Automatic oestrus detection using a camera-software device and oestrus detector strips in dairy cattle at pasture

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General Abstract

This study aimed to develop an automated system of oestrus detection building on the widely utilised technique of tail paint, which assists effective and accurate oestrus detection. A camera-software device (CSD) and oestrus detector strips (ODS) were tested in this study. This system has extended the technique of tail painting and modified it so that the CSD can automatically detect, read and interpret paint removal optically using digital technology.

A clinical trial involving 480 New Zealand dairy cows grazing pasture was conducted to determine the efficiency of ODS with CSD compared to traditional farm management comprising visual observation and tail paint and to the tail paint technique alone as scored by an observer in the milking shed. Tail paint readings were classified into four categories 1-25%, 26-50%, 51-75%, 76-100% of tail paint removed. Visual observation on the two groups was conducted for 30-45 minutes before morning and afternoon milking and at other times when work was occurring near the cows. Milk samples were collected for progesterone (P4) analysis. The sensitivity, specificity, predictive values and accuracy of oestrus detection were compared. The confirmed pregnancy diagnosis and artificial insemination (AI) results were used as one standard to allow comparison of the different oestrus detection methods. When P4 results became available, they were integrated into the performance standard (a strong level of agreement was found between P4 results and oestruses that were confirmed by pregnancy diagnosis $\kappa=0.74$). Standardised reproductive analysis for each group was conducted using DairyWin™ farm records.

The test sensitivity, specificity, positive predictive value (PPV) and overall accuracy for the CSD group were higher than those for traditional farm management (comprising tail paint and visual observations; $p<0.0063$; $p<0.001$, $p<0.0001$, $p<0.0001$ respectively based on pregnancy diagnosis (PD) outcome for confirmation the occurrence of oestrus; $p<0.039$, $p<0.01$, $p<0.0001$, $p<0.0001$ respectively based on PD outcome and P4 combined to confirm the occurrence of oestrus). They were also higher than for tail paint use alone ($p<0.004$, $p<0.0001$, $p<0.05$, $p<0.0001$ respectively; based on PD outcome for confirmation of the occurrence of oestrus). Negative predictive value (NPV)

didn't differ between CSD and traditional farm management (p=0.28 based on PD outcome for confirmation of occurrence the oestrus and p=0.55 based on PD outcome and P4 results combined for confirmation of the occurrence of oestrus) and was significantly higher (p<0.0001) when compared to the NPV of tail paint alone. The pregnancy rate and non-return rate (49 day) to first service by artificial insemination were higher (72%, 71% respectively; p<0.05) in the CSD group than that in the control group (39%, 47% respectively). CSD application significantly influenced the proportion conceiving from planned start of mating (PSM) until the end of the artificial breeding season (p= 0.044).

The study showed that the CSD system can satisfactorily detect oestrus in seasonally calving dairy herds grazing pasture. With the positive influence that the CSD had on this farm's performance it appears that the CSD offers the potential to increase conception rate in similar herds if AI is timed using the results of CSD oestrus detection.

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Chapter One

The oestrous cycle and oestrus detection devices in cattle.

Abstract

The effects of detection of oestrus and its importance on improving reproduction and thus production in dairy cows are reviewed. Emphasis on economic impacts of traits like health and reproduction have increased compared to the emphasis on milk yield in dairy cows. Oestrus detection is commonly performed by visual observation during feeding and milking. In large dairy herds oestrus detection is difficult due to the short time available to observe individual cows given the large number of cows present. Computer use in monitoring cows and the technical progress associated with it have made automation of oestrus detection possible. A practical and efficient method for farmers, which provides a high degree of accuracy with minimal cost and effort, is yet to be found. Such a system will save time and labour during the mating season and will ensure that mounted cows are identified, thus enabling dairy farmers to introduce high quality genetics to their herds by using semen from proven sires. The system would also provide accurate information on the reproductive status of the herd to farmers and veterinarians. An automated system of oestrus detection, building on the widely utilised tail paint technique that can detect oestrus effectively and accurately and which must be compatible with automated milking systems offers a promising area for development.

KEY WORDS: Review, dairy cattle, automated oestrus detection, tail paint.

1.1. Introduction

Dairy farming in New Zealand is predominantly pasture-based and herds are calved seasonally, with the breeding period being restricted to approximately twelve weeks, comprising of four to eight weeks of artificial breeding followed by a period of natural mating using herd bulls (Macmillan and Asher, 1990). Cows not conceiving during this twelve-week period are usually culled. Reproductive failure is one reason for the removal of cows from New Zealand dairy herds. Therefore, the development of strategies to aid in the reproductive management of New Zealand dairy herds is vital to allow farmers to optimise reproductive performance and to minimise cow wastage (Harris, 1989; Hanlon, 1995).

In New Zealand, the average dairy cow must have a calving interval of around 365 days to maintain a calving pattern that ensures that peak feed requirements for the herd are synchronised with the seasonal pasture production peak in the spring of the year. Within the 365 day inter-calving interval, 282 days are required for pregnancy leaving only about 83 days for cows to resume normal cycling and conceive (Holmes and Wilson, 1987; Quinn, 1997).

New Zealand farmers receive among the lowest milk payouts in the world, which has a direct impact on the performance and efficiency of dairy production required when compared to other international producers. Thus pasture, generally considered to be the least expensive source of feed for cattle, is used to meet the bulk of dairy cows' feed requirements. The New Zealand climate creates a distinct pattern of pasture availability throughout the year and this in turn determines the seasonality of calving in dairy systems in New Zealand through managing calving patterns and times of drying off (Holmes and Wilson, 1987).

One of the most limiting factors in artificial insemination (AI) programs is the inability to achieve proper detection of oestrus in cows or heifers. In dairy cattle where artificial insemination is used to breed the females, herdsmen must recognise and interpret the signs of oestrus in cows. Proper timing of AI is necessary to accomplish a high percentage of conceptions in cows that are bred artificially.

During the period of artificial breeding, errors of oestrus detection will cause a significant effect on the reproductive performance of the herd (Xu, McKnight, Vishwanath, Pitt and Burton, 1998). The errors may be either a failure to detect cows in oestrus (low efficiency), or errors in oestrus detection (low accuracy) (Macmillan and Curnow, 1977).

New technologies to accurately detect oestrus play important roles in reproductive management strategies for commercial dairy operations. Combining an oestrus detection system with a management decision to quickly initiate AI service improves reproductive efficiency and pregnancy rate by increasing the AI service rate and decreasing the interval between AI services. Failure to observe cows in oestrus (false negatives) spreads the breeding season and increases the costs of production through reduced reproductive efficiency. Detection of non-oestrus cows (false positives) is undesirable because it decreases conception rate. A high rate of false positive results diminishes the usefulness and cost effectiveness of any system used for oestrus detection (Cordoba, Sartori and Fricke, 2001).

Considerable research has been conducted on factors contributing to the efficiency with which cows are detected in oestrus. One of the key factors is the skill of the people performing the detection (Esslemont, 1974). With an AI program, people assume the same responsibility for accurately detecting oestrus and achieving the proper timing of insemination as a bull does in a natural breeding situation. A challenge for a farmer is to determine which cows are in standing oestrus and when breeding should occur (Imtiaz, Hussain, Fuquay and Younas, 1992; Folman et al., 1979; Lindsay, 1996).

Williamson, et al. (1972) and Stevenson and Britt (1977) both reported that approximately 50% of oestrus periods were being missed by farm workers. Lack of diligence in oestrus detection in New Zealand seasonally calving dairy herds is a major limitation to effective herd breeding management. With labour continuing to be limiting factor for most dairy producers, an oestrus detection aid or system that is accurate and can totally replace visual detection would be highly desirable. The advent of electronic methods and computerisation has enabled recent research and development efforts to be focused on tools that provide continuous oestrus detection and high efficacy. If systems are produced, they will have to be economical and practical (Macmillan and Moller, 1975). The reduction in staff per head of cattle and need for reproductive efficiency in modern dairy farming make a reliable system that is capable of accurately detecting oestrus highly desirable.

1.1.1. Breeding and reproductive performance of New Zealand dairy herds

Reproductive performance at a high level is essential to maintain the profitability of dairy enterprises. Consequently, it is a focus of dairy management and veterinary herd health programs in most successful dairy operations. When the average age of heifers at first calving is 24 versus 30 months, lifetime milk production potential is increased and replacement-rearing costs decrease substantially. Earlier conception following calving results in fewer days dry and a higher average daily milk production. Efforts to attain these reproductive goals are consistent with progress toward the overall objective of achieving the maximum amount of milk yield per day of cow life (Shearer, 2003).

Reproductive problems in herds may be simple or multifactorial and complex. If difficulties are associated with a few cows which may have a history of problems then the solution to the problem may be fairly simple: increase culling of these cows for reproductive reasons. If the underlying causes are related to management, feeding, or nutritional factors; identifying and solving problems may be more difficult. An increase in the number of days to first breeding after calving lengthens calving intervals and is frequently a result of inadequate oestrus detection. Under most circumstances oestrus detection problems are related to observation failure. However, weather extremes, poor footing or slippery conditions, negative energy balance and many other factors can influence the intensity of normal oestrus behaviour and make it more difficult to detect cows in oestrus (Shearer, 2003).

Milk production and genetic improvement of dairy cows are heavily dependent on high reproductive efficiency. This influence is manifest through the number and quality of available replacements and involuntary culling mainly because of reproductive failure (Xu et al., 1998). The diagram below in Figure

1.1 shows the factors that influence reproductive performance in New Zealand dairy herds.

1.1.1.1 The influence of previous seasons

The seasonal effect takes place because of the annual pattern of interaction between animals and pasture. The effect can be positive or negative and influences a wide range of on farm factors such as cow condition, calving pattern and somatic cell count (Quinn, 1997). Each season has a carry over effect on the following season. A feed shortage in one season that causes cows not to reach their target body condition score, has an effect on oestrus cycling and conception rate in the following season (Macmillan and Asher, 1990). Thus, management of dairy farms to minimize any negative carry over effect and to maximize the positive effects of satisfactory performance is of great importance (Quinn, 1997).

1.1.1.2 Calving pattern

The productivity of New Zealand herds is significantly affected by calving pattern. A herd's requirement has to be matched with the pasture available which necessitates a compact calving pattern (Holmes and Wilson, 1987). Cows must calve at least 60 days before the start of mating to achieve a 21-day submission rate of 94% (Hayes, 1997). Calving ≤40 days before the start of mating drops this to 80% and calving within 20 days will drop it to 50%. A reduced interval from calving to start of breeding occurs mainly as a result of failure to submit cows for breeding causing late conception dates in the previous season's mating period (Burke, 2003).

Induction of calving and culling of cows that calve late will result in a more compact calving pattern. Early abortions in the herd will cause a spread in the calving pattern and fewer replacements (Quinn, 1997). Induction of calving may extend a cow's survival in a herd for at least a lactation and reduce the cost of reproductive wastage (Macmillan, 1995).

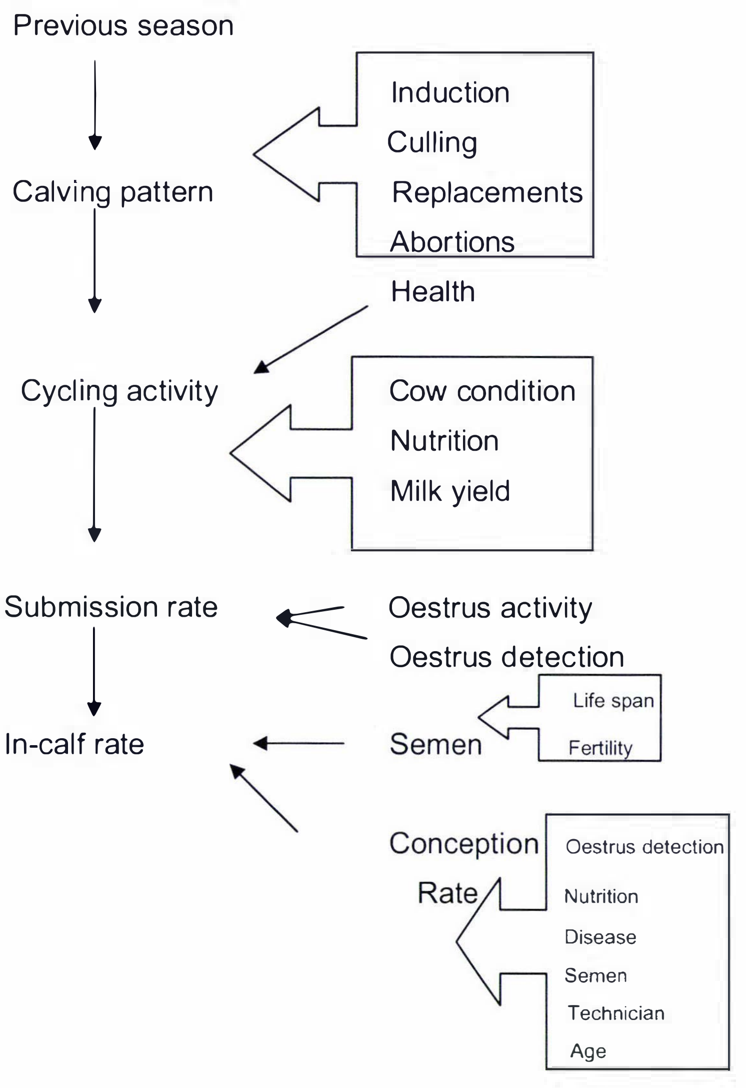


Figure 1.1: Factors affecting reproductive performance in New Zealand dairy cattle.

Source: (Quinn, 1997), p2.

1.1.1.3 Cycling activity, submission rate, conception rate and in-calf rate

A period of anoestrus follows calving for a period 30–40 days and allows the normal healthy cow's reproductive tract to recover with an absence of the normal cyclical pattern (Holmes and Wilson, 1987; McDougall, 1993). The postpartum anoestrus interval and conception rates are adversely affected by nutrient quality that is either low in energy or protein. That effect is expressed mainly by high yielding cows when the total feed allowance does not meet the cow's requirements (Holmes and Wilson, 1987).

O'Farrell (1974) showed that cows with poor body condition score at calving needed a longer period to conceive (113 days) compared to their herd mates (<80 days). Ducker (1984) also showed that lower conception rates occurred amongst cows found to be too thin or too fat at artificial insemination, compared to cows of moderate condition score. King (1991) reported that improved cow condition score was the only factor associated with a reduction in the interval from the start of mating to conception when anoestrus cows were treated earlier in the breeding season. Anoestrus can also be affected either directly or indirectly by cow health status and illness that may arise from the reproductive tract such as retained foetal membranes, or a disease which significantly affects cow body condition post-partum (Holmes and Wilson, 1987).

Macmillan and Moller (1975) stressed that proper feeding and mating management is a key to success in New Zealand grassland farming. Records for 28,202 cows showed that the average calving interval was 364 days under New Zealand conditions. Results from weighing of herds in the Waikato indicated that high initial milk production and efficient breeding was achieved when adult cows achieved average weights (Table 1.1).

Harris and Kolver (2001) showed that New Zealand dairy herds' fertility has declined as the proportion of overseas Holstein Friesian dairy cows genetics has increased. This deterioration in cow fertility has become a major problem in dairy cattle production and it is becoming apparent that high milk yielding cows are more difficult to get in calf (Olori and Galesloot, 2000). This negative association between production and reproduction (fertility) has been observed in several dairy breeds of cattle(Hermas, Young and Rust, 1987; Harris and Pryce, 2004).

The issue of nutrition and high milk yield is under debate and many theories have arisen such as, high yielding cows have more difficulty conceiving than lower producers (Ducker, 1984) and inadequate nutrition is one of the underlying causes of poor fertility in dairy cows (Esslemont, Bailie and Cooper, 1985). Harrison, Ford et al. (1990) suggested that high milk production reduces the intensity of behavioural oestrus with normal ovarian function, which in turn may reduce oestrus detection.

Table 1.1: Average body weight of Waikato dairy herds in relation to achievement of higher initial milk production.

	Jersey cows (three herds)	Friesian-jersey cows (two herds)
At drying off	327 kg /721 lbs	351kg /773 lbs
Mid drying period	340 kg /749 lbs	358 kg /788 lbs
Before calving	357 kg /789 lbs	372 kg /819 lbs

Source: (Macmillan and Moller, 1975).

The plasma glucose concentration of cows did not vary between those that conceived and those that did not conceive within the same genetic level and no significant difference occurred in milk yield and feed intake (Snijders et al., 2001) which suggests that poor reproductive performance may not be a direct consequence of high milk production (Olori and Galesloot, 2000).

Submission rate is defined as the percentage of cows in a herd detected in oestrus and served in a 21-day or in a 28-day interval from the planned start of mating. The target for the 21-day submission rate is 90%. Achieving a high submission rate requires efficiency and accuracy in oestrus detection in artificially inseminated herds and the resumption of ovarian activity postpartum (Macmillan, 1972; King, 1991).

Senger, et al. (1988) showed using plasma progesterone measurement at the time of oestrus that up to 20% of the cows in the United States may be inseminated at the wrong time. Under field conditions, fertilisation failure accounts for 20% of reproductive failure (Ayalon, 1978; Hunter, 1994; Xu et al., 1998). Factors such as sires, semen processing, site of insemination and sperm dosage among others also affect fertilization failure (Nadir, Saacke, Bame, Mullins and Degelos, 1993; Hunter, 1994; Xu et al., 1998).

Failure to return to service within a specified period (e.g. 49 days) as a result of pregnancy is termed a non-return to oestrus. Conception requires a fertile oestrous cow and a competent technician using semen of high quality. The accuracy of non-return rates depend on efficient post-service oestrus detection (King, 1991). The rate at which cows become pregnant in a dairy herd is commonly called the "in-calf" rate. It is defined as the proportion of eligible cows in a herd that conceive within a defined time period. The in-calf rate is affected by two factors. One is the conception rate (the proportion of services that result in a conception) and the second is the submission rate which is influenced by the occurrence and detection of oestrus. Dairy cow fertility is commonly measured by calculating the percentage of cows that conceive after their first AI service which is known as the first service pregnancy rate (Fricke, 2000).

In cases where oestrus detection efficiency appears to be adequate but conception failure is the problem, other factors need to be considered. For example, infertility may be due to oestrus detection inaccuracies, poor semen quality, faulty insemination technique, early embryonic death, reproductive tract infection, cystic ovarian disease or other causes (Shearer, 2003).

All of the factors shown in Figure 1.1 affect the number of cows which successfully become pregnant. The in-calf rate is the total number of cows that become pregnant in a specific period and each factor, if below ideal, will have a negative impact in the current and next season's herd reproductive performance (Holmes and Wilson, 1987; Quinn, 1997).

