P4; ng/ml) determined by radioimmunoassay.**

P4	Sensitivity %	Specificity %
2	98	98
3	98	99
4	94	100
5	82	100

Table 4.4 **Comparison between milk progesterone concentrations (ng/ml) by radioimmunoassay (RIA) one day after CIDR-B removal and pregnancy status at rectal palpation 42 days after artificial insemination.**

Progesterone ≤ 3 ng/ml		Progesterone > 3 ng/ml	
Pregnant Cows	Non-pregnant Cows	Pregnant Cows	Non-pregnant Cows
2	181	80	1
(2.4%)	(99.5%)	(97.6%)	(0.5%)

( ) = Percentage of animals

Table 4.5 **Percentage (%) of non-pregnant (NP) and pregnant (P) animals at 3 concentrations of progesterone determined by radioimmunoassay.**

	Concentration of Progesterone		
Status	< 1	1 to 3	> 3
NP	95	4.9	0.5
P	0	2.4	97.6

Table 4.6 **Progesterone classification into low and high level by using Best test and radioimmunoassay (RIA).**

	Level of Progesterone by RIA			
	Low		High	
Progesterone Test	n	%	n	%
B.E.S.T.	124	90.3	63	85.5

Table 4.7 **Distribution of samples by concentrations of progesterone (P4) in milk as determined by RIA (non-pregnant and pregnant), after being classified by BEST test.**

Test	Progesterone Concentrations (ng/ml)							
	≤ 2		> 2 to 4		> 4 to 10		> 10	
	NP	P	NP	P	NP	P	NP	P
BEST	113 (92.2%)[a]	8	0 (100%)	3	6	30 (80%)	2	25 (92%)

NP = non-pregnant
P = pregnant
a = Percentage of samples classified correctly

Table 4.8 **Sensitivity and specificity for different concentrations of progesterone in plasma (P4; ng/ml) determined by Radioimmunoassay.**

P4	Sensitivity %	Specificity %
1	100	95
2	98	98
3	93	100

Table 4.9 **Means (SEM) of plasma progesterone concentrations (ng/ml) by radioimmunoassay (RIA) of non-pregnant and pregnant cows at one day after CIDR-B removal**

Status	N° of Animals	Progesterone ng/ml[a]
Non-pregnant	181	0.3 ± 0.1
Pregnant	79	7.7 ± 0.6

Probability a = $P < 0.001$

REFERENCES

ALLEN, R. M., REDSHAW, M. R. and HOLDSWORTH, R. (1980). A comparison of triated and iodinated tracers in the radioimmunosaay of progesterone in cow milk. **J. Reprod. Fertil.** 58: 89-93.

ALLEN, S. E. and FOOTE, R. H. (1988). An enzyme-linked immunoassay of milk progesterone as a diagnostic aid in embryo transfer programs. **Theriogenology.** 29: 893-903.

ARNSTADT, K. I. and CLEERE, W. F. (1981). Enzyme-immunoassay for determination of progesterone in milk from cows. **J. Reprod. Fertil.** 62: 173-180.

BALL, P. J. H. (1978). The relationship of age and stage of gestation to the incidence of embryo death in dairy cattle. **Res. Vet. Sci.** 25: 120-122.

BALL, P. J. H. (1980-81). Milk progesterone profiles and the diagnosis and treatment of subfertility in dairy cattle. **Proc. Brit. Cattle Vet. Assoc.** 67-69.

BENMRAD, M. and STEVENSON, J. S. (1986). Gonadotropin-releasing hormone and prostaglandin F 2 alpha for postpartum dairy cows: estrus, ovulation, and fertility traits. **J. Dairy Sci.** 69: 800-811.

BISHOP, C. A., BOND, C. P. and ROBERTS, C. (1976). Early diagnosis of nonpregnancy in cattle: the first eighteen months of a commercial service. **Br. Vet. J.** 132: 529-533.

BOOTH, J. M. (1980). Milk progesterone pregnancy testing in cattle and other species. **Proc. 9th Int. Cong. Anim. Reprod. Artif. Insem.** 2: 109.

BOOTH, J. M. (1980-81). Cystic ovaries: milk progesterone levels. **Proc. Brit. Cattle Assoc.** 71-75.

BULMAN, D. C. and LAMMING, G. E. (1978). Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. **J. Reprod. Fertil.** 54: 447-458.

CHANG, C. F. and ESTERGREEN, V. L. (1983). Development of a direct enzyme immunoassay of milk progesterone and its application to pregnancy diagnosis in cows. **Steroids.** 41: 173-195.

COX, N. M., THOMPSON, F. N. and CULVER, D. H. (1978). Milk progesterone to predict reproductive status in a commercial dairy herd. **J. Dairy Sci.** 61: 1616.

DAVIES, J. (1983). The milk progesterone test for pregnancy diagnosis in cattle. **Proc. Brit. Cattle Vet. Assoc.** 39-41.

DICK, A. R. (1990a). Controlled breeding management through the strategic use of the CIDR-B intravaginal device for cows in herds with a daily milk quota. In **Studies on the use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 3: 127-185.

DICK, A. R. (1990b). The use of tailpaint and an aerosol raddle to monitor oestrous behaviour of animals after different synchrony treatments. In **Studies on the use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 2: 80-126.

DOBSON, H., MIDMER, S. E. and FITZPATRICK, R. J. (1975). Relationship between progesterone concentrations in milk and plasma during the bovine oestrous cycle. **Vet Rec.** 96: 222-223.

DUIRS, G. F., MACMILLAN, K. L., RHODES, A. P., BARNES, D. R. and TAUFA, V. K. (1986). CIDR: Concepts in breeding management. **Proc. Dairy Cattle of the N. Z. Vet. Assoc.** 127-136.

EDDY, R. G. (1983). The use of prostaglandin analogue cloprostenol and the milk progesterone test to control breeding policy in one dairy herd. **Br. Vet. J.** 139: 104-108.

EDDY, R. G. and CLARK, P. J. (1987). Oestrus prediction in dairy cows using and ELISA progesterone test. **Vet Rec.** 120: 31-34.

ELMORE, R. G. (1986). Using rapid progesterone assay kits to detect open cows. **Veterinary Medicine/October.** 969-972.

ELMORE, R. G. (1987). Better reproductive management through rapid progesterone assay kit technology. **Veterinary Medicine/January.** 84-87.

FAGAN, J. G. and ROCHE J. F. (1986). Reproductive activity in postpartum dairy cows based on progesterone concentrations in milk or rectal examination. **Ir. Vet. J.** 40: 124-131.

FOOTE, R. H., OLTENACU, E. A. B., KUMMERFELD, H. L., SMITH, R. D., RIEK, P. M. and BRAUN, R. K. (1979). Milk progesterone as a diagnostic aid. **Br. Vet. J.** 135: 550-558.

FOOTE, R. H. (1988). Using rapid progesterone assays in embryo transfer programs. **Veterinary Medicine/June.** 617-621.

FOULKES, J. A., COOKSON, A. D. and SAUER, M. J. (1982). AI in cattle based on dailey microtitre plate enzymeimmunoassay of progesterone in whole milk. **Br. Vet. J.** 138: 515-521.

HEAP, R. B., HOLDSWORTH, R. J., GADSBY, J. E., LAING, J. A. and WALTERS D. E. (1976). Pregnancy diagnosis in the cow from milk progesterone concentration. **Br. Vet. J.** 132: 445-464.

HEINONEN, K., RANTASALMI, K. and ALANKO, M. (1988). Milk progesterone samples in identifying cycling dairy cows. **Acta Vet. Scand.** 29: 245-248.

HOFFMAN, B., HAMBURGER, R., GÜNZLER, O, KORNDÖRFER, L. and LOHOFF, H. (1974). **Theriogenology.** 2: 21-28.

KIRWOOD, R. N., LAPWOOD, K. R., SMITH, W. C. and ANDERSON, I.L. (1984). Plasma concentrations of LH, prolactin, oestradiol-17B and progesterone in sows weaned after lactation for 10 or 35 days. **J. Reprod. Fertil.** 70: 95-102.

KUMMERFELD, H. L., OLTENACU, E. A. B. and FOOTE, R. H. (1978). Embryonic mortality in dairy cows estimated by non-returns to service, estrus, and cyclic milk progesterone patterns. **J. Dairy Sci.** 61: 420-422.

LAMMING, G. E. and BULMAN, D. C. (1976). The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. **Br. Vet. J.** 132: 507-517.

LAMMING, G. E., DARWASH, A. O. and BACK, H. L (1989). Corpus luteum function indairy cows and embryo mortality. **J. Reprod. Fertil.** 37: (suppl.): 245-252.

LESLIE, K. E. and BOSU, W. J. K. (1983). Plasma progesterone concentrations in dairy cows with cystic ovaries and clinical responses following treatment with fenprostalene. **Can. Vet. J.** 24: 352-356.

MACMILLAN, K. L. and CURNOW, R. J. (1977). Tailpainting: a simple form of oestrus detection in New Zealand dairy herds. **N.Z. Exper. Agric.** 5: 357-361.

MACMILLAN, K. L., FIELDEN E. D., MORRIS, G. R. and CURNOW, R. J. (1977). Factors influencing A.B. conception rates , IX-pregnancy rates to first insemination: mating with a herd sire compared with artificial insemination. **N. Z. Exper. Agric.** 5: 265-271.

MACMILLAN, K. L. (1988). Non-infectious factors affecting reproductive performance of dairy herds. **Dairy Cattle Reproduction Research Workshop.** Werribee, Nov. 15-17.

MACMILLA, K. L., TAUFA, V. K., BARNES, D. R., DAY, A. M. and HENRY, R. (1988). Detecting oestrus in synchronized heifers using tailpaint and aerosol raddle. **Theriogenology.** 30: 1099-1114.

MELROSE, D. R. (1979). The need for, and possible methods of application of, hormone assay techniques for improving reproductive efficiency. **Br. Vet. J.** 135: 453-459.

MEYERS, P. J., ELMORE, R. G., VARNER, D. D., BLANCHARD, T. L., SHULL, J. W. and TODD, J. (1988). Use of a rapid progesterone assay in a beef cattle estrus synchonization program. Theriogenology. 29: 1285-1294.

MOLLER, K., LAPWOOD, K. R. and MARCHANT, R. M. (1986). Prolonged service intervals in cattle. **N.Z. Vet J.** 34: 128-132.

NAKAO, T., SUGIHASHI, A., SAGA, N., TSUNODA, N. and KAWATA, K. (1983). Use of milk progesterone enzyme immunoassay for differential diagnosis of follicular cyst, luteal cyst and cystic corpus luteum in cows. **Am. J. Vet. Res.** 44: 888-890.

NEBEL, R. L., WHITTIER, W. D., CASSELL, B. G. and BRITT, J. B. (1987). Comparison of on-farm and laboratory milk progesterone assays for identifying errors in detection of estrus and diagnosis of pregnancy. **J Dairy Sci.** 70: 1471-1476.

NEBEL, R. L. (1988). On-farm milk progesterone tests. J. Dairy Sci. 71: 1682-1690.

NEBEL, R. L., ALTEMOSE, D. L., MUNKITTRICK, T. W., SPRECHER, D. J. and McGILLIARD, M. L. (1989). Comparisons of eight commercial on-farm milk progesterone tests. Theriogenology. 31: 753-764.

PENNINGTON, J. A., SCHULTZ, L. H. and HOFFMAN, W. F. (1985). Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding: a field study in dairy cattle. **J. Dairy Sci.** 68: 2740-2745.

QUINLAN, T. J. (1986). Progesterone E.I.A. assay in dairy cattle. **Proc. Dairy Cattle Soc. of the N.Z. Vet. Assoc.** 5th Seminar 26-27 November, p. 109-126.

REIMERS, T. J., SMITH, R. D. and NEWMAN, S. K. (1985). Management factors affecting reproductive performance of dairy cows in the northeastern United States. **J. Dairy Sci.** 68: 963-972.

RUIZ, F. J., OLTENACU, P. A. and SMITH, R. D. (1989). Evaluation of on-farm milk progesterone tests to determine non-pregnant cows and to prevent insemination errors. **J. Dairy Sci.** 72: 2718-2727.

SREENAN, J. M. and DISKIN, M. G. (1983). Early mortality in the cow: its relationship with progesterone concentration. **Vet Rec.** 112: 517-521.

STANLEY, C. J., PARIS, F., WEBB, A. E., HEAP, R. B., ELLIS S. T., HAMON, A. W. and BOOTH, J. M. (1986). Use of a new and rapid milk progesterone assay to monitor reproductive activity in the cow. **Vet Rec.** 18: 664-667.

TUNGSUBUTRA, V. and FRANCE, J. T. (1978). Serial changes in plasma levels of progesterone, unconjugated oestradiol and unconjugated oestriol in normal pregnancy. **Aust. N. Z. Jl. Obstet. Gynaec.** 18: 97-103.

VAN CLEEFF, J., MACMILLAN, K. L., THATCHER, W. W. and LUCY, M. C. (1989). Estrous synchronization and fertility in heifers treated with CIDR before and after insemination. **J. Anim. Sci.** 67:(suppl. 1):383.

VAN de WIEL , D. F. M., KAMONPATAMA, M., NGGRAMSURIJAROY, C., KOOPS, W. and SINGHAJAN, S. (1982). Enzyme immunoassay of milk progesterone: its application to estrus confirmation and early pregnancy diagnosis in cattle. **Vet Q.** 4: 72-78.

VOLLER, A., BARTLETT, A. and BIDWELL, D. E. (1978). Enzyme-immunoassay with special reference to ELISA techniques. **J. Clin. Path.** 31: 507-520.

WIJERATNE, M. V. S. (1973). A population study of apparent embryonic mortality in cattle with special reference to genetic factors. **Anim. Prod.** 16: 251-259.

WIMPY, T. H., CHANG, C. F., ESTERGREEN, V. L. and HILLERS, J. K. (1986). Milk progesterone enzyme immunoassay: modifications and a trial for pregnancy detection in dairy cows. **J. Dairy Sci.** 69: 1115-1121.

WISDOM, G. B. (1976). Enzyme-immunoassay. **Clin. Chem.** 22: 1243-1255.

WORSFOLD, A. I., BOOTH, J. M., WELLS, P. W., HUDDART, A. C. and STANLEY, C. J. (1987). The evaluation of a new rapid milk progesterone test as an aid to improving dairy herd fertility. **Br. Vet. J.** 143: 83-87.

CHAPTER 5

ULTRASONIC IDENTIFICATION OF OVARIAN FOLLICLES AND RESPONSE PATTERNS IN ANOESTROUS DAIRY COWS TREATED WITH CIDR DEVICES AND PMSG AT TWO STAGES OF THE POST-PARTUM PERIOD

ABSTRACT

This study was designed: i) to characterize the dynamic changes of follicles in the ovaries by using ultrasonography at two different post-partum periods (early, 25 days and late, 50-55 days post-partum) as well as changes associated with CIDR treatment and with an injection of PMSG, and ii) to measure post-treatment response rates in oestrus and ovulation in anoestrous dairy cows associated with changes in plasma progesterone concentrations at device removal.

Ovarian function was monitored during the treatment programme and after device removal, as well as the oestrous response patterns during the 2 weeks from the end of treatment. Treated cows (n = 18) received a CIDR device for 10 days with an injection of 400 IU of PMSG at device removal. Control cows (n = 10) did not receive any treatment.

The population of follicles classified as classes 1 (< 6 mm diameter), 2 (6 to 9 mm diameter) and 3 (> 9 mm diameter) varied between cows in both post-partum periods, but the average number of follicles did not differ significantly between day 25 (early) and 50-55 (late) for treatment and control groups. The average number of follicles in each class did not increase significantly in early post-partum cows during the treatment period in either treated or untreated contemporary animals, but in the late post-partum period, the average number of class 1 follicles increased in the animals of the treated group ($P < 0.05$). However, when the comparisons in treatment groups were done between early and late post-partum period, the average number of class 2 follicles and the total number of follicles were increased at CIDR removal ($P < 0.05$; $P < 0.01$, respectively), while in the control cows, the average number of class 1 follicles and the total number of follicles were also increased at the time of device removal ($P < 0.01$).

The average number of class 1 follicles in the early post-partum period increased significantly in the treatment group irrespective of whether or not animals displayed oestrus or ovulated with corpus luteum (CL) formation after treatment with CIDR/PMSG.

In the early post-partum period, the diameter of the largest unovulated luteinized follicle in treated cows which displayed oestrus and/or ovulated increased significantly during CIDR treatment, and its growth continued after the device was removed. Normal class 3 follicles did not increase during this period and some of them ovulated after CIDR device removal and formed a (CL).

In the early and late post-partum period, there were in most cows (treated and control) a CL and a luteinized follicle or a luteinized follicle alone in one ovary, and a luteinized follicle in the opposite ovary, at scanning on day 14 after device removal. Normal follicles commenced development within a follicular wave in the presence of a large unovulated luteinized follicles.

There was a significant variation in plasma progesterone concentrations at CIDR

removal, with values ranging from 0.8 to 15.8 ng/ml in the early post-partum period, and from 0.8 to 6.9 ng/ml for cows treated in the late post-partum period.

Only 25% and 33.3% of the treated cows in the early and late post-partum period displayed oestrus, respectively. However, 55% and 50% of the treated and control cows which had not displayed oestrus, actually ovulated and formed a CL in the early post-partum period. There was a significant percentage of untreated cows which had formed a CL, but did not display oestrus.

Further research has to be focused on the study of the endocrinological patterns of LH and FSH in anoestrous cows during the post-partum period and the factors which affect treatment response, and the interactions of the nutritional status, body condition and ovarian function during the post-partum period especially to measure the ovarian response at the different degrees of nutritional anoestrus.

INTRODUCTION

Post-partum anoestrus is the most common form of infertility in New Zealand dairy herds (Macmillan and Day, 1987). The condition in primarily a consequence of having a concentrated calving pattern in late winter with cows receiving a sole diet of pasture, mainly ryegrass (Macmillan et al., 1990b). It is known that in early lactation, cows experience stress from milk production with a reduced appetite and substantial loss in body condition (Macmillan and Day, 1987). If seasonal conditions result in a delay in the onset of the period of rapid pasture growth in the spring, then cows will experience a prolonged interval of underfeeding in the post-partum period and in early lactation (Macmillan et al., 1990b).

Long periods of anoestrus can affect submission rate ([SR] Macmillan et al., 1975). SR is defined as the percentage of cows in a herd presented for insemination during a selected period of time from the start of the seasonal breeding programme. A high SR is necessary for a compact breeding programme in seasonal herds, and to maintain optimum calving intervals in year-round herds (O'Farrel, 1984). SR is affected if a herd has a spread calving pattern. The average interval between calving and first oestrus also varies between herds. This variation contributes to the proportion of animals in a herd which have not re-commenced normal ovarian activity by the start of the seasonal artificial breeding (AB) programme. Moreover, poor conditions for pasture growth tend to be a recurring problem in some herds, consequently increasing anoestrum and prolonging the interval to conception. A spread calving pattern will result in the following year, especially if slow pasture growth also delays induction therapy for the later calving cows. These animals are less likely to be inseminated in the first weeks of the breeding season and may be less fertile to first insemination (Macmillan, 1989).

Pregnant mare serum gonadotrophin (PMSG) was discovered 60 years ago, and it has been a commonly used preparation for inducing ovulation (Cole and hart, 1930 see Short et al., 1988). The use of exogenous progesterone, particularly when its removal is followed by the injection of PMSG has been the most successful treatment for induction of follicular growth and ovulation (Mulvehill and Sreenan, 1977). In New Zealand, one early report showed that treatment with a progestagen sponge for 7 days and 1000 IU of PMSG at sponge removal produced a 4 week pregnancy rate of 38% (Fielden et al., 1976). The controlled internal drug release device (CIDR) was commercially available in 1986, and it was tested first that year with cows diagnosed as having post-partum anoestrum associated with non-cyclic ovarian activity 3 weeks after the start of the seasonal AB programme. The results of these trials showed that the combination of CIDR and PMSG significantly increased the incidence of oestrus within 7 days of device removal, and the fertility of that oestrus was normal (Macmillan and Day, 1987). The CIDR provides a period of progesterone priming which precedes gonadotrophin stimulation with PMSG to produce follicle maturation and ovulation.

In summary, the use of CIDRs to treat anoestrus in lactating dairy cows conducted in the last 4 years has shown that: a period of progesterone priming for 7 days was preferable to 4 days, but a 10-day CIDR treatment was as effective as the 7-day treatment. PMSG-use at device removal was necessary in most cases, and 400 IU was the most suitable dose. Around 80% of treated cows ovulated within 14 days of device removal, but the percentage detected in oestrus and inseminated was variable. On average, 23% of treated animals which ovulated were not detected in oestrus and inseminated within 14 days of CIDR removal. The variation in response patterns between herds was too great to justify fixed time insemination at a single post-treatment interval. The preferred alternative was to re-examine all cows not detected in oestrus about 14 days after device removal, and to then treat those which had ovulated with prostaglandins (PGF) and those with no palpable ovarian response with a CIDR.

Previous studies based on rectal palpations at weekly intervals, starting at 4 to 7 days post-partum, indicated that there was follicular development in the early post-partum period. Moller (1970) studied ovarian activity after calving in general and in two groups of grazing dairy cows subjected to different management (milked and suckled cows). In that study, small follicles (< 10 mm) were found in both suckled and milked cows during the first 3 weeks after calving, and until their first ovulation which was 42.3 ± 16.2 days post-partum in milked cows and 64.9 ± 15.8 days in suckled cows. Larger follicles > 15 mm) become more common with time post-partum. Morrow et al. (1968) studied post-partum ovarian activity in dairy cows fed hay, corn silage and grain to meet production requirements, and reported follicular development that progressed until first ovulation (15.0 ± 3.9 days) in cows with a normal parturition. The follicle diameter ranged from 0.5 to 1.5 cm. Other studies reported that there were large (> 8 mm) follicles in the early post-partum period, and the number of medium follicles (4 to < 8 mm) increased until 42 days after calving in acyclic suckled beef cows (Spicer et al., 1986). There was growth of small antral follicles between 15 and 35 days after calving in dairy cows (Dufour and Roy, 1985).

Diagnostic ultrasonography has a relative short history in the area of domestic animal reproduction. A transrectal real-time or dynamic imaging ultrasound scanning of the cow's reproductive tract and ovarian structures allow the operator to view images of structures which normally can be only palpated (Pierson and Ginther, 1984). Peter and Bosu (1988) used this technique to assess the influence of uterine infections and follicular development on the response to GnRH in post-partum dairy cows. Large follicles were absent in anoestrous post-partum dairy cows during the first 60 days post-partum, but ovaries contained follicles of 4 to 8 mm diameter. However, in cows which had ovulated by day 15 post-partum, follicles ≥ 10 mm were observed before or by day 12 post-partum. Another report, studying the time of resumption of follicular activity in the early post-partum period in dairy cows (Savio et al., 1990a), reported that during the post-partum anoestrous period, follicular development was characterized by the growth and regression of small (≤ 4 mm), and medium-sized (5-9 mm) follicles which were present by day 5 after calving until a dominant follicle (> 10 mm) was eventually detected. Finally, Chaimongkol (1990) monitored ovarian activity during the post-partum period and showed that in lactating dairy cows which calved in spring, the population of follicles did not increase significantly between the second and seventh weeks after calving in acyclic cows.

The study of dynamic changes of the patterns of follicles growth in the post-partum during and after CIDR/PMSG treatment has not been reported. Therefore, the aim of this study was to characterize the dynamic changes of the follicles in the ovaries by using ultrasonography during this period in two different periods of the post-partum and to measure post-treatment response rates in terms of oestrus and ovulations in anoestrous dairy cows. In addition, plasma progesterone concentrations (PPCs) were measured in blood samples taken before device insertion and at removal in treated cows, and at equivalent times in contemporary controls.

LITERATURE REVIEW

Post-partum Period.

Parturition is followed by a period of acyclicity in most mammals. This is the post-partum anoestrous period.

It is initially a time for recovery from pregnancy and parturition. Two of these functions are:

1) Genital tract function, and

2) Hypothalamic-pituitary ovarian function.

This period ends with the first post-partum oestrus at which pregnancy is possible followed by formation of a corpus luteum (CL) with a normal lifespan to facilitate maternal recognition of pregnancy (Malven, 1984).

1. Genital tract function.

1.1 Uterine involution.

Uterine involution is the recovery process of the uterus from pregnancy and parturition.

The time required for involution of the bovine uterus usually ranges from 3 to 7 weeks, but may vary widely (Sloss and Dufy, 1980). However, from other previous studies (Gier and Marion, 1968; Wagner and Hansel, 1969) in which palpable or histologic changes were used as a criteria, it appears reasonable to consider that final involution is completed about day 40 after calving. Moller (1970) studied uterine involution in 2 groups of cows subjected to different management. The cows were milked twice daily, or each suckled 3 or 4 calves. No differences were found between groups (31.5 ± 3.0 vs 31.6 ± 4.2 days, respectively).