1.2 Literature review

1.2.1 Oestrus, fertility and artificial insemination (AI)

A cow is fertile only when an egg has been released (or ovulated) from the ovary. This occurs about 10-18 hours after the period called standing oestrus ends. Sperm need time in the cow's reproductive tract before they are capable of fertilizing the egg, a period during which the sperm undergoes capacitation, so insemination should occur several hours before ovulation. This means that for the highest fertility, cows or heifers should be inseminated in the latter two-thirds of oestrus or within a few hours after cows have gone out of oestrus.

Insemination normally occurs approximately 12-18 hours after the cow first shows standing oestrus (Hammond, 1927; Hurnik, King and Robertson, 1975; King, Hurnik and Robertson, 1976; Roditan, King, Subrod and Pongpiachan, 1996).

The surest sign of oestrus is when a cow or heifer permits other animals to mount her while she remains standing. This is the best sign of a cow's fertile period. Therefore, the most productive means of determining which cows are in standing oestrus is to observe the cattle carefully for about 30 minutes at least thrice per day. More frequent observations may also be beneficial whenever it is practical. Oestrous synchronization will aid in accurate oestrus detection and reduce the number of days on which oestrus detection must be done (King et al., 1976; Esslemont, Glencross, Bryant and Pope, 1980; Britt, Scott and Armstrong, 1986).

1.2.2 Influence of oestrus detection

Reproduction is a vital factor in determining the efficiency of animal production. In dairy herds, the goal of ever increasing milk yields is often pursued to the exclusion of other factors. However, a cow will only begin to lactate effectively after calving and milk yield will eventually cease unless she calves again. The reproductive process is thus of a vital importance to lactation (Peters and Ball, 1987).

Pregnancy rate to AI in lactating dairy cows has decreased from 66% in 1951 (Spalding, Everett and Foote, 1974) to about 40% in 1997 (Butler, Cherney and Elrod, 1995; Pursley, Kosorok and Wiltbank, 1997). However it remains at 70% in heifers, not changing during the same period (Spalding et al., 1974; Foote, 1975; Pursley et al., 1997). This reflects that cow fertility is a major cause for poor reproductive efficiency in dairy herds, which could be improved by improving the conception rate to artificial insemination and this is directly influenced by the accuracy of oestrus detection (Fricke, 2000).

The duration of oestrus is dependent on several factors and there appears to be diurnal pattern because cows seem to show oestrus mostly at night (Peters and Ball, 1987). The most common approach is to breed cattle a few hours after they are detected in oestrus by observing mounting behaviour. Therefore, failure to detect oestrus in a timely and accurate manner greatly limits reproductive performance in dairy herds (Nebel et al., 2001).

Infertility occurs in all herds but reproductive problems may become more obvious with AI use since mating related events are recorded and summarised. Animal attendants often blame breeding failures on reproductive abnormalities such as acyclicity, silent oestrus, follicular defects or insemination deficiencies. However, the inability to detect oestrus so that females can be mated at the optimum time is often overlooked and yet is the most frequent cause whenever AI is the principal mating procedure. People responsible for executing insemination procedures need a comprehensive understanding of reproductive function and sexual behaviour to appreciate how important their oestrous detection activities are in contributing toward the success of an entire breeding and production program (Esslemont et al., 1980).

Incorrect timing of insemination causes failure of fertilization (Xu et al., 1998). In normally cycling cows, the fertilisation rate to a single insemination at the appropriate time with semen of proven fertility can be higher than 90% (Sreenan and Diskin, 1986; DeJarnette, Saacke, Bame and Volger, 1992; Nadir et al., 1993).

Indications of poor oestrus detection include few oestrus periods observed and recorded before first service, excessive intervals from the end of the voluntary waiting period to first service, between services and a high incidence of non-pregnant, cycling cows at the time of pregnancy diagnoses (Kastelic, 2001). Esslemont and Ellis (1974) showed that the calving to first service interval and the first service to conception interval are dependent on both the oestrus detection rate and the average conception rate of the herd. Studies utilizing milk or blood progesterone assays indicate that 5 to 30% of inseminations occur in cows that are not in oestrus (Senger, 1994). This results in marked economic losses.

Reproductive inefficiency in lactating cows is a source of frustration to dairy producers since it reduces dairy farm profitability. Reproductive inefficiency in lactating dairy cows substantially reduces the efficiency of artificial insemination in dairy operations (Senger, 1994). Anoestrus at the start of a breeding season is a serious reproductive inefficiency in New Zealand dairy herds (Xu and

Burton, 1996). In well-managed herds the percentage could vary between 10% and 35% (Xu, Burton, Burton and Macmillan, 1995). The variation in the percentage of anoestrus cows is multifactorial, since factors like nutrition and feed supply during the pre-partum and post-partum periods, date of calving in relation to planned start of calving, number of induced cows and high stocking rates all result in delayed initiation of oestrus (McDougall, 1993).

Oestrus detection efficiency has been estimated to be less than 50% in most dairy farms (Senger, 1994). In New Zealand synchronised herds, detection of oestrus percentage was found to be 54.4% and a conception rate of 37% (McDougall, 2003). This inefficiency in oestrus detection not only increases the interval to first insemination but can increase the average interval between services to 40-50 days (Stevenson and Call, 1983).

The use and accuracy of oestrus detection aids has a direct impact on the reproductive performance of commercial dairy herds. Table 1.2 shows the dramatic difference between effective and poor oestrus observation and their impact on the proportion of the herd pregnant at 200 days postpartum (Quinn, 1997). With a detection rate of 80% only 3% of the herd are not pregnant and will return to oestrus after 200 days postpartum. If a 20% detection rate is achieved, 50% of the herd will be found not to be pregnant and 20% will not have been observed in oestrus.

Table 1.2: The effect of different rates of oestrus detection on reproductive performance.

	Percentage of oestrus detected		
Description	**20%**	**50%**	**80%**
Cows not pregnant by 200 days, %	48	13	3
Cows not inseminated by 200 days, %	21	<1	0

Source: (Heersche and Nebel, 1994), p2756.

In New Zealand dairy herds, cows are milked twice daily during spring which provides the opportunity for farmers and farm-workers to observe oestrus at least twice daily, so any cow that has been obviously ridden between milkings should be mated after the end of the next milking.

Checking cows for oestrus between milkings may reduce the number of errors in oestrus detection by reducing the number of cows that are missed and will therefore increase submission rates (Macmillan and Moller, 1975). In New Zealand, there are two types of errors regarding oestrus detection, errors in diagnosis and errors in identification. These errors arise through herd owners adopting a liberal attitude in their interpretation the signs of oestrus, in their attempt to reduce the level of errors of omission (King, 1991).

Recently, research using radio-telemetric monitoring showed that lactating dairy cows poorly express oestrous behaviour when compared with dairy heifers. Old textbooks showed the duration of oestrous behaviour in dairy cattle to be about 18 hours. More recent studies by Dransfield et al. (1998) estimated oestrous behaviour to last 7.1±5.4 hours. Conception for each cow requires a high rate of oestrus detection combined with a high level of accuracy (Boyd, 1984). Insemination outcome and pregnancy increase with an increase in oestrus detection rate and accuracy while calving interval decreases. Falsely detected oestrus causes financial loss through failure to conceive, embryonic and foetal loss and resulting reduced milk production. These breeding failures also retard the genetic progress of herds (Lehrer, Lewis and Aizinbud, 1992; Eradus, Scholten and UdinkTen Cate, 1998).

1.2.3 Oestrus cycles and oestrus detection aids

1.2.3.1 Oestrus cycle

Puberty is when the reproductive tract and secondary sex characteristics achieve a mature adult form. This may occur as early as at 5 months old in smaller dairy breeds and usually occurs in all breeds by 12 months of age (Shearer, 2003). Bovine reproduction is not seasonal and oestrus cycles continue throughout the year (Bloomfield, 1987; Quinn, 1997). The time between periods of oestrus is defined as an oestrus cycle (Bearden and

Fuquay, 1980). The length of the cycle averages approximately 21 days. Values within the range of 18–24 days are considered normal (Macmillan and Asher, 1990).

1.2.3.2 Hormonal interactions and control of the oestrus cycle

Reproduction in mammals is controlled by a complexity of interacting hormones secreted from various endocrine glands. The regulation centre of the genital tract (sex centre) is located in the hypothalamus in the central nervous system. Hormones secreted from the hypothalamus and the pituitary gland control ovarian activity. The hypothalamus and pituitary receive feedback from the ovarian steroid hormones progesterone and oestradiol-17-beta (Figure 1.2). Other regulation centres, for other parts of the body, are located next to the sex centre. The different centres influence each other because of the existence of neural bindings (Bloomfield, 1987).

Gonadotrophin-releasing hormone (GnRH) is produced in the hypothalamus and its' release is affected by number of stimuli which include light, temperature, smell (pheromones), blood pressure, nutrition, illness (Bloomfield, 1987; Holmes and Wilson, 1987). GnRH causes the pituitary to release luteinizing hormone (LH) and follicle stimulating hormone (FSH). Other hormones produced from the hypothalamus either inhibit or induce the release of additional pituitary hormones such as Growth Hormone, ACTH, TSH, MSH and Prolactin. Although these hormones have some effect on reproduction, the focus here will be on FSH and LH because they regulate more directly the oestrous cycle (Larson, 2000).

Hormones produced by the ovarian structures have a direct effect on the hypothalamus, as do the hormones produced from the adrenal glands and the placenta. Raised levels of the steroid hormones oestrogen and progesterone act as inhibitors of the hypothalamic secretion of GnRH, but a baseline level is necessary or stimulatory to GnRH secretion (Larson, 2000). GnRH is secreted in pulses from the hypothalamus into the pituitary portal blood supply to stimulate the synthesis and secretion of LH and FSH from the anterior pituitary gland, which when transported in blood to the ovaries performs specific functions. The major function of FSH is to stimulate the growth of the ovarian follicle and the production of oestrogen by the ovary. Another function is to act with LH to bring about the maturation of the follicle. LH stimulates the growth of the ovarian follicle and the production of oestrogen by the ovary. It also stimulates the growth and maintenance of a corpus luteum (CL) in the metoestrus phase from the luteal cells of the ruptured follicle (Ducker, Bloomfield and Morant, 1986; Bloomfield, 1987; Jolly, 1993).

Oestradiol-17-beta and progesterone are the two major ovarian hormones and their pattern of secretion is cyclical in healthy non-pregnant animals (Figure 1.3). The role of oestradiol-17-beta is to prepare the reproductive tract for conception, causing characteristic signs of behavioural oestrus and preparing for the pre-ovulatory surge of LH. The natural progesterone prepares the uterus for receiving the embryo, maintains the pregnancy, suppresses the ovarian cycle and regulates pituitary gonadotrophin secretion (Bloomfield, 1987).

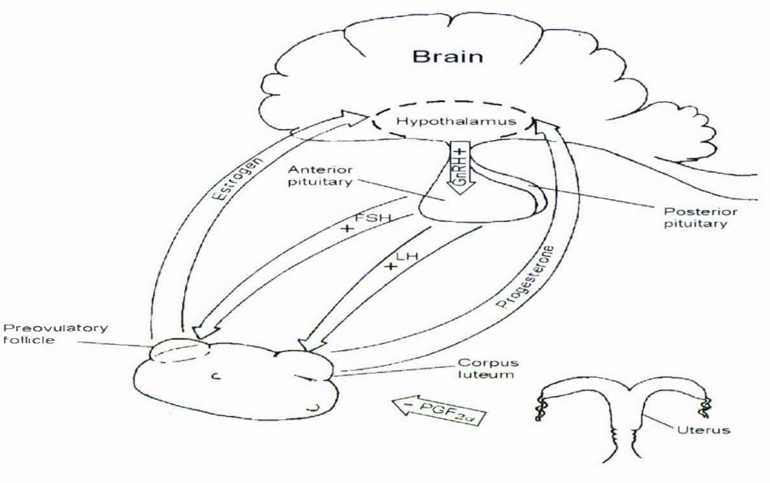


Figure 1.2: Hormonal control of ovarian cycle
Source: (Larson, 2000) the bovine oestrus cycle.

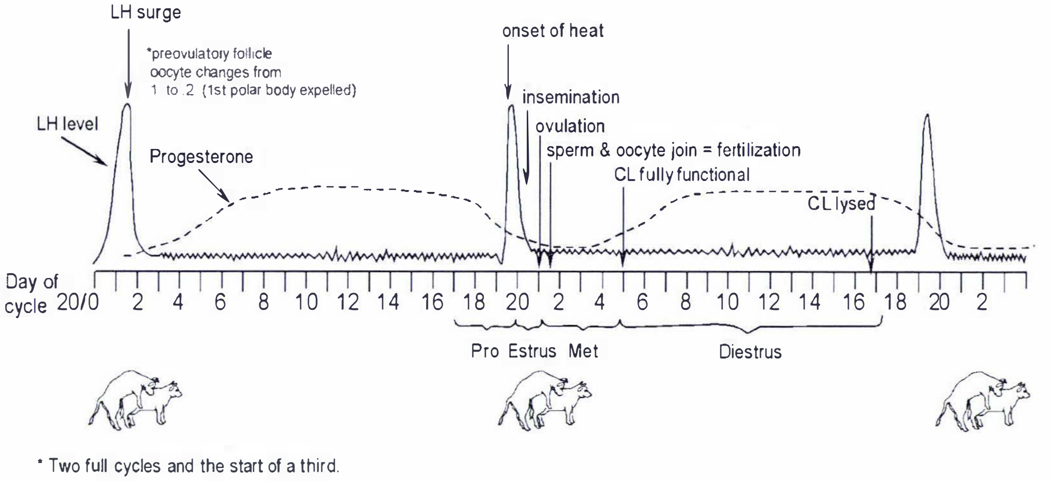


Figure 1.3: The bovine oestrus cycle.

Source: (Larson, 2000) the bovine oestrus cycle.

Table 1.3: Stages of the bovine oestrous cycle.

Stage	Cycle day	Duration	Event
Oestrus	0	10–12 hours	Mature follicle high levels oestrogen LH surge
Metoestrus	1-3	5-7 days	Ovulation (with in12-18 hrs) formation of CH, no response to prostaglandin
Dioestrus	5-18	10-15 days	Mature corpus luteum. High levels of progesterone
Pro-oestrus	19-21	3 days	CL regressing maturing follicle. Rising oestrogen

Source: (Shearer, 1992), reproductive Anatomy and Physiology of Dairy Cattle

1.2.3.3 Stages of the oestrous cycle

The oestrus cycle may be broken down into four phases: pro-oestrus, oestrus, metoestrus and dioestrus. The pro-oestrus and oestrus phases are primarily under the influence of oestrogen and are associated with growth of the Graafian follicle. Metoestrus and dioestrus are associated with growth of the corpus luteum and are primarily under the influence of progesterone as shown in Table 1.3 and Figure 1.3 (Larson, 2000; Shearer, 2003).

Pro-oestrus

The pro-oestrus phase occurs about 2 to 3 days prior to the onset of oestrus and is characterised by follicular growth and rising oestrogen levels which increase the blood supply to the reproductive tract resulting in oedema and swelling of the entire tract. Glands of the cervix and vagina are stimulated to increase secretory activity and produce a thin vaginal discharge (Larson, 2000; Shearer, 2003).

Oestrus

Following the end of pro-oestrus, oestrus is the period when sexual behaviour appears. Behavioural manifestations have been discussed above and they result from oestrogen acting on the central nervous system. After about 14 to 18 hours, signs of oestrus begin to wane so oestrus activity in cows can be easily missed unless they are observed frequently (Maatje and Rossing, 1976; Metz, Wiersma, Rossing and Van Den Berg, 1987; Lehrer et al., 1992; Larson, 2000; Firk, Stamer, Junge and Krieter, 2002b; Shearer, 2003).

Metoestrus

The phase following oestrus is metoestrus that lasts about 2 to 3 days. The cow does not ovulate until after she goes out of oestrus. Therefore, metoestrus is the period in which ovulation and the formation of the corpus luteum (CL) occurs (Bearden and Fuquay, 1980).

Dioestrus

This is the period of the oestrus cycle during which there is a functional CL which develops as a result of changes that start immediately following ovulation. The CL develops into a fully functional organelle that secretes progesterone. If pregnancy does not occur, the CL remains functional only up to about day 17 or 18 after ovulation and then it regresses, permitting a new oestrous cycle to be initiated (Bearden and Fuquay, 1980; Larson, 2000; Shearer, 2003).

1.2.3.4 Oestrus detection aids

Signs of oestrus include standing to receive mounting or mounting of other cows. Oestrus animals are active, nervous, restless, bawling, walking and searching (Williamson et al., 1972). Many signs and behaviours have been described when cows are seen in oestrus including ruffling of the rump hair, vulval relaxation, moisturization and change of colour to erythematous, combined with a clear mucus discharge coming out from the vulva. Sensitivity of the rump to pressure which elicits standing, sniffing and licking of the vulva of other cows, tail playing and chin resting and rubbing are all typical signs. Standing to be mounted is taken to accurately indicate that cows are suitable for insemination (Williamson, 1980; King, 1991).

The major purpose of oestrus detection is to identify the appropriate time to inseminate cows so they can achieve conception. Standing to be mounted is considered to be the most important sign of oestrus (Foote, 1975; French, Moore, Graham and Long, 1989; Allrich, 1993).

Oestrus detection is a major challenge for the cattle breeding industry. Livestock farmers need a non-invasive automated system that accurately indicates ovulation. The system needs to be cost-effective, simple to use and provide rapid information at an animal's side (Senger, 1994). Two important indices to measure the success of oestrus detection are detection efficiency (Number of correct detections / total number of oestrus periods)X 100 and detection accuracy (Number of correct detections / Number of correct and false positive detections) X 100 (Lehrer et al., 1992).

Despite their cost effectiveness, non-automated oestrus detection methods, like mount detectors, teaser animals and continuous radio recording are often of limited use, due to a lack of time or lack of willingness to allocate the time needed for their daily updating (Lehrer et al., 1992). Many new tools offer the potential to improve conception rates via better timing of insemination by identifying when oestrus occurs. The trade-off for reducing labour though is usually a larger capital investment for equipment and ongoing equipment repair plus maintenance costs.

Table 1.4 shows briefly a summary of the advantages and disadvantages of available detection methods and aids, also showing a reported range of efficiency and accuracy for each method and its estimated cost in US$ (Rae, 2001).

1.2.3.5 Records and visual oestrus detection

Commonly, oestrus detection is performed by visual observation. This is particularly difficult on large dairy farms because of the short observation periods per cow that occur mainly during feeding and milking. Oestrus detection may be time consuming and increases labour costs by 30% to achieve an efficiency of 50 - 70 % (Eradus, Rossing, Hogewerf and Benders, 1992). Visual observation is the most common method utilised and standing immobility was the most important sign used by dairy farmers for oestrus detection. A standard indicator for cows in oestrus is their willingness to stand while being mounted (Williamson, 1980; Eradus et al., 1992). This may be sufficient to enable accurate detection in small herds but it is generally not sufficient in large herds (Williamson et al., 1972).