A number of factors have been reported by Marion et al. (1968) to affect the duration of involution of the bovine uterus:

i) It is prolonged with advancing parity, possibly because of greater uterine trauma with each successive parturition;

ii) It is prolonged in winter as compared with spring or summer;

iii) Lactation accelerates the involution rate (Schirar and Martinet, 1982);

iv) Difficult parturitions or dystocia associated with the birth of a very large calf, periparturient trauma, or a retained placenta delay uterine involution;

v) Bacterial contamination of the uterus, whether occurring during parturitional difficulties or later, delay involution of the uterus; and,

vi) Hormones such as prostaglandin have been considered to be involved in involution of the uterus (Kindahl et al., 1982). Oxytocin may be involved in involution but no specific effects have been reported. Ovarian hormones are not involved in delayed uterine involution (except with progesterone in pyometra). Hormone therapy with oestrogen or progestin has little or no effect on the involution process (Marion et al., 1968).

1.2 Involution process.

i) Phase one of uterine involution.

The process of uterine involution in cows begins with expulsion of placental tissue and fluids. Uterine tissue and fluid continue to be expelled for several days or weeks, even when the placenta is promptly expelled after parturition.

The tissue loss involves shedding, disintegration, and dissolution of decidual tissue; reduction of endometrial vascularity; regression of endometrial glands; and reduction of endometrial cell numbers, and myometrial cell volumes. Contractions of the myometrium aid these processes, which proceed more rapidly in the non-pregnant areas of the puerperal uterus.

ii) Phase two of uterine involution.

The second phase is called uterine recovery. This phase involves regeneration of the endometrial epithelium, including the glandular epithelium. This process begins almost immediately after calving in those areas, such as the intercaruncular areas, which are the least seriously damaged. More time is required for regression of maternal caruncles in the pregnant horn and for re-growth of their epithelium.

Although several processes are involved in uterine involution and recovery, they appear to progress simultaneously in different parts of the uterus.

2. Hypothalamic-pituitary ovarian function.

The recovery of the hypothalamic-pituitary ovarian axis appears to involve a number of sequential events during the post-partum period. The accomplishment of each event in the recovery process appears to depend on the successful completion of the preceding event. The sequential series involves the recovery from pregnancy state, escape from the induced inhibition of gonadotropin, initiation of ovulation followed by luteal development, and the occurrence of behavioural oestrus with ovulation.

Cows experience a period of infertility which is characterized by anovulation and anoestrus following the birth of a live calf. If a cow is to produce a calf at least once each year, it must conceive within 83 days post-partum, and maintain the pregnancy to term.

2.1 Pituitary hormones.

Luteinizing hormone (LH) is secreted from the pituitary gland in episodic bursts. Each secretory pulse is a response to a secretory pulse of gonadotropin-releasing hormone (GnRH) from the hypothalamus.

There is evidence in ovariectomized cows (OVX) of an inherent rhythm of LH secretory episodes which are characteristic for individual animals (Rahe et al., 1982). These pulses normally occur once every hour. It is likely that a similar rhythm exists for follicle stimulating hormone (FSH) although it is not proven, (McNatty, 1988). The inherent rhythmicity of both gonadotrophin is almost certainly due to an identical rhythm of episodic GnRH release from the hypothalamus.

In the intact cycling animal, LH secretory pulsing at a frequency of once per hour is not achieved until 44 to 96 h before oestrus. Environmental (day length, temperature, nutrition), physiological (pregnancy, lactation), and gonadal factors all act to modulate LH peak frequency. The number of LH pulses varies from 4 to 12 per 24 h during the luteal phase but it increases to between 16 and 30 pulses every 24 h during the follicular phase. In the early post-partum period, the LH pulse frequency and amplitude are both low. Thus, the early post-partum period is characterized by inhibition of LH secretion. This inhibition is coincident with reduced pituitary stores of LH (McNatty, 1988), low biological activity of the secreted LH (Weesner et al., 1987), and a decreased responsiveness of the pituitary to GnRH (Mawhinney et al., 1979). Also. the decreased LH production is probably due to a reduction in GnRH release (McNatty, 1988). Other factors which influence the patterns of LH release are the time of year and stress. Pulse frequency is lower in cycling cows in winter than spring (McNatty et al., 1983), and stress has been demonstrated to inhibit both LH peak frequency and amplitude (Rasmussen and Malven, 1983).

There is no evidence in dairy cows for any marked inhibition of FSH secretion or reduction in pituitary FSH content apart from the periparturient and the early post-partum (<6 days) period (Moss et al., 1985).

It is known that milking stimuli increase prolactin (PRL) release (Tucker, 1971),

and that PRL can inhibit pituitary LH secretion in some species (Smith, 1980). However, the elevated PRL concentrations in lactating cows were abolished by pharmacological means without an alteration in post-partum LH release, or to the duration of the post-partum period (Kaltenbach et al., 1977; Schallenberger et al., 1978). PRL plasma concentrations were the same in milked and suckled cows (Smith et al., 1981). The PRL concentration was associated with increased milk yields in dairy cattle (Akers et al., 1980). Anoestrus length was observed by some (Chang et al., 1981), but not others (Webb et al., 1980) to be closely correlated with plasma PRL concentrations.

Injecting PRL (Forrest et al., 1980) or bromocryptine (Williams and Ray, 1980) as an inhibitor of PRL release, influenced neither the variations of LH or FSH concentrations, nor the resumption of ovarian activity after calving. The local ovarian effect of this hormone on the synthesis of progesterone (McNatty, 1977, see Harret 1986), and on follicular development (Tsai-Morris et al., 1983) was reported. Hyperprolactinaemia is a common condition in lactating cows, but its role in the decreased ovarian follicular response to LH is not known (McNatty, 1988).

2.2 Adrenal cortex.

It has been shown that cortisol can inhibit LH secretion (Li and Wagner, 1983). Plasma corticosteroid concentrations may be increased in cows suckling calves and as a result of stress or disease. However, it remains to be determined whether the small increases in endogenous cortisol during suckling of cows are sufficient to inhibit release of LH (Faltys et al., 1983). In addition to hormones such as PRL and cortisol which circulate systemically, neural afferent tracts conveying sensory stimuli from suckled mammary glands may directly inhibit GnRH neurons within the hypothalamus (Malven, 1984). However, another report has failed to confirm glucocorticoid release during suckling in intact animals (Convey et al., 1983).

2.3 Uterus.

The metabolite of prostaglandin F2 α (13, 14-dehydro, 15 keto-PGF 2 alpha; PGFM) is released for 10-20 days after calving in the cow. The release may reflect the degree of endometrial damage and/or repair (Kindahl et al., 1982).

There is a positive correlation between the duration of this increased concentration of PGFM, and the time taken for the uterus to involute completely (Eley et al., 1981). Another report has suggested that the ovary-uterine axis exerts an inhibitory effect on LH secretion during the early post-partum period, as hysterectomy resulted in a rapid increase in plasma gonadotrophin concentrations in cows (Schallenberger et al., 1984). It has been also shown that PGFM concentrations return to their basal levels before the first ovulation post-partum (W.W. Thatcher, see Peters and Lamming, 1986).

2.4 Mammary gland.

The ruminant mammary gland has an endocrine function in addition to its exocrine function. During the peri-parturient period the gland secretes PGF and oestradiol-17β (Moule-Walker et al., 1983). Therefore, an endocrine role for the mammary gland in controlling ovarian function post-partum would not be surprising, although it has not been demonstrated (Peters and Lamming, 1986). However, it was hypothesized that the control of the milk ejection reflex must be different from those that suppress gonadotropin secretion (involving oxytocin released from the posterior pituitary [Williams, 1990]).

2.5 Effects of steroids on LH and FSH release.

Low levels of oestradiol decreased plasma LH concentrations in OVX cows by inhibiting LH pulse amplitude (but not frequency), and FSH in a dose dependent manner. Progesterone, at constant levels, decreased plasma LH but not amplitude. In the presence of progesterone, oestradiol decreased LH by reducing LH pulse frequency as well as amplitude. It seems likely that the suppressive effects of oestradiol are mediated by decreasing the frequency of GnRH pulses, and by decreasing the responsiveness of the pituitary to GnRH (McNatty, 1988). Progesterone seems to act by altering GnRH pulse frequency. The sensitivity of the hypothalamic-pituitary axis to the inhibitory effects of oestradiol and progesterone are enhanced by the brain opioid systems (Hanzen, 1986).

Plasma levels of oestradiol 17β are low after calving and highly variable before the first ovulation. Progesterone is not a major steroid in the blood of the post-partum cow before the first ovulation. Frequently, the first luteal phase is short, and this is invariably due to an inappropriate levels of gonadotrophin secretion during the follicular and periovulatory phase preceding ovulation.

In addition to the negative feedback effects of oestradiol and progesterone on gonadotrophin release, oestradiol may also provoke a preovulatory-like peak of gonadotrophin release similar to that observed around oestrus. This positive feedback effect of oestrogen shortly before oestrus, stimulates the capacity of the pre-ovulatory follicle to secrete oestradiol up to a rate of up to 50 ng per minute. The rate of oestradiol secretion causes this preovulatory-like peak of LH. This positive feedback mechanism is critical for ovulation-induction.

An inadequate output of follicular oestradiol, or an inadequate quantity of LH in response to the positive feedback signal result in an abnormal process of ovulation or CL formation and/or function (McNatty, 1988). Failure of the oestradiol feedback is a phenomenon which commonly occurs in cows during the post-partum period.

2.6 Progesterone priming and its effect on silent ovulations in the post-partum period.

The final event in the recovery sequence for the hypothalamic-pituitary ovarian axis is the concurrence of external signs of oestrus and ovulation.

In many cases, the first ovulation is followed by the formation of a short-lived CL. In others, ovulation may even fail to occur. A number of ovulations, followed by a silent ovulation, may occur during the post-partum period. The juxtaposition of silent ovulations and short luteal phases is suggestive of a common aetiology.

Previous reports have shown a requirement for progesterone pretreatment when inducing either oestrous behaviour (Carrick and Shelton, 1969), or precocious ovulation and normal luteal function in heifers (Gonzalez Padilla et al., 1975).

Hunter et al. (1987) have shown that a brief exposure to progesterone was sufficient to decrease the incidence of short luteal phases in anoestrous ewes. Thus, it appears that the lack of antecedent progesterone priming is responsible for the frequent ovulation failure and lack of oestrus at the end of the post-partum periods in ruminants. The brief exposure to elevated progesterone in cows may serve to prime the ovulatory and oestrus manifestation processes, ensuring that the next LH peak leads to an ovulation that is accompanied by external signs of oestrus and followed by a normal luteal phase.

Since progesterone pretreatment does not influence the magnitude of the LH peak in response to oestradiol 17β in heifers (Gonzalez Padilla et al., 1975) or to GnRH injection in anoestrous ewes (Legan et al., 1985), its effect on subsequent luteal activity must result from a direct action on the ovary. It was found that follicles from primed anoestrous ewes had greater oestradiol production, testosterone concentrations and granulosa cell LH binding capacity than follicles from non-primed animals (Hunter et al., 1987). Legan et al. (1985) suggested that progesterone may synchronize follicular development to enhance the LH peak-release mechanism.

Carrick and Shelton (1969) also suggested that progesterone alleviates the post-partum oestrogen refractoriness induced during late gestation in cows. Hunter et al. (1987) demonstrated the ability of progesterone to increase follicular oestradiol synthesis. Thus, by increasing ovarian oestradiol production, progesterone priming could also enhance oestrus.

Each successive post-partum ovulation in dairy cows appears to have a greater probability of being associated with oestrus (Lamming et al., 1981; Fonseca et al., 1983).

2.7 Follicle growth and pituitary hormones.

Follicles > 1 mm diameter are responsive to pituitary gonadotrophic hormones and to exogenous gonadotrophin such as pregnant mare serum gonadotrophin (PMSG), and FSH-p (a porcine pituitary extract of FSH). The thecal cells have specific receptors for LH, PMSG or the LH contaminant in FSH-p. These cells secrete androstenedione and testosterone in response to binding with any of these mentioned hormones. The number of LH receptors and the level of androgen production in response to LH remain unchanged throughout follicle development. However, the production of androgen increases with the diameter of the follicle due to a progressive increase in the number of thecal cells. FSH preparations with LH contamination can produce an excessive ovarian response at high doses. Granulosa cells have specific receptors for FSH. These cells bind FSH-like hormones such as PMSG.

The individual granulosa cells become increasingly responsive to FSH as a follicle matures. This is due, at least in part, to an increased coupling of the FSH receptor to the intracellular signalling system. The result is an increased synthesis of an aromataze enzyme which is essential for the follicular synthesis of oestradiol-17β, consequently inducing oestrous behaviour and ovulation. The FSH-dependent aromatizing enzyme system also permits the cell to metabolize androgens to oestradiol.

The time taken for the follicle to grow to periovulatory size is relatively rapid. This rapid growth is in part, a consequence of the increasing intrafollicular concentrations of oestradiol-17β. The collective actions of FSH and oestradiol lead to the induction of LH receptors on granulosa cells.

The presence of LH receptors on granulosa cells, together with FSH receptors, lead to a greater coupling to the intracellular signalling system and a more rapid phase of cell differentiation. Moreover, the development of LH receptors on granulosa cells prepares them for the ovulatory peak of LH in blood and their transformation into LH-dependent progesterone-secreting luteal cells (McNatty, 1988).

2.8 Follicle maturation and gonadotrophin secretion before ovulation.

The interrelationships have been examined in some detail in normal cycling cows, but not to the same extent in post-partum cows. The limited studies reinforce the hypothesis that an adequate level of LH secretion is essential for the maturation of follicles to ovulation (Spicer et al.,1986). Oestrus and the preovulatory LH peak occur some 40-60 h after an injection of prostaglandins (PGF) to cows in the luteal phase, with ovulation occurring some 12 to 48 h after the onset of oestrus. The follicle destined to ovulate after luteolysis is more likely to be > 5 mm diameter and probably > 8 mm diameter, although the follicle which ovulates may not be the largest non-atretic follicle at the time luteolysis occurs (McNatty, 1988).

All non-atretic follicles > 5 mm diameter are responsive to LH and FSH and have the capacity to synthesize oestradiol. More than 90% of follicular oestradiol originates from the granulosa cells. However, they depend upon another cellular

source for the steroid precursor androstenedione or testosterone, since they are incapable of synthesizing these androgens (McNatty et al., 1984). In contrast, more than 90% of follicular androstenedione or testosterone originates from thecal cells which are incapable of synthesizing oestradiol (McNatty et al., 1984). Thus, to synthesize adequate amounts of follicular oestradiol, there needs to be sufficient LH to stimulate thecal androgen synthesis. In the cow, this LH requirement involves a minimum pulse frequency of between 1 and 2 pulses every 2 h, with a pulse amplitude of between 1 and 5 ng/ml. In response to the rich local source of oestradiol precursor provided by the thecal cells, the granulosa cells produce oestradiol, with the largest non-atretic follicle producing the largest amount of oestradiol, since it has the greatest number of granulosa cells and highest levels of aromataze activity (McNatty, 1988).

The role of FSH during the preovulatory period is unclear. Plasma levels of FSH decline after luteolysis. Suppression of FSH inhibits or delays oestrus and/or ovulation (Quirk and Fortune, 1986b). Presumably, FSH in cows is crucial to sustain or enhance oestradiol formation by granulosa cells. Large follicles (>8 mm diameter) are more sensitive to FSH than small follicles (< 5 mm diameter) as judged by the ability of granulosa cells to produce cyclic adenosine monophosphate. A decline in FSH secretion after luteolysis probably inhibits oestradiol formation by all but the most FSH sensitive follicles (Henderson and McNatty, 1987).

When the progesterone concentrations decline after luteolysis, a marked change in LH pulse frequency is observed together with a decline in FSH secretion concomitant with an increase in oestradiol secretion, mainly from the largest non-atretic follicle. Over the same period, the increased LH pulse frequency causes increased androstenedione and testosterone production from most non-atretic follicles (> 1 mm diameter). The increased androgen production is greater than that of oestradiol, and may enhance oestrous and sexual behaviour. About 40-60 h after luteolysis, the preovulatory rise in oestradiol provokes the preovulatory peak of LH leading to down regulation of thecal androgen synthesis. When the oestradiol synthesis has fallen abruptly, LH enhances progesterone secretion from the granulosa cells, follicular rupture, expulsion of the ovum and CL formation.

3. Anoestrus.

Post-partum anoestrus is the most common form of infertility in New Zealand dairy herds (Macmillan and Day, 1987).

Long periods of anoestrus can affect submission rate [SR] (Macmillan et al., 1975). SR is defined as the percentage of cows in a herd presented for insemination during a selected period of time, usually 3 or 4 weeks. It is affected if a herd has a spread calving pattern. The average interval from calving to first oestrus also varies between herds. This variation contributes to the proportion of animals in a herd which have re-commenced their ovarian activity by the start of the seasonal artificial breeding programme. Moreover, poor conditions for pasture growth tend to be a recurring problem in some herds, increasing anoestrus and prolonging the interval to conception. A spread calving pattern will result in the following year, especially if slow pasture growth delays induction therapy for

the later calving cows. These animals are less likely to be inseminated in the first weeks of the mating season and may be less fertile to first insemination.

Ovarian activity has been induced in cattle by such hormonal treatments as injections of vitamins and minerals, human chorionic gonadotrophin (HCG), PMSG, GnRH and progestagen. However, none of these treatments has been completely effective in cattle (Short et al., 1988). PMSG was discovered 60 years ago, and it has been the most commonly used preparation for inducing ovulation (Cole and Hart, 1930; see Short et al., 1988).

The use of exogenous progesterone, particularly when its withdrawal is followed by the injection of PMSG, has been the most successful treatment for induction of follicular growth and ovulation. Mulvehill and Sreenan (1977) used progesterone and Cronolone (SC-98880; G.D. Searle & Co) impregnated intravaginal sponges which were inserted for 9 days, together with an i.m. injection of progesterone and Cronolone + oestradiol benzoate on the day of insertion. PMSG at a dose of 750 IU was given to half of the cows in each group at the time of sponge removal. Animals were inseminated on a fixed time basis at 48 and 72 after sponge removal. Fertility data, based on the calving rate from the fixed inseminations was 57% in the progesterone treated animals, and 71% with the use of PMSG.

In New Zealand, one early report showed that treatment with a progestagen sponge for 7 days and 1000 IU of PMSG at sponge removal produced a 4 week pregnancy rate of 38% (Fielden et al., 1976). A controlled internal drug release device (CIDR) was commercially available in New Zealand in 1986, and the first anoestrous cows which were treated experimentally with these devices were in the breeding programme of that year. The device was first tested with cows diagnosed as having post-partum anoestrus associated with non-cyclic ovarian activity 3 weeks after the start of the seasonal artificial breeding programme. The results of these trials showed that the combination of CIDR and PMSG significantly increased the incidence of oestrus within 7 days of device removal, and the fertility of that oestrus was normal (Macmillan and Day, 1987).

In the following season (Day and Taufa, 1988), the results with CIDRs were highly variable, with some herds obtaining very good responses and others with poor responses.

In general, the oestrous response was lowest in 2 year old (55.5% in oestrus within 15 days of diagnosis), increasing to 87.5% in mature cows. Also, rates of response to oestrus slightly increased with post-partum interval but varied from 62.5% in October to 89.5 in December. Many cows which were not detected in oestrus had ovulated after CIDR treatment. Trials including 10 and 12-day treatment periods and different dose rates of PMSG were also evaluated.

In summary, trials conducted in the last 4 years, on the use of CIDRs to treat anoestrus in lactating dairy cows have shown that:

i) priming progesterone for 7 days was preferable to 4 days, but the 10-day CIDR treatment was as effective as the 7-day

period;

ii) PMSG-use at CIDR removal was necessary in most cases, and 400 IU was the most suitable dose;

iii) around 80% of treated cows ovulated within 14 days of device removal, but the percentage detected in oestrus and inseminated was variable;

iv) on average, 23% of treated animals which ovulated were not detected in oestrus and inseminated within 14 days of CIDR removal;

vi) the variation in response patterns between herds was too great to justify fixed time insemination at a single post-treatment interval; and,

vii) the preferred alternative is to re-examine all cows not detected in oestrus within 14 days of device removal, and to then treat those which have ovulated with PGF and re-treat those with no palpable ovarian response with a CIDR.

4. Ultrasound and Animal Reproduction.

Diagnostic ultrasound instrumentation has been available to the medical community since the early 1970's. In the area of domestic animal reproduction, diagnostic ultrasonography has a relative short history. The transrectal real-time or dynamic imaging ultrasound scanning of the cow's reproductive tract and ovarian structures allows the operator to view images of structures which normally can be only palpated (Pierson and Ginther, 1984).

Ultrasound scanning of the bovine ovaries and the diagnostic reliability of this technique was first reported by Pierson and Ginther, 1984). Changes in growth patterns of the follicles in the ovary with the aid of daily ultrasound scanning have also been studied (Savio et al., 1988).

Ultrasonography is beneficial when rectal palpation of the genital tract as well as hormone analysis do not give the desired information about normal or pathological ovarian activity. Images of the ovaries using ultrasound scanning can give additional information about the follicle population, the presence of a functional CL, follicles, and luteinized cysts. Moreover, haematomas in the ovaries, ovarian responses to hormonal treatment can be identified (Chupin and Procureur, 1983; Thayer et al., 1985). Also, ultrasound is an aid to a superstimulation regime as a complementary step in the procurement of

transferable embryos (Pierson et al., 1988), and identifying abnormalities in the ovarian region. Other applications of ultrasonography in cows, besides the study of ovarian structures, include early pregnancy diagnosis (Hansen and Delsaux, 1987), verification of the age, sex and/or viability of the foetus, and aspiration of an oocyte (Pieterse et al., 1988). A correct interpretation of the physiological structures in the ovaries visualized by ultrasonography is of primary importance to make effective use of this modern technique in bovine gynaecology (Pieterse, 1989). These advantages have potential promise for the use of ultrasound technology by practitioners as well as for research purposes (Kahn and Leidl, 1989).

OBJECTIVES

The objectives of the following experiment were:

a) to characterize the dynamic changes of the follicles in the ovaries by using ultrasonography during and after CIDR-B insertion, together with an injection of PMSG in two different stages of the post-partum period (early and late) in anoestrous lactating dairy cows;

b) to measure changes in plasma progesterone concentrations from insertion to removal of CIDR devices in anoestrous cows; and,

c) to measure treatment response rates in terms of oestrus and/or ovulation.

MATERIAL AND METHODS

Animals.

This experiment was carried out with cows in two commercial seasonal supply dairy herds in the Manawatu area of New Zealand. The animals were Friesian cows were aged from 3 to 9 years and grazed ryegrass-white clover pastures throughout the study. Groups of lactating non-cycling cows (n = 28) calving between September and October 1989 were studied at two different times in the post-partum period.

Oestrus detection was monitored in both treatment and control groups during and after the treatment, by twice daily observation, with each observation lasting at least 30 minutes and carried out in conjunction with the use of tailpaint and raddle as aid in oestrus detection (Macmillan et al., 1988). Oestrus was defined as that period during which a cow would stand to be ridden by its herdmates. The animals were considered to be or have been in oestrus when raddle was removed and varied amounts of paint has been rubbed off.

Experimental Protocol.

A controlled internal drug releasing device (Eazi-Breed CIDR^{tm}-B; CHH Plastic Moulding Co., Hamilton, N.Z.) containing 1.9 g progesterone in a silicone elastomer (10% w/w) was used with each treated cow in this experiment.

Pregnant Mare Serum Gonadotrophin (PMSG, as Folligon, Intervet, Chemavet Division Phamaco Ltd, N.Z.) was also used.

Animals in each treatment group received the following treatment:

CIDRs were inserted for 10 days on day 25 (trial 1; 1st herd) or day 55 (trial 2; 2nd herd) of the post-partum period, respectively.

Each animal was injected intramuscularly in the rump with PMSG (2 ml containing 400 I.U.), when the CIDR-B was removed.

Animals in the respective control groups did not receive any treatment.

In Trial 1, 12 non-cycling cows were included in the treatment group, and 10 in the control group.

In Trial 2, 6 non-cycling cows were included in the treatment group, and 5 in the control group.

Blood Collection and Analysis.

Blood samples for progesterone (P4) measurements were collected as follows:

i) Treatment groups:

a) Immediately before CIDR-B insertion and removal.

ii) Control groups:

At the same times as when a CIDR-B was inserted or removed from a cow in the treatment group.

These samples were collected by coccygeal venepuncture and assayed to check luteal ovarian activity post-partum and the concentrations of progesterone at the end of treatment.

Each sample (8 ml) was drawn into a heparinised tube (Nipro-New Tube System, Vacuum Blood Collecting System, Nissho Corporation, Osaka, Japan) which were put on ice. Samples were centrifuged within 2 h at 5 °C for 20 minutes at 3000 xg . The extracted plasma was stored at - 20 °C until measured by specific radioimmunoassay (RIA) for P4.

Those animals with levels above 1 ng/ml before CIDR-B insertion were excluded from the experiment because they were considered to have significant luteal ovarian activity post-partum, and may have previously recovered, spontaneously from the condition.

Hormone Assay.

Plasma concentrations of progesterone were measured by the method of Kirwood et al. (1984).

Progesterone. Determinations were made on 500 µl samples from the blood collection. Samples were extracted with 5 ml toluene:hexane (1:2 v/v). The plasma was frozen overnight, and the solvent then decanted into clean tubes, dried under air and redissolved in 500 µl ethanol. Duplicate 100 µl samples of ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 µl samples of standard ethanolic solutions of progesterone (P-1030:Sigma Chemical Co., St Louis, Missouri, U.S.A.) with concentrations corresponding to plasma progesterone levels of 0.625-40 ng/ml. A mixture containing antiserum (courtesy of Dr J. T. France) at a final dilution of 1:18000 (Tungsubutra & France, 1978); [1,2,6,7-H] progesterone(TRK 413, Amersham, Bucks, U.K.) at 10000 c.p.m./100 µl; phosphate-buffered saline containing 0.02 m-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by vol.) was added (600 µl) to each tube and vortexed. After overnight incubation at 4 C, 600 µl of 2.5% (W/V) charcoal (Norit A; A.H. Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and then incubated at 4 C for 10 minutes. Tubes were then centrifuged at 3000 g for 10 minutes at 4 C. The

supernatant was decanted into scintillation vials and 6 ml toluene-trition scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter.

Assay samples were processed in a single assay in which the limit of sensitivity was 0.07 ng/ml, and the intra-assay coefficient of variation was 9.5%.

Ultrasound Examination.

Ovarian follicular populations were examined using a transrectal real time linear array ultrasound (Aloka, Echo Camera, Multicrystal Scanner, model SSD-210-DX, Japan) with a 5.0 megahertz probe.

Size and number of ovarian follicles with antral diameters equal to or greater than 2 mm were recorded as well as the size and number of unovulated luteinized follicles (follicles with unclear fluid, and heavily echogenic borders), and corpus luteum. The follicles were classified in three categories described as follows:

Small : <6 mm (Class 1).

Medium: from 6 to 9 mm (Class 2).

Large : >9 mm (Class 3).

In the treatment groups, ovaries were examined by ultrasonic scanning:

1) when the CIDR was inserted;

2) on day 8 after CIDR insertion;

3) at the time a CIDR was removed;

4) on the first day after a CIDR was removed; and

5) on the 14th day after a CIDR was removed.

In the control groups, ovaries were examined by ultrasonic scanning on the same days as the CIDR was inserted and removed from cows in the treatment groups. No sedative was used with an animal which was simply restrained in a single race during the scanning. The method for examining the ovaries by ultrasonography was adopted from previous reports (Quirk et al., 1986a; Sirois and Fortune, 1988).

The routine procedure for each examination was as follows:

i) faecal material was removed manually from the rectum before examination;

ii) the transducer was inserted into the rectum;

iii) each ovary was separately located and scanned several times, and in more than one plane. When it was necessary, the image was frozen on the screen and the size of a follicle measured; and,

iv) The probe was dipped in antiseptic solution between examinations of different animals.

Each ultrasonography procedure was recorded on videotape (Panasonic-VH-NV-E180SP). The video recorder was National AG-6200-EN, Matsushita Electric Industrial Co., Ltd Japan. The tape was reviewed on the screen of the scanner, and diagrams of the relative positions of the follicles in relation to other ovarian structures were drawn for each ovary. This allowed individual follicles to be identified on successive days. When the image of the follicle being scanned was not spherical, the diameter was estimated by averaging the longest and shortest diameters. All follicles were measured with a calliper calibrated against the scale provided with the ultrasound unit. All ultrasonographic examinations, and the reviews of the videotapes were performed by one operator.

Statistical Analysis.

Data were analyzed using the Panacea Database Management System (PAN Livestock Services Ltd. Department of Agriculture, University of Reading, P.O. Box 236, Reading, Berkshire, England). The numbers of follicles within each class and the total number per animal were analyzed. Also included in the analysis was the effects of oestrous response to treatment, and ovulation after treatment. Follicular diameter classes (class 1, 2 and 3), and diameter of the largest follicle were also analyzed.

RESULTS

Level of Production and Body Condition Score.

The level of production in herd 1 (Trial 1) during 1989 was 148.3 kg of milk fat/per animal/ year. In herd 2 (Trial 2) it was 135 kg of milk fat/per animal/year. The condition scores varied from 3.0 to 4.0 for the animals involved in both herds (Holmes and Wilson, 1987).

Follicular Status of the Ovaries at Initial Examination.

Early post-partum.

The follicular status of the ovaries at the time CIDRs were inserted into each animal was that although the ovaries were small (under 10 mm along the longest axis), there were always follicles present. The average number of class 1 follicles per animal was 13.3 ± 0.6 with a range of 10.0 to 15.0 follicles. Not all the cows had at least one class 2 follicle at scanning. Normal (seen as fluid-filled structures, which appear on the screen as black [nonechogenic] areas by a defined, relatively smooth outline; Figure 5.1) and luteinized (as a follicle [black on the screen] lined by an irregular enlarged echogenic layer [grey on the screen] with unclear fluid; Figure 5.2) class 3 follicles were found in non-cycling cow. There were 6 cows with luteinized follicles from a total of 12 animals.

Contemporary control cows had small ovaries. The average number of class 1 follicles was 13.8 ± 0.9 with a range of 8.0 to 17.0 follicles. Also, there were class 2 and class 3 follicles in all the ovaries. Normal and luteinized class 3 follicles were found in the ovaries of these animals. From a total of 6 animals, there were 2 cows with luteinized follicles.

Late post-partum.

There were small follicles present in the ovaries of every cow at initial scanning. The average number of class 1 follicles was 13.5 ± 2.7 with a range of 3 to 20 follicles. There were also class 2 and 3 follicles present including normal and luteinized class 3 follicles. There were 3 cows with a luteinized follicles from a total of 6 animals.

Similarly, there were follicles in the small ovaries of every contemporary control cow. The average number of class 1 follicles was 16.7 ± 3.4 with a range of 13.0 to 19.0 follicles. Follicle classes 2 and 3 were present on the ovaries at the moment of scanning. Normal and luteinized class 3 follicles were identified in each of these animals. The proportion of animals with a luteinized follicle were 2 cows from a total of 4 animals.

There were no differences in ovary size in the early or late post-partum period. Also, the average number of follicles in each class was not significantly different

between post-partum stages. However, although it was not significant, the total number of follicle was slightly higher in the late than in the early post-partum period, and there was an increase in the number of class 2 follicles (Table 5.1).

Follicle Number during Treatment.

Early post-partum.

The average number of follicles in each class was not significantly altered during the CIDR treatment in treated animals (Table 5.2). The size of the largest follicle did not increase during the CIDR treatment in which the diameter was 13.7 mm at CIDR insertion vs 12.9 mm at device removal. The total number of follicles was not significantly altered during treatment. However, in untreated contemporary animals some cows were not considered for analysis because thus had developed significant luteal activity which indicated spontaneous recovery from the condition (4 cows from a total of 10 animals). The results from the 6 non-cycling cows remaining showed that the average number of each follicle class and the total number of follicles were not significantly altered, but the size of the largest follicle was significantly increased (12.5 mm at day 25 post-partum vs 15.2 mm at day 35 after calving; $P < 0.10$).

Late post-partum.

The average number of class 1 follicles increased significantly during the period when CIDRs were inserted into cows in the treated group ($P < 0.05$; Table 5.3). However, the number of small follicles did not change in the untreated group.

Class 2 and 3 follicles did not increase in either treated or untreated animals, and the average size of the largest follicle did not increase during this period of time in both treatment and control groups (12.0 mm and 11.8 mm at the first examination [days 50-55 post-partum] vs 11.0 mm and 11.3 mm at the second examination [days 60-65 post-partum], respectively).

When the comparisons in treatment and control groups were made between early and late post-partum period, the results showed that in the treatment groups, the average number of class 2 follicles and the total number of follicles were significantly increased at CIDR removal in the late compared with the early post-partum period (Table 5.4). However the average number of class 1 follicles was increased but not significantly. In the control cows, the average number of class 1 follicles, and the total number of follicles were significantly increased at the time equivalent to device removal in the late compared with the early post-partum period. Moreover, the population of follicles in both treatment and control groups was increased in the late post-partum period, compared with the early post-partum period.

Follicle Changes before Oestrus.

Early post-partum.

In treated anoestrous cows, the average number of class 1 follicle per animal increased significantly 1 day after the CIDR was removed from 15.0 ± 1.2 at CIDR removal to 21.1 ± 1.1 one day later ($P < 0.01$; Table 5.5). The average numbers of class 2 and 3 follicles did not change during this period, and the size of the class 3 luteinized follicles increased significantly after CIDR removal and injection, from 14.6 ± 1.1 at removal to 18.1 ± 0.7 one day later ($P < 0.01$; Table 5.5).

In the anoestrous cows which displayed oestrus after CIDR + PMSG treatment, the average number of follicles per animal in each class at CIDR removal was similar to cows not displaying oestrus, but the diameter of class 3 luteinized follicles was slightly greater in animals displaying oestrus ($P < 0.13$; Table 5.6).

These oestrous cows also had a greater increase in class 1 follicles by one day after device removal (15.3 ± 0.8 vs 23.6 ± 1.4; $P < 0.001$; Table 5.6). However, the average sizes of class 3 luteinized and normal follicles were not increased. The results for the non-oestrous cows are also presented in Table 5.6. In these cows, the average number of follicles in each class, and the size of the largest follicle were similar at CIDR removal and one day after device removal.

The average number of follicles in each class was similar at CIDR removal among cows which ovulated or did not ovulate after CIDR/PMSG treatment. However, the size of the large luteinized follicle increased significantly in cows which ovulated compared with cows which did not have an ovulation by 7 days after removal ($P < 0.10$; Table 5.7). After treatment, the number of class 1 follicles increased significantly in early post-partum cows which had an ovulation and formed a CL (14.5 ± 1.1 vs 23.4 ± 1.4; $P < 0.001$; Table 5.7). Although it was not a significant difference, these cows also had an increase in the number of class 3 follicles after treatment (1.6 ± 0.3 vs 2.3 ± 0.6).

Late post-partum.

The average number of follicles in each class and the size of the normal and luteinized class 3 follicles did not increase after CIDR/PMSG treatment (Table 5.8).

In treated cows displaying oestrus with ovulation and formation of a CL after treatment, the number of follicles in each class was similar at CIDR removal compared with cows which did not display oestrus and did not ovulate by 7 days after device removal. The size of the normal and luteinized class 3 follicles did not increase significantly. Cows which did not display and ovulate also did not increase the follicles classes 1, 2 and 3 and the size of normal and luteinized follicles after treatment (Table 5.9).

Plasma Progesterone Profiles.

Early post-partum.

Average plasma progesterone concentrations (PPC) at the time of CIDR insertion and at removal in non-cycling cows in the early (Trial 1) and late (Trial 2) post-partum period are presented in Table 5.10. The average PPC before CIDR insertion in the treatment group was 0.4 ± 0.1 ng/ml compared with an average concentration of less than the detectable limit of the assay (< 0.1 ng/ml) in the control group.

The average PPC before CIDR removal for treated cows was 4.5 ± 1.3 ng/ml. In the untreated control group at the same sampling time it was 0.3 ± 0.1 ng/ml.

Late post-partum.

The average PPC before CIDR insertion in the treatment group was 0.3 ± 0.1 ng/ml, compared with 0.4 ± 0.1 ng/ml at the same time of sampling in the untreated control group (Table 5.10). Before CIDR removal concentrations in the treatment group were 2.4 ± 0.9 compared with 0.4 ± 0.1 ng/ml in the untreated control group (P> 0.10).

The concentrations of progesterone in the control cows which had a luteinized follicle present at the sampling when the CIDR device was removed in treatment groups (n = 6), varied from 0.1 ng/ml to 1.6 ng/ml.

Oestrous Response and Ovulation after Treatment.

Early post-partum.

The 22.7% of cows which displayed oestrus in the early post-partum period comprised 25% of the treated cows vs 33.3% of control animals.

One third of the treated group were in oestrus within seven days after CIDR/PMSG. None of the cows in the control group were in oestrus during the same period of time.

The percentage of cows which had not displayed oestrus, but had ovulated and formed a CL was 53.8% for cows in the early post-partum period, being 55.5 and 50.0% in treated and control cows respectively.
After removal of the CIDR and PMSG injection, 3 of the 12 cows were in oestrus within the first 7 days after treatment, and 2 of these 3 had an ovulation and formed a CL. Moreover, these 2 cows had a luteinized follicle in the other ovary as identified by ultrasonography at scanning on the 14th day after CIDR removal. The third cow did not ovulate.

There were 5 cows which did not display oestrus, but a CL was identified by ultrasonography when they were scanned 14 days after CIDR removal. In 3 of these 5 cows, a luteinized follicle was identified by ultrasonography at the same time in the other ovary.

In the 4 remaining cows in the treatment group, each cow had a luteinized follicle identified by ultrasonography 14 days after device removal.

Only 2 out of 6 cows displayed oestrus in the contemporary control group, and in one of these cows a CL was identified. In the remaining cow a luteinized follicle was identified by ultrasonography on the 14th day after device removal.

There were 4 cows which did not display oestrus, but a CL was identified in 2 of them.

The 2 remaining cows in the group did not display oestrus and had not ovulated by the time of scanning performed 14 days after device removal.

Late post-partum.

Treated cows in the late post-partum period did not ovulate without displaying external signs of oestrus. However, all of the untreated cows which ovulated formed a CL without any external signs of oestrous.

After removal of the CIDR and subsequent PMSG injection, 2 cows were in oestrus within the first 7 days after treatment. A CL was identified in both of these cows by ultrasonography at scanning 14 days after device removal.
A luteinized follicle was identified by ultrasonography at the same time, in the remaining 4 cows which did not display oestrus.

Contemporary control cows did not display oestrus, but each of them had formed a CL in one ovary, and a luteinized follicle in the other ovary.

DISCUSSION

Follicular Status of the Ovaries at Initial Examination.

In the present study, follicles in classes 1, 2 and 3 were identified by ultrasonography at initial examination in early and late post-partum periods in anoestrous cows.

The population of follicles varied between cows in both early and late post-partum periods, but the average number of follicles per animal and in each class at day 25 and 50-55 for treatment and control groups was similar (Tables 5.2 and 5.3). This result is in agreement with the data of Chaimongkol (1990), although in the present study the average number in each follicle class was less. Chaimongkol (199) reported that the population of follicles, identifiable by ultrasound examinations, did not increase significantly between the second and seventh weeks after calving in acyclic dairy cows. The finding that class 2 and 3 follicles were present in these anoestrous cows is in agreement with a previous report indicating that there were large (> 8 mm) follicles in early post-partum in acyclic suckled beef cows (Spicer et al., 1986). However, these data differ from findings in another report (Peter and Bosu, 1988), who indicated that large follicles were absent in post-partum anoestrous cows.

Follicle Number during CIDR-B Treatment.

The average number of follicles in each class was not significantly increased in early post-partum cows during the treatment period, in either treated or untreated contemporary animals. However, in the late post-partum period, only the average number of class 1 follicles was significantly increased in animals in the treated group (Table 5.3).

The increased number of small follicles in this period was due to the fact that during this time the anoestrus cows were in a period of follicular growth. It is known that the circulating concentrations of LH are lower in acyclic post-partum animals than in cyclic animals, but the circulating concentrations of FSH are not suppressed (Nett, 1987). Therefore, the class 1 follicles which are more FSH dependent, could develop in these cows. Moreover, the early post-partum period is characterized by lower biological activity of the LH which is secreted from reduced pituitary stores (Weesner et al., 1987; McNatty, 1988), as well as a decreased responsiveness of the pituitary to GnRH (McNatty, 1988).

On the other hand, the population of follicles in both treatment and control groups increased in the late post-partum period. This can be explained because there is a gradual increase in the frequency of LH pulses and in plasma LH concentrations with time post-partum. Moreover, the secretion of gonadotrophin stimulates follicular growth and production of oestradiol (Peters and Lamming, 1986). Therefore, concurrent with these changes there is a progressive recovery of the anoestrus condition.

Follicle changes before Oestrus.

The average number of class 1 follicles in the early post-partum period increased significantly in the whole treatment group irrespective of whether or not animals displayed oestrus or ovulated with CL formation after the treatment with CIDR/PMSG (Tables 5.5, 5.6 and 5.7). This is because the small follicles were responsive to pituitary hormones, and to exogenous hormones such as PMSG. The class 2 and 3 follicles which were present did not respond. These medium and large follicles are responsive to FSH and LH, and also are capable of synthesizing oestradiol-17β. However, during post-partum anoestrus, these follicles can fail to secrete adequate amounts of oestradiol which is essential for the induction of oestrous behaviour and ovulation, and often fail to reach preovulatory size, thus causing a decreased ovarian follicular response to LH (McNatty, 1988).

The lack of response to PMSG in each class of follicles in the late post-partum period can be explained as follows:

The functional competence of the hypothalamus and pituitary is normally decreased for a 10 to 70-day period after calving (McNatty, 1988; Short et al., 1990). The primary deficit that exists in post-partum cows is low concentrations of LH (Short et al., 1990). On the other hand, the duration of the post-partum anoestrus is related (either in part or in various combinations) to breed, body condition at calving, time of year, age of cow, levels of nutrition, lactation, suckling, dystocia, uterine pathology and chronic debilitating disease (Roberts, 1971; Kaltenbach, 1980; Lamming et al., 1981; Tucker, 1982; McNatty et al., 1983; Chauhan et al., 1984; Montgomery, 1986; Hanzen, 1986; Peter and Lamming, 1986; Wright et al., 1987). The cows included in the present study were in deep anoestrus at the time of CIDR/PMSG treatment. Therefore, the sensitivity of the hypothalamic-pituitary axis was in a complete state of suppression and less responsive to PMSG.

In the early post-partum period, the diameter of the largest unovulated luteinized follicle in treated groups which displayed oestrus and ovulated was significantly increased during CIDR treatment and its uncontrolled growth was extended after the device was removed (Tables 5.5, 5.6 and 5.7). Also, these unovulated luteinized follicles had the largest diameter in the ovaries. However, the diameter of normal class 3 follicles did not increase during this period, and some of them ovulated after CIDR removal and formed a CL.

There were two consistent findings in this study:

a) after device removal in both treated groups, and also in the contemporary control group, class 3 follicles grew, and at scanning on day 14 after device removal there was a CL in almost all cows and luteinized follicles, or luteinized follicles alone in one ovary and a luteinized follicle in the opposite ovary.

b) normal follicles commenced development within a follicular wave in the presence of large unovulated luteinized follicles.

The following is offered as a possible explanation for the uncontrolled growth of luteinized follicles, and for the fact that few normal class 3 follicles did ovulate, with most of them becoming luteinized:

After parturition, the peripheral plasma levels of oestradiol and progesterone are low before first ovulation. In addition to the suppressive effects of these hormones on gonadotrophin release, (especially LH), oestradiol-17β can cause a periovulatory-like peak of LH (and to a lesser extent of FSH). This feedback mechanism is critical for induction of ovulation. If the preovulatory follicle has matured sufficiently then this is communicated to the hypothalamic-pituitary axis via oestradiol-17β and the pituitary releases an ovulatory peak of LH at the time most appropriate to the follicle.

An inadequate level of either follicular oestradiol or LH in response to this positive feedback signal results in an abnormal ovulation or CL formation and/or function. The failure of oestradiol feedback is a phenomenon which commonly occurs in cows during the post-partum period (McNatty 1988).

The extent of follicular development in the presence of a large luteinized follicle could indicate that the regulatory mechanism associated with follicular dominance was not present in those luteinized follicles. Therefore the mechanism of control of follicular growth and development was not altered. This probably can be only related to the luteinization process within the follicle.

Plasma Progesterone Profiles.

The average PPC at CIDR removal in anoestrous treated cows in the early post-partum period was 4.5 ± 1.3 ng/ml, with values ranging from 0.8 to 15.8 ng/ml (Table 5.10). Treated cows at CIDR removal in the late post-partum period had a mean of 2.4 ± 0.9 ng/ml, with values ranging from 0.8 and 6.9 ng/ml. The significantly higher variation in PPCs at CIDR removal in the early post-partum can be explained as follows:

During the early post-partum period, there is a combination of factors such as the stress of high production levels with a reduced appetite and loss in body condition. On the other hand, a previous report indicated that levels of feeding and body weight were associated in ewes with altered metabolic clearance rates of progesterone from the body and mobilization of stores of progesterone (Payne et al., 1987). Therefore it is logical to suppose that in this period, the degree of anoestrus differed according to liveweight and different degrees of nutritional stress during early lactation. The variation in concentrations of progesterone in untreated control cows at day 35 of the post-partum period was associated with different degrees of luteinization within the population of follicles which were able to secrete small amounts of progesterone (Schirar and Martinet, 1982).

Oestrous Response and Ovulation after Treatment.

From the results of this study, few animals displayed oestrus in both post-partum periods, and the number of animals which had not displayed oestrus but had

ovulated and formed a CL was quite variable.

There are also results from other studies. In a concurrent study with cows in year-round herds, only 8% of cows were diagnosed as non-cycling. When these cows were treated with a CIDR and PMSG, 53% were inseminated within 10 days of treatment. This percentage varied between herds, ranging from 40% to 63%. However, another 24% ovulated but were not observed in oestrus. In one seasonal herd, 12% were diagnosed as non-cycling, and the 71% of them were inseminated after treatment (Dick, 1990a). In another seasonal herd, cows were also treated with a 10-day CIDR/PMSG in which only 5 cows out of 10 were observed in oestrus within 7 days after treatment. The 5 cows which did not display behavioral signs of oestrus included 2 animals which had a CL identified by ultrasonography at scanning. No response to treatment was observed in the 3 remaining animals (Dick, 1990b).

In general, there was a significant percentage of untreated cows which had formed a CL, but did not display oestrus. It was especially notable with the cows in the late post-partum period (Trial 2) in which none of the cows displayed external signs of oestrus, but all of them ovulated and formed a CL. The high incidence of ovulation without oestrus can be explained partly by the organization of the oestrus detection which was considered to be less satisfactory than usual.

Although the data in this study is not enough to support definitive conclusions, it does show the following:

1) The use of CIDR/PMSG treatment was not as successful as one would have expected. It produced some positive response in terms of oestrous behaviour and formation of a CL in the late post-partum period, where the cows were in deep anoestrus. Moreover, it is known that the response to CIDR/PMSG treatment is quite variable, possibly because the condition of anoestrus as seen in New Zealand dairy herds under these conditions is essentially nutritional anoestrus rather than simple post-partum or lactational anoestrus (Macmillan et al., 1990a).

2) The high percentage of untreated cows which did not display external signs of oestrus with first ovulation post-partum, would suggest that an ovulation without any external signs of oestrus may still be physiologically normal in the post-partum period in cattle (Zemjanis, 1980; Savio et al., 1990b; Chaimongkol, 1990; Kyle et al., 1990). However, the factors influencing oestrous expression and the way in which a detection aid is used cannot be ignored.

It was suggested that the absence of progesterone priming is responsible for the frequent lack of oestrus and ovulation failure (Carrick and Shelton, 1969; Gonzalez-Padilla et al., 1975). On the other hand, it has also been shown that progesterone priming is necessary to increase follicular oestradiol synthesis and the development of LH receptors on granulosa cells in anoestrous ewes (Hunter et al., 1986; Hunter et al., 1987) and cows (Inskeep et al., 1988). Moreover, there was a good correlation between plasma levels of oestradiol-17β and the incidence of hyperactive behaviour (Rhodes and Randel, 1978). This implies that progesterone treatment is able to take greater advantage of the ovulatory peak of LH that triggers ovulation and luteinizes the follicle. Thus, by increasing ovarian oestradiol and LH receptors in the preovulatory follicle, progesterone priming could

enhance oestrus. In this present study, treated groups did not have a significantly higher proportion of cows in oestrus after CIDR/PMSG treatment, compared with the control groups. Therefore, the postulated effects of the necessary priming of progesterone to enhance oestrus was not observed.

This may mean that the exposure to progesterone may not have been adequate to prime the ovulatory system in anoestrous cows in which the inadequate or inappropriate patterns of LH release seem more closely related to the presence or absence of ovarian activity in post-partum cows (McNatty, 1988; Short et al., 1990).