Visual observations, aided by tail painting, is the main method of oestrus detection used in New Zealand (Macmillan and Curnow, 1977; Macmillan, Taufa, Barnes, Day and Henry, 1988). This technique utilises a strip of paint applied over the tail head and regular observation through the day and milking hours for evidence of paint removal due to oestrous behaviour. The disappearance or disruption of the paint strip is taken as an indication of the occurrence of previous mounting activity. Using this oestrus detection aid and visual observation under New Zealand conditions, a conception rate of around 65% can be achieved during the first 21 days of a mating season (Xu and Burton, 1996; Xu and Burton, 1997; Xu et al., 1998).

Results reported from previous studies about oestrus detection were largely obtained by visual observation. Detecting oestrus by observation is accurate, but the efficiency ranges from 50% to 70% (Rorie, Bilby and Lester, 2002). Fulkerson et al. (1983) detected only 56% of cows in oestrus by observing at milking time in a herd of 140 cows and 66% when observation was combined with tail paint, an efficiency was found of 80% for tail paint used in combination with observations. Stevenson and Britt (1977) reported an accuracy of 68% and an efficiency of 51% by observation of standing oestrus in 88 Holstein cows.

Table 1.5 shows the distribution of oestrus periods over the 24 hours of a day. The proportion of cows in oestrus during a 24 hour period can be influenced by the season of the year, with more cows showing oestrus at night during hot weather and more during the day in cold weather. Housing conditions also influence the distribution of the display of oestrus during a 24 hour period. Oestrus can occur at any time, which is important to remember when evaluating methods of oestrus detection (Jeffrey and Duane, 1989).

One of the most important aids to oestrus detection is an effective recording system. Stockmen must keep complete and accurate records of all details related to oestrus events for every cow on the farm. These should assist in determining a cow's status if any of the cows is suspected to be in oestrus, by allowing a manager to check back to see if she was in oestrus approximately 21 days previously. If so, this will help to confirm the occurrence of oestrus.

Observational experiences in dairy enterprises have led to recognition that mounting activity (Figure 1.4) is the most reliable sign for oestrus (Foote, 1975). However, non-oestrous cows also must stand if there is no possibility of escape (Williamson et al., 1972). The accuracy of visual observations is compromised because most mounting activity occurs at night (Hurnik et al., 1975; Esslemont and Bryant, 1976), which reduces the efficiency of visual observation. In New Zealand dairy farming, most of the observations of cows happen during daylight and in some cases, occur only when cows are shifted from one paddock to another or are held in yards during milking times.

There are many factors affecting the efficiency of visual aids to detect oestrus. Major factors are the time of observation and the observer's experience in distinguishing oestrus signs and also the proper identification of cows that manifest these signs. Williamson et al. (1972) described many signs of oestrus but farm workers visually detected only 58% of oestrus occurrences (Williamson et al., 1972; Liu and Spahr, 1993; At-Taras and Spahr, 2001). A 30-minute observation period, both as early in the morning and as late in the evening as practical, are an essential minimum for adequate oestrus detection. The factor that is most critical to the success of detection by observation is the motivation of the human observer (Rae, 2001).

Table 1.4: Advantages and disadvantages of oestrus detection aids.

Detection Method	Advantages	Disadvantages	Range Reported (%) Efficiency	Accuracy	Cost
Observation	Observation of animals' behaviour.	Time commitment is required.	51 - 94	50 - 98	Time
Tail Paint/ chalk	Simple and inexpensive.	Repeated paint or chalk application is required.	66+		$1 / cow
KaMaR™ detection	Relatively simple and inexpensive.	Detectors can be lost and erroneously triggered.	80+		$1 - 2 / cow head
Chin ball marker on bulls and androgenised cows.	Bulls and androgenised cows are very effective detectors.	Animals and equipment must be maintained.	79+		$150 plus ink
Continuous video recording.	A continuous visually recorded record of activity is produced.	Placement and maintenance of video equipment. Video evaluation time.	56 - 94	50	$400 - 800 plus videotape
Hormonal assay-progesterone.	It is best able to confirm oestrus detection errors.	Poor accuracy of detection.	60 - 100	<50	$ 4 - 10 / cow
Electrical impedance of vaginal mucus	A quick, simple procedure in gathered cows.	Large variations in values require continual monitoring which can be impractical.	65 - 82	57 - 82	$ 50 - 300 /probe.
Elevation in body or milk temperature.	A quick, simple procedure in milked cows.	Large variations in values require continual monitoring.	50 - 74	55 +	
Radio telemetric activity monitor.	Continuous monitoring of activity.	Cost and maintenance demands of equipment and sensors.	62 - 81	22 - 100	Individual cow and system costs.
Radio telemetric mount detector.	Continuous mount detection.	Cost and maintenance demands of equipment and transmitters.	89 - 92	88 - 100	$ 4260

Source (Rae, 2001), pp10-11.

Table 1.5: Distribution of oestrus occurrence in 24 hours.

Time	Cows showing oestrus signs
6 a.m.-noon	22%
Noon-6 p.m.	10%
6 p.m.-midnight	25%
Midnight-6 a.m.	43%

Source: (Jeffrey and Duane, 1989), estrus (Heat) detection guidelines.

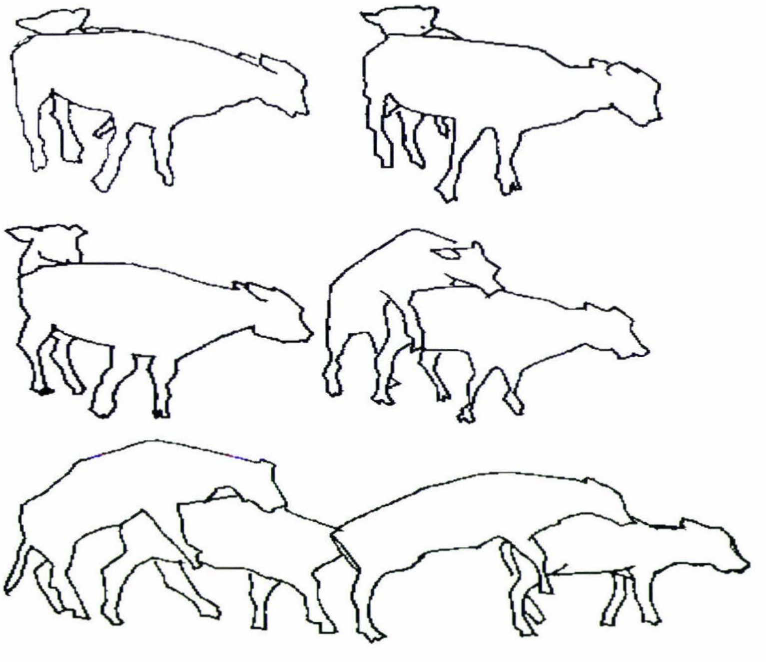


Figure 1.4: The sequence of oestrus mounting behavior in cattle.
Source: (Esslemont et al., 1985), p74.

To make visual detection more efficient, the number of observation periods should be increased and the length of each expanded. Timing these periods should include night time observations to ensure that a reliance on observations during milking or feeding periods does not occur (Williamson et al., 1972; Sambraus, 1978; Roth, Schlunsen and Schon, 1987; Le Blanc, Leslie, Ceelen, Kelton and Keefe, 1998).

1.2.3.6 Progesterone

Progesterone tests can be used to detect oestrus and to provide a presumptive diagnosis of pregnancy in cattle. The concentration of progesterone can be measured in milk and blood serum or plasma. The concentration of progesterone strongly correlates with the reproductive status and the various stages of the oestrus cycle can be detected (Bloomfield, 1987).

The concentration of progesterone in milk in the luteal phase is normally higher than 1.0 ng/ml (Heckman et al., 1979). From days 10 to 17 of the oestrus cycle, concentrations of 8.1 to 10 ng/ml can be measured. After day 17 the concentration declines rapidly from 10 to 3 ng/ml (Lehrer et al., 1992). During ovulation a concentration of 0.75 ng/ml exists for 3 days (Kerr and McCaughey, 1984). A cow with cystic ovarian disease has a higher average concentration of 2.6 ng/ml. A three week pregnant cow has a concentration of 19 to 25 ng/ml (Gartland, Schiavo, Hall, Foote and Scott, 1976), whereas a non pregnant cow would be expected to have concentrations typical of oestrus 3 weeks after breeding.

Progesterone tests can be used to detect oestrus and may provide supportive evidence of early pregnancy, but disadvantages in using progesterone for these purposes are that it is expensive and labour intensive. Progesterone testing is more accurate in determining non–pregnancy than pregnancy, due to factors such as embryonic loss occurring later than the time of testing, which leads to false pregnancy diagnosis as shown in Figure 1.5 (Bloomfield, 1987) as well as other causes of high progesterone such as uterine disease, atypical oestrus cycle lengths and improper timing of sampling.

Methods for measuring progesterone concentration are:

Radioimmuno assay (RIA)

The RIA-test is done in a laboratory. The test uses a solid phase radioimmunoassay. Milk (0.1 ml) is poured into tubes coated with a specific progesterone antibody. A buffer with I-125 labelled progesterone is added and rinsed away after three hours. The samples are then measured in a gamma spectrometer (Houwelingen, van Stijnen and Strik, 1995).

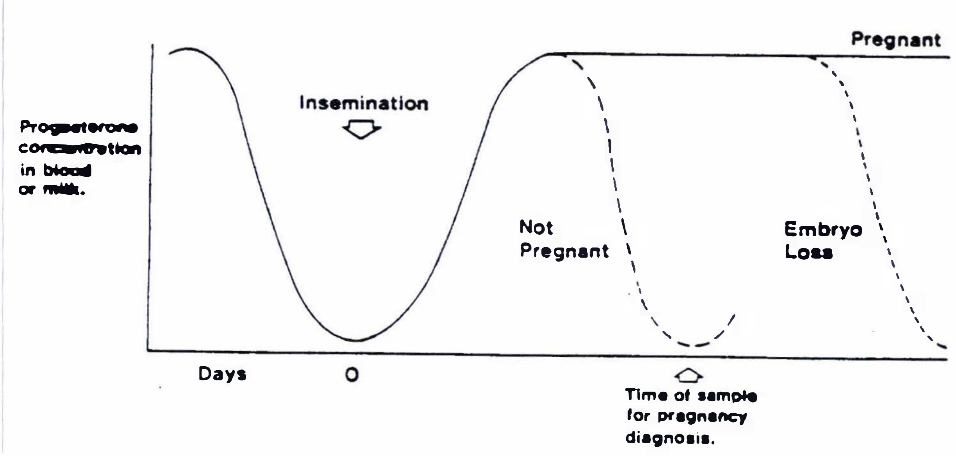


Figure 1.5: Use of progesterone measurement in milk for pregnancy diagnosis.

Source: (Bloomfield, 1987), p72.

Radioimmunoassay depends on the ability of an antibody to bind its antigen. In order to quantify the antigen; a radioactive antigen and a non-radioactive antigen compete for a limited number of binding sites on a specific antibody. As more non-radioactive antigen is introduced, less binding sites are available for the radioactive antigen thus yielding a method of quantifying the antigen (Anon, 1994).

In the RIA assay, a limited amount of specific antibody is reacted with the corresponding hormone labelled with a radioisotope. The addition of an increasing amount of the hormone causes a corresponding decreasing fraction of the added labelled hormone to be bound to the antibody. After separation of the bound from the free labelled hormone, the amount of the radioactivity is evaluated and used to construct a standard curve against which the unknown samples are measured (Anon, 1994).

The detection rate for early pregnancy with the RIA-test is 68.4% for day 19 and 83.8% for day 21. The detection rate for non-pregnancy is 84.6% for day 19 and 100% for day 21 (Kastelic, 2001). A sensitivity of up to 95.73% was reported and early pregnancy diagnosis accuracy of pregnancy and non-pregnancy of 89.4 and 100%, respectively (Cai, Xu and Xu, 2001).

Enzyme-linked immunosorbent assay test (ELISA)

Commercial test kits are available to detect oestrus on the farm. It takes 15 minutes to test a milk sample. Colour intensity is related to the progesterone concentration in the milk sample. ELISA-test has an accuracy of 100%, if a ≤0.5 ng/ml concentration is used to indicate oestrus (Houwelingen et al., 1995).

Procedures for performing ELISA milk progesterone tests are specific for each company producing a test but the principles of the assays are similar. The principles are the same as for RIA, but with ELISA an enzyme is used to label progesterone rather than a radioactive isotope. Some ELISA tests are designed to determine if the progesterone concentration is low or high rather than to provide a precise concentration as with RIA and the ELISA produces a colour reaction that depends on the conjugate-substrate employed. The assay utilizes the principle of competitive absorption of milk progesterone to an antibody specific to progesterone which in turn is coated on plastic tubes, then a progesterone enzyme conjugate is added (Alkaline phosphatase or β galactosidase,or horseradish peroxidase) which bind to the unbound antibody sites. Once that step is accomplished then the substrate and a developer is added and the amount of colour developed is inversely proportional to the concentration of progesterone in milk (Nebel, 1988).

Latex agglutination test (LA)

This test rapidly quantifies the concentration of progesterone utilizing progesterone monoclonal antibodies and latex beads coated with progesterone. Antibodies and progesterone coated latex beads are mixed together on a reaction slide. As the mixture diffuses through narrow channels, the latex beads and solution react with each other and produce a thin film. If the sample progesterone concentration is high, then progesterone antibody binding occurs and no agglutination resulting in a smooth film appearing in the test chamber. If the progesterone concentration is low, then the progesterone coated latex beads cluster, resulting in a grainy film in the slides' chamber (Nebel, 1988).

Progesterone assay may be done on milk samples collected from lactating cows from 21 to 24 days post-breeding and the non-pregnant pregnancy status of the animal identified. The accuracy of the test is 91–100% in detecting non-pregnancy and 67-88% for pregnant cows (Booth and Hollandsworth, 1976; Foote et al., 1979; Booth, 1980; Nebel, 1988).

Milk progesterone concentration can potentially be affected by many factors, such as the time of collection of samples, stage of the oestrus cycle, breed of the cow, storage of the samples and milk fat concentration. Jerseys have a higher concentration of progesterone in the milk than Brown Swiss, Guernsey and Holstein-Friesian cows. The samples need to be stored at 4 to minus 10 degrees and must be analysed within two weeks after collection (Keown and Duane, 1996).

The progesterone concentration of milk increases by 3ng/ml for every percentage of milk fat. Early in pregnancy the progesterone concentration is the same as during the luteal phase. The progesterone concentration subsequently increases until 200 days of pregnancy (Keown and Duane, 1996).

1.2.3.7 Movement detectors

A cow in oestrus tends to be more restless than usual. The increased movement associated with oestrus has been measured experimentally by means of pedometers (Kiddy, 1977). It is more difficult to observe oestrus activity in cows which are tied up, so the type of housing affects the degree of restlessness that can be displayed and observed. As freedom and comfort improve the degree of activity increases and provides an increased chance to detect oestrous cows.

The detection efficiency of pedometers ranges from 60% to 100% and when used in combination with observations, they increase efficiency and reduce false positive detections (Williams, Yver and Gross, 1981; Phillips and Schofield, 1988; Secchiari, Romagnoli, Mele and Ferramosca, 1998). Cohen, Eisenberg, Amir, Etam and Aizinbud (1990) reported an accuracy of 92% for pedometers. Lehrer et al. (1992) reported that observation for 60 minutes, four times daily or 30 minutes, six times daily is more effective than pedometry. Shorter observation periods or less frequent observations are less effective than pedometry.

The efficiency of pedometry for oestrus detection varies more between cows than between groups and it is much improved if supported by visual observation periods of 30 minutes or 1 hour, 1-3 times a day (Pennington, Albright and Callahan, 1986). Lehrer, et al. (1992) reported that, in detection of overt oestrus, pedometry is more efficient than two and as efficient as four visual observations per day (Table 1.6).

Roth (1987) pointed out that the preferable oestrus detection system depends mainly on the housing system. Pastured cows had 218% higher activity during the time that they were in oestrus than in other intervals (Farris, 1954). The activity of cows in free-stall or comfort stall barns during oestrus was 3.9 and 2.8 times respectively, greater than at other stages of the cycle (Kiddy, 1977).

Management plays a major role in the efficacy of pedometry and also oestrus detection of mounting behaviour. Minimizing crowding and providing a firm footing enhances mounting activity. The efficiency of pedometry varies from 60-100% with accuracy from a low of 22% up to 100%. Low accuracy results from high false positives, which are due to technical and managerial limitations and environmental conditions (Peter and Bosu, 1986).

Two commercially available activity meters are:

- *Cowtracker*®, manufactured by Bou-Matic, which is an activity meter attached to the cow by a neckband.

- *AfiACT*®, manufactured by Germania Dairy Automation, is a pedometer that is attached to the leg.

Oestrus detection 24 hours a day is possible with these devices. The pedometers and activity meters indicate when increased activity occurs. They can decrease labour costs if used properly. Pedometers can be integrated with electronic animal identification systems. Limitations of electronic pedometers are that increased activity is not always associated with oestrus and that they are expensive (Firk et al., 2002b).

1.2.3.8 Temperature measurement

The normal temperature of cows is approximately 38.6°C. Body temperature tends to drop one or two days before oestrus and to rise again by 0.1-0.5°C to a short-level peak on the day of oestrus (Boyd, 1984; Lewis and Newman, 1984;

Peters and Ball, 1987; Geers, Puers, Goedseels and Wouters, 1997). Such changes in body temperature can be monitored through sensors implanted in the ear or under the skin and these sensors transmit the body temperature on a continuous basis (Maatje and Rossing, 1976). The costs and the possible discomfort to the animal probably outweigh the advantages of using these devices.

Table 1.6: Efficiency / accuracy of oestrus detection using pedometers, compared with two or three visual observations per day.

Pedometers (efficiency/accuracy)	Visual Observation (efficiency/accuracy)	Reference
78/100 to 96/88	45	Pennington, 1986
76	35	Peter and Bosu, 1986
91/92	94/94	Cohen et al. 1990
76/56 to 87/22	60	Gauger et al. 1991
61/87 to 86/?	42	Liu and Spahr, 1991
91/87	46	Pulvermacher and Wiersma, 1991
96/95	81	Schofield et al. 1991

Source: (Lehrer et al., 1992), p3.

Detection of oestrus through milk temperature measurements became possible with modern electronics (Fordham, Rowlinson and McCarthy, 1988). Measurement of body temperature was found to be more possible and more practical through milk than by rectal temperature measurement (Boyd, 1984; Roth et al., 1987). A slight variation was found in the temperature between the quarters but the overall temperature of the milk was 0.14°C less than the rectal temperature (Schlunsen, Roth, Schon, Paul and Speckmann, 1987).