CONCLUSIONS

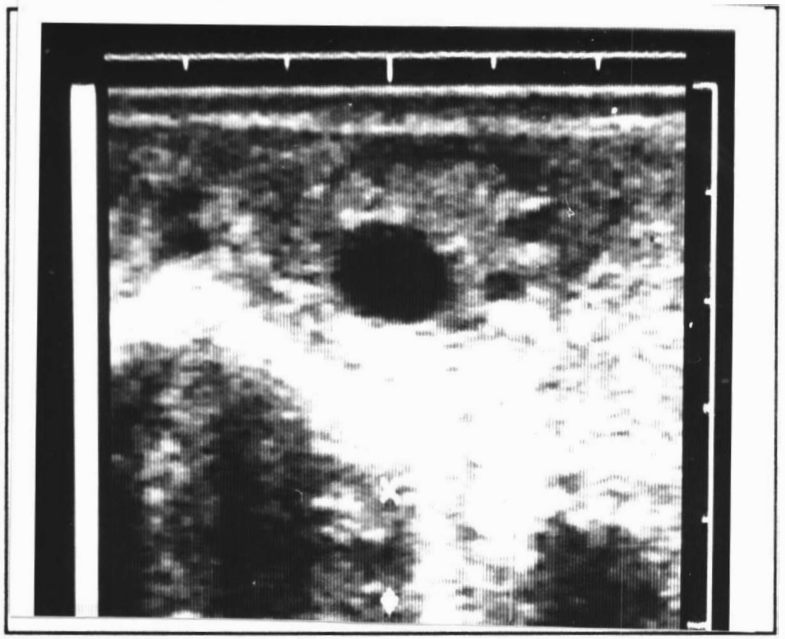
These results show that the treatment did create some differences in the patterns of follicular development at the early and late post-partum periods, and the response to CIDR/PMSG treatment was quite variable. This was possibly because the condition of anoestrus is essentially nutritional anoestrus rather than simple post-partum or lactational anoestrus. The distinction has to be made between these forms and its combination. Moreover, in early lactation when negative energy balance is common, this deficit apparently affects the hypothalamus and the secretion of pituitary gonadotrophin, and this ultimately affects follicular growth on the ovary. When CIDR/PMSG treatment is used to stimulate ovarian activity (including hypothalamic sensitisation to oestrogen to produce release of LH and ovulation), then ovulation may occur without oestrus and inadequate follicle development may sometimes occur after treatment.

Further studies have to focus on:

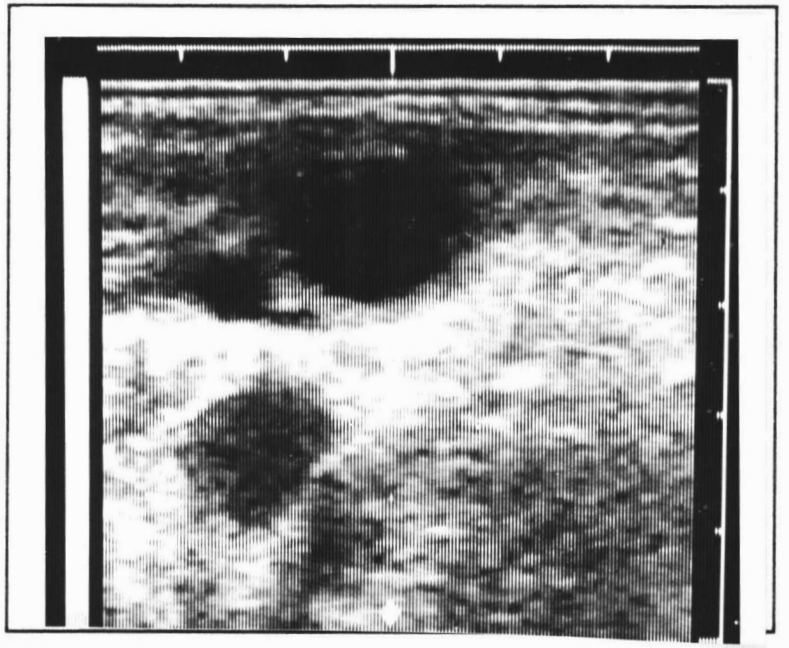
i) the importance of identifying factors which affect the treatment response (resulting in ovulation with oestrus, ovulation without oestrus or no endocrine response at all) and to consequently reduce the variation between herds in treatment efficacy;

ii) The endocrinological characteristics of anoestrous cows at the post-partum period; and,

ii) the interrelationship between nutritional state of the animal before and at calving, and post-partum follicular activity, since the ovarian response appears to be related to post-partum stage as well as to the severity and duration (short or long term) of nutrient deprivation.



A



B

Figure 5.1 Ultrasound images of follicles at various stages of the oestrous cycle. Follicles approximately 2 to 20 mm are visible on the ovaries (A and B). Scale on the right image is in centimetres.

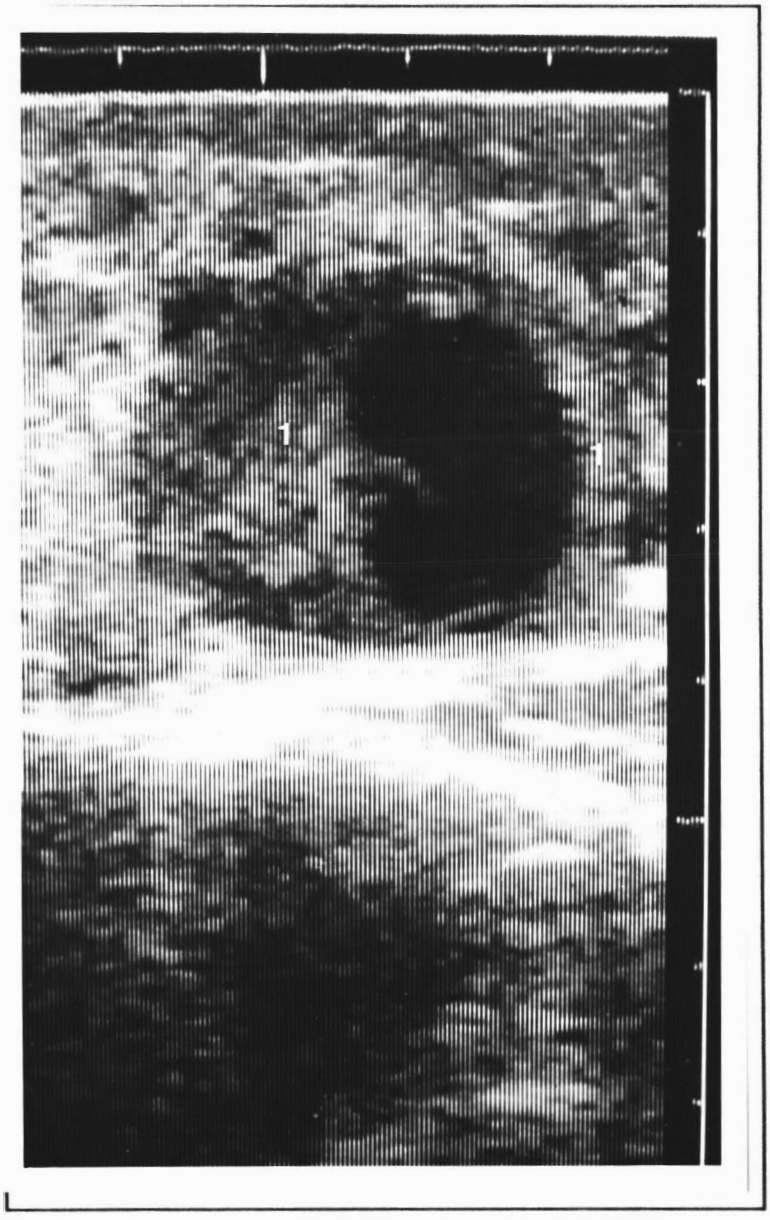


Figure 5.2 Ultrasound image of a luteinized follicle in a non-cycling cow at day 14 after treatment with CIDR/PMSG. 1 = luteinized cells. Scale on the right image is in centimetres.

Table 5.1 **Average number of ovarian follicles in classes 1, 2 and 3 and total number of follicle at initial examination in treated cows and at the same time for the contemporary control cows in the early and late post-partum (PP) period in anoestrous cows.**

		Treatment Group	Control Group
Post-partum Period	Class Follicle	Mean (SEM)	Mean (SEM)
Early[a]	1	13.3 0.6	13.8 1.1
	2	1.2 0.2	1.3 0.4
	3	1.3 0.3	1.0 0.1
	Total	15.8 0.6	16.1 0.8
Late[b]	1	13.5 2.7	16.7 3.4
	2	2.0 0.6	2.7 0.2
	3	2.0 0.1	1.0 0.1
	Total	17.5 1.7	20.4 1.7

a = In treated cows CIDR-B was inserted from 25 day PP
b = In treated cows CIDR-B was inserted from 50-55 day PP

Table 5.2 **Average number of ovarian follicles per animal in classes 1, 2 and 3 and total number of follicles during CIDR-B insertion in treated cows and in the same period for contemporary controls cows in early post-partum period (PP) in anoestrous cows.**

		Treatment Group (12)	Control Group (6)
Day PP	Class	Mean (SEM)	Mean (SEM)
25[a]	1	13.3 0.6	13.8 1.1
	2	1.2 0.2	1.3 0.4
	3	1.3 0.3	1.0 0.1
	Total	15.8 0.6	16.1 0.8
35[b]	1	15.0 1.2	15.8 1.0
	2	1.7 0.3	2.0 0.2
	3	2.0 0.5	1.2 0.2
	Total	18.7 1.0	18.0 0.8

a= In treated cows CIDR-B was inserted for 10 days from 25 days PP
b= In treated cows CIDR-B was removed from 35 days PP
( ) number of animals

Table 5.3 **Average number of ovarian follicles in classes 1,2 and 3 and total number of follicles during the period of CIDR-B insertion in treated cows and in the same period for contemporary control cows in late post-partum period (PP) in anoestrous cows.**

		Days Postpartum	
Group	Class	1st examn 50-55[b]	2nd examn 60-65[c]
Treatment (6)	1	13.5 2.7[a]	19.6 2.0
	2	2.0 0.6	2.8 0.5
	3	2.0 0.1	1.8 0.1
	Total	17.5 1.7	24.2 1.3
Control (4)	1	16.7 3.4	21.6 3.7
	2	2.7 0.2	3.0 1.5
	3	1.0 0.1	1.8 0.1
	Total	20.4 1.7	27.4 2.6

a = Days PP differ $P < 0.05$
( ) number of animals
b = In treated cows CIDR-B was inserted for 10 days from day 50-55 PP
c = In treated cows CIDR-B was removed from day 60-65 PP

Table 5.4 **Average number of ovarian follicles per animal in classes 1, 2, and 3 and total number of follicles during CIDR-B insertion in treated cows and in the same period for contemporary control cows in the early and late post-partum (PP) period in anoestrous cows.**

Group	PP	Class Follicle	CIDR-B Insertion	Device Removal
			Mean (SEM)	Mean (SEM)
Treatment (12)	Early	1	13.3 (0.6)	15.0 (1.2)
		2	1.2 (0.2)	1.7[b] (0.3)
		3	1.3 (0.3)	2.0 (0.5)
		Total	15.8 (0.6)	18.7[c](1.0)
Treatment (6)	Late	1	13.5 (2.7)	19.6 (2.0)
		2	2.0 (0.6)	2.8 (0.5)
		3	2.0 (0.1)	1.8 (0.1)
		Total	17.5 (1.9)	24.2 (1.3)
Control[a] (6)	Early	1	13.8 (1.1)	15.8[c](1.0)
		2	1.3 (0.4)	2.0 (0.4)
		3	1.0 (0.1)	1.2 (0.2)
		Total	16.1 (1.3)	19.0[c](1.4)
Control (4)	Late	1	16.7 (3.4)	21.6 (3.7)
		2	2.7 (0.2)	3.0 (1.5)
		3	1.0 (0.1)	1.8 (0.1)
		Total	20.4 (1.8)	27.4 (2.1)

( ) number of animals
a = In control cows the 1st examn was at the day of CIDR insertion in treatment groups and the 2nd at device removal
b = post-partum period differ $P < 0.05$
c = post-partum period differ $P < 0.01$

Table 5.5 **Average number of class 1 (< 6 mm), class 2 (6 to 9 mm), and class 3 (> 9 mm) of ovarian follicles and the average size of the largest normal and luteinized class 3 follicle at CIDR-B removal and one day later in anoestrous treated cows in the early post-partum period (Trial 1).**

Group	N° of Cows	Class Follicle	C I D R - B Removal Mean (SEM)	1 Day after Removal Mean (SEM)
Treatment	12			
		1	15.0[a] (1.2)	21.1 (1.1)
		2	2.0 (0.3)	1.5 (0.2)
		3	1.2 (0.2)	1.8 (0.4)
		Size LF	14.6[a] (1.1)	18.1 (0.7)
		Size NF	12.4 (0.9)	12.0 (1.1)

a= Days differ P< 0.01
LF= Luteinized class 3 follicle
NF= Normal class 3 follicle

Table 5.6 **Average number of class 1 (< 6 mm), class 2 (6 to 9 mm), and class 3 (> 9 mm) of ovarian follicles and the average size of the largest normal and luteinized class 3 follicle at CIDR-B removal and one day later in anoestrous treated cows displaying (+) or not (-) oestrus after CIDR/PMSG treatment in the early post-partum period (Trial 1).**

Sub-group Treatment	N° of Cows	Class Follicle	CIDR-B Removal Mean (SEM)	1 Day after Removal Mean (SEM)
Oestrus +	3	1	15.3[a] (0.8)	23.6 (1.4)
		2	1.6 (0.6)	1.2 (0.1)
		3	2.0 (0.4)	2.0 (0.8)
		Size NF	11.1 (1.0)	10.6 (0.6)
		Size LF	18.5[b] (0.7)	18.9 (0.5)
Oestrus -	9	1	15.1 (1.5)	16.9 (1.2)
		2	1.7 (0.4)	1.7 (0.3)
		3	2.0 (0.5)	1.8 (0.5)
		Size NF	12.3 (1.1)	12.7 (1.6)
		Size LF	15.4 (0.7)	17.5 (1.1)

a = Days differ $P < 0.001$
b = Sub-groups differ $P = 13$
LF = Luteinized class 3 follicle
NF = Normal class 3 follicle

Table 5.7 **Average number of ovarian follicles in class 1 (< 6 mm), class 2 (6 to 9 mm), and class 3 (> 9 mm), and the average size of the largest normal and luteinized class 3 follicle at and 1 day after CIDR-B removal in anoestrous treated cows which subsequent formed (+) or did not form (-) a corpus luteum (CL) after CIDR/PMSG treatment in the early post-partum period (Trial 1).**

Sub-group Treatment	N° of Cows	Class Follicle	CIDR-B Removal Mean (SEM)	1 Day after Removal Mean (SEM)
CL +	7	1	14.5[a] (1.1)	23.4 (1.4)
		2	1.3 (0.3)	1.0 (0.2)
		3	1.6 (0.3)	2.3 (0.7)
		Size NF	12.2 (1.1)	10.8 (0.4)
		Size LF	18.3[b] (0.5)	18.8 (0.6)
CL -	5	1	16.2 (2.2)	18.6 (0.2)
		2	2.2 (0.6)	2.2 (0.4)
		3	2.0 (1.1)	1.3 (0.3)
		Size NF	14.4 (0.9)	13.3 (2.2)
		Size LF	14.8 (1.4)	17.2 (1.6)

a = Days differ $P < 0.001$
b = Sug-groups differ $P < 0.10$
LF = Luteinized class 3 follicle
NF = Normal class 3 follicle

Table 5.8 **Average of class 1 (<6 mm), class 2 (6 to 9 mm), and class 3 (> 9 mm) of ovarian follicles and the average size of the largest normal and luteinized class 3 follicle at CIDR-B removal and 1 day later in anoestrous treated cows in the late post-partum period (Trial 2).**

Group	N° of Cows	Class Follicle	CIDR-B Removal Mean (SEM)	1 Day after Removal Mean (SEM)
Treatment	6	1	19.6 (2.0)	20.0 (2.7)
		2	2.8 (0.5)	3.6 (0.9)
		3	1.0 (0.9)	1.9 (0.7)
		Size NF	10.0 (1.0)	10.0 (1.1)
		Size LF	14.0 (0.2)	13.8 (0.5)

NF = Normal class 3 follicle
LF = Luteinized class 3 follicle

Table 5.9 **Average number of class 1 (< 6 mm), class 2 (6 to 9 mm), and class 3 (> 9 mm), and the average size of the largest normal and luteinized class 3 follicle at CIDR-B removal and 1 day later in anoestrous treated cows which displayed oestrus and formed a corpus luteum (CL) (+) or did not display oestrus and form a CL after CIDR/PMSG treatment in the late post-partum period (Trial 2).**

Sub-group Treatment	N° of Cows	Class Follicle	CIDR-B Removal Mean (SEM)	1 Day after Removal Mean (SEM)
Oestrus + CL +	2	1	18.5 (2.5)	19.5 (1.5)
		2	3.0 (0.8)	3.0 (0.9)
		3	1.0 (0.7)	1.8 (0.8)
		Size NF	9.0 (0.7)	10.0 (0.6)
		Size LF	14.5 (1.0)	13.5 (1.1)
Oestrus - CL -	4	1	20.2 (2.8)	20.2 (4.2)
		2	2.7 (0.7)	3.2 (1.2)
		3	1.0 (0.5)	0.8 (0.3)
		Size NF	11.0 (0.9)	10.1 (0.6)
		Size LF	13.7 (0.2)	14.0 (1.4)

NF = Normal class 3 follicle
LF = Luteinized class 3 follicle

Table 5.10 **Average plasma progesterone concentrations (PPC; ng/ml) at the time of CIDR-B insertion and removal in treated and in the same period for contemporary control in anoestrous cows in the early and late post-partum period (PP).**

	Early Post-Partum Period (Trial 1)			Late Post-Partum Period (Trial 2)		
Group	Number of Animals	Day PP[a] Mean (SEM)	Day PP Mean (SEM)	Number of Animals	Day PP[b] Mean (SEM)	Days PP Mean (SEM)
Treatment	12	0.4 (0.1)	4.5 (1.3)	6	0.3 (0.1)	2.4 (0.9)
Control	6	< 0.1	0.3 (0.1)	4	0.4 (0.1)	0.4 (0.1)

a = In treated cows CIDR-B was inserted on Day 25 PP for 10 days.
b = In treated cows CIDR-B was inserted on Day 50-55 PP for 10 days.

REFERENCES

AKERS, R. M., GOODMAN, G. T. and TUCKER, H. A. (1980). Clearance and secretion rates of prolactin in dairy cattle in various physiological states. **Proc. Soc. Exp. Biol. Med.** 168: 115-119.

CARRICK, M. J. and SHELTON,J. N. (1969). Oestrogen-progesterone relationships in the induction of oestrus in spayed heifers. **J. Endocrinol.** 45: 99-109.

CHAIMONGKOL, C. (1990). Studies on reproductive performance in New Zealand dairy cows and some factors which may influence reproductive efficience. **Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 1: 1-112.

CHANG, C. H., GIMENEZ, T. and HERNRICKS, D. M. (1981). Modulation of reproductive hormones by suckling and exogenous gonadal hormones in young beef cows postpartum. **J. Reprod. Fertil.** 63: 31-38.

CHAUHAN, F. S., MGONGO, F. O. K. and KESSY, B. M. (1984). Recent advances in hormonal therapy of bovine reproductive disorders: a review. **Vet. Bull.** 54: 991-1009.

CHUPIN, D. and PROCURER, R. (1983). Prediction of bovine ovarian response to PMSG by ultrasonic echography. **Theriogenology.** 19: 119.

CONVEY, E. M., TUCKER, H. A. and SHORT, R. E. (1983). Acute effect of suckling on gonadotropin, prolactin and glucocorticoid concentrations in serum of intact and ovariectomized beef cows. **Theriogenology.** 20: 661-674.

DAY, A. M. and TAUFA, V. K. (1988). CIDR-B; Some observations and feedback from 1987. **Proc. of the Dairy Cattle Society of the New Zealand Veterinary Assoc.** 5: 193-201.

DICK, A. R. (1990a). Controlled breeding management through the strategic use of the CIDR-B intravaginal device for cows in herds with a daily milk quota. In **Studies on the use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University thesis, Palmerston North, New Zealand. Chapter 3: 127-187.

DICK, A. R. (1990). The use of tailpaint and an aerosol raddle to monitor oestrous behaviour of animals after different synchrony treatments. In **Studies on the of use of the CIDR intravaginal device for reproductive management of dairy cattle. Master of Philosophy thesis,** Massey University, Palmerston North, New Zealand. Chapter 2: 80-126.

DUFOUR, J. J. and ROY, G. L. (1985). Distribution of ovarian follicular populations in the dairy cow within 35 days after parturition. **J. Reprod. fertil.** 73: 229-235.

ELEY, D. S., THATCHER. W. W., HEAD, H. H., COLLIER, R. J., WILCOX, C. J. and CALL, E. P. (1981). Periparturient and postpartum endocrine changes of conceptus and maternal unit in Jersey cows bred for milk yield. **J. Dairy Sci.** 64: 312-320.

FALTYS, G. L, FOGWELL, R. E., SHORT, R. E. and CONVEY, E. M. (1983). Influence of suckling on secretory patterns of LH and concentrations of cortisol in lactating beef cows. **J. Anim. Sci.** 57: (suppl.): 335.

FIELDEN, E. D., MACMILLAN, K. L. and MILLER, K. (1976). The pre-service anoestrous syndrome in New Zealand dairy cattle. **Bovine Practitioner.** 11: 10-14.

FONSECA, F. A., BRITT, J. H., McDANIELS, B. T., WILK, J. C. and RAKES, A. H. (1983). Reproductive traits of Holstein and Jerseys. Effect of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. **J. Dairy Sci.** 66: 1128-1149.

FORREST, D. W., FLEEGER, J. L., LONG, C. R., SORENSON, A. M., HARMS, R. G. (1980). Effect of exogenous prolactin an peripheral luteinizing hormone levels in ovariectomized cows. **Biol. Reprod.** 22: 197-201.

GIER, H. T. and MARION, G. B. (1968). Uterus of the cow after parturition: involutional changes. **Am. J. Vet. Res.** 29: 83-95.

GONZALEZ PADILLA, E., NISWENDER, G. D. and WILTBANK, J. N. (1975). Puberty in beef heifers. II. Effect of injections of progesterone and estradiol-17B on serum LH, FSH and ovarian activity. **J. Anim. Sci.** 40: 1105-1109.

HANSEN, C. and DELSAUX, B. (1987). Use of transrectal B-mode ultrasound imaging in bovine pregnancy diagnosis. **Vet. Rec.** 121: 200-202.

HANZEN, Ch. (1986). Endocrine regulation of postpartum ovarian activity in cattle: a review. **Reprod. Nutr. Develop.** 26: 1219-1239.

HENDERSON, K. M. and McNATTY, K. P. (1987). Factors influencing ovulation rate in sheep. **Proc. Asia and Australasian Assoc. Animal Prod.** 130-133.

HUNTER, M. G., SOUTHEE, J. A., McLEOD, B. J. and HARESIGN, W. (1986). Progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes. **J. Reprod Fertil.** 76: 349-363.

HUNTER, M. G., SOUTHEE, J. A. and HARESIGN, W. (1987). The effect of progesterone on follicular functions in onoestrous ewes. In: **Follicular**

Growth and Ovulation Rate. eds. J. F. Roche and D. O'Callaghan. Martinus Nijhoff Publishers, The Netherlands. 163-176.

HOLMES, C. W. and WILSON, G. F. (1987). Feeding the herd: Feed requirements and feeding levels. Milk production from pasture. Butterworths of New Zealand Ltd [New ed.] Agricultural Books 1 v. 24-32.

INSKEEP, E. K., BRADEN, T. D., LEWIS, P. E., GARCIA-WINDER, M. and NISWENDER, G. D. (1988). Receptors, for luteinizing hormone and Follicle-stimulating hormone in largest follicles of postpartum beef cows. Biology of Reproduction. 38: 587-591.

KALTENBACH, C. C., JONES, K. R., PAYNE, E., SMITH, J. F., TERVIT, H. R. and WELCH, R. A. S. (1977). Effect of CB-154 in post-partum anestrus in beef cows. J. Anim. sci. 45: (suppl.): 173.

KALTENBACH, C. C. (1980). Initiation of puberty and postpartum estrus in beef cattle. In: Current Therapy in Theriogenology. 164-168. ed. D. A. Morrow. W. B. Saunders Company, Philadelphia.

KAHN, W. and LEIDL, W. (1989). Ultrasonic characteristics of pathological conditions of the bovine uterus and ovaries. Diagnostic Ultrasound and Animal Reproduction. eds. M. M> Taverne and H. Willemse. Kluwer Academic Publishers. 53-65.

KINDHAL, H., EDQVIST, L. E., LARSOON, K. and MALMQVIST, A. (1982). Influence of prostaglandin on ovarian post-partum. In: Factors Inluencing Fertility in the Postpartum Cow. 173-196. eds. H. Karg and E. Schallenberger. Matinus Nijhoff Publishers.

KIRKWOOD, R. N., LAPWOOD, K. R., SMITH, W. C. and ANDERSON, I. L. (1984). Plasma concentrations of LH, prolactin, oestradiol-17B and progesterone in sows weaned after lactation for 10 or 35 days. J. Reprod. Fertil. 70: 95-102.

KYLE, S. D., CALLAHAN, C. J. and ALLRICH, R. D. (1990). The effects of early postpartum progesterone treatment on the expression of estrus at first ovulation in dairy cattle. American Dairy Sci. Assoc. J. Dairy Sci. 73: (suppl. 1): 178.

LAMMING, G. E., WATHES, D. C. and PETERS, A. R. (1981). Endocrine patterns of the post-partum cow. J. Reprod. Fertil. 30: (suppl.): 155-170.

LEGAN, S. J., I'ANSON, H., FITZGERALD, B. P. AND AKAYDIN, M. S. (1985). Importance of short luteal phases in the endocrine mechanism controlling initiation of oestrus cycles in anoestrous ewes. Endocrinology. 117: 1530-1536.

LI, P. W. and WAGNER, W. C. (1983). In vivo and in vitro studies on the effect of adenocorticotropic hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biol. Reprod. 29: 25-37.

MACMILLAN, K. L., FIELDEN E. D. and WATSON, J. D. (1975). The anoestrous syndrome in New Zealand dairy herds. II. Some factors influencing submission rates in taranaki herds. **N. Z. Vet. J.** 23: 4-8.

MACMILLAN, K. L. and DAY, A. M. (1987). Treating the non-cycling cow. **Proc. Ruakura Farmers Conf.** 39: 65-68.

MACMILLAN, K. L., TAUFA, V. K., BARNES, D. R., DAY, A. M. and HENRY, R. (1988). Detecting oestrus in synchronized heifers using tailpaint and aerosol raddle. **Theriogenology.** 30: 1099-1114.

MACMILLAN, K. L., DAY, A. M. and TAUFA, V. K. (1990a). Anoestrum update. **Proc. Ruakura Farmers Conf.** 42: 107-114.

MACMILLAN , K. L., TAUFA, V. K. and DAY, A. M (1990b). Response patterns among anoestrous dairy cows treated with CIDR devices and PMSG as folligon or pregnecol, unpublished.

MALVEN, P. V. (1984). Pathophysiology of the puerperium: Definition of the problem. **Proc. Int. Cong. on Anim. Reprod. and AI.** 3: 1-7.

MARION, G. B., NORWOOD, J. S. and gIER, H. T. (1968). Uterus of the cow after parturition: Factors affecting regression. **Am. J. Vet. Res.** 29: 71-75.

MAWHINNEY, S., ROCHE, J. F. and GOSLING, J. P. (1979). The effects of oestradiol benzoate (OB) and gonadotrophin releasing hormone (GnRH) on reproductive activity in beef cows at different intervals post-partum. **Annls. Biol. Anim. Biochim. Biophys.** 19: 1575-1587.

McNATTY, K. P., HEATH, D. A., HENDERSON, K. M. LUN, S. HEATH, D and MONTGOMERY, G. W. (1983). Seasonal differences in ovarian activity in cows. **J. Endocrinol.** 102: 189-198.

McNATTY, K. P., HEATH, D. A., HENDERSON, K. M., LUN,S., HURST, P.R., ELLIS, L. M., MONTGOMERY, G. W., MORRISON, L. and THURLEY, D. C. (1984). Some aspects of thecal and granulosa cell function during follicular development in the bovine ovary. **J. Reprod. Fertil.** 72: 39-53.

McNATTY, K. P. (1988). Reproductive endocrinology of the postpartum cow. **Proc. Dairy Cattle Soc. of the N.Z. Vet. Assoc.** 5th Seminar 22-24 June, p. 161-174.

MOULE-WALKER, F. M., DAVIS, A. L. and FLEET, I. R. (1983). Endocrine activity of the mamary gland: oestrogen and prostaglandin secretion by the cow and sheep mamary glands during lactogenesis. **Brit. Vet. J.** 136: 171-177.

MOLLER, K. (1970). Uterine involution and ovarian activity after calving. **N. Z. Vet. J.** 18: 140-145.

MONTGOMERY, G. W. (1985). The effects of season on reproduction in beef

cows: A review. **Proc. N. Z. Soc. Animl Prod.** 45: 43-48.

MORROW, D. A., ROBERTS, S. J. and McENTEE, K. (1968). Postpartum ovarian activity and involution of the uterus and cervix in dairy cattle. I. Ovarian activity. **Cornell Vet.** 59: 134-154.

MOSS, G. E., PARFET, J. R., MARVIN, C. R., ALLRICH, R. D. and DIEKMAN, M. A. (1985). Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. **J. Anim. Sci.** 60: 285-293.

MULVEHILL, P. and SREENAN, J. M. (1977). Improvement of fertility in post-partum beef cows by treatment with PMSG and progestagen. **J. Reprod. Fertil.** 50: 323-325.

NETT, T. M. (1987). Function of the hypothalamic-hypophysial axis during the post-partum period in ewes and cows. **J. Reprod. Fertil.** 34: (suppl.): 201-213.

O'FARREL, K. L. (1984). Oestrous behaviour, problems of detection and relevance of cycle lengths. In: **"Dairy Cow Fertility"** eds. R. G. Eddy and M. J. Tucker, Brit. Vet. Assoc. Edit. Services, london 47-59.

PAYNE, E., SMITH, J. F., McWAN, L. T. and COPE, B. C. (1987). Relationships of liver microsomal enzymes and nutritional effects on ovulation in the ewe. **Proc. 4th AAAP Animal Sci. Cong.** Hamilton, N. Z. p. 239.

PETER, A. R. and LAMMING, G. E. (1986). Regulation of ovarian function in the post-partum cow: An endocrine model. **Vet. Rec.** 118: 236-239.

PETER, A. L. and BOSU, W. L. (1988). Influence of intrauterine infections and follicular development on the response to GnRH administration in postpartum dairy cows. **Theriogenology.** 29: 1163-1175.

PIERSON, R. A. and GINTHER, O. J. (1984). Ultrasonography of the bovine ovary. **Theriogenology.** 21: 495-504.

PIERSON, R. A., KASTELIC, J. P. and GINTHER, O. J. (1988). Basic principles and techniques for transrectal ultrasonography in cattle and horses. **Theriogenology.** 29: 3-20.

PIETERSE, M. C., KAPPEN, K. A., KRUIP, Th A. M. and TAVERNE, M. A. M. (1988). Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. **Theriogenology.** 30: 751-762.

PIETERSE, M. C. (1989). Ultrasonic characteristics of physiological structures on bovine ovaries. In: **Diagnostic Ultrasound and Animal Reproduction.** eds. M. M. Taverne and H. Willemse. Kluwer Academic Publishers. 37-51.

QUIRK, S. M., HICKEY, G. J. and FORTUNE, J. E. (1986a). Growth and regression of ovarian follicles during the follicular phase of the oestrous

cycle in heifers undergoing spontaneous and PGF-2∝-induced luteolysis. **J. Reprod. Fertil.** 77: 211-219.

QUIRK, S. M. and FORTUNE, J. E. (1986b). Plasma concentrations of gonadotrophins preovulatory follicular development and luteal function associated with bovine follicular fluid-induced delay of oestrus in heifers. **J. Reprod. Fertil.** 76: 609-621.

RAHE, C. H., HEEGER, J. L. and HARMS, P. G. (1982). Evidence for an inherent rhythm in pulsateli LH release in ovariectomized cows. **Theriogenology.** 18: 573-581.

RASMUSSEN, D. D. and MALVEN, P. V. (1983). Effects of confinement stress on episodic secretion of LH in ovariectomized sheep. **Neuroendocrinology.** 36: 392-396.

RHODES, R. C. and RANDEL, R. D. (1978). Reproductive studies of Brahman cattle. I. Behavioural effect of various dose levels of estradiol-17B upon ovariectomized brahman, Brahman x Hereford cows. **Theriogenology.** 9: 429-434.

ROBERTS, S. J. (1971). **Veterinary Obstetrics and Genital Diseases.** 2nd ed S. J. Roberts, Ithaca.

SAVIO, J. D., KEENAN, L., BOLAND, M. P. and ROCHE, J. F. (1988). Patterns, of growth of dominant follicles during the oestrous cycle of heifers. **J. Reprod. Fertil.** 83: 663-441.

SAVIO, J. D., BOLAND, M. P., HYNES, N. and ROCHE, J. F. (1990a). Resumption of follicular activity in the early post-partum period of dairy cows. **J. Reprod. Fertil.** 88: 569-579.

SAVIO, J. D., BOLAND, M. P. and ROCHE, J. F. (1990b). Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. **J. Reprod. Fertil.** 88: 581-591.

SCHALLENBERGER, E., SCHAMS, D. and ZOTTMEIER, K. (1978). Response of lutropin (LH) and follitropin (FSH) to the administration of gonadoliberin (GnRH) in pregnant and post-partum cattle including experiments with prolactin suppression. **Theriogenology** 10: 35-53.

SCHALLENBERGER. E., WALTERS, D. L., OSCHMANN, S. L. and MEYER, H. D. (1984). Endocrine changes during the early postpartum period in dairy cattle. **Proc. Int. Cong. on Anim. Reprod. and AI.** IV: III-9.

SCHIRAR, A. and MARTINET, J. (1982). Postpartum ovarian activity and its interaction with the uterus in resuming cyclic activity postpartum. **Factors Influencing Fertility in the Postpartum Cow. eds.** H. Karg and Schallenberger, Martinus Nijhoff Publishers, The Netherlands. 67-94.

SHORT, R. E., STAIGMILLER, R. B. and BELLOWS, R. A. (1988). Hormonal

treatment to induce ovulation. **Proc. Int. Cong. on Anim. Reprod. and AI.** 1: 147-154.

SHORT, R. E., BELLOWS, R. A., STAIGMILLER, R. B., BERARDINELLI, J. G. and CUSTER, E. E. (1990). Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. **J. Anim. Sci.** 68: 799-816.

SIROIS, J. and FORTUNE, J. E. (1988). Ovarian follicular dynamic during the oestrous cycle in heifers monitored by real time ultrasonography. **Biology of Reproduction.** 39: 308-317.

SLOSS, V. and DUFFY, J. H. (1980). Uterine involution in cattle. Handbook of Bovine Obstetrics. **Williams and Wilkins,** Baltimore, p. 208.

SMITH, M. S. (1980). Effects of suckling stimuli on Prl and LH secretion in some species. **Federation Proc.** 39: 2571-2576.

SMITH. J. F., PAYNE, E., TERVIT, H. R., Mc GOWAN, L. T., FAIRCLOUGH, R., KILGOUR, R. and GOOLD, P. G. (1981). The effect of suckling upon the endocrine changes associated with anoestrous in identical twin dairy cows. **J. Reprod. Fertil.** 30: (suppl.): 241.

SPICER, L. J. and ECHTERNKAMP, S. E. (1986). Ovarian follicular growth, function and turnover in cattle: a review. **J. Anim. Sci.** 62: 428-451.

THAYER, K. M., FOREST, D. M. WELSH, T. H. Jr. (1985). Real-time ultrasound evaluation of follicular development in superovulated cows. **Theriogenology.** 23: 233.

TSAI-MORRIS, C. H., GHOSH, M., HIRSHFIELD, A. N., WISE, P. M. and BRODIE, A. M. J. (1983). Inhibition of ovarian aromatase by prolactin in vitro. **Biol. Reprod.** 29: 342-346.

TUCKER, H. A. (1971). Hormonal response to milking. **J. Anim. Sci. 32 (suppl.): 137-141.**

TUCKER, H. A. (1982). Seasonality in cattle. **Theriogenology.** 17: 53-59.

TUNGSUBUTRA, V. and FRANCE, J. T. (1978). Serial changes in plasma levels of progesterone, unconjugated oestradiol and unconjugated oestriol in normal pregnancy. **Aust. N. Z. Jl. Obstet. Gynaec.** 18: 97-103.

WAGNER, W. C. and HANSEL, W. (1969). Reproductive physiology of postpartum cow. I. Clinical and histological findings. **J. Reprod. Fertil.** 18: 493-500.

WEBB, R., LAMMING, G. E., HAYNES, N. B. and FOXCROFT, G. R. (1980). Plasma progesterone and gonadotropin concentrations and ovarian activity in postpartum dairy cows. **J. Reprod. Fertil.** 59: 133-143.

WEESNER, G. D., NORRIS, T. A. FORREST, D. W. and HARMS, P. G. (1987).

Biological activity of luteinizing hormone in the peripartum cow: least activity at parturition with an increased throughout the postpartum interval. **Biol. Reprod.** 37: 851-858.

WILLIAMS, G. L. and RAY, D. E. (1980). Hormonal and reproductive profiles of early postpartum beef heifers after prolactin suppresion or steroid-induced luteal function. **J. Anim. Sci.** 50: 906-917.

WILLIAMS, G. L. (1990). Suckling as a regulator of postpartum rebreeding in cattle: A review. **J. Anim. Sci.** 68: 831-852.

WRIGHT, I. A., RHIND, S. M., RUSSEL, A. J. F., WHYTE, T. K., McBEAN, A. J. and McMILLEN, S. R. (1987). Effects of body condition, food intake and temporary calf separation on the duration of the post-partum anoestrous period and associated LH, FSH and prolactin concentrations in beef cows. **Anim. Prod.** 45: 395-402.

ZEMJANIS, R. (1980). Anestrus in cattle. In: **Current Therapy in Theriogenology**. ed. D. A. Morrow. W. B. Saunders, Philadelphia.

APPENDIX

I. Operation Manual for Aloka Echo Camera Model SSD-210DXII

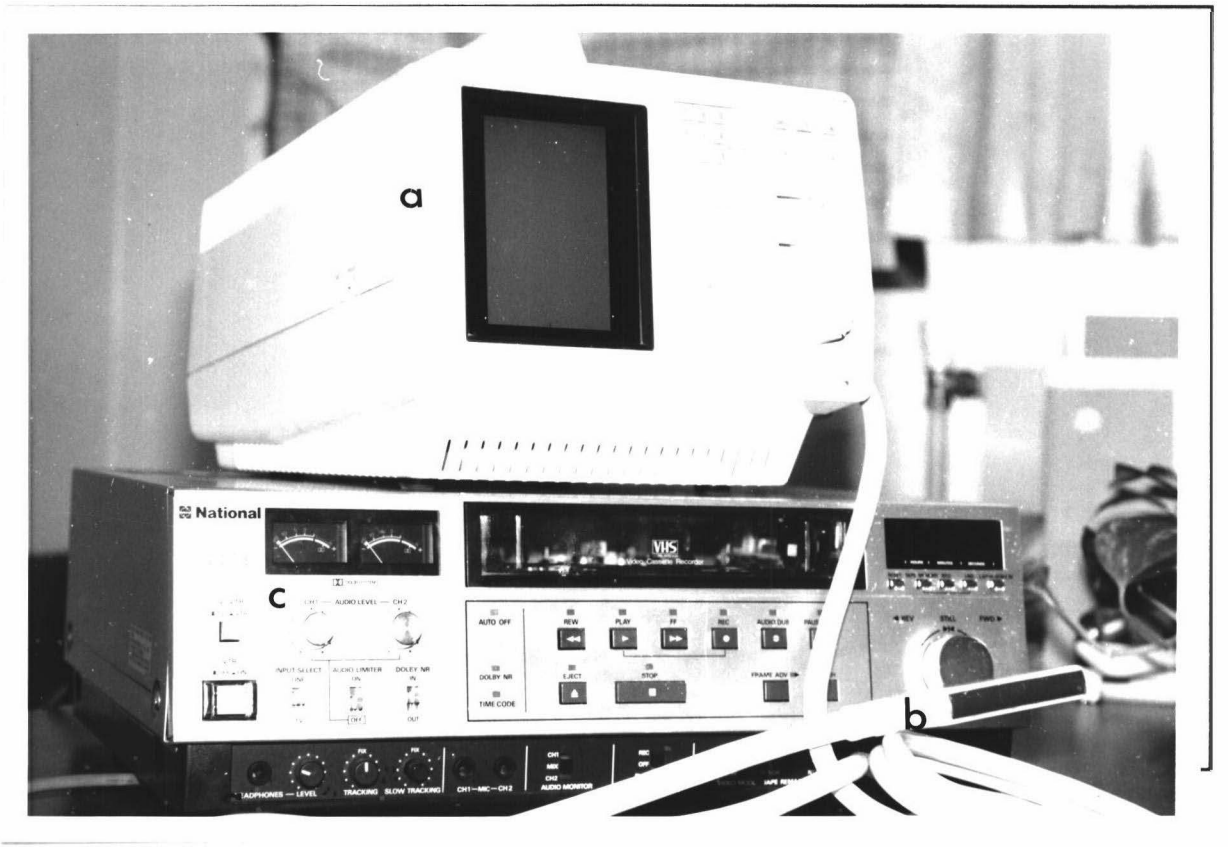


Figure 6.1 A transrectal real time linear array ultrasound scanner (a), equipped with a 5.0 MHz rectal probe (b), and a video recorder (c).

CONTENTS

CONTENTS (continued)

1. INTRODUCTION

SSD-210DXII is designed to be compact, lightweight and portable. Its compactness and portability facilitate carrying it to patient's bedside, operating rooms and for out-of-hospital visits.

Its high performance will be particularly efficient for abdominal diagnosis and obstetrical/gynecological examinations. As SSD-210DXII employs a standard TV-scan video system which allows the image to be recorded by a VTR.

Also SSD-210DXII is equipped with useful functions such as distance measurement system, patient's ID code display and image freezing system.

Thus SSD-210DXII will expand the field of activity and enable easy access to ultrasound data.

2. CAUTIONS

Operate the equipment only after becoming sufficiently familiar with its handling and operation.

2.1 SSD-210DXII is a medical equipment and is to be operated by medical field personnel only.

2.2 Keep the equipment away from:

(1) Water splashing
(2) High humidity
(3) Bad ventilation
(4) Direct sunlight
(5) Dust
(6) Salty or Sulphurate air
(7) Chemical drugs or gas
(8) Strong vibration or shock (also during transportation)

2.3 Before operating, check that:

(1) The capacity, frequency and voltage of the power source are suited to the equipment.

(2) The equipment is normal and operates safely.

(3) Grounding is complete.

Also, note that:

(4) Noise on image may occur if the equipment is operated in a location near a generator room, X-ray equipment, broadcasting stations or underneath power transmission lines.

(5) Operating with other equipment, may cause abnormal image.

2.4 During operation

(1) Continuously monitor the equipment and the patient. If the equipment malfunctions, immediately turn it off and remove its power cord from the wall receptacle so that the patient is protected.

(2) Do not allow patient to touch the equipment or other electrical instruments.

(3) Do not seal the ventilation holes of the equipment.

2.5 Using probe

(1) A probe is fragile. Do not drop, vibrate, or bump the probe or the system.

(2) The probe can be connected with or disconnected from the equipment only while the system power is off.
Confirm that connector is firmly connected.

(3) Do not dip the probe in any liquid.

(4) Do not heat the probe.

(5) Do not strongly bend or pull the probe cable to avoid damage to the cable.

(6) Use ultrasound gel for coupling agent. Other materials such as oil may damage the probe and/or probe cable.

(7) Make the probe clean by wiping out ultrasound gel on the probe after each usage. Use neutral cleanser or alcohol for cleaning.

2.6 After operation

(1) Turn off the power switch.

(2) The power cord must be disconnected by holding the plug.

(3) Clean the equipment and the probe.

2.7 Moving the equipment

(1) Disconnect the power cord and wind it securely around the power cord winding posts on the rear panel of the equipment.

(2) Wear the wrist strap of the probe to avoid dropping the probe.

(3) Do not drop, vibrate, or bump the probe or the system. Hand carry the system while traveling by aircraft.

2.8 In case of improper conditions or equipment malfunctions, turn the system power off immediately and remove the power cord from the wall receptacle. Always contact the service personnel.

2.9 Periodically check the equipment for malfunctions.

2.10 Do not disassemble the equipment or the probe.

3. SPECIFICATIONS

Scanning method:	Electronic linear scanning
Display mode:	Real-time or frozen B-mode (Dual-frame imaging is possible)
Standard probe: (UST-5020)	Scanning width: 107 mm, Frequency: 3.5 MHz
Diagnostic depth:	Maximum 279 mm (Varies with tissue attenuation)
Display range:	186 mm (normal), 279 mm (long)
Frame rate:	At long 10 frames/sec 20 frames/sec (frame rate ; Up) At normal 15 frames/sec 30 frames/sec (frame rate ; Up)
Acoustic scanning lines: (per frame)	339 lines (at normal) 226 lines (at long)
Focusing method:	Transmission/reception electronic focusing and acoustic lens
Dynamic range:	50 dB (fixed)
Gain:	0 to 100 dB, continuously variable
STC:	NEAR GAIN: -80 to -10 dB FAR GAIN: 0 to 5 dB
Measuring function	Push key controlled, Two-channel distance measurement
Character display:	Numerics and symbols (M.F.) for ID code display with maximum of 16 charactors
Gray shades:	16 gray shades
Display monitor:	5.5-inch diagonal
Video output:	TV-scan method*, 525 lines/30 frames or 625 lines/25 frames

Power requirement:	100/120/220/240 V 50/60 Hz, approx. 60 VA		
Dimensions:	Approx. 25 (W) x 20 (H) x 36.5 (D) cm		
Weights:	Approx. 8 Kg (including standard probe)		
Environmental conditions in storage:	a.	Temperature:	-10°C to +60°C
	b.	Relative humidity:	30% to 95%
Environmental conditions in operation:	a.	Temperature:	+10°C to +40°C
	b.	Relative humidity:	30% to 85%

* Other monitor than the specified models by Aloka may be adjusted or modified to connect with this equipment.

** The specifications may be subject to change without notice for improvement.

4. SYSTEM CONFIGURATION

4.1 Standard components

		Quantity
Main unit USI-102		1
Probe UST-5020		1
Accessories:		1 set
Grounding wire NCC-0850	1	
Ultrasound gel	1	
Spare fuse	2	
Operation manual	1	
Guide book for Examination	2	

4.2 Options

(1) Linear Probes

a.	UST-5021	For general(wide view-field probe)
b.	UST-589-5	For pediatrics and shallow part
c.	UST-5023P	For puncture needle
d.	UST-558-5	For small part
e.	UST-5810I-5	For intraoperate(I-shaped type)
f.	UST-5810T-5	For intraoperate(T-shaped type)
g.	UST-5511TU-7.5	For intraoperate(T-shaped type)
h.	UST-5511I-7.5	For intraoperate(I-shaped type)
i.	UST-658-5	For prostate gland(transrectum)
j.	UST-5813-5	For horses and cows(transrectum)*
k.	UST-5034	For horses (transrectum)*

* Not for human

(3) Puncture adaptor

a.	MP-2389	For standard probe UST-5020

(4) Photographing equipment

a.	ACR-7510	Pistol type polaroid camera
b.	ACR-5010	Photographing hood for 35mm SLR camera ** Camera body and TXP mount are separately prepared

(5) External monitor

a.	IP-0902-TV	9-inch

(6) Mobile cart

a.	RMT-210	
b.	RMT-210S	Tall mobile cart with 9-inch monitor

(7) Video tape recorder

Any Video tape recorder can be connected.
*** Some low quality VTRs provide poor reconstructed images.

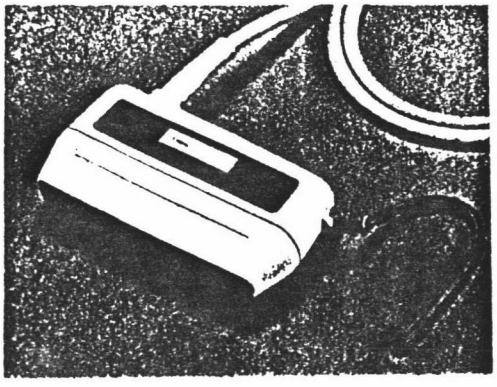
(8) Remote freeze switch

a.	MP-2345	Footswitch for freezing
b.	Hand-grip remote switch for freezing MP-2397	

4.3 Description of optional probes

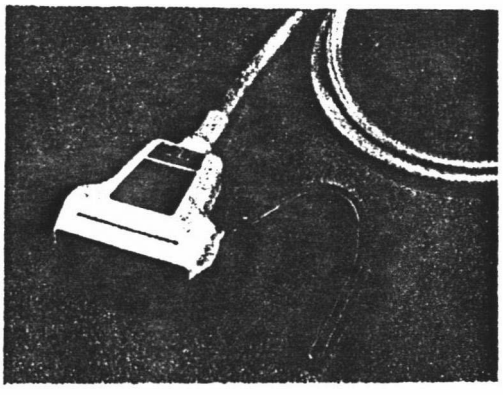
Many kinds of optional probes are provided, which widen the fields of examination, and a most suitable probe can be selected for the best imaging. The probes can be easily connected or disconnected. As the scanning width varies according to the probe, diagnostic range also differs depending on the probe connected.