Firk et al. (2002b) reported oestrus detection with milk temperature had an efficiency of 50% and accuracy of 55%. This results in an unacceptable number of false positive detections. Boyd (1984) pointed out that an increase of body temperature is not a specific indication oestrus. Systemic and local inflammatory reactions might also cause increased body temperatures and environmental temperature also had a greater influence on body temperature than did oestrus (Lewis and Newman, 1984). The housing system also affects the body and milk temperatures markedly (Firk et al., 2002b).

Accurate and timely temperature measurements that reflect the animal's status, require consideration of the way that the temperature is taken (rectally, via implants or through milk temperature). The environment, the location of the implants, the variation in temperature of different quarters or the housing of the animal can also influence the efficacy of temperature based automated oestrous detection. Brehme, Ahlers et al., (2001) reported that the temperature in an implant was 1.1-1.6°C higher than the rectal measurement, with a large fluctuation (±0.65°C) of temperature in the animal at pasture and approximately 0.45°C change in body temperature during immobility or lying activity (Metz et al., 1987; Brehme, Scherping, Stollberg and Warner, 1997).

These variations and the direct effect of the environment, housing system and other factors such as animal status, make this method impractical for oestrus detection (Metz et al., 1987; Firk, Stamer, Junge and Krieter, 2002a).

1.2.3.9 Odours

Pheromones produced at the time of oestrus are sensed by and attract bulls. These odours are found in milk, urine, blood and cervico-vaginal mucus where they are believed to be the strongest (Kiddy, Mitchell and Hawk, 1984).

Dogs trained to sniff samples of mucus have been used with considerable success in the USA, achieving 80% correct identification with 6% false positives (Kiddy et al., 1984). Later work using swabs of mucus, urine, milk and blood plasma reported a higher response rate of 90+% with 8% of false positives. The use of dogs for oestrus detection may be convenient but training is expensive and difficult due to variations between different animals (Lake, 1987).

1.2.3.10 Vaginal resistance measurement

During oestrus there is an increase in the volume and the ionic content of vaginal mucus that makes it better able to conduct electricity. A fall in electrical resistance normally lasts for no more than 24 hours. Readings vary from one cow to another so repeated observations on individuals are required to identify increases in conductivity (Peters and Ball, 1987). Since routine daily oestrus detection with a probe is required this technique becomes impractical and is liable to cause inflammation that in it self can alter conductivity measurements.

Cysts, ulcers and inflammation were found to affect the electrical resistance of vaginal mucus (Boyd, 1984). The conductivity of vaginal mucus is influenced by several non-oestrus parameters which makes it unsuited to routine oestrus detection (Brehme et al., 2001).

1.2.3.11 Synchronisation

Oestrus can be synchronised by shortening the luteal phase with $PGF_{2\alpha}$ or by extending the luteal phase artificially with progestins (Richardson, Hensley, Marple, Johnson and Stevenson, 2002). Intravaginal progesterone releasing vaginal inserts (CIDR-B, Pfizer Animal Health, Auckland, New Zealand) produce tight synchrony of oestrus after treatment for 6-10 days (Xu and Burton, 2000a).

Different oestrus synchronisation techniques have been described for use in cattle to enable oestrus detection and insemination over a restricted time (Folman, Kaim, Herz and Rosenberg, 1984; Ferguson and Galligan, 1993; Hanlon, 1995). This has a favourable effect and reduces the percentage of non-pregnant cows (Ferguson and Galligan, 1993; Hanlon, 1995).

When oestrus detection efficiency is low, an opportunity to improve efficiency is provided by synchronisation of the occurrence of oestrus in the cows. Synchronisation may start 35 days before the breeding period. The cows get two injections of prostaglandin $F_{2\alpha}$ which then stimulates regression of the corpus luteum (Macmillan, 1972). When cows are synchronised, increased oestrus activity can be predicted thus allowing farm staff to concentrate their oestrus detection effort in that period or all cows can be inseminated by appointment without reference to oestrus (Hanlon, 1995; Dransfield, Nebel, Pearson and Warnick, 1998).

1.2.3.12 Closed circuit television

Given suitable confined housing, or a loafing area where sexually active cows can be relied upon to congregate, it is effective to view cows in oestrus using a closed circuit television. Activities can be recorded then played back at high speed and slowed to identify cows which are in oestrus.

Disadvantages of this system include problems with the correct identification of cows in oestrus and the fact that this application is only possible when cows are held in free stall barns (Boyd, 1984).

1.2.3.13 Teaser animals

Teasers are animals which take an interest in an oestrus cow and in so doing, help to make the stockman aware that the cow is in oestrus. A common drawback of the various types of teasers is that they tend to cultivate favourites among oestrus cows and may completely ignore others that are also in oestrus (Peters and Ball, 1987).

The use of androgenised cows has several advantages, including safe presence, facilitate more mounting of oestrus cows and reduce false non-oestrus cows detection (Nix, Spitzer and Chenoweth, 1997). Another advantage is their availability, since an androgenised cow can be induced by administering testosterone to cull cows that then become sexually active. Dairy farms with a small number of cows benefit most from teaser oestrus detection animals because the number of cows in oestrus at one time is small. If there is a bull on the farm it is helpful to house him near the cows. A cow in oestrus is likely to seek the bull and thus identify herself (Peters and Ball, 1987).

The use of chin-ball marking devices (CMD) in combination with teaser animals enables oestrus detection without constant observation. A CMD is a halter with a built-in freely rotating ball-pen containing an ink or marker unit. The CMD is worn on the chin of teaser animals. When the teaser animal mounts a cow, the CMD makes a mark parallel to the vertebrae of the mounted cow (Nebel, Dransfield, Jobst and Bame, 2000). The cows should be examined for mount marks twice a day.

Stevenson and Britt (1977) showed that androgenised cows gave a detection efficiency of 70% while Kiser, Britt and Ritchie (1977) reported that androgenised cows gave a high detection percentage when combined with visual observations and were as efficient as surgically altered bulls. Foote (1975) highlighted that the use of male teasers is more reliable than androgenised cows as they provide a positive identification if equipped with a marking device which will provide more accurate identification. It was previously claimed that their presence would stimulate the onset of oestrus (Skinner and Bonsma, 1964).

Fulkerson et al. (1983) reported an oestrus detection efficiency of 88% with twice daily observations in combination with hormone-treated steers with chin ball markers. The use of hormone-treated steers is most effective in combination with behavioural observations. McCaughey and Martin (1980) reported an accuracy of 95% using epididymectomised bulls in a herd for periods of 30 minutes several times a day. Stevenson and Britt (1977) reported an efficiency of 52% and an accuracy of 79% using a testosterone-treated heifer with chin-ball marker in 88 Holstein cows.

1.2.3.14 Tail paint

Tail paint is the most used oestrus detection aid in New Zealand within seasonal dairy herds, especially on farms with more than 300 cows. This aid increases the average pregnancy rates by reducing the incidence of detection errors (Macmillan, 1972; Williamson, 1980).

Tail painting indicates standing activity and can also be used to detect silent ovulation and non-cycling cows (Ducker, Haggett, Fisher, Bloomfield and Morant, 1983; Dransfield et al., 1998). Advantages of tail paint as an oestrus detection aid are that the paint is inexpensive and there is 24-hour monitoring of mounting activity. Challenges are the occurrence of many false positive detections, a requirement for mounting and the labour costs of painting and observing cows (Macmillan, 1972; Williamson, 1980; Ball, Cowpe and Harker, 1983; Kerr and McCaughey, 1984).

Tail paint provides a cheaper alternative to other methods, which can be effective under suitable conditions (Peters and Ball, 1987). Experiments in commercial and experimental herds showed that tail paint correctly identified significantly more oestrus periods (81.5 %) than did stockman (70.2 %) during their routine observations (Ball et al., 1983). It also aided in identifying the animals during the night and it was simple to apply (Ridler, 1976). A detection efficiency and accuracy of over 90% was reported when using tail paint as an aid for oestrus detection (Cavalieri, Eagles, Ryan and Macmillan, 2003a).

Ball et al. (1983) and Ducker et al, (1986), reported an unacceptably high incidence of tail paint being rubbed off at the wrong time. The efficacy of detectors, paint or paste will vary with herds under different housing and management systems. Falsely detected oestrus occurred mainly when many cows were in oestrus or the marked cow rubbed herself.

Although the tail paint technique of oestrus detection relies on secondary signs of oestrus (caused by mounting behaviour) rather than direct observation, it is an effective method of detecting oestrus in synchronised heifers (Hanlon, 1995). Macmillan et al. (1988) found that only 0.8% of synchronised heifers ovulated without being detected in oestrus using a modified tail painting technique for oestrus detection. A combination of tail paint and visual observation resulted in a detection rate of 98.4%, with 2.4% of falsely detected oestruses (Xu et al., 1998).

1.2.3.15 Oestrus mount detectors and pressure-sensing radio-telemetric devices

The obvious clear sign of sexual receptivity in the cow is standing to be mounted. To observe this behavioural sign is a management challenge as it occurs during only 1% of the time period during oestrus (Pennington et al., 1986), making it exceptionally difficult to detect unless sufficient dedicated time is employed to record such an event or visual observation is supplemented by employing a device that can record the event.

KaMaR™ oestrus detectors are pressure sensing patches fitted on the sacrum or tail head of the cow and secured by glue. Each consists of an outer white tube and an inner phial of red dye which is discharged when subjected to pressure from a mount lasting for three or more seconds (Esslemont et al., 1985). The KaMaR™ mount detector devices were found to increase the efficiency of oestrus detection to up to 90% (Williamson et al., 1972) compared to 56% without devices.

Matemaster detectors were produced by Delta Plastics (Palmerston North, New Zealand). This detector was a plastic device with a dye-filled reservoir attached to a 2x8 cm extension. It was glued to the tail head. Pressure exerted by a mounting cow forced the dye from the reservoir into the extension. More than 4cm dye displacement was necessary for accurate oestrus detection.

A study was done to evaluate the effectiveness of oestrus detection using KaMaR™ (K) and/or Hot Flash (HF) rump-mounted detectors and androgenised cows (AC). Accuracy for detecting standing oestrus, based on low milk P4, was 97.4% for control animals detected with the help of androgenised cows, 87.9% for K and 81.1% for HF (Gwazdauskas, Nebel, Sprecher, Whittier and Mcgilliard, 1990). Using the KaMaR™ mount detector with a teaser bull, the efficiency of oestrus detection averaged 100% vs. 71.4% using the bull alone (Huang, Chen, Poun, Young and Huang, 1987). Another study was conducted to determine the efficacy of some aids (tail painting, KaMaR™ detectors and a chin-ball mating device (CMD)) for detecting oestrus in 72 synchronised Bunaji cows. Unaided visual observation, tail painting, KaMaR™ and CMD detected 52.2, 82.6, 82.6 and 76.8% of oestruses (Mai, Ogwu, Eduvie and Voh, 2002).

Despite the relative success of oestrus mount detectors, an obvious disadvantage is the cost of their use (Foote, 1975). Esslemont et al. (1985) estimated that the KaMaR™ patches are approximately seven times more expensive than tail paint. Another disadvantage for oestrus mount detectors was their loss, as reported by Williamson et al. (1972) which was 26% and more than 40% in longer studies (Gwazdauskas, Lineweaver and McGilliard, 1983). The increase of false positive detectors in close confinement is another disadvantage (Donaldson, 1968; Foote, 1975) which can lead to some interpretation problems (King, 1991).

A commercial electronic oestrus detection system (HeatWatch™; DDx Inc., Denver CO, USA) has been available for several years. A radio-telemetric sensor attached to each cow consists of a plastic case (5.3 x 8.1 x 1.8 cm) containing a pressure sensor, a miniature radio transmitter and a lithium battery.

Each device is secured in a water-resistant pouch, attached to a 35 x 20cm nylon mesh patch that is glued with contact-type (e.g. back tag) adhesive to the hair of the tail head region. When a herd-mate mounts and activates the pressure sensor for a minimum of 2 seconds, a radio wave is produced, indicating sensor identification, date, time and duration of sensor activation. Transmitted signals are received by an antenna (within 400m of the sensors) and sent to a buffer. The buffer is either attached to a microcomputer or to a printer. When attached to a microcomputer, the software generates both fixed reports and individual cow files. Two management lists are generated: 1) Suspect oestrus (cows receiving <3 standing events within a user-defined time interval); and 2) Standing oestrus (cows receiving >3 standing events within a 4 h interval (Kastelic, 2001).

Xu, McKnight, et al. (1998) reported on a study that lasted for six weeks during a breeding season in New Zealand, where the efficiency of oestrus detection using the HeatWatch™ system versus visual observations/tail paint was 91.7 and 98.4 respectively ($p \leq 0.05$) with an accuracy of 100 and 97.6% (not significantly different). It was noted that 5 to 10 percent of the oestruses were not detected in the HeatWatch™ group due to the premature detachment of the transmitters from the cows (Kastelic, 2001).

Cavalieri and Macmillan, (2002) reported on a study to measure the agreement between the characteristics of oestrus when measured using visual and radio-telemetry methods. Despite the high efficiency of both methods, radio-telemetry was found to be affected by a lag-period. This means that if the intensity of mounts increases the accuracy of recording the characteristics of these mounts decreases because of the lag-period which makes the system sometimes impractical.

This technology is indicated in herd that has a high proportion of non-pregnant cows but is very expensive to apply on the entire herd. It also has a high maintenance cost and labour input. To reduce these costs, the same company introduced the Mountcount™ system that is a manual version of the HeatWatch™ sensor unit that requires manual reading and evaluation (Kastelic, 2001).

1.2.4 The application of electronics to cattle management - in brief

The introduction of automation and electronic technology in dairying, such as on-line animal data capture and processing, requires modification of management methods. It is expected that the number of cattle farms will continue to decrease with an associated increase in herd sizes. Husbandry methods will therefore become more intensive which will prompt the development of new automated means that the farmer will employ to supervise and control herd production and profit (Lydehoj, Hagelso, Northeved, Nilsson, Jensen and Staun , 1983).

Automation means the control of processes, data collection and analysis into useful information to assist management. In several countries the following elements are used in on farm computer systems (Postma, 1983):

- Cow Identification
- Daily milk yield recording
- Automatic milking sampling
- Measuring the cow's body temperature
- Detection of defective milk quality
- Feed ration calculations
- Isolation of cows
- Individual concentrate feeding
- Stock-keeping
- Recording body weight
- Animal activity
- Attention lists for production and health management
- Life history
- Production outline
- Financial figures

Farmers will need to be provided with better tools to control and manage herds. These tools and control technology have been applied in five main areas of cattle businesses so far, as illustrated in Table 1.7 (Cobben, Mouthuy and Jaspar, 1987).

Table 1.7: Application of control electronics to the dairy and beef cattle industries.

Area	Application
Environmental control	Temperature, humidity, air velocity, CO2 content.
Animal health	Pulse rate, blood pressure, body and milk temperature, milk conductivity, respiration rate.
Nutrition	Food intake, rumen pH sensor, weight, milk quality (protein, fat), stool content (pH).
Fertility	Oestrus detection, pregnancy detection, calving monitors.
Production control	Weight, meat fat content, milk quality (protein, fat).
Integrated management systems	Sensors, Identification, systems computers.

Source: (Cobben et al., 1987), pp 149-150.

1.3 Purpose of this study:

An extensive literature search into oestrus detection methods revealed that a practical and efficient method for farmers, which provides a high degree of accuracy with minimal cost and effort, is yet to be found. The goal of this study was to develop an automated system of oestrus detection building on the widely utilised tail paint technique that can detect oestrus effectively and accurately. The developed system must be compatible with automated milking systems.

A desired system will save time and labour during the mating season and will ensure that cows in oestrus are identified, thus enabling dairy farmers to introduce high quality genetics to their herds by using semen from proven sires. Such a system will also provide accurate information on the reproductive status of the herd to farmers and veterinarians.

Thus the problem statement for this research is: oestrus detection in New Zealand dairy farms is limiting the productive performance among dairy herds. With the shift towards more automatic milking systems, the need for an automated system using an improved oestrus detection method is now required.

The null hypothesis to be tested in this study states that:

An automated tail-paint reading technique to detect oestrus in cows operates with the same accuracy and efficiency as traditional oestrus detection.

1.4 Objectives

The objectives of this research are to:

Develop a system that can efficiently detect previous mounting activity using an enhanced tail-paint technique and image analysis.

Develop an image analysis system that reliably detects the tail-paint based mounting detector device and accurately measures tail paint removal.

Automate the reading of the tail-paint oestrus detection technique using an imaging system and a computer to assess and interpret the image. The system must accurately detect oestrus depending on the automated sensing of the amount of paint removed from a reflective strip placed over the sacral region of cows during the breeding season, in order to increase the efficacy of oestrus detection and minimise cow wastage in New Zealand.

Chapter Two

Testing the accuracy of a camera-software device and modification of oestrus detector strips.

Abstract

This chapter describes the camera-software device (CSD) and oestrus detector strips (ODS). This model has extended the technique of tail painting and modified it by using painted reflective strips, so that a CSD can automatically detect, read and interpret it optically. Two cameras (Sony™ SX900, Intel™ web cam) and a range of paints and reflective strips were tested initially in a laboratory and then in the field. Reflective strip durability was also tested. A video image processing software (VIPS) was modified so that within VIPS a script was developed to automatically identify the presence of a potential ODS, based on a threshold of light intensity in the captured image being exceeded. Correct adjustment of the reflected image of the ODS allows the correct identification and measurement of contaminated indicator and painted areas. Three pilot trials were conducted to obtain insights about ODS composition and their desired location on the cows' rumps, camera compatibility, proto-type VIPS program, model accuracy and to test the efficacy of the glue used under prevailing environmental conditions. The Sony™ camera has more powerful features and gave a more accurate interpretation of ODS status than the Intel web cam. It was found to be accurate for reflective areas ranging from 0% to 100% of the total strip areas. Zylone sheen paint was the most suitable of the paints tested to apply on the ODS for oestrus detection. The location where the ODS should be applied, is on the sacral region of the cow, as it was found to be a point of acceptable mounting intensity. The Ados F2 contact adhesive was found to have satisfactory adhesive powers after preliminary field testing. The Sony camera didn't need any adjustment to enable recognition or accurate measurement of the area of paint removal from the ODS placed over the sacral region of the cows rump using Ados F2 glue and direct identification and evaluation by the CSD using these components proved to be satisfactory.

KEY WORDS: Camera-software device, oestrus detector strips, tail paint, automation

2.1. Introduction

Inadequate oestrus detection can cause major financial losses on intensive dairy farms through increased cow wastage, reduced submission rates and wasted artificial inseminations. Thus, an easy and effective oestrus detection aid is a potentially important technology to provide assistance in these production systems. The importance and effect of oestrus detection and benefits of oestrus detection aids have been reviewed in chapter one.