(1) Wide view-field probe UST-5021



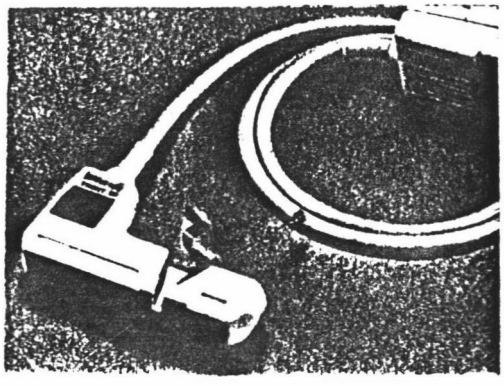
a.	Frequency:	3.5 MHz
b.	Scanning width:	125 mm
c.	Display distance:	217 mm (normal) 283 mm (long)
d.	Application:	Abdomen, OB/GY
e.	Cable length:	Approx. 1.4 m
f.	Weight:	Approx. 430 g (excluding cable and connector)

(2) Pediatric probe UST-589-5



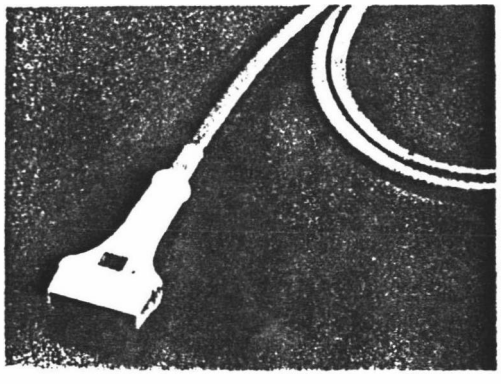
a.	Frequency:	5 MHz
b.	Scanning width:	56 mm
c.	Display distance:	98 mm (normal) 147 mm (long)
d.	Application:	Pediatrics Shallow part
e.	Cable length:	Approx. 1.4 m
f.	Weight:	Approx. 170 g (excluding cable and connector)

(3) Puncture probe UST-5023P



a.	Frequency:	3.5 MHz
b.	Scanning width:	107 mm
c.	Display distance:	186 mm (normal) 279 mm (long)
d.	Application:	Puncture needle
e.	Cable length:	Approx. 1.4 m
f.	Weight:	Approx. 450 g (excluding cable and connector)

(4) Carotid artery probe UST-558-5

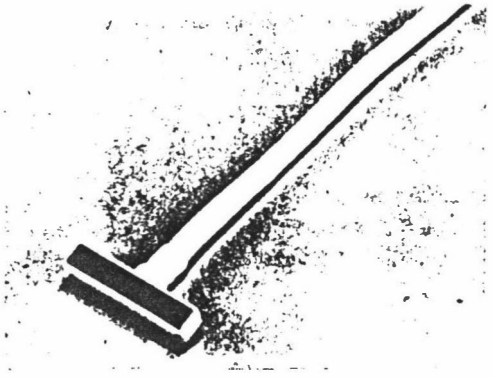


a.	Frequency:	5 MHz
b.	Scanning width:	34 mm
c.	Display distance:	59 mm (normal) 88 mm (long)
d.	Application:	Carotid artery Small part
e.	Cable length:	Approx. 1.4 m
f.	Weight:	Approx. 80 g (excluding cable and connector)

(5) Intraoperate probe UST-5810I-5
(I-shaped type)

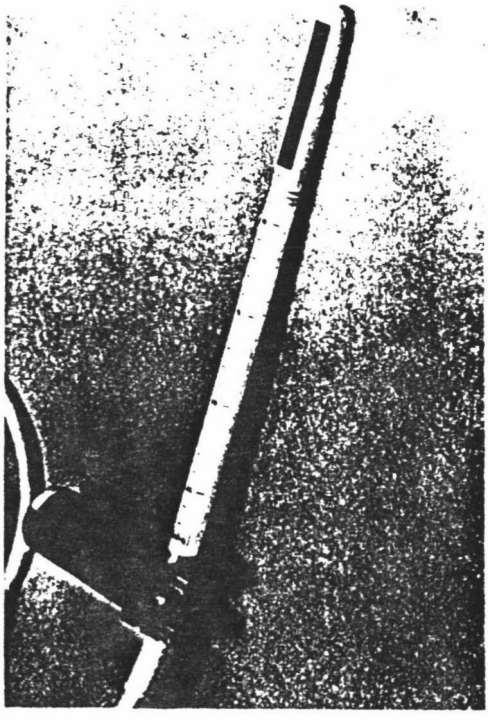
a.	Frequency:	5 MHz
b.	Scanning width:	56 mm
c.	Display distance:	98 mm (normal) 147 mm (long)
d.	Application:	Intraoperative
e.	Cable length:	Approx. 2 m
f.	Weight:	Approx. 70 g (excluding cable and connector)

(6) Intraoperate probe UST-5810T-5
(T-shaped type)



a.	Frequency:	5 MHz
b.	Scanning width:	56 mm
c.	Display distance:	98 mm (normal) 147 mm (long)
d.	Application:	Intraoperation
e.	Cable length:	Approx. 2 m
f.	Weight:	Approx. 70 g (excluding cable and connector)

(7) Transrectum probe UST-658-5



a.	Frequency:	5 MHz
b.	Scanning width:	56 mm
c.	Display distance:	98 mm (normal) 147 mm (long)
d.	Application:	Prostate gland
e.	Cable length:	Approx. 1.4 m
f.	Weight:	Approx. 200 g (excluding cable and connector)

(8) Intraoperate probe UST-5511TU-7.5
(T-shaped type)

a. Frequency: 7.5 MHz

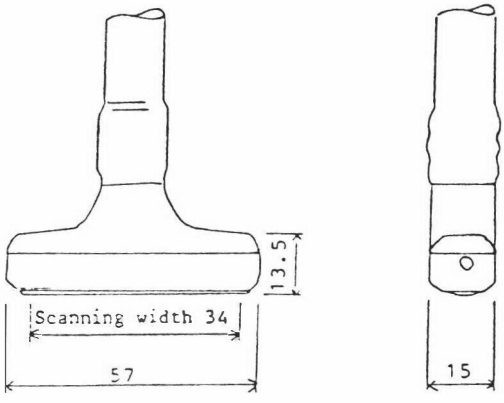
b. Scanning width: 34 mm

c. Display distance: 59 mm (normal)
88 mm (long)

d. Application: intraoperation

e. Cable length: Approx. 2 m

f. Weight: Approx. 20 g
(excluding cable
and connector)



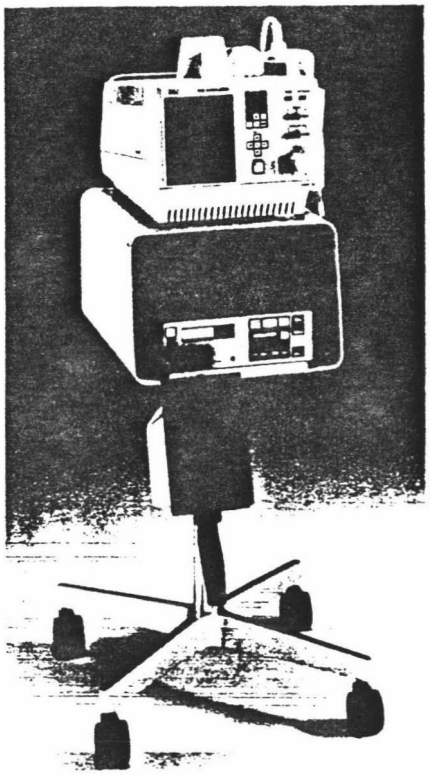
(Unit: mm)

4.4 Description of mobile cart

(1) RMT-210

RMT-210 is the mobile cart for using SSD-210DXII with the operator sitting. RMT-210 can mount pistol type polaroid camera ACR-7510 or photographing hood for 35 mm SLR camera ACR-5010 together with Video Tape Reocorder.*

* The size of the VTR which can be mounted on the RMT-210 is smaller than 28 cm (W) X 30 cm (D) X 18 cm (H).

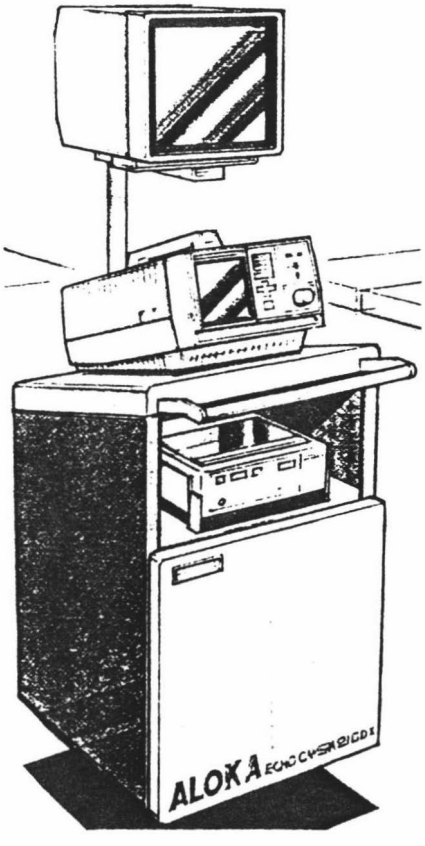


(2) RMT-210S

RMT-210S is tall mobile cart with 9-inch monitor for using SSD-210DXII with the operator standing.
Also RMT-210S is suitable for intraoperational use.
RMT-210S can mount polaroid photographing unit SSZ-108-P or photographing unit for 35 mm SLR camera SSZ-108-35 and Video Tape Recorder.**

** The size of VTR which can be mounted on the RMT-210S is smaller than 35 cm (W) X 35 cm (D) X 16 cm (H).

** RMT-210S requires to be connected to AC power outlet for 9-inch monitor.



5. DESCRIPTION OF EACH PORTION

5.1 Name of each portion

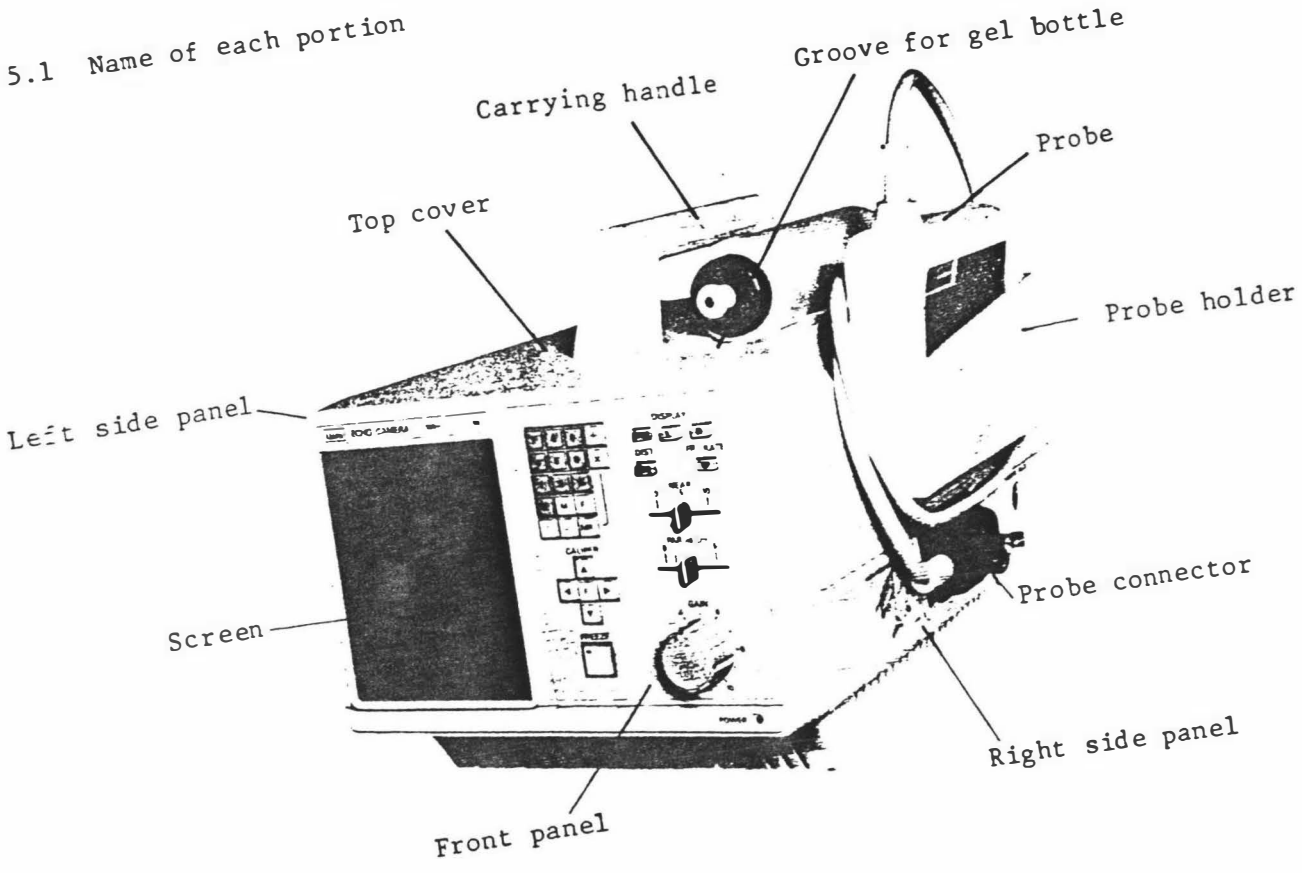


Fig. 5-1

5.2 Front panel (Open page 5-6 for the picture of the panel.)

(1) Ten-key (0 - 9)

Displays numerals on the monitor screen.

(2) Symbol key (M, F)

Displays M (for male) and F (for female) on the monitor screen.

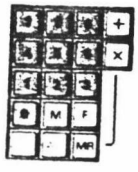


Fig. 5-2

(3) Clear key

C: Erases all the characters in the ID area.

AC: Erases all the characters in the ID area and the date area.

(4) Caliper mark selector

MR: Changes caliper mark which moves by the CALIPER control. MR means "Mark Reference".

+: Displays + caliper mark for distance measurement.

x: Displays x caliper mark for distance measurement.

(5) CALIPER control

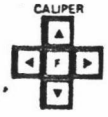


Fig. 5-3

Moves the caliper mark in the direction of the arrow.

When adjacent two arrow keys are pressed together, the caliper mark moves at an angle between the two arrows (diagonally).

F: When the F key and one or two arrow keys are pressed together, the caliper mark moves faster.

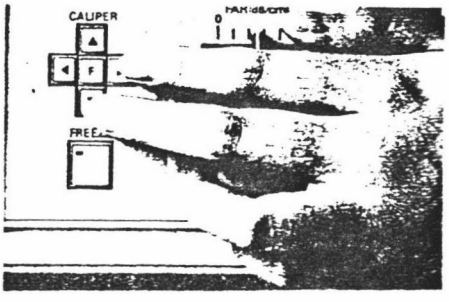


Fig. 5-4

(6) FREEZE switch

Fig. 5-5

Freezes the displayed image. Pressing this switch on freezes the image on the monitor screen instantly.

(7) Image format selector

Fig. 5-6

Selects single- or dual-frame display.

SINGLE: Displays single frame image.

L: Displays real-time image in the left frame in dual-frame display.

R: Displays real-time image in the right frame in dual-frame display.

(8) DIST (Distance) switch

To change the diagnostic distance.
Pressing this switch to ON (▭)
make the diagnostic distance long.

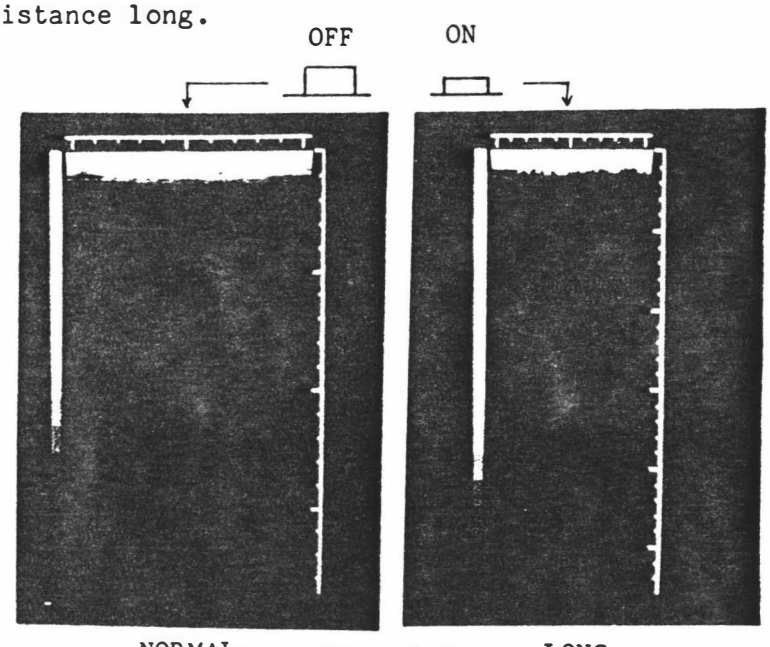


Fig. 5-7

Table. 5-1 Diagnostic range

(unit in mm)

Probe	Scanning Width	Diagnostic Range	
		Normal	Long
UST-558-5	34	59	88
UST-589-5 UST-658-5 UST-5010I/T-5 UST-5511T/TU/I-7.5	56	98	147
UST-5020 UST-5023P	107	186	279
UST-5021	125	217	283

* This function does not work in the FREEZE mode.

** UST-5020 is a standard component.
Other probes are options.

(9) FR RATE (Frame Rate) switch

To select frame rate.
Pressing this switch on increases the frame rate.

Table 5-2 Frame Rate

Probe	DIST Switch	FR RATE Switch	
		OFF	ON
UST-558-5	OFF	15	30
	ON	15	30
UST-589-5 UST-658-5 UST-5010I/T-5 UST-5511I/T/ TU-7.5	OFF	15	30
	ON	15	30
UST-5020 UST-5023P	OFF	15	30
	ON	10	20
UST-5021	OFF	12	24
	ON	10	20

(frames/second)

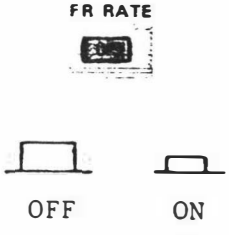


Fig. 5-8

(10) NEAR gain control

To compensate for sensitivity in the area near to the body surface.
Sliding the knob to the right increases sensitivity in the area near to the skin line and the displayed echos will become bright.

Fig. 5-9

(11) FAR gain control

To compensate for sensitivity in the area far from the body surface. Sliding this knob to the right increases sensitivity in the deeper regions and they will be displayed more brightly.

(12) GAIN knob

Increases or decreases system sensitivity.
Turning this knob clockwise displays whole echos more brightly.

(13) Pilot lamp

Lights up in green when power is turned on.

(14) Monitor screen

Displays a cross-sectional image.

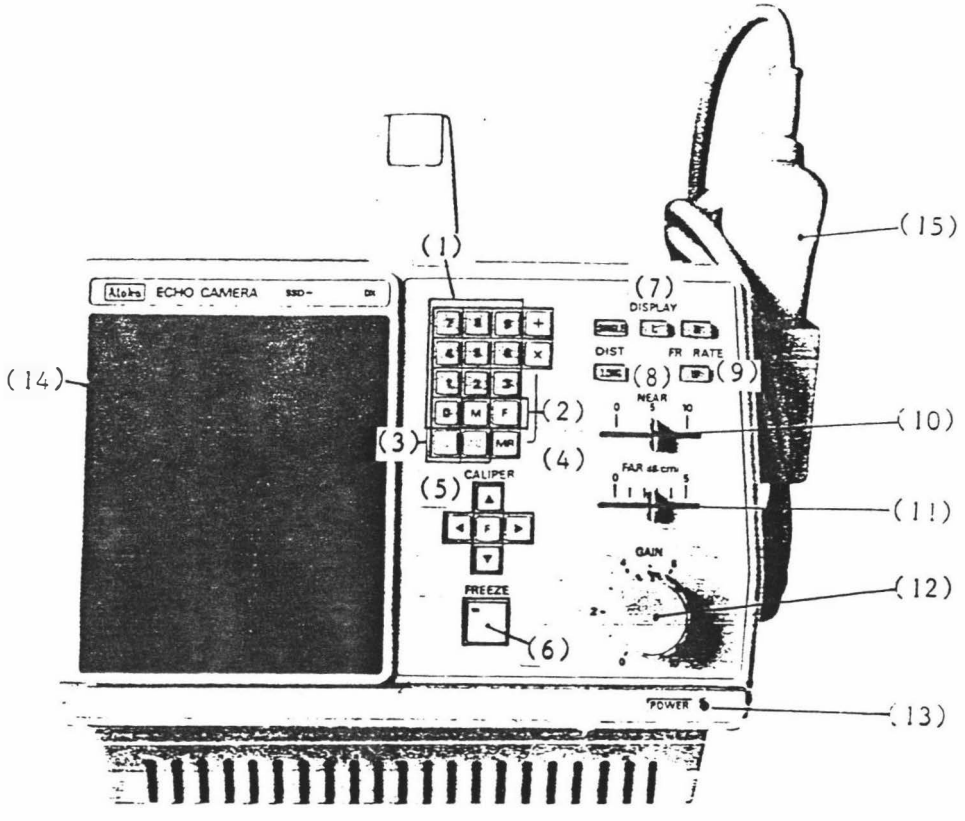

(1)
(2)
(3)
(4)
(5)
(6)
(7)
DISPLAY
(8)
(9)
(10)
(11)
(12)
(13)
(14)
(15)
ECHO CAMERA
DIST
FR RATE
NEAR
FAR
CALIPER
GAIN
FREEZE
POWER

Fig. 5-10

5.3 Left side panel

(1) CONT control

To adjust the contrast of images on the monitor screen.

Raising the contrast provides an image with sharply defined light and shades, but weak echos tend to be lost.

Lowering the contrast provides a hazy image but enables the display of all echos ranging from weak to strong.

(2) BRIGHT control

To adjust brightness of images on the monitor screen.

(NOTE) The CONT and BRIGHT controls have been adjusted at the factory.

Adjustment is not required in general use.

A small screwdriver is required for the adjustment.

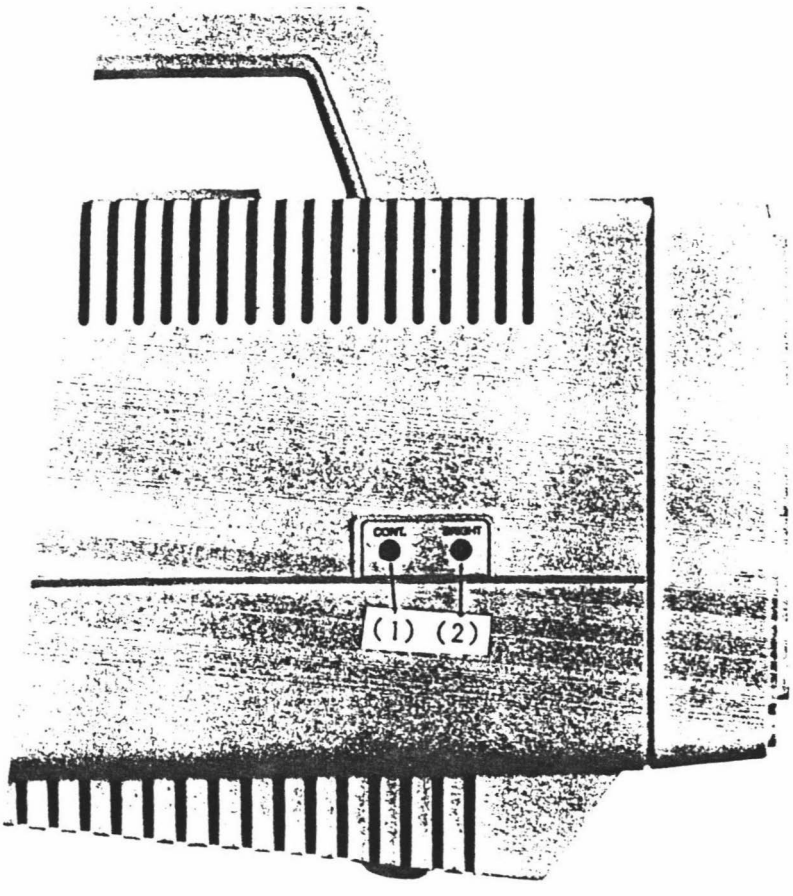


Fig. 5-11

5.4 Top panel

(1) POWER switch

To actuate the power of the equipment. Leave it off when the equipment is not in use.

(2) Groove for gel bottle.

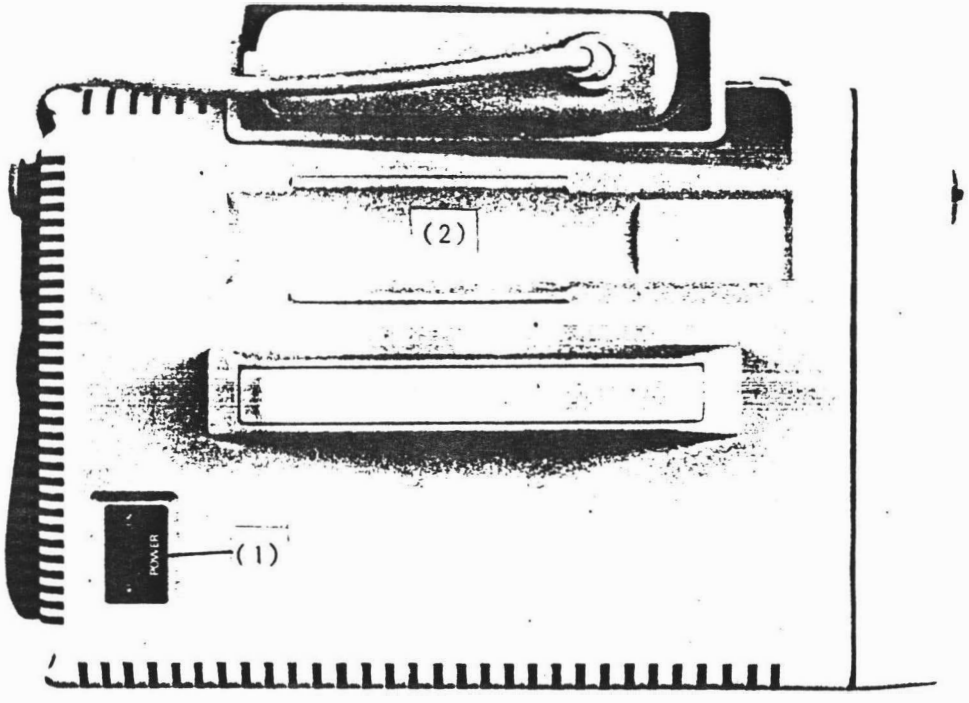


Fig. 5-12 TOP VIEW

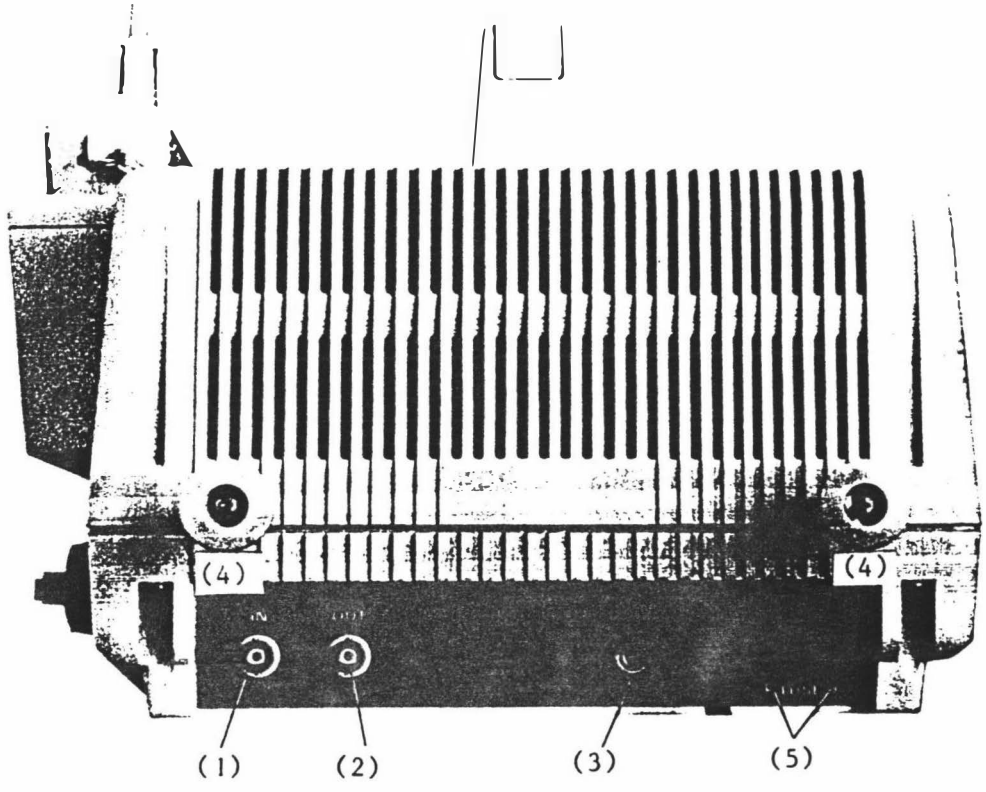


Fig. 5-13 REAR VIEW

5.5 Rear panel

(1) Video input connector

To receive the play back video signal from a VTR.

Playback image will automatically be displayed on the screen when the connected VTR is in the playback mode.

(2) Video output connector

To deliver the video signal to a video tape recorder or external monitor.

(3) GROUND connector

To ground the equipment chassis. When two-line power cord is used, or grounded wall outlet is not available, ground the terminal by the grounding wire.

(4) Power cord winding post

Wind the power cord around the posts prior to carrying the equipment.

(5) Fuse holder

Power line fuses are contained.
Note: Fuses are to be replaced by service personnels only.

5.6 Right side panel

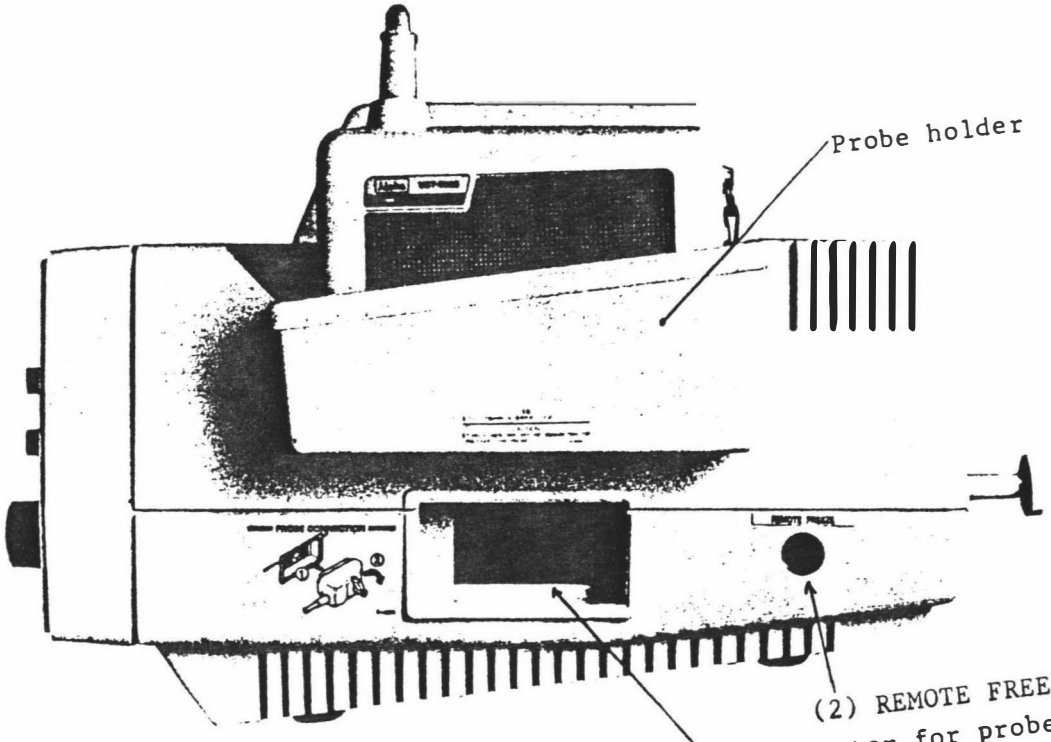


Fig. 5-14

(1) Connector for probe

Refer to 6.1 Probe connection.

(2) REMOTE FREEZE switch

Connector for remote freeze switch (option).

5.7 Probe

The probe is to contact the patient's skin for transmitting and receiving of ultrasonic beams.
The probe is fragile.
Do not drop, vibrate, or bump the probe.

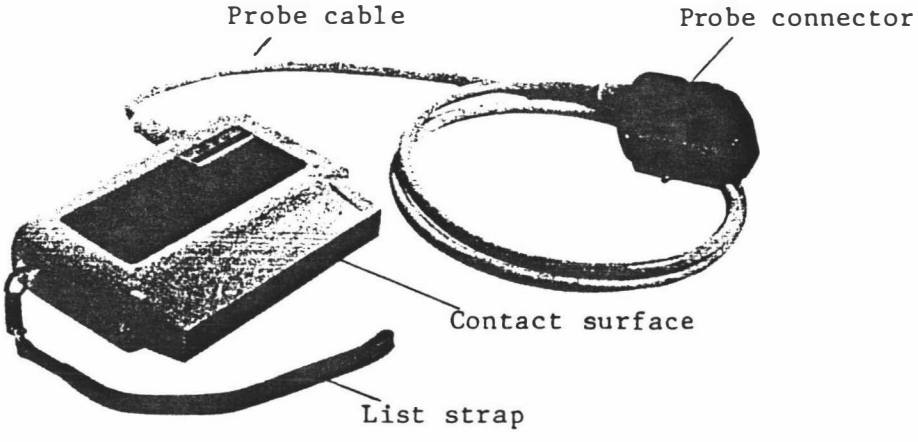


Fig. 5-15

CAUTION:	Turn the system power off before connecting or disconnecting the probe.

6. OPERATING PROCEDURE

(WARNING) Ensure that the system is correctly and securely grounded before operating.

6.1 Probe connection

Handle the probe with care. Do not drop, vibrate, or bump the probe.

Turn the system power off before connecting or disconnecting the probe.

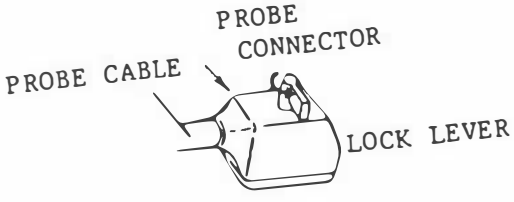


Fig. 6-1

(1) Turn the lock lever of probe connector counterclockwise.

(2) Plug the connector into the receptacle completely.

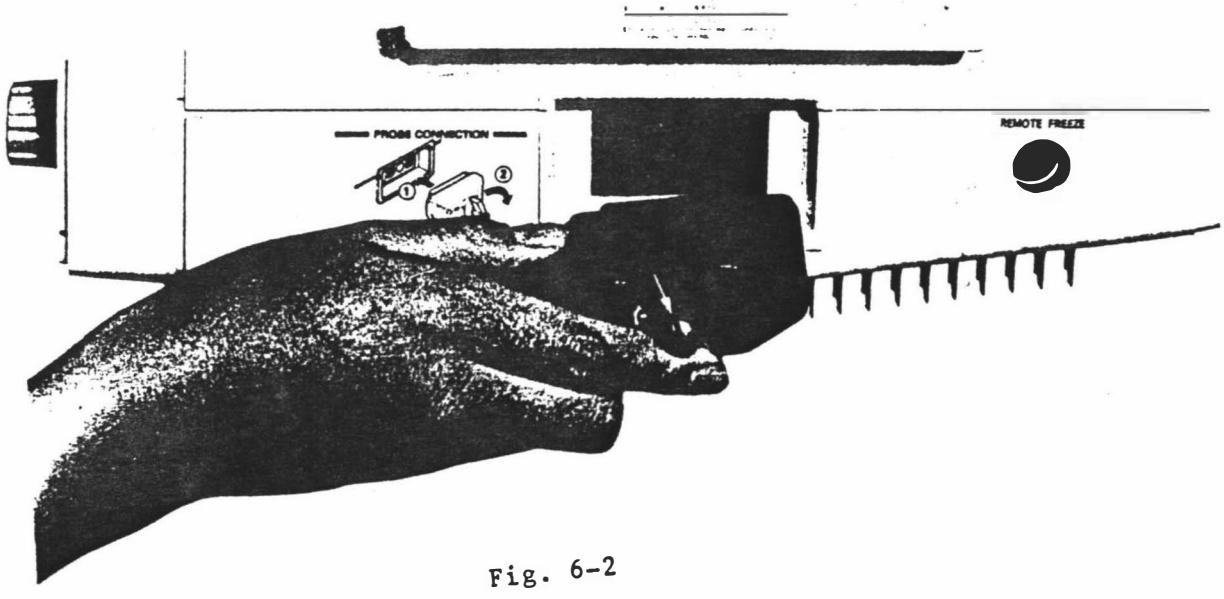


Fig. 6-2

(3) Turn the lock lever clockwise by hand as far as it will go.

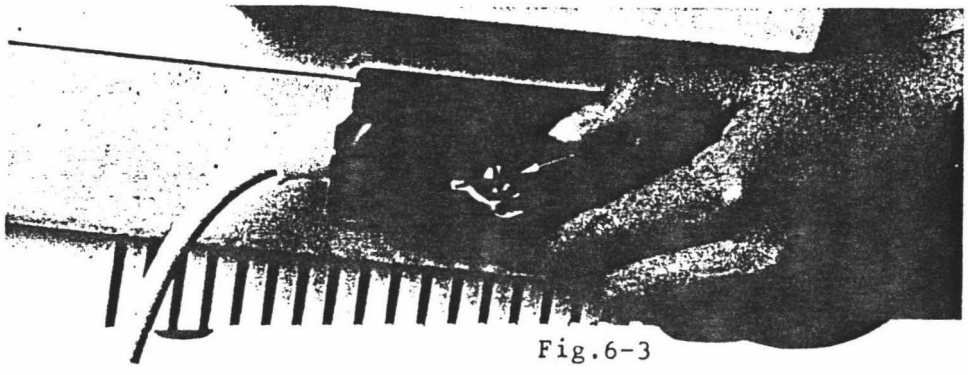


Fig.6-3

(4) Confirm that probe connector does not come off.

6.2 Power-on procedure

(1) Ensure that the system power switch is turned off.

(2) Connect the system power cord to a suitable wall receptacle.

(3) Turn the system power switch on. The power lamp will illuminate (green).

(4) After about 30 seconds delay, the monitor will display an image.

6.3 Initial setting

(1) Remove the protective cover from the contact surface of the probe, at the initial use.

(2) Set individual knobs on the front panel as follows:

NEAR	5
FAR (dB/cm)	2.5
GAIN	5 to 6
FR RATE	OFF
DIST	OFF
DISPLAY	SINGLE: ON

Fig. 6-4

6.4 Standard operation

(1) Apply ultrasound gel on the examination area of the patient. Place the probe on the examination area.
A cross-sectional image will be displayed on the monitor screen.

The positional relationship between the front mark on the probe and the monitor image is as shown in Fig. 6-5.

(2) Observe the image on monitor and adjust the NEAR, FAR, and GAIN controls to obtain an optimum cross-sectional image.

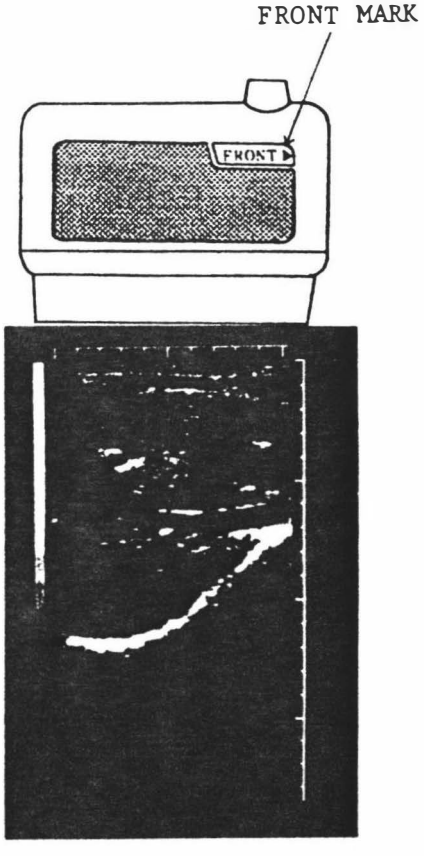


Fig. 6-5

(3) To get clear image of an interested region, try to change patient's posture and condition of breath.

(4) When a desired image is obtained, hold the probe in position and press the FREEZE switch to freeze the image.

NOTE: If the freeze switch is pressed while moving the probe, a clear image may not be obtained.

(5) Pressing DIST swich, far field of body can be observed.

To observe moving tissue such as fetal heart, press FR RATE switch. Frame rate will be increased.

For dual-frame imaging and image storage, see next page and onward.

(6) You may now measure the distance of the area of concern or take a picture of the image.
(Refer to chapter 7 or 9, for each operation).

(7) After each usage, wipe off the ultrasound gel and put the probe in the probe holder.

NOTE: The probe becomes warm during operation. This is normal and is not a malfunctions.

6.5 Dual-frame imaging

(1) Press the DIST switch to ON (▭). An long-range image will be displayed.

(2) Press the L or R switch of the DISPLAY selector.

When the L switch is pressed down, left image is real-time and right image is frozen.

When the R switch is pressed down, right image is real-time and left image is frozen.

(3) To restore single-frame mode, press the SINGLE switch.

NOTE: The dual-frame mode functions only when the DIST switch is in ON position (▭).

When the FREEZE switch is ON, both of the images will freeze.

When the FREEZE switch is OFF, an image with ▽ mark on it is real-time and the another one is frozen.

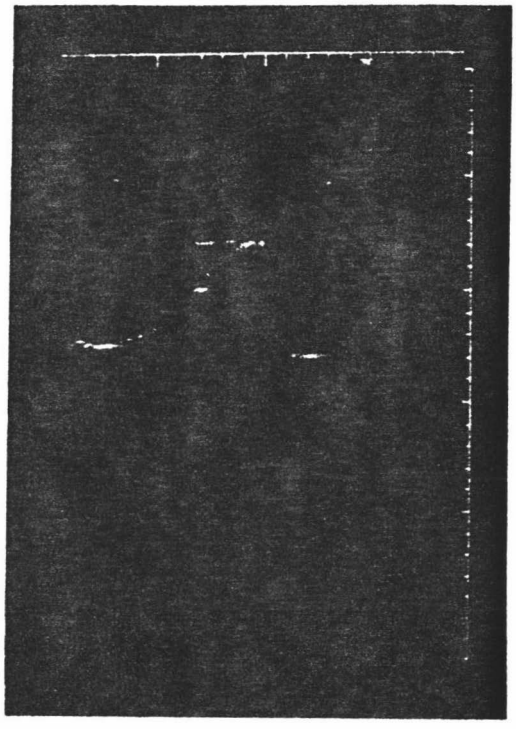


Fig. 6-6

6.6 Image storage

When the DIST switch is in OFF position (⌐¬), images can be stored in different two memories.
The L and R switches select the memory in which the image is stored.

(1) Turn the DIST switch to OFF (⌐¬).

(2) Press the L switch to ON (▭).

(3) Display an image.

(4) Press the FREEZE switch to ON (lit).

(5) Press the R switch to ON (▭).
The image is stored in the "L" memory.

(6) Press the FREEZE switch to OFF (unlit).
A real-time image will be displayed instead of the former image.
Display an image.

(6) Press the FREEZE switch to ON (lit).
The image is stored in the "R" memory.

(7) Pressing the L switch displays the image stored in the "L" memory.

Pressing the R switch displays the image stored in the "R" memory.

NOTE: Do not turn off the FREEZE switch when changing the L and R switch, or the image will be erased from the memory.

(8) To restore a real-time image, turn off the freeze switch.

NOTE: The image-storage function works only when the DIST switch is in OFF position (⌐¬).

7. CALIPER OPERATION

(1) Press the + key of the Caliper Mark Selector.
Then a + mark will appear at the center of the monitor screen and the caliper read-out of CAL+; 00.0 CM, appears at the bottom of the screen.

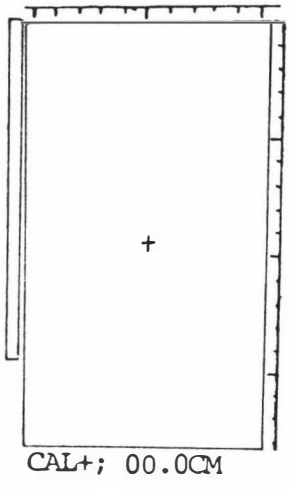


Fig. 7-1

(2) Operate the CALIPER control to position the + mark at the starting point of the measurement.
The + mark will move in the direction of the arrow key pressed.

To move it diagonally, press two arrow keys together.

To move it fast, press the F key and arrow key(s) together.

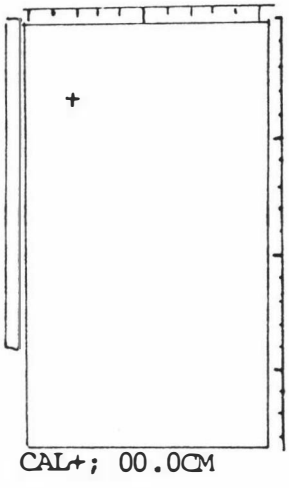


Fig. 7-2

(3) Press the MR key momentarily.

(4) Press an arrow key. A small claiper mark remains at the point and a large caliper mark will move by arrow keys.

The caliper readout starts to indicate the distance between the small and large marks.

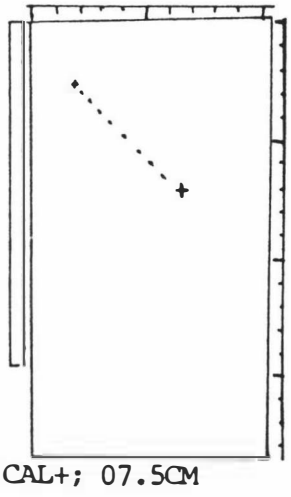


Fig. 7-3

(5) Position the large mark at the point ending the measurement. The distance is displayed at the right of CAL+; in cm at the bottom of the monitor screen.

(6) Touch the MR key once changes the mark which moves by the arrow key from large to small or small to large.

(7) Pressing the x key displays x caliper mark, and the distance measurement is possible in the same manner as the + mark. The distance measured is displayed at the right of CALx;.

(8) To erase the + mark and the result of the measurement, press the + switch once.

To erase the x mark and the reault of the measurement, press the x switch once.

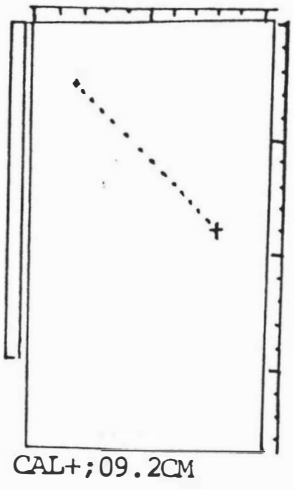


Fig. 7-4

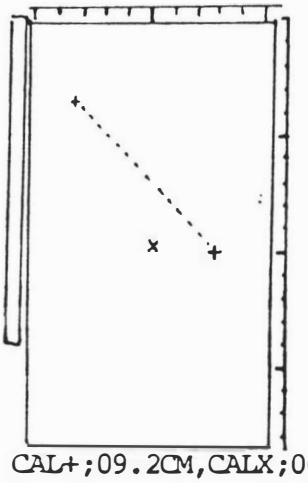


Fig. 7-5

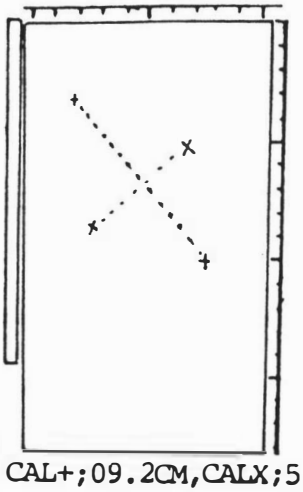


Fig. 7-6

8. ID Code display

8.1 Introduction

SSD-210DXII can display the date of examination, identification number, sex, and age of patient on the bottom of the monitor screen.
Key in the date first.