Oestrus detection aids should be accurate, have low running costs and labour requirements, yet be able to serve and function in large pastoral farms. This study has extended the technique of tail painting and modified it by using painted reflective strips, so that a camera-software device (CSD) can automatically detect, read and interpret it optically.

In a previous study (Williamson and Butler, Unpublished Data) a suitable reflective strip and a paint combination that was readily detected optically was investigated. A range of paints and reflective strips were tested. The paints on the reflective strips were initially tested in a laboratory and then in the field. The paint was painted on the strips and once dry it was repeatedly scratched with a screwdriver and rubbed forcefully in wet and dry condition to test its durability.

Williamson and Butler (Unpublished Data) tested the reflective strip durability under field conditions and both Zylone sheen paint (water based low sheen acrylic, Resene Paint Limited, Palmerston North, New Zealand) and 3M Scotchlite reflective strip (3M 9920, 3M New Zealand Ltd, Auckland, New Zealand) were selected. The reflectivity and measurement of the pilot oestrus detection strips, both intact and after being rubbed from cows' mounting behaviour were captured by a video-computer link on a dairy farm, then measured in a laboratory using a black and white CCD camera and video image processing software (VIPS).

The version of the VIPS program and CCD camera used initially caused difficulties in automatically identifying the reflective strips and measuring the amount of paint removed from the oestrus detector strip (ODS), especially when the strip was contaminated with faeces.

The camera-software device (CSD) is based on the use of tail paint with modification and technological extension to allow automatic reading and interpretation. The automation is based on the optical detection of the oestrus detection strips and an assessment of their condition. This technique offers great potential as a means to automate oestrus detection. There are potentially two places where errors can occur when using this technique in oestrus detection. Firstly, the percentage of paint removed from the ODS when an oestrous cow is mounted may not accurately reflect the degree of oestrus behaviour. Secondly, the measurement of the paint removed and interpretation applied by the software used in the process may have inherent errors.

To address the first point, a field trial was conducted in the spring breeding season of 2003 at Massey University dairy unit 4, Palmerston North to determine the efficacy of ODS in detecting oestrus in cows. The study is detailed in chapter three. To test for potential sources of error in the camera software system, a trial was conducted in winter 2003 at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University Palmerston North. This study is a major part of this chapter.

This chapter also details additional investigations extending the previous work on the reflective strip, paint and the use of different paints to test their durability and suitability for the intended purpose of the study under field conditions. The chapter also details an investigation into the development of reflective strip to enable it to be detected and measured accurately using 2 digital video cameras (Sony™ SX-900; Intel web cam™). Both of these cameras support video streaming and still-image capture at a resolution of 640x480 pixels and a variable lens view angle.

The objectives of this chapter are:

To describe and discuss key components of the CSD system and the mechanisms of its function and data processing.

To obtain insights about the oestrus detector strips and the paint used as a part of them. Also to test the proto-type image analysis software (VIPS), designed to automatically detect, read and interpret painted ODS. Further aims were to identify the location of the strips on the cows' rumps that provided the most *reliable results and to test the efficacy under environmental conditions of the glue used to attach strips.*

To test the accuracy of the CSD in measuring known and controlled paint removal from ODS and to set limits to assist in their interpretation.

2.2. ODS and CSD model

2.2.1. Oestrus detector strips (ODS)

ODS comprise a reflective tape (3M 9920, 3M New Zealand Ltd, Auckland, New Zealand) and Zylone sheen paint (water based low sheen acrylic, Resene Paint Limited, Palmerston North, New Zealand). Selection of these materials was made according to their light reflection characteristics, flexibility and durability.

2.2.1.1. The reflective tape

The reflective tape (Figure 2.1) comprises three layers (Figure 2.3), a fabric layer, reflective layer and reflective surface protection layer. The fabric layer acts as the skeleton for the reflective strip and should be flexible and enduring plus attach well by the adhesive material applied to secure the strip to the cow's rump. The reflective layer reflects light largely towards the source of light exposure (retro-reflection). This helps minimize distortion from extraneous light sources and maximises the intensity of the applied light that is reflected. The third layer is made of transparent non-reflective material to protect the reflective layer from being worn to minimise unfavourable effects on the reflection criteria. The reflective strips are rectangular with dimensions 150mm long by 50 mm wide. The advantage of this shape will be explained later in this chapter.

2.2.1.2. The paint

The search for the ideal paint to cover the reflective strip was carried over from the primary work of Butler (Unpublished Data). The paint is required to have a low light reflection index, durability so that it does not rub off easily, flexibility with movement and to possess reliable adhesion to the surface it is applied to so that it does not peel.

Zylone sheen paint (water based low sheen acrylic, Resene Paint Limited, Palmerston North, New Zealand) was selected. A variety of other paints was also investigated as will be described in section two of this chapter. The physical properties of Zylone paint are shown in Table 2.1.

2.2.1.3. Oestrus detector strip characteristics

The paint is applied to cover most of the reflective area of 100mm x 50mm on the strip leaving reflective areas of 50mm x 25mm at both ends of the strips that act as indicators in the process of optical detection by the camera-software device (Figures 2.2, 2.3).

The amount of paint applied is just sufficient to block the reflection (thin film of paint) of the strip (Figure 2.2) and does not interfere with strips' flexibility when applied to a cow's rump, as movement may occur at the desired location. Painting of the ODS is preferably over 24 hours before strip application to cows, although the paint dries within two hours.

An ODS is secured to a cow's rump using Ados F2 glue (CRC industries New Zealand Ltd, Auckland, New Zealand). This glue was previously tested amongst a variety of commercially available glues and was found to provide superior adhesion for the required purpose (Williamson and Butler, Unpublished Data). A glue layer was added to the cows and ODS before application. Prior to the application of glue to the cows' rumps, these areas were carefully brushed and cleared of any loose hair or debris to achieve optimum adhesion.

In the process of optical identification, indicators at the ends of the strips act as markers that indicate strip presence and enable the software associated with the CSD to identify the ODS within the image, when the intensity of the reflected light from the indicator area exceeds a threshold level. Also the shape of the ODS differentiates it from the cow's hair surrounding it, or other objects in the environment, especially those that are capable of reflecting light. The software identifies the strip by its shape and aspect ratio of height (H) x width (W) which should be 3 X 1.

Table 2.1: The physical characteristics of Zylone sheen paint.

Criterion	Comments
Vehicle type	Novel high polymer combination
Solvent	Water
Finish	Low sheen
Dry time	45 min at 18 °C
Dry film thickness	34 microns at 11 sq. metres/litre
Heat resistance	Thermoplastic
Chemical resistance	Good
Abrasion resistance	Very good
Solvent resistance	Fair
Toxicity	Non-toxic (Dry Film)
Durability	Excellent
Thinning and cleaning up	Water
Performance	Easily cleaned, burnish resistant, clean splatter free application

min= minutes

Figure 2.1: Oestrus detector strip (ODS) reflective strip (3M Scotchlite reflective strip tape 9920, 3M Auckland, NZ).

Figure 2.2: The structure of an oestrus detector strip.

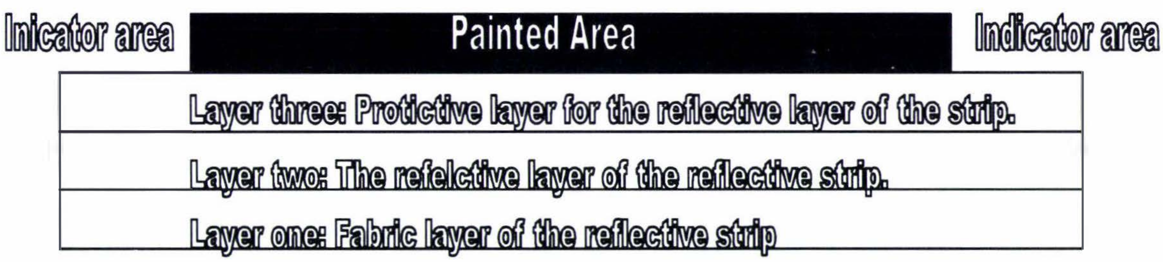


Figure 2.3: Cross section of an oestrus detector strip.

2.2.2. Camera and light source

The specification for a camera to suit the purposes of this study was based on it having a lens view that covered the area of interest on the cow's rump. The resolution and image definition should have a minimum resolution of 640 x 480 pixels for sufficient clarity in identifying the ODS and determining paint removed. The camera should also be capable of frame image capture while video streaming.

Initially an Intel™ web camera was used because of its low cost. While this camera performed adequately in the laboratory, several problems were experienced during field tests. The first was that the web camera had a relatively wide angle lens and low depth of field that potentially would cause problems because of the variability in the height of cows. For optimum measurement with a wide angle lens, the camera to ODS distance is critical and this could not be maintained within a suitable range. A second problem with the web camera was that the true resolution was actually less than 640 x 480 pixels which reduced the accuracy further. A third problem resulted from a bug within the camera's software driver that resulted in image capture becoming unreliable after about one hour of operation.

A Sony™ firewire camera (Sony™ SX-900) was then trialled. This camera has a separate lens so a more suitable focal length can be selected. Although the camera has significantly higher resolution than necessary, this helped in the trials because it enabled digital zooming to adjust the apparent focal length in the software, allowing optimum image clarity and facilitating identification of the ODS. The field of view covered by the settings and the lens is 1500mm x 1500mm which adequately covers the amount of free movement that a cow has in the milking parlour and the individual variation in size between cows.

The ODS was illuminated by a wide angle 40W tungsten halogen light, positioned to cover the view area. This exposed the ODS to a medium intensity light source which overpowered reflected light from the environment and minimised distortion of reflected light. The light source was mounted perpendicular to the ODS resulting in even exposure of the ODS to the light which facilitated strip identification by ready recognition of the indicator areas.

Image capture was set up (in the field) to capture three successive frames per cow. This enabled the system to avoid missing the identification of an ODS on cows standing on a rotary milking platform which could result from platform movement and the variation between the cows in terms of their heights and widths. The camera was connected using a cable via a firewire card into a computer (NEC Versa M320). Utilizing the firewire card, a single frame image could be obtained from the camera. Images were analysed to interpret if they were images of ODS adhered to the cows. Images of the ODS (intact and with paint removed) could be identified and interpreted using VIPS software. The setup of the automated oestrus detection system used is shown in Figure 2.4.

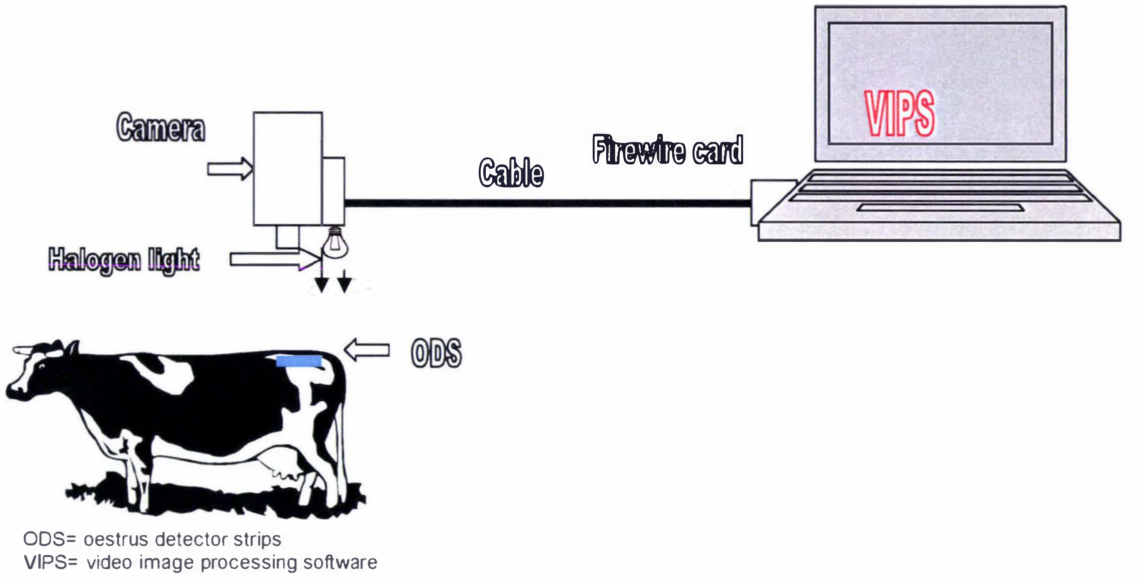


Figure 2.4: Camera-software device (CSD) component setup.

2.2.3. VIPS (Video Image Processing Software)

VIPS is an image analysis software program written by Dr. Donald Bailey (Institute of Information Science and Technology, Massey University) (Bailey and Hodgson, 1988). Within VIPS a script was developed to automatically identify the presence of a potential ODS, based on a threshold of light intensity in the captured image being exceeded.

When the ODS passed under the camera and the image was captured, the data was transferred to VIPS for analysis. When the light hits the ODS, the strip indicator areas reflect the light with highest intensity and appears as the brightest part in the image field. The automatic threshold setting of light intensity is necessary to discriminate strip identification and measurement in the presence of contamination such as dirt and manure. Correct adjustment of the reflected image of the ODS allows the correct identification and measurement of contaminated indicator and painted areas. Figure 2.5 shows examples of the level of contamination from dirt and manure that could affect the accurate reading of an ODS.

The Intel web camera had automatic gain control which automatically compensated for variations in light levels. The Sony camera had a fixed aperture and exposure, which were manually set close to optimum values for ODS detection. To compensate for variations in reflection, the VIPS software automatically adjusted the threshold level based on the image. The automatic threshold setting to identify the ODS operates by analysing the intensity histogram of a successfully captured image and then finding an optimum segmentation or separation point to separate the exposed reflective ODS part from the rest of the image, which then leads to potential ODS recognition for further identification.

The bright areas are then analysed for their shape to determine if they conform to those of an ODS, based on the area and aspect ratio. To allow for some distortion in the ODS (the rump of the cow is not flat and may not be perpendicular to the line of sight of the camera) the aspect ratio must be in the range 2-4.

Once the ODS is identified, the program eliminates the indicator areas by subtracting their areas from the total ODS image area. The remainder is a known area and the software then measures the area of the reflective strip from which paint has been removed (if any paint has been removed) as a proportion of the total painted area on the ODS (Figure 2.2). An advantage of measuring the exposed area as a proportion of the total painted area instead of as a value in mm^2, is that the camera does not need to calibrate for the variable camera distance above the cow for each ODS image as the area ratio remains correct independent of the height of the cows.

2.2.4. Experimental laboratory and field setup of ODS and CSD

A model of the sacral region of the cow was made to test the efficacy of the imaging system and computer based recognition and analysis system under various conditions. The model used was made from a paper cast using white glue over a skeleton of an adult cow to give the approximate shape of the sacral region of the cow. As the cast was dried, it was filled with foam (Gorilla® foam, Holdfast New Zealand Ltd, Hamilton, New Zealand) to give support and to maintain the shape of the cast. Finally, a piece of rug with long surface fibres dyed black and white in a Holstein cow hair pattern was glued over the cast to give it a realistic appearance.

The experimental setup shown in Figure 2.6 was used to obtain the images. ODS were placed over the cast in the area of the sacral spine. The camera was placed vertically approximately 1500 mm over the ODS at a 90° angle. The scene viewed by the camera which was mounted on a camera tripod to secure the required heights, was illuminated as described in section 2.2.2. In the field setup, it was impossible to position the camera at a 90° angle to the strips because of a pipe that was positioned exactly over the ODS location and which obscured the upper part of the ODS. This hindered the detection of the ODS. The camera setup was therefore adjusted off the perpendicular to allow complete exposure of the ODS to the camera and light source, thus allowing its identification and interpretation by VIPS. Images were captured as described in section 2.2.2.

2.3. Investigation of the most suitable paint type, location of ODS and accuracy testing of the CSD

2.3.1. Trial one: a preliminary trial of automatic detection of the ODS.

2.3.1.1. Purpose of the trial

This trial was conducted in order to obtain insights about ODS and the paint used as a part of them. It also was conducted to test our proto-type computer program (VIPS), designed to automatically detect the ODS and to measure the paint removed from them. Further aims were to determine the desired location of the strips on the cows' rumps and to test the efficacy of the glue used under prevailing environmental conditions.

2.3.1.2. Material and methods

This experiment was performed at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Veterinary Large Animal Teaching Unit (VLATU), Palmerston North campus. Heifers (n=18) that were 16 months old and were of mixed breeds, (Friesian, Hereford, Friesian-Hereford and Angus) were used. The heifers were grazed together at pasture and were provided with supplementary hay to cover their daily feed requirements. They had free access to water. They were monitored before use in the trial for their oestrous cyclic behaviour and all were confirmed to be cycling regularly and normally.

The ODS were prepared as described in section 2.2.1.2-3. On the day of ODS application, the lumbo-sacral area of the cows was brushed to remove any excess hair or other debris to ensure a clean adhesion. Ados F2 glue (CRC industries New Zealand Ltd, Auckland, New Zealand) was applied on the ODS and the lumbo-sacral area of the cows and when tack dry, ODS were adhered to the cows. The cows then were released to pasture for 21 days and were visually observed twice daily, at 6 am and 6 pm for 30--45 minutes.

Records were made of any primary oestrus signs (being mounted), which were graded as positive and secondary signs (mounting other cows, sniffing and licking of the vulva) which were graded as suspicious. Animals that had a percentage of paint removed and were not confirmed by primary or secondary signs of oestrus were considered as not being in oestrus.

Replacement with a new ODS occurred when an ODS was missing or showed paint removal, regardless of whether the cow had been observed in standing oestrus. A visual estimation of the worn portion of the painted area on the ODS was done to evaluate the location and shape of the paint removed and then the percentage of paint removed was estimated using VIPS software and the Intel™ web cam in the field and then repeated in the laboratory.

2.3.1.3. Results

Table 2.2 below summarises the oestrous behaviour and the efficacy of the painted strips in detecting oestrus in cows. It also shows that the glue utilised had the desirable adhesion properties.

Fourteen of ODS were confirmed by visual observations as indicating cows being in oestrus. Of the positive ODS, 7 were associated with positive visual observation of mounting behaviour and equivalent VIPS estimation of worn paint between 1-10%, 4 had 11-50% of paint removed and 3 had 51-100% of paint removed. In cows showing only secondary oestrus signs, 1 had 11-50% and 1 had 0% of paint removed. The same percentage was found for the category that was not detected by visual observations. This indicates that most of the oestruses showing standing or mounting behaviour had less than 50% of paint removed. No strips were lost or needed to be re-glued throughout the 21day trial period and at the time of collecting the strips, the glue's adhesive strength remained excellent.

2.3.1.4. Discussion

The initial test of the paint and the camera/VIPS program in experiment one revealed problems that needed to be overcome. These problems were:

2.3.1.4.1. Paint thickness over the ODS

It was obvious that ODS with thicker paint layers were worn off when the cow was first seen to have been mounted. Those with a thin paint layer took a longer time for the paint to wear off and the paint was not removed so readily.

ODS that had an extremely thin layer of paint could not be differentiated by the program from ones that had already had paint worn off, as the reflective surface of the strip appeared through the paint, misleading the program (Figure 2.7).

2.3.1.4.2. Location of the ODS

The strips needed to be placed on the sacral area of the cows closer to the caudal end, rather than the lumbar end as the paint was worn mainly from the caudal end of the strips (Figure 2.7 red circle). The positioning of strips may explain the high number of ODS having a low percentage of paint removed and the 2 ODS from suspected and negative cows without positive oestrus detection, especially in the absence of a reliable confirmatory gold standard such as a measure of milk or blood progesterone. Another possible reason for non-detection of oestrus is that the intensity and duration of oestrus is lower and shorter in heifers than that in mature cows, preventing oestrus detection by the ODS and reducing the chance of observation of the cows with a short oestrus display.

2.3.1.4.3. Gloss of the paint

The paint is a low sheen paint, which means that it has low degree of gloss. The gloss was enhanced when mounting occurred and when part or none of the paint was removed. The gloss at times confused the program because it increased the reflectivity of the paint even when paint was not removed from the ODS and was interpreted as removed paint on occasions.

2.3.1.4.4. The camera

The camera used in this study (Intel™ web cam) was unable to consistently distinguish removed paint from the enhanced glossiness due to mounting which lead to errors in reading the paint and the strips.

The problems that were encountered in experiment one directed the course of the research at that stage to further investigate suitable paint. A variety of paints was chosen, based on the characteristics of the first paint. Desirable characteristics identified were an ability to resist environmental conditions and to provide elasticity with a low degree of glossiness. A camera with improved capabilities and a higher resolution to enable the program to identify the strips and determine the area of paint removed in order to obtain accurate readings with low error was also shown to be desirable.

Table 2.2: The efficacy of the painted oestrus detector strips (ODS) in detecting oestrus as confirmed by visual observation of mounting behaviour at oestrus detection twice daily for 30-45 minutes.

ODS status	Oestrus status		
	Positive (Number of cows)	Suspected (Number of cows)	Negative (Number of cows)
Paint worn 0 %	0	1	1
Paint worn 1-10%	7	0	0
Paint worn 11-50%	4	1	1
Paint worn 51-100%	3	0	0
Number of ODS falling off	0	0	0
Total number	14	2	2

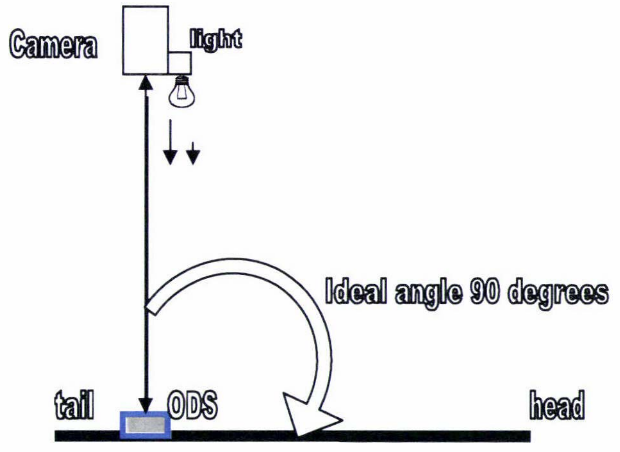
-ODS = oestrus detector strip
-Positive = cow showing primary oestrus signs (being mounted)
-Negative = cow showing secondary oestrus signs, mounting other cows, sniffing and licking of the vulva
-Suspected = animals that had a percentage of paint removed and were not confirmed by primary or secondary signs of oestrus

a) Uncontaminated oestrus detector strip before application on cows

b) Manure contaminated oestrus detector strip

c) Dirt contaminated oestrus detector strip

Figure 2.5: Uncontaminated oestrus detector strips (ODS) before application on cows (a) and contaminated ODS (b,c), positive for oestrus, successfully identified and read. Experiment 4, Dairy Unit 4, Massey University.



ODS=oestrus detector strip

Figure 2.6: Schematic diagram of the system used to obtain images of the oestrus detector strips (ODS) using a cow model. The camera/light and oestrus detector strips are perpendicular to each other.

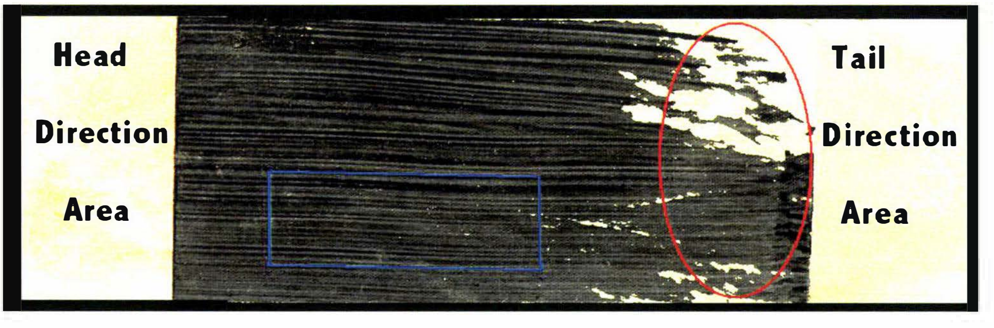


Figure 2.7: Positive oestrus detector strip (ODS) showing the location of mounting intensity (red circle) and the longitudinal reflections through the paint of the strip (blue rectangle).

2.3.2. Trial two: paint type and ODS location-troubleshooting trial of oestrus detector strips.

2.3.2.1. Purpose of the trial

This trial was conducted to overcome the increased paint surface gloss when mounting activity occurred, by seeking an alternative paint, modifying paint application on the ODS and adjusting the ODS position. In addition, the trial was conducted to test ongoing modifications of the VIPS program and the camera in order to adjust its sensitivity in identifying and reading the oestrus detector strips.

2.3.2.2. Material and methods

This experiment was performed at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Veterinary Large Animal Teaching Unit (VLATU), Palmerston North campus. Heifers (n=18) that were 16 months old and were of mixed breeds, (Friesian, Hereford, Friesian-Hereford and Angus) were used. The heifers were grazed together at pasture and were supplied with hay daily to cover their feed requirement. Animals had a free access to water. They were monitored before use in the trial and their oestrous cyclic behaviour confirmed they were cycling regularly and normally.

Various paints (n=6) were selected for testing and for comparison with the previously used paint. They were selected based on their low gloss and drying speed. Paints tested were:

- Apcolite (Water Based, Acrylic Gloss Finish, Apco coatings, Asian Paints Limited. Australia),
- Timbacryl (Water Based, Dulux Low Sheen Acrylic, Dulux New Zealand an Orica New Zealand Limited Business),
- Black Board Paint (Oil Based, Wattyl Blackboard Black, Wattyl Limited, NZ),
- Zylone 20 (Water Based, Flat Acrylic, Resene Paints Limited, NZ),
- Zylone Spacecote (Water Based, Flat Acrylic, Resene Paints Limited, NZ),

- Zylone Sheen (Water Based, Low Sheen Acrylic, Resene Paints Limited, NZ).

The reflective strips (3M 9920, 3M New Zealand Ltd, Auckland, New Zealand) were painted with these paints 48 Hours before application to the cows. Attention was given to the amount of paint applied over the strips to ensure it was sufficient to mask the reflective surface of the strips when the paints dried. Another consideration was the speed of drying. It is desirable that the paint used has a short desiccation time with high flexibility over the strip being achieved which makes the paint more durable and not easy to peel off when bending strips at application or through movement of a cow's tail head.

The resulting gloss and number of paint coats applied to achieve the desired outcome were also important, as these paints were initially chosen because of their low gloss and suitability for outdoor use.

Before application of ODS, the sacral area of the cows was brushed to remove any loose hair or other debris to assist adhesion. After strip application the cows were released to pasture for 42 days and were observed twice daily, at 6 am and 6 pm for 30–45 minutes. In this trial, ODS reflective ends were protected during preparation for application with a soft adhesive sealing tape to minimise the risk of the reflective area being damaged by the Ados F2 glue (CRC industries New Zealand Ltd, Auckland, New Zealand) solvents.

Three strips of each of the paints were prepared in order to use them on the heifers (n=18) through a trial and error process intended to achieve elimination of paint with unwanted characteristics and replacement with strips prepared with better performing paint.

Detection and measurement of paint removal from ODS was made using VIPS with both an Intel™ webcam and a Sony™ firewire camera (SX-900) in field and laboratory conditions. Both cameras were tested to ensure that they could operate over three continuous hours.

2.3.2.3. Results

The following paints were excluded: -

- Apcolite (Water Based, Acrylic Gloss Finish, Apco coatings, Asian Paints Limited. Australia),
- Timbacryl (Water Based, Dulux Low Sheen Acrylic, Dulux New Zealand an Orica New Zealand Limited Business),
- Black Board Paint (Oil Based, Wattyl Blackboard Black, Wattyl Limited, NZ),
- Zylone 20 (Water Based, Flat Acrylic, Resene Paints Limited, NZ).

Both Apcolite and Timbacryl painted strips needed a second coat when dried due to visible longitudinal reflection lines being seen through the strips' painted area resulting from the paint brush tips. The other painted strips initially looked suitable with no reflection visible through the paint. It took about 2 hours for the paints to dry and they all dried within approximately the same time. After 24 hours the strips were ready for use.

The following two paints were fit to be used in for the purpose of making ODS for the trial:

- Zylone Spacecote (Water Based, Flat Acrylic, Resene Paints Limited, NZ),
- Zylone Sheen (Water Based, Low sheen Acrylic, Resene Paints Limited, NZ).

2.3.2.4. Discussion

Apcolite and Timbacryl were excluded because they needed a double coating to fully mask the strips' reflective area. The excessive hardness and strong adhesion of the paint to the strip surface was the reason why Zylone 20 was excluded. On the other hand, the blackboard paint was too soft when fully dry and peeled off the strips when they were placed on the cows due to movement that was not associated with the cows being mounted.

Both Zylone space coat and Zylone sheen paints showed reliable adhesion and an acceptable response when a heifer was mounted. Neither of these paints had an increase in the gloss resulting from being mounted. The removal of paint that exposed the reflective strip occurred over the whole painted area of the strip, indicating that the sacral area of the cow is suitable for ODS application and it is a point that was subjected to acceptable mounting intensity to activate the strips by removing paint from them.

The rubbing of the paint was evenly distributed over the entire strip and none of the positive strips had paint removal distributed on less than 50% of the total painted area. The Sony™ camera showed better results in detecting ODS and a better identification of the margin between the painted area and the index areas of ODS. The Intel™ camera had problems identifying the ODS and the paint-index area margin and those problems were more evident after 1 hour of operation.

Zylone sheen acrylic when applied more thickly showed a better paint removal response than other paints tested and there was no increase in glossiness when the paint was subjected to rubbing during mounting by cows as measured by VIPS, unlike when it was applied more thinly as was described in section 2.3.1.2. The increased paint thickness did not affect ODS flexibility. Thus Zylone sheen is the most suitable of the paints tested to apply on the ODS for oestrus detection. The location where the ODS should be applied, is on the sacral region of the cow in the midline, as it was found to be a point of acceptable mounting intensity. The camera (Intel™ web cam) performance in accurately distinguish removed paint has improved slightly after suitable changes were made to VIPS software. Further technical work on the software and camera driver compatibility (see section 2.2.2) need to be introduced and then tested (next section).

2.3.3. Trial three: testing the accuracy of the CSD in measuring paint removal from the ODS.

2.3.3.1. Purpose of the trial

The purpose of this trial was to test the accuracy of the CSD in measuring a known and controlled removal of paint from the oestrus detector strips.

2.3.3.2. Material and methods

This experiment was performed in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North. Scotchlite (3M

9920, 3M New Zealand Ltd, Auckland, New Zealand) reflective strips of 150mm x 50mm were used. With the aid of a masking tape (3M Scotch masking tape, 3M New Zealand Ltd, Auckland, New Zealand) strips were painted using Zylone sheen paint (water based low sheen acrylic, Resene Paint Limited, Palmerston North, New Zealand). Then, known areas of paint were removed representing different proportions of the strip. These were created by painting over known areas of masking tape mounted on the strips. The masking tape was then removed with the overlying paint creating known areas of reflective surface.

For reading the known areas of reflective surface, the CSD was used. A cow rump dummy was used to simulate the usual placement of the ODS (as described in section 2.2.4).

In order to identify more adequately the areas of paint removal to be tested, information from the previous field experiments was used to simulate the frequency of the naturally occurring removal of paint that occurred in the field as seen in Table 2.3. The table shows that a high percentage of ODS had less than 50% of paint removed from their surface and out of those, 69% had less than 25% removed as measured by the CSD.

Measurements were taken when areas of removed paint were 0%, 5%, 10%, 15%, 20%, 25%, 35%, 40%, 45%, 50%, 60%, 75%, 80%, 90%, 95% and 100%. Each of these areas was built with rectangles and squares to resemble a pattern observed on cows with strips. Each strip was measured three times with the CSD and individual values were recorded (n=48).

The controlled area representing paint removal and those estimated by the CSD were adjusted to linear regressions using Microsoft Excel 2000 (Microsoft Excel® for Windows 1989-2001). The intercept was generally forced to zero. However, when a poor adjustment was obtained, the intercept was estimated and included in the equation.

2.3.3.3. Results

The linear regression of the proportion of paint removed from ODS and the areas measured by the CSD using the Intel™ camera is shown in Figure 2.8. A close relationship between the proportion of the actual and estimated areas was obtained (R^2=0.92, p<0.001). The slope obtained in the regression means that the CSD overestimated the area by 12% overall. However, by visual assessment of the observation points in the regression it was possible to identify a different behaviour of the predicted values when the proportion of exposed was up to 50%. To test this, separate regression equations were fit for the two proportions of area ranges. The results are shown in Figure 2.9.

By splitting the data set, a better regression was obtained (see R^2). In the 0–50% range, the oestrus detection device overestimated by 35% (Figure 2.9) and in the case of 50–100%, the intercept had to be added to obtain an acceptable R^2 (Figure 2.9). Although a high correlation was obtained after adjustment (R^2 =0.99). An outcome of this intercept is that when the actual area is 0%, then the CSD would be predicting 44%! A careful use of this prediction should therefore be done but it should also be remembered that this level of wear is outside of the predicted zone of interest (Tables 2.2; 2.3).

The linear regression of the proportion of actual area of paint removal from the ODS and the area measured by the CSD using the Sony™ (SX-9200) camera is shown in Figure 2.10. A much better relationship between the actual exposed and estimated areas was obtained (R^2=0.99, $p<0.001$) than that obtained by using the Intel™ camera (R^2= 0.92, $p<0.001$). The slope obtained in the regression means that the CSD overestimated the exposed area by 0.3% overall (Figure 2.10).

2.3.3.4. Discussion

The slope obtained in the regression in experiment number three means that the CSD overestimated the area of paint removal by 12% when using the Intel™ web cam. However, by visual assessment of the observation points on the regression plot it was possible to identify a different behaviour of the predicted values when the area of the removed paint was less than and up to 50%. This over estimation appeared to occur because of the poor capability of the camera to overcome the glare of the light reflected from the reflective material that showed beyond the borders of the painted area or the areas of removed paint. This problem resulted from limitations in the automatic exposure control within the camera and limited resolution.

The Sony camera had more powerful features and a lens that eliminated this problem and gave a more accurate measurement and interpretation of ODS status. Despite a slight overestimation of 0.3% resulting from reflection glare of the ODS index area or the area where paint was removed, it was found to be constant for reflective areas ranging from 0% to 100% of the total strip area.

The Sony camera did not need any adjustment to enable recognition or accurate measurement of the area of paint removal from the ODS and direct identification and evaluation by the CSD using this camera proved to be highly satisfactory (Figure 2.10).

The present results were obtained within a controlled and fixed situation when utilising the strips. Field-testing of CSD is required in order to proceed with an evaluation of this technology before its application as a potential commercial product can be recommended.

Table 2.3: Frequencies of paint removal areas on the oestrus detector strips (ODS) as recorded during field trials.

Exposed area (ODS)	Frequency of cases
0–10 %	47%
11–25%	22%
26– 50%	11%
51–75 %	8%
76–95%	11%
96–100 %	0%

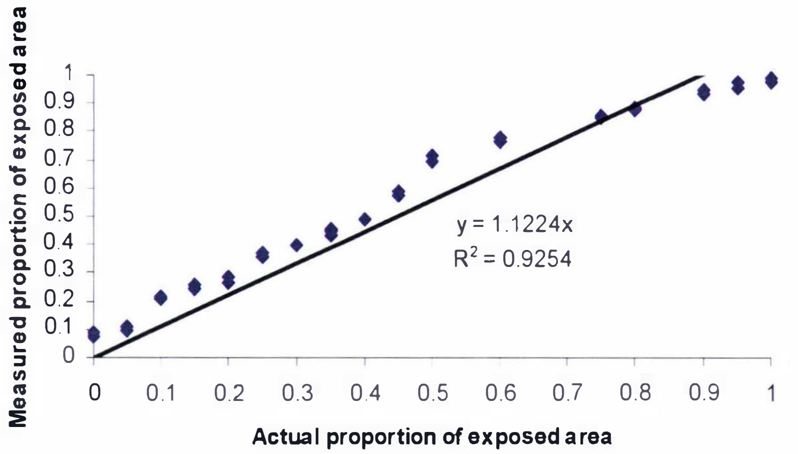


Figure 2.8: Linear regression of the proportion of actual area exposed of oestrus detector strips (ODS) and the proportion of area measured by the camera-software device (CSD) using the Intel camera (n=48).

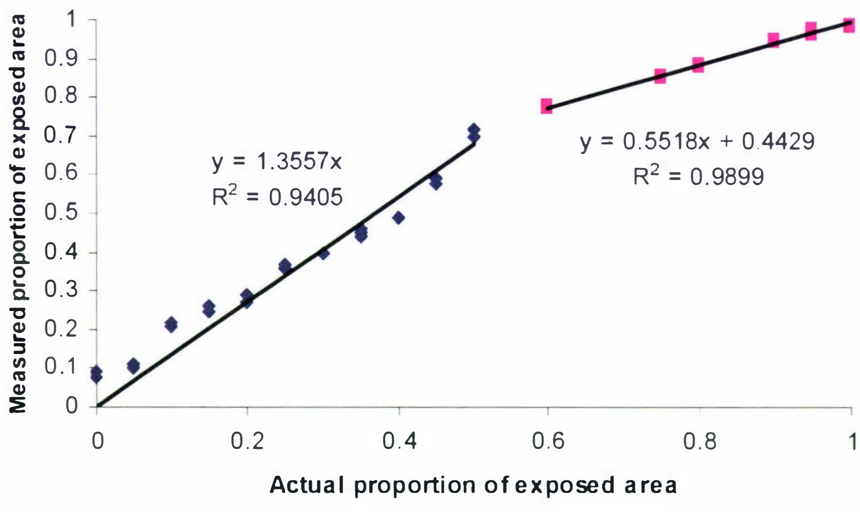


Figure 2.9: Linear regression of the proportion of known area of paint removed from ≤50% (diamonds) and >50% (triangles) of ODS and the proportion of area measured by the CSD using the Intel camera (n=30, 18 respectively).