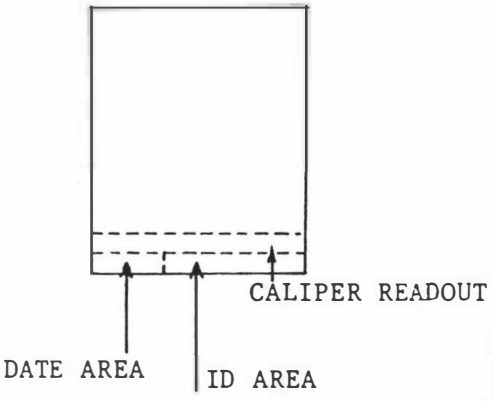


Fig. 8-1

8.2 Date input

(1) When power is turned on, a cursor appears at the lower left of the monitor screen.

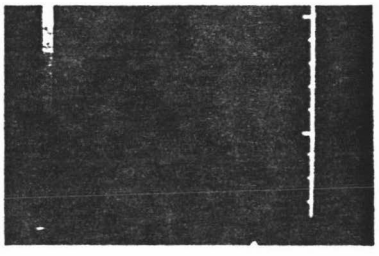


Fig. 8-2

(2) Key in the date by two digits each for day, month, and year from the ten-key.
When day or month is one digit (from 1 to 9), put a zero in front of it for a two-digit indication.
A period is automatically displayed every two digits.
When the date input is completed, a colon is displayed automatically.

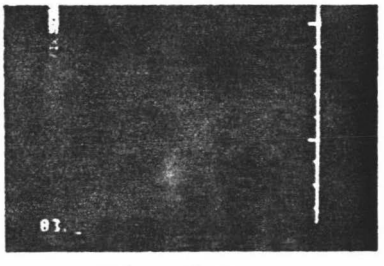


Fig. 8-3

(3) When correcting the date, press the AC key and re-enter the date.

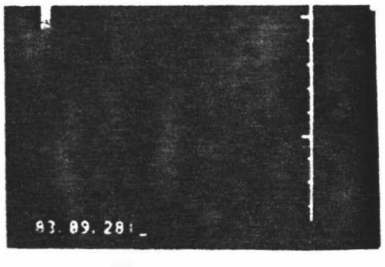


Fig. 8-4

8.3 ID Code input

(1) Key in the patient number, sex, and age from the ten-key (within nine letters).

(2) To change or correct the patient information, press the [C] key and re-enter the ID.

Pressing the [C] key does not erase date indication.

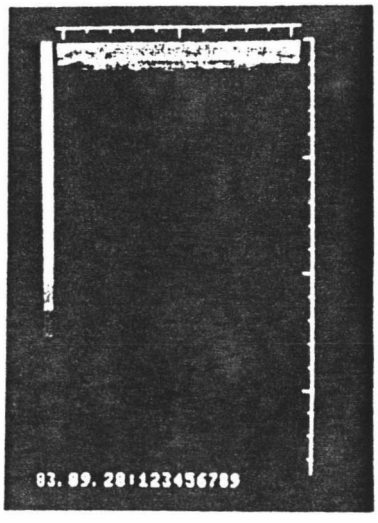


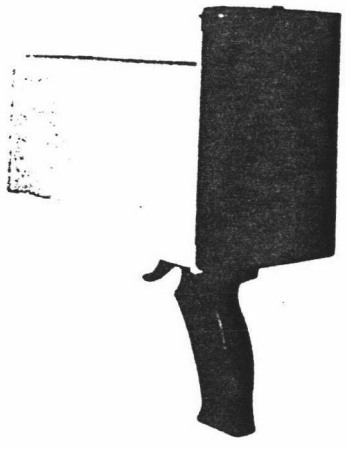
Fig. 8-5

8.4 Correction

(1) The [AC] key clears all the display of date, patient number, sex, and age.

(2) The [C] key clears the patient information only.

9. PISTOL TYPE POLAROID CAMERA (OPTION)



The optional ACR-7510 pistol type Polaroid camera enables the recording of data on Polaroid films.

* Polaroid is the registered trademark of the Polaroid Corporation.

9.1 Photographing conditions

The conditions are adjusted at the factory for type 611 land film. When readjustment is required, follow the procedures below:

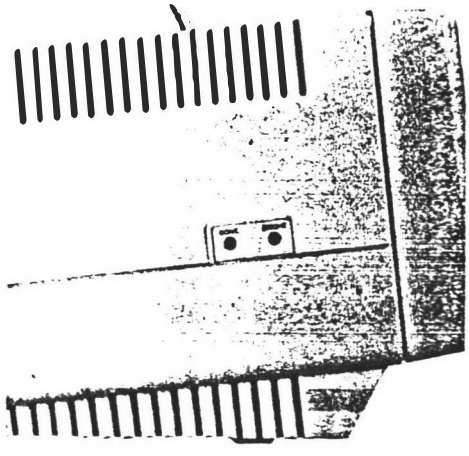


Fig. 9-1

(1) Set the camera stop at F5.6 and shutter speed at 1/8 (for type 611 land film).
When using type 667 or 107 land film, set camera stop at F16 and shutter speed at 1/8.

(2) Set the CONT control, on the left side panel, to its center position.

(3) Adjust the BRIGHT control, on the left side panel, so that the darkest portion of the cross-sectional image or the gray-scale bar can be slightly recorded on the film.

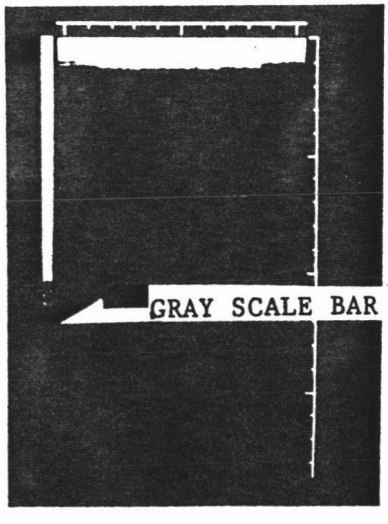


Fig. 9-2

(4) Adjust the CONT control, on the left side panel, so that the gray-scale bar can be recorded on the film with no saturation of intensity.

(NOTE) You may need several trials to obtain the correct setting.
The image contrast and brightness for photography is different from the setting that is best for the eye.

9.2 Film loading

(1) Open the polaroid camera door.

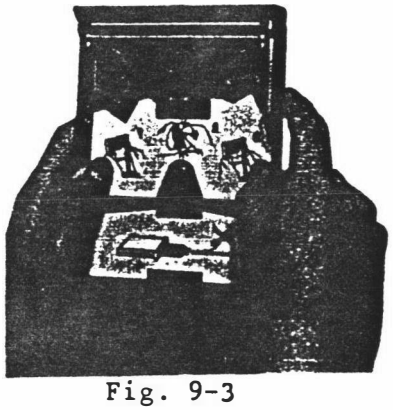


Fig. 9-3

(2) Before loading a film, inspect the developer rollers inside the camera door, to be sure they are clean. Remove all glue or pieces of paper.

(3) Hold the film pack by its edges only. If the center of the pack is pressed, the film can be damaged.

(4) Slide the pack at an angle into the camera back and push it down flat so that it snaps into place. Be sure the white tabs are not caught between the pack and the camera.

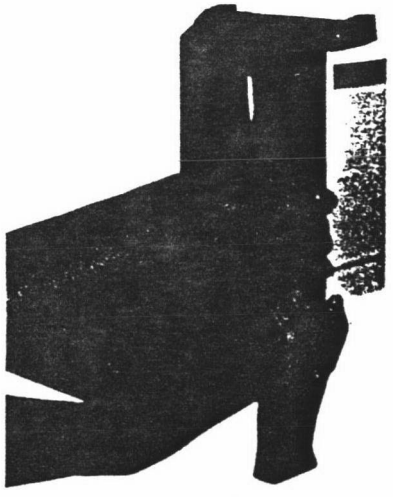


Fig. 9-4

(5) Close and latch the camera door.

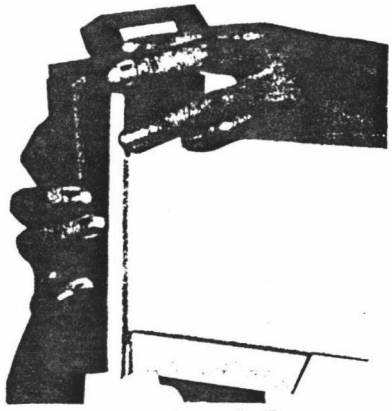


Fig. 9-5

(6) Pull the black safety cover all the way out of the camera.

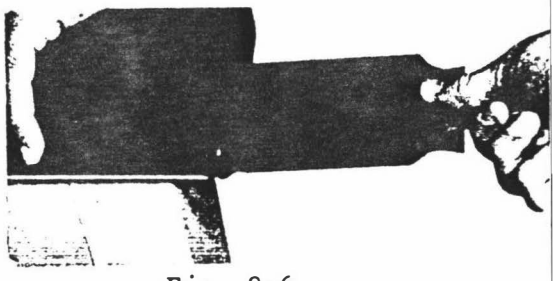


Fig. 9-6

9.3 Make the exposure

(1) Display the cross-sectional image on the monitor screen.

(2) Freeze the cross-sectional imag with the FREEZE switch.

(3) Set the camera hood on the monitor and push the shutter release button on the camera.

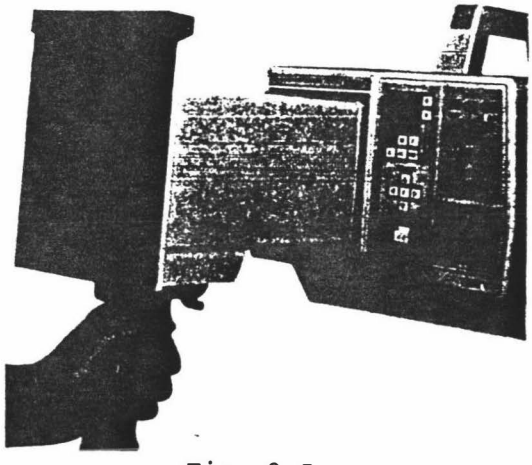


Fig. 9-7

9.4 Process the picture

(1) Grip the center of the white tab and pull it all the way out of the camera. A yellow tab will appear.

(NOTE) If no yellow tab appears, do not pull another white tab (See section 9.7).

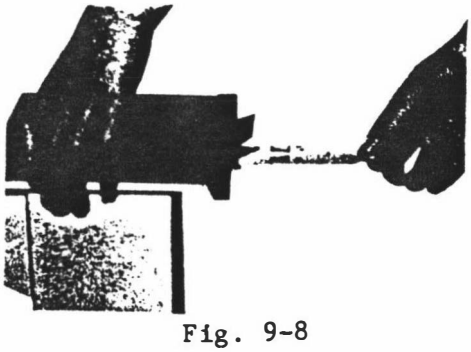


Fig. 9-8

(2) Without delay, grip the center of the yellow tab and pull it straight, at moderate speed, with a smooth uninterrupted motion, all the way out. Begin timing immediately.

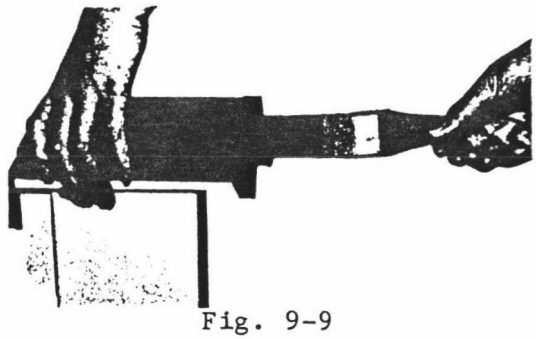


Fig. 9-9

9.5 Processing time and temperature

The temperature of the film during processing is important. Process pictures for the time shown in Fig. 9-9, but not longer. Over-processing may result in a loss of information in the dark areas of the print. In addition, extended processing (over 3 min.) may damage the print surface. A picture processed for too short a time will have dull grays, mottle, and little contrast.

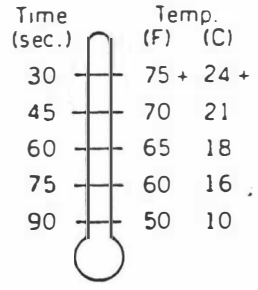


Fig. 9-10

9.6 Separating print from negative

(1) After the recommended development time, quickly separate the print from the negative. Start at the end nearest the yellow tab.

(WARNING) Avoid contact with the developer chemicals.

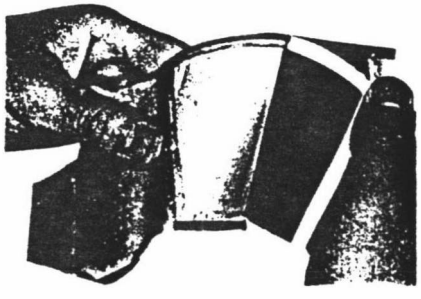


Fig. 9-11

(2) Fold the negative, moist side in, and dispose of it properly.

(NOTE) In low humidity, prints may curl. Do not attempt to straighten them by bending. Instead, place them in an area of higher relative humidity (about 45 percent), until they are flat.

9.7 Removal of jammed film

If the yellow tab does not appear after the white tab is pull out, the film is jammed in the camera.

Remove the jammed film according to the procedures below:

(1) Open the camera door but do not pull out the film pack out.

(2) Remove the jammed film.

(3) Check the developer roller inside the camera door and remove any paper or excessive glue.

(4) Set the white tab of the next film.

(5) Close and latch the camera door.

The film is ready for the next exposure.

10. PHOTOGRAPHING HOOD (OPTION)

Photographing hood ACR-5010 with 35 mm SLR camera enables recording data on 35 mm films.

* 35 mm SLR camera body and TXP mount are not included in the components of ACR-5010. Prepare them separately.

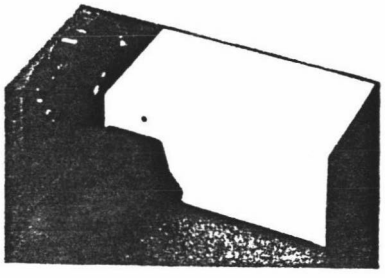


Fig. 10-1 ACR-5010

10.1 Film

We recommend to use 400 ASA film.
In the case of using 100 ASA film, pay special attention to focus adjustment.

10.2 Camera setting

Set the camera stop at F8 and shutter speed at 1/8 (for 400 ASA film).
In the case that 100 ASA film is used, set camera stop at F5.6 and shutter speed at 1/8.

10.3 Make the exposure

(1) Have the cross-sectional image for photography displayed on the monitor screen.

(2) Hold the cross-sectional image frozen with the FREEZE switch.

(3) Set the camera hood on the monitor, and push the shutter release button on the camera.

11. CARRYING THE EQUIPMENT

When carrying the Echo Camera model SSD-210DXII, note the following:

(a) Wind the power cord around the winding posts on the rear panel.

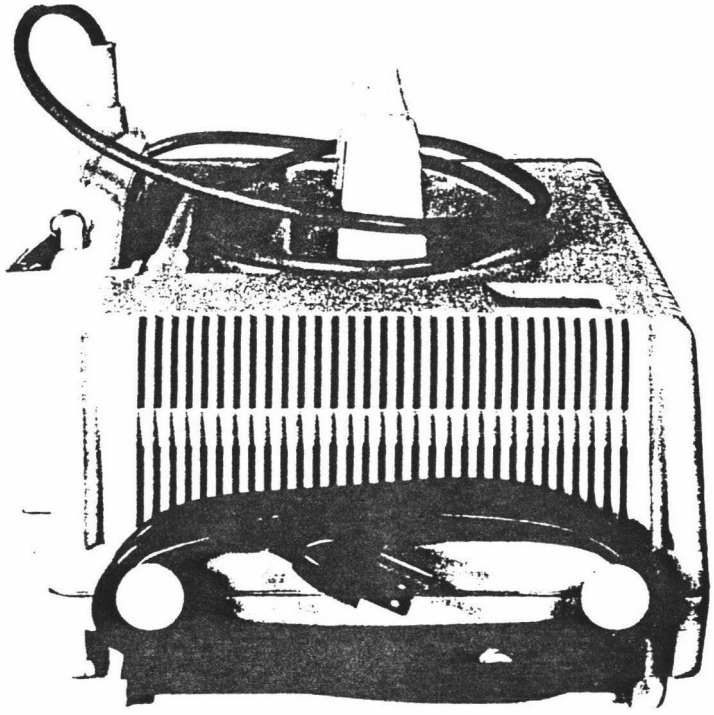


Fig. 11-1

(b) Wind the probe cable around the carrying handle.

(c) A gel bottle may be placed in the groove in the top of the system. Endure that the bottle top is sufficiently tightened to prevent leakage of gel.

(d) Wear the wrist strap of the probe and grip the handle. (See Fig. 11-2 and 11-3.)

(e) Do not drop, vibrate, or bump the probe or the system.

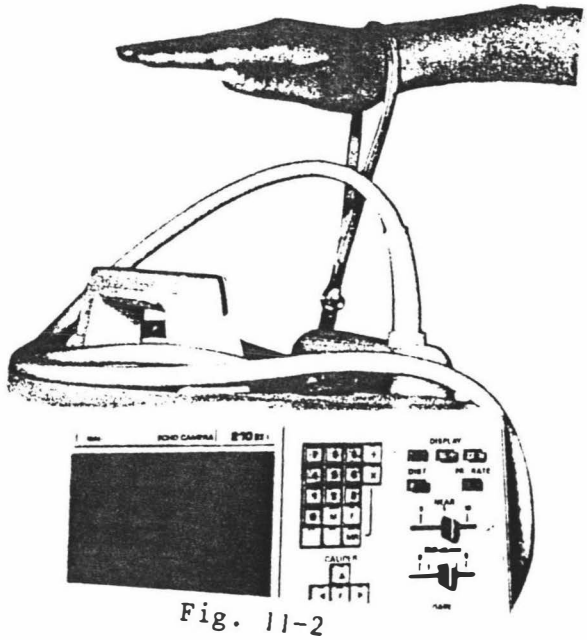


Fig. 11-2

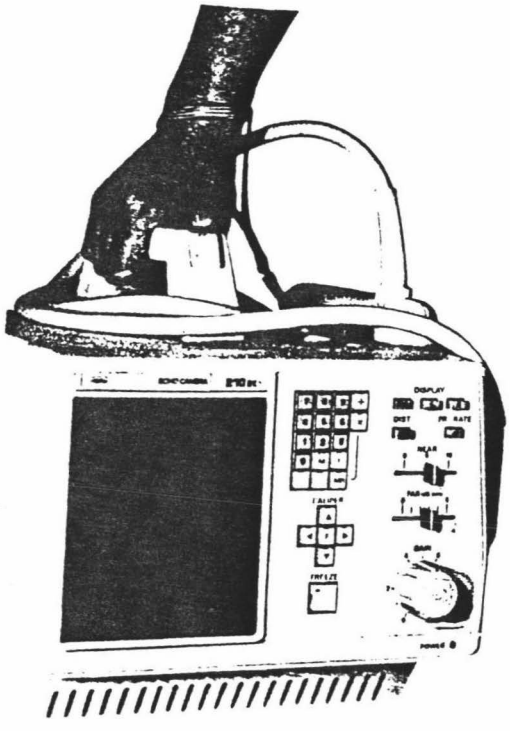


Fig. 11-3

12. CONNECTIONS OF VIDEO TAPE RECORDER (option)

Any video tape recorder may be connected to the equipment.
The connections is as shown below:

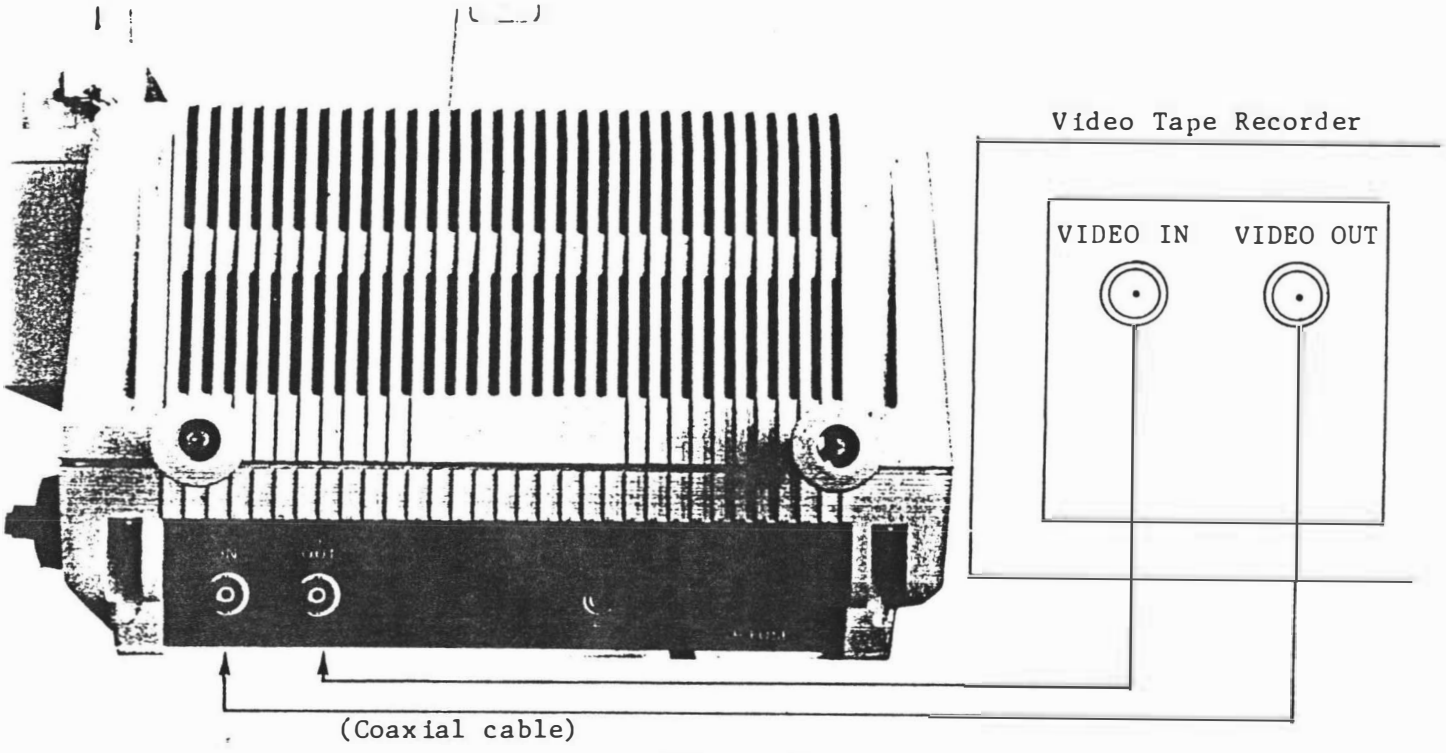


Fig. 12-1

A playback image will automatically be displayed when a connected video tape recorder is in the playback mode.

NOTE: Prepare two coaxial cables:
One end of each cable should have a BNC connector (for the system) and the other end of each cable depends on the VTR to be connected.

Small VTR can be mounted on the mobil cart (option) as shown in the figure below:

The size of VTR which can be mounted on the cart is 28 cm (W) X 30 cm (D) X 18cm (H).

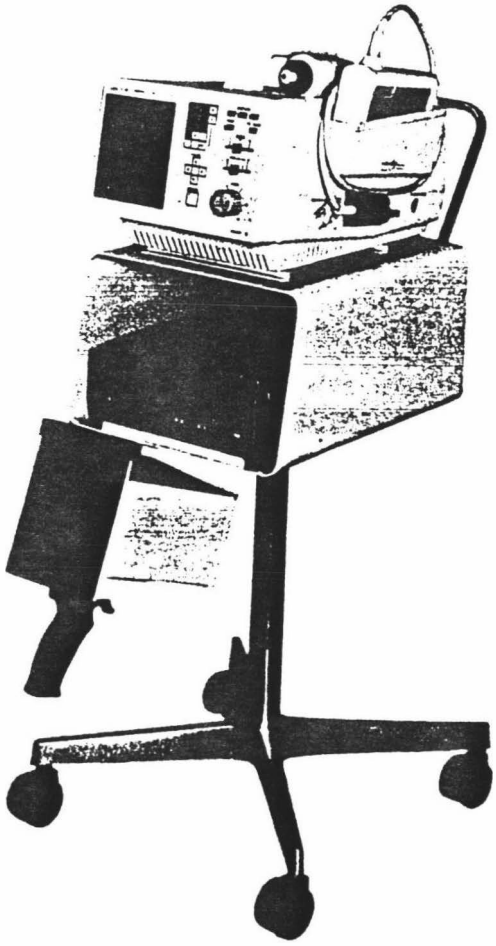


Fig. 12-2