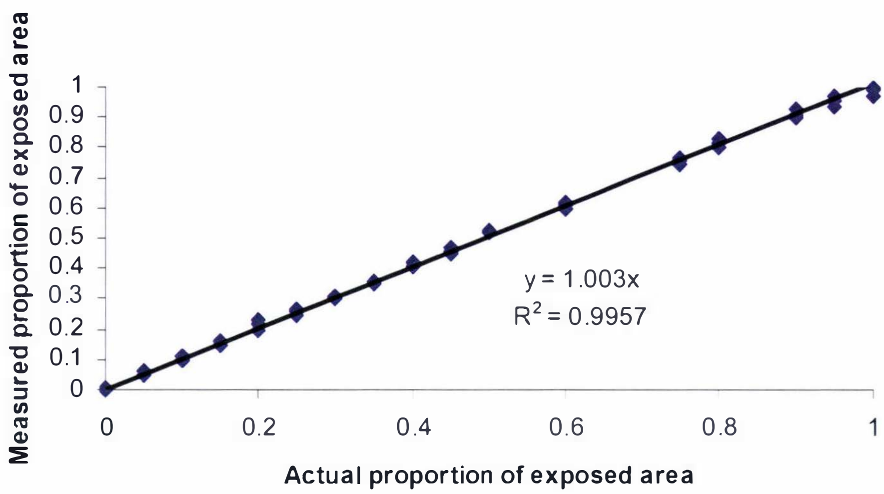


Figure 2.10: Linear regression of the proportion of paint area removed from the ODS and the proportion of area measured by the camera-software device using the Sony camera (n=48).

Chapter Three

Comparison of a camera software system and normal farm management for detecting oestrus in dairy cattle at pasture.

Abstract

The aim of this trial was to compare the sensitivity, specificity, predictive values and accuracy of detection of oestrus using a camera-software device (CSD) or normal farm management of visual observation and the use of tail paint. A group of cows (n=240) monitored by a CSD were fitted with oestrous-detection strips while cows in a control group (n=240) were monitored by the use of the conventional tail painting technique and visual observations of mounting activity carried out by the farm staff. These were the routine oestrus detection practices of the farm. Visual observation was conducted in both groups. The confirmed pregnancy diagnosis results to artificial insemination (AI) were used to confirm oestrus. Reproductive analysis was conducted utilising DairyWin farm records. The sensitivity ($p<0.0063$), specificity ($p<0.001$), positive predictive value ($p<0.0001$) and overall accuracy ($p<0.0001$) of the tests for the CSD group were significantly higher than for the control group being 85%, 99.6%, 88% and 99% compared with 78%, 98%, 51% and 98% respectively. The negative predictive value did not differ significantly between groups ($p=0.28$). The conception rate to first service by AI was higher ($p<0.05$) in the CSD group (72%) than in the control group (39%). Application of a CSD significantly increased the proportion of cows that conceived ($p=0.044$). Under normal farm management, more cows were drafted for AI and bred when they were not in oestrus. Cows detected in oestrus using the CSD system achieved improved reproductive performance, indicating that the CSD system was satisfactory for detection of oestrus in a seasonally-calving dairy herd grazing on pasture and improved the oestrus detection performance in this herd that had relatively poor detection of oestrus in the control group.

KEY WORDS: Oestrus detection, dairy cattle, automation, camera-software device, oestrus detection strip, tail paint.

3.1. Introduction

Detection of oestrus is an important task that needs to be conducted effectively in artificially bred dairy herds to ensure adequate reproductive performance of the herd. As detailed in chapter two, an oestrus detection system was designed to optically identify and read oestrus detection strips fixed to cows in order to automatically and accurately detect mounting. Early testing showed success in automatically identifying the presence of ODS and automatically determining that paint had been removed from the strips indicating that cows had been mounted.

Observing cows for signs of oestrus and inseminating them at the correct time are necessary steps for effective reproductive management. Artificial insemination (AI) became one of the most important agricultural technologies of the Twentieth Century and most dairy producers adopted some aspects of this technology in order to maintain competitive agricultural businesses (Nebel, 1988).

Inefficiency of reproductive performance in dairy herds is a source of frustration for producers and also substantially reduces potential profits in dairy enterprises. Increases in the size of herds and milk yield have been implicated as a contributors to the decreased reproductive efficiency experienced by many dairy farms (Nebel et al., 2000).

The major factor limiting optimum reproductive performance on many farms is a failure to detect oestrus in a timely and accurate manner (Senger, 1994; Guilbault, Pothier, Twagiramungu and Sirard, 1998; Fricke, 2000).

The objectives of work reported in this chapter were:

To determine the accuracy and the efficacy of ODS in association with CSD in detecting oestrus.

To compare detection using ODS and the CSD to normal farm management using visual observation and tail painting combined.

To determine the threshold of the proportion of paint removed from the CSD to allow an interpretation that oestrus has occurred when the paint removed from the ODS was measured by the CSD.

3.2. Materials and methods

The experiment was conducted in the spring breeding season of 2003 at the Massey University number 4 dairy unit, which operates commercially in the Manawatu district of New Zealand. The experiment took place between the 6th of October and the 4th of December of 2003.

Four hundred and four 2-13 year old cows of mixed breed (Friesian, Holstein, Jersey and their crossbreeds; calved >26 days from planned start of mating (PSM; 20th October 2003) were used in the study. Before the trial started, animals' reproductive histories were checked for abnormalities (retained foetal membranes, abortion and dystocia). All animals selected were condition scored and reproductively sound animals with a body score not less than 3 (scale of 1-8; mean body condition score = 4.0).

Ninety-three of the initially selected animals were two year old heifers (23%) and the rest of the animals ranged from 3 to13 years old, as shown in Table 3.1. Animals' condition scores ranged from 3.0 to 7.0 out of 8 (Table 3.2).

Selected animals were randomly allocated to one of two groups using the randomisation function of Microsoft Excel 2000 (Microsoft Excel® for Windows 1989-2001 English version). The randomisation was stratified by age, condition score and days in milk for the animals. The outcome was then analyzed using the one way analysis of variance (ANOVA) and cross-tabulation functions of the SPSS© program (Apache software foundation, SPSS© SPSS Inc. 12.01 for Windows, 2001, USA) and showed no significant difference between the groups (p=0.259) in these measures. All animals were found eligible for the trial and were randomly allocated to the groups. Seventy-six further suitable cows were later added to the trial, by alternate allocation to the groups comprising 49 late calving cows (cows calved 6th October to 25th October 2003) and 27 new cows that had entered the herd.

Table 3.1: Percentages by age of trial animals.

Age (Years)	2	3	4	5	6	7	8	9	10	>10
Percentage	23	18	15	12	11.5	5.5	4	4.5	3.5	3

Table 3.2: Condition score of trial animals (Scale 1 to 8*).

Condition score [a]	Number of cows	Percentage
3.0–3.9	215	45
4.0-5.9	186	39
6.0-7.0	79	16
Total	480	100

[a] mean body condition score=4.0
* independent scoring was carried on by Professor Colin Holmes, Massey University IVABS, Palmerston North.

The two experimental groups were designated as the control group (n=240) and a CSD group (n=240). Within the CSD group, two methods of detection of oestrus were applied on the cows simultaneously, comprising detection by the farmer and detection by the CSD. Animals grazed together at pasture with free access to water. The farm manager managed the herd so that the pasture allowance was adequate for maintenance and production requirements in accordance with the normal management of the farm.

The trial was conducted from 6th October to 4th December of 2003. Cows were milked twice daily at 5:30 am and 3:00 pm using a rotary platform milking parlour. Animals of both groups were inspected twice daily at milking to assess the status of their tail paint, oestrus detector strips and to note CSD data. Animals were handled in agreement with protocols approved by the Massey University Animal Ethics Committee (MUAEC 03-114, Palmerston North).

For the control group, two techniques of oestrus detection were applied, comprising a conventional tail painting technique and visual observations of oestrus activity carried out by the farm staff; These were the routine oestrus detection practices of the farm. Visual observations of behaviour were made with no regard to the groups and all groups were in the same herd. The visual observations checked for obvious signs of oestrus like standing to be mounted, mounting other cows, head mounting, vaginal discharge and the behavioural changes of restlessness, sniffing, licking, chin-resting and chin-rubbing. If the farm manager believed that a cow was in oestrus, she would be inseminated regardless of tail paint or CSD findings.

Oestrus was detected in the CSD group using both ODS-CSD and tail paint and visual observations (tail paint was applied caudal to ODS and posteriorly covering the midline, on the rump of each animal). Herdsmen marked cows that were observed or suspected to be in oestrus at the morning milking of each day and drafted them for breeding with the cows observed at the previous evening milking. A decision on which cows from the drafted cows were to be inseminated was then made by the farm manager.

Fifty-nine animals had a reproductive treatment as they were not cycling between the 14th to 23rd of November. All treated cows were included in the analysis as they were found to be distributed approximately equally (31 CSD group and 28 control group) between the two groups as follows:

- 36 cows with an 8 day program of CIDR (Pfizer EAZI-BREED™ CIDR® (progesterone) Cattle Inserts, with 1mg Oestradiol benzoate (ODB; CIDEROL™ Bomac Laboratories Ltd, Auckland, NZ) at day 8; 21 cows CSD group and 15 cows control group).
- 11 cows with Prostaglandin injections (Estroplan injection 2ml intramuscular injection, Parnell Laboratories NZ Ltd, New Zealand. 7 cows CSD group and 4 cows control group).
- 4 cows with intra-uterine Lugol's iodine (2 cows CSD group and 2 cows control group).
- 8 cows with unspecified treatments (1 cow CSD group and 7 cows control group).

3.2.1. Tail painting, ODS and visual observations

The farmer used three colours of tail paint which was maintained by re-touching weekly. Blue was used when a cow calved and was being watched for the first postpartum oestrus, red when the cow was confirmed cycling then green when artificially inseminated. If a cow returned to oestrus after insemination but still within the artificial breeding period it was re-inseminated and repainted with green paint.

On the first day of the breeding season commercially available tail paint (Tell tail oil-based tail paints, fluorescent oestrus detection tail paint, FiL New

Zealand Ltd; Mount Maunganui, New Zealand) was reapplied as a strip 18–21 cm long and 5–6 cm wide from the sacral area, posteriorly covering the midline, on the rump of each animal in the control group. The paint was applied using a brush, initially against the direction of the hair and then with the direction of the hair to achieve a smooth surface (Kerr and McCaughey, 1984; Williamson, 1980).

A cow was considered by the farmer to be in oestrus based on tail paint alone when more than 75% of tail paint was removed. Cows suspected to be in oestrus were checked for mounting marks such as skin abrasion and hair removal over the sacrum in addition to tail paint removal before being drafted. If a cow was visually observed bulling then it was drafted regardless of tail paint status. When the paint was believed by the farm manager and workers to be removed for any other reason, the cow was repainted and sent back with the herd.

ODS comprise Scotchlite (3M 9920, 3M New Zealand Ltd, Auckland, New Zealand) reflective strips of 150mm x 50mm, painted with Zylone sheen paint (water based low sheen acrylic, Resene Paint Limited, Palmerston North, NZ), glued to cows using Ados F2 glue (CRC industries New Zealand Ltd, Auckland , New Zealand). ODS were placed initially on the sacro-coccygeal area of the CSD group animals' rumps (ODS location 1, Figure 3.5), after adequate brushing and after glue had been applied to the area to insure adhesion (see chapter two).

The camera for the CSD readings was mounted in an area of the parlour with low variation in light and at approximately a right angles with the ODS within a range of 120–150 centimetres in order to make the measurements of the ODS and amount of paint removed more reliable (see chapter two).

The CSD measured and reported the proportion of paint removed as a percentage. A cow was considered to be in oestrus if it had 10% or more paint removed or if the ODS itself was missing (on the assumption that removal occurred from intensive mounting behaviour).

Images of passing cows and strips were captured at three second intervals which allowed three frames per cow to be obtained as the rotary platform progressed under the camera (see chapter two). In the absence of electronic identification, manual collation and recording of the animal's identification and the reading of the proportion of paint removed was required. The ODS were maintained weekly, which involved cleaning the very dirty ones and replacing the missing ones during an afternoon milking. The ODS that showed a positive reading were removed from cows and new ones applied after four days.

Cows in both groups were observed in the paddock for 30–45 minutes before milking and at random times, while they travelled from the paddocks to the milking shed and in the yards during each milking (for approximately 3 hours each milking).

3.2.2. Inseminations

Inseminations occurred once daily after the morning milking on cows identified in oestrus at the previous evening milking and at milking on the same morning. Insemination occurred about 19 hours after detection of oestrus for cows detected in the evening and 4 hours after detection of oestrus for those detected in the morning. The farm management made all decisions on which cow to inseminate. Once a cow was inseminated it was sent back to graze with the herd. After 8 weeks of artificial breeding, bulls were introduced to the herd for four weeks.

3.2.3. Pregnancy diagnosis as a measure of the accuracy of oestrus detection

Pregnancy diagnosis by rectal palpation and/or ultrasound examination was conducted on the 27th and 28th of January 2004, which was 51 and 52 days after the end of the breeding season. The pregnancy diagnosis (PD) results were used as a standard outcome to allow comparison the ability of the oestrus detection methods used to correctly identify oestrus (Figure 3.1).

When a cow was confirmed to be pregnant by palpation and/or ultrasound examination to a service on a date as recorded in the herd's AI records, that oestrus was regarded as a true one. A reverse count of 21 days (±3 days) from an AI that produced a confirmed pregnancy was made and if oestrus was detected by any of the methods in that time period, that oestrus also was considered as a true oestrus and recorded. The oestrus detection methods that detected oestrus on these days were credited with having correctly recognised the oestrous event. If there was no oestrus detected in that time period or by a further reverse count for another 21±3 days, the insemination was considered to have occurred at the first oestrus.

No oestrus observed after the confirmed date of conception was considered to be a true one. An oestrus was regarded as being a true one if a cow was found pregnant to a recorded oestrus and she was previously cycling with an interval between recorded oestruses of 18 to 24 days or 36 to 48 days before the recorded oestrus leading to pregnancy (Figure 3.1).

3.2.4. Contingency 2x2 tables

Contingency 2x2 tables were constructed for each group to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for each oestrus detection method based on the oestruses confirmed by the pregnancy diagnosis (PD) results as described above.

Test sensitivity was calculated for a given oestrus detection method as the proportion of the cows detected in oestrus that were confirmed in oestrus by the method (number of true-positive results/ (number of true-positive results + number of false-negative results)) and test specificity was calculated for a given oestrus detection method as the proportion of the cows that were not detected in oestrus that were selected as not in oestrus by the method (number of true-negative results/(number of true-negative results + number of false-positive results)).

The PPV of an oestrus detection method was calculated as the probability that a detected oestrus is occurring in a cow that is in oestrus (number of true-positive results / (number of true-positive results + number of false-positive results)). The NPV was calculated as the probability that non-detection of oestrus occurred for a cow not in oestrus (number of true-negative results / (number of true-negative results + number of false-negative results)).

Test accuracy or overall accuracy was calculated as the measure of the true findings (true positive and true negative results of a test) divided by all test results. This is also termed as the efficiency of the test ((True positives + true negatives "all cases truly identified by a test") / (true positives + false positives + true negatives + false negatives i.e. "total population")) (Harrison and Braunwald, 1987).

3.2.4.1. Data analysis for contingency tables

For the purpose of analysis, oestrus observations were limited to one per cow per day. For example, when a cow was detected by a given method as being in oestrus, a one was recorded for that method on that day, else zero. If the same cow was detected in oestrus again on the following day, she was again recorded as one (else zero). Thus, each cow received a 1/0 value for every day in the trial for which oestrus information was available.

3.2.4.1.1. Statistical analysis

Statistical analyses were conducted to compare the different oestrus detection methods utilising SPSS© (Appache Software Foundation, SPSS© SPSS Inc. 12.01 for Windows, English version, 2001), initially using descriptive statistics cross-tabulation, one way analysis of variance and also using SAS® (SAS Institute Inc 8.02. 1999-2001, Cary NC, USA) for logistic regression. The pregnancy information used in this trial (PD) refers to the use of pregnancy to confirm oestruses that matched the criteria as described above and is not intended to reflect the pregnancy percentage of the herd.

The data set had two groups designated as the CSD group and control group. Within the CSD group, two methods of oestrus detection were applied on the cows simultaneously being farmer detection and detection by CSD. Thus two datasets were created. The first was used to run the analysis between different oestrus detection methods applied to the two different groups in the herd. The second dataset was used to run the analysis between the two treatments applied on the same cows within CSD group, i.e. observation by farmer and the findings of the CSD).

Sensitivity and specificity were modelled using logistic regression as the log-odds of the probability of a test result given true oestrus status, stratified by method of oestrus detection. Thus, the response was whether or not a cow was detected in oestrus by one of two methods on a particular day and independent effects were the 2 x 2 strata of true oestrus (1/0) x method of detection (method A/B). Predictive values were modelled as the log-odds of the probability of true oestrus status given the result of the method of detection. Predicted probabilities and the individual contrasts to compare sensitivities and specificities (or predictive values, respectively) between detection methods were calculated from these four strata for each method of detection. The model also included independent effects for parity, body condition score and days in milk at planned start of mating. To adjust for repeated observations between subsequent oestrus observations on the same cow, cow identification number was used as a class statement in the repeated option of *proc genmod* in SAS. In the case of the first dataset where the two methods were applied to the same cow simultaneously, a matched analysis was run that included method of detection nested within cow as a repeated effect (Allison, 1999). After running each model, the covariate effects parity, body condition score and days in milk at PSM were removed one by one and the relative changes in model coefficients were calculated. A relative change of more than 15% was interpreted as a confounding effect of that variable on sensitivity/specificity, or predicted values, respectively (Dohoo, Martin and Stryhn, 2003). Statistical significance was declared at $p<0.05$.

Survival analysis was used to assess the effect of the treatments on the risk of conception from ten days before the PSM; as CSD recording started 10 days to PSM, over the period of mating of 55 days.

3.2.5. Reproductive analysis

A herd reproductive analysis was conducted using DairyWin™ software (DairyWIN™ 2001 v99.91.148, Massey University, Palmerston North, New Zealand). Data from the herd were loaded into DairyWin™ and the control and CSD groups were identified and tagged in the program. Reproductive indices were calculated by the program in a standard way using records of PSM, oestrus detection dates and PD results for each cow.

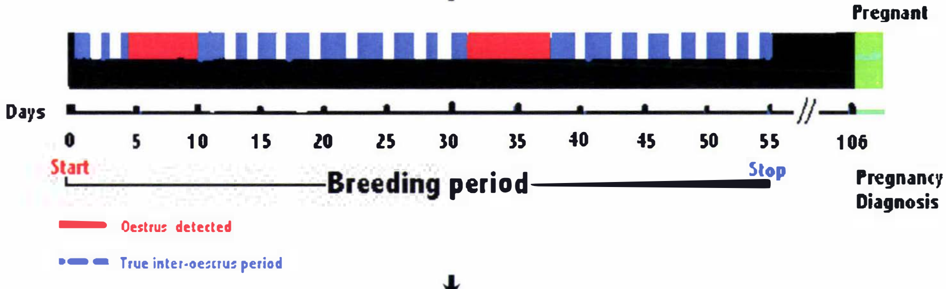
Reproductive performance, including cows calved within 40 days of PSM, submission rates to AI, conception rates to dates since calving and non-return rates were calculated using standard DairyWin reports. The submission rate indicates the cumulative proportion of cows submitted to breeding within a given time period after the PSM for the herd. Non-return rates are an indirect estimate of pregnancy rates in which a non-return to service is the failure to record a subsequent oestrus or breeding within a specified time period (Williamson et al., 1972).

Reports of calving, submission, non-return and pregnancy rates, as well as analyses of the interval to return to oestrus, were evaluated for errors and corrections were made if data entry errors or incorrect PSM or oestrus detection dates or PD dates were identified. Submission rates, return intervals and in-calf rates for the two groups were then subjected to Chi-squared (χ^2) analysis for statistical significance.

Select cows that are PD positive from control and CSD groups

↓

For each selected cow identify true oestrus through AI records by reverse counting from PD positive day 18 to 24 days or 36 to 48 days through the AB period.



↓

A true positive is credited for each method if detection occurred in the predicted oestrus periods and is credited for that method until a cow is no longer in oestrus. A true negative is credited if no detection occurred between the inter-oestrus periods.

↓

Contingency tables constructed for each group and method of detection to calculate methods sensitivity, specificity, accuracy, PPV and NPV

↓

Comparison of Sensitivity, specificity, accuracy, PPV, NPV of detection of oestrus (CSD vs. control, CSD vs. farmer-CSD, Farmer –CSD vs. control) was performed using Logistic regression, with a p-value based on the likelihood ratio statistics.

Figure 3.1: Diagram explaining the utilization of pregnancy diagnosis results and artificial insemination records of cows for the analysis and identification of true oestrus period/s (▬ ▪) and true inter-oestrus period/s (▬) for the trial cows during the artificial breeding of 55 days. It also explains the statistical test performed to compare sensitivities, specificities, accuracy, positive predictive values (PPV) and negative predictive values (NPV) of the treatment applied for oestrus detection.

3.3. Results

Fifty-seven cows from the control group were not able to be used in this analysis because they did not have pregnancy diagnosed, including 7 cows that were sold or died. Twenty-seven cows including 7 cows that were sold or died were excluded from the CSD group analysis because they were detected by the oestrus detection methods applied but showed no evidence of pregnancy to enable oestrus to be confirmed in the described way.

Cross-tabulations of PD results and oestrus detection aids are shown in Table 3.3. The control group (Farmer) that was detected by visual observations and tail painting and confirmed by PD had a total number of 201 oestruses detected. In the CSD group, the total numbers of oestruses detected by CSD or the farmer (using visual observations and tail paint) and subsequently confirmed by PD were 341, 292 respectively.

The test sensitivity for the farmer in the control group was 78% (201 oestruses, 95% CI: 73% to 82%) which is equivalent to a false negative proportion of 22% (57 oestruses). Test specificity was 98% (95% CI: 97.7% to 98.3%) which is 2 % false positive results as shown in Tables 3.3; 3.5. In the CSD group, the test sensitivity for the CSD was higher than for the farmer in the CSD group (85% {341 oestruses}, 95% CI: 80% to 87%), 72% {292 oestruses}, 95% CI: 67% to 76%, $p<0.0001$)). This equates to 15% (65 oestruses) and 28% (114 oestruses) of false negative results by the CSD and farmer respectively in the CSD group, ($p<0.0063$).

Test specificity was also higher for CSD (99.6%; 95% CI: 99.4% to 99.7%) than farmer in CSD group (98.1%; 95% CI: 97.8% to 98.3%) which is consistent with a lower proportion of false-positive results from the CSD than from the farmer in both the CSD and control group ($p<0.001$). The CSD had a higher overall accuracy than the controls and the farmer in CSD group ($p<0.0001$) and also a higher PPV ($p<0.0001$) than the farmer in both groups. The NPV for detection of oestrus was not different between methods of detection ($p=0.28$) as is seen in Tables 3.4 and 3.5.

The sensitivity ($p=0.13$), specificity ($p=0.59$), PPV ($p=0.13$), NPV ($p=0.28$) or accuracy ($p=0.08$) of farmer detection of oestrus did not differ in the control group and in the CSD group. Parity, body condition score and days in milk at the PSM were excluded from the analysis as they did not have a significant effect ($p>0.05$).

The percentage of cows calved <40 days at the PSM ($p=0.071$) and submission rates ($p>0.05$) of both the control and CSD groups did not differ significantly (Table 3.6; Figure 3.2). The non-return (NR) rate to first service by artificial insemination was higher ($p<0.001$) in the CSD group than in the control group for the first service and the total service NR percentages (CSD 71%, 74%; control 47%, 57%). The CSD group had a higher ($p<0.05$) proportion of 2-17 day return intervals (RI) (32%) and a lower ($p<0.05$) proportion of 18-24 day (56%) and 39–45 day (1%) return intervals than the control group (21%; 64%; 3% respectively). The in-calf % was higher ($p<0.0001$) for the CSD group over 4 week (70%) and 8 week (90%) periods than that of the control group (44%; 70% respectively; Table 3.6).

The CSD group had higher conception rates and a lower services per conception ratio than the control group as is shown in Table 3.7. Both the percentage of cows not in calf by PSM + 165 days (Table 3.6; $p<0.001$) and the cumulative proportions of cows not pregnant over time, controlled for the effects of age, condition score and days from calving to PSM using survival analysis were lower in the CSD than in the control group (Figure 3.4; $p=0.044$).

Mounting behaviour caused the loss of 72% (245) ODS; this resulted from the intensive mounting behaviour (results shown in chapter two illustrated that none of the ODS was lost spontaneously without major mounting activity having occurred. Table 3.6 results also confirm that finding). Of the lost ODS, 40% were found lost with a previous reading of 0%. 46% a reading between 1 and 5% and 14% a reading between 5-10%. Thus 5% paint loss could be considered as being diagnostic of oestrus since there is indication that intense mounting behaviour happened after this point was reached (Table 3.8).

As the observations proceeded the ODS were relocated further cranially over the lumbar area (a point of minimal contact from mounting, from ODS location 1 to ODS location 3 as shown in Figure 3.5). The caudal part of the ODS was placed at the level of tuber coxae (hip pins) in an attempt to reduce their loss. The relocation was found to be impractical after three days (27^{th} October-morning milking 30th October 2005) for monitoring and could lead to significant data loss, thus a further relocation was made to the lumbo-sacral area (Location 2; Figure 3.5) where the cranial part of ODS was placed at the level of the hip bones (Tuber coxae). The latter relocation showed much better results than location 3 in relation to the detection of mounting activity, paint removal and a slight improvement in reducing ODS loss compared with location 1.

Table 3.3: Results for oestrus detection aids for eligible cows in the control and camera-software device groups (n=183; 213 respectively) over the artificial breeding period of 55 days as confirmed by pregnancy diagnosis results and artificial insemination records.

Group*	Oestrus detection aid	Method results	Oestrus status confirmed by pregnancy diagnosis results	
			Oestrus	Not in oestrus
Control	Visual observation and tail paint (Farmer)	Oestrus	201	194
		Not in oestrus	57	9665
CSD	CSD	Oestrus	341	48
		Not in oestrus	65	11209
	Visual observation and tail paint (Farmer)	Oestrus	292	219
		Not in oestrus	114	11038

- Data for both groups was collected on a day by day basis, twice a day in the morning and evening and then pooled into one day observations for each cow. Cows that did not show evidence of pregnancy were excluded from the analysis. Data loss resulted from cows occasionally failed to attend at milking time (52 entries)

Table 3.4: Test sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the camera-software device (farmer, CSD) and control (farmer) groups with 95% confidence intervals for recording of oestrus detection.

Group[a]	Oestrus detection method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Control	Farmer	78	98	51	99.3
	95% CI	73 to 83	97.7 to 98.3	46 to 56	99.2 to 99.6
CSD	CSD	85 [b]	99.6 [c]	88 [d]	99.4[e]
	95% CI	80 to 87	99.4 to 99.7	84 to 91	99.3 to 99.6
	Farmer	72	98.1	57	99
	95% CI	67 to 76	97.8 to 98.3	53 to 61	98.8 to 99.2

Sensitivity= (number of true-positive results/ (number of true-positive results + number of false-negative results))
Specificity= (number of true-negative results/(number of true-negative results + number of false-positive results))
PPV= (number of true-positive results / (number of true-positive results + number of false-positive results)).
NPV= (number of true-negative results / (number of true-negative results + number of false-negative results))
[a] Data for both groups were collected on a day-by-day basis, twice a day in the morning and evening and then pooled into 1-day observations for each cow. Cows that did not show evidence of pregnancy were excluded from the
[b] $p<0.0063$ to farmer-control, $p<0.0001$ to farmer-CSD
[c] $p<0.001$ to farmer-control; $p<0.001$ to farmer-CSD
[d] $p<0.0001$ to farmer-control and farmer-CSD
[e] $p=0.28$ to farmer-control and farmer–CS

Table 3.5: Overall accuracy of a camera-software device (farmer and camera-software device (CSD)) and the control (farmer) in detecting oestrus and 95% confidence interval (CI).

Group	Oestrus Detection Method	Overall Accuracy[a] %
Control	Farmer	98
	95% CI	97.2 to 97.8
CSD	CSD	99[b]
	95% CI	98.8 to 99.2
	Farmer	97
	95% CI	96.8 to 97.4

[a] Overall accuracy = ((True positives + true negatives "all cases truly identified by a test") / (true positives + false positives + true negatives + false negatives i.e. "total population"))
[b] $p<0.0001$ to farmer-control and farmer-CS

Table 3.6. DairyWIN reproductive monitor report for control and camera-software device groups in the period from 20th October 2003 to 6th December 2003.

Reproductive monitor		Group		
		Control	CSD	Target
Submission rates	% Calved <40 days at PSM $^{p=0.071}$	26%	19%	10%
	21Day submission rate $^{p>0.05}$	76%	75%	90%
	28Day submission rate $^{p>0.05}$	81%	81%	92%
Return intervals	Return intervals: % 2 - 17 days $^{p=0.01}$	21%	32%	13%
	Return intervals: % 18 - 24 days $^{p=0.01}$	64%	56%	69%
	Return intervals: % 39 - 45 days $^{p=0.01}$	3%	1%	7%
	Ratio of (18 - 24 cyc):(39 - 45 cyc) $^{p=0.01}$	22:1	42:1	9:1
Conception rates	1st Service 49-Day NR $^{p<0.01}$	47%	71%	61%
	Total services 49-Day NR $^{p<0.01}$	57%	74%	61%
	1st service pregnancy rate $^{p=0.044}$	39%	72%	60%
	Total services pregnancy rate $^{p<0.01}$	46%	70%	60%
	Services per conception $^{p<0.01}$	2.2	1.4	1.7
In-calf rates	4-week in-calf rate $^{p<0.001}$	44%	70%	57%
	8-week in-calf rate $^{p<0.001}$	70%	90%	86%
	% Not in calf by PSM + 165 days $^{p<0.001}$	27%	10%	7%
	Calving to conception interval (days) $^{p<0.001}$	84	77	83

cyc cycle; NR non return rate; PSM= planned start of mating

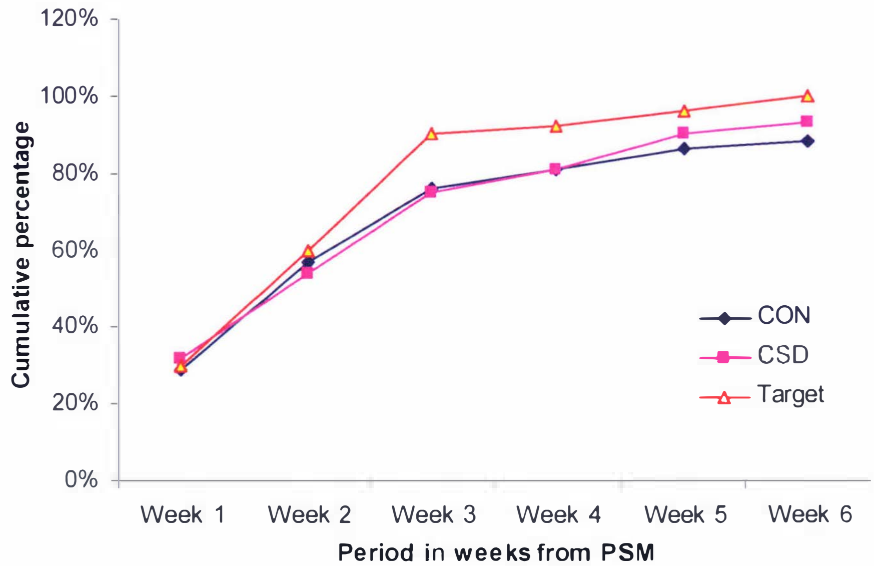


Figure 3.2: Submission rates for camera-software device (CSD) and control group (CON) and DairyWIN targets from planned start of mating (PSM) 20th October 2003 – 3rd January 2004.

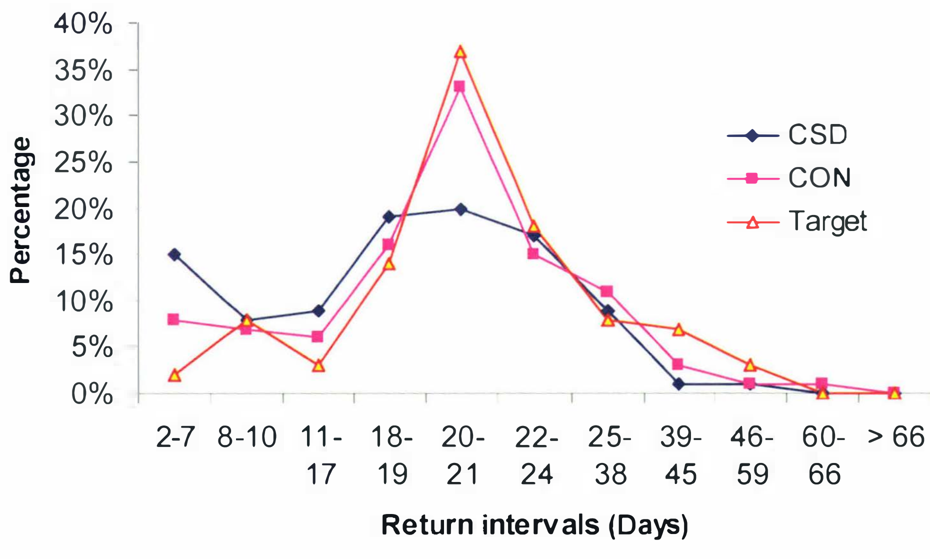


Figure 3.3: Return interval analysis for camera-software device (CSD) and control group (CON) from planned start of mating 20th of October 2003 -3rd January 2004.

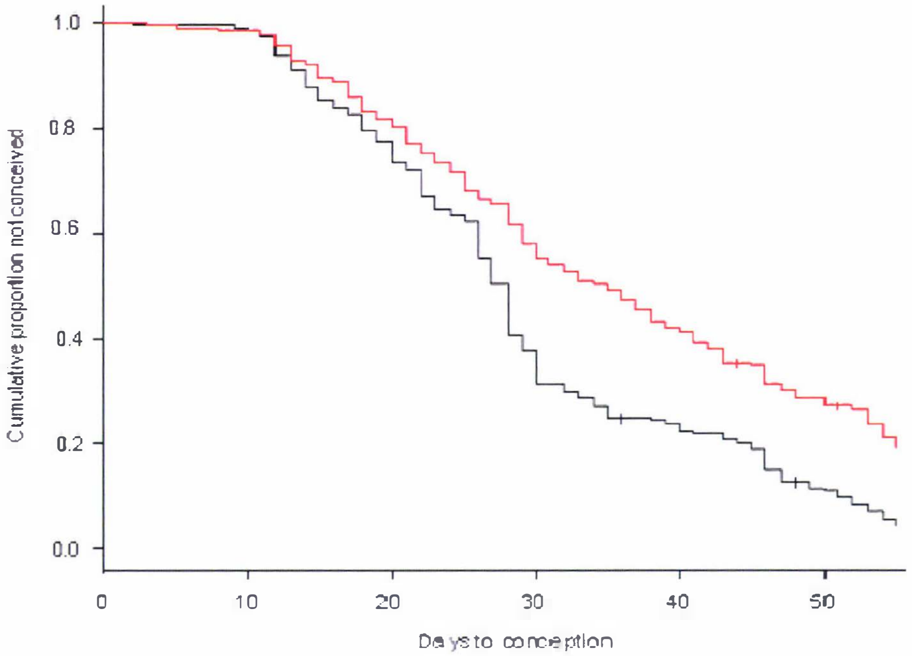


Figure 3.4: Kaplan-Meier survival analysis of the cumulative proportion of cows still to conceive at each day from 10 days before planned start of mating, for camera-software device group (CSD; black line; n= 213) and control group (red line; n=183) stratified by age, days from calving to planned start of mating and condition score.

Table 3.7: Conception rate analysis by days since calving for camera-software device (CSD) and control (CON) groups. Service dates between 20th October 2003 3rd January 2004.

Days calved at service		1st Service CSD	1st Service CON	2nd Service CSD	2nd Service CON	3rd+ Service CSD	3rd+ Service CON	Total services CSD	Total services CON	18-24 D Ser. CSD	18-24 D Ser. CON	Ser./conc. CSD	Ser./conc. CON
< 40 days	No. of cows	18	22	1	2	0	0	19	24	14	21	1.6	2.0
	Conception %	61	45	100	100	-	-	63	50	86	57		
40-59 days	No. of cows	35	54	6	16	11	0	42	70	38	60	1.2	1.8
	Conception %	80	56	83	50	100	0	81	54	89	63		
60-79 days	No. of cows	77	86	16	32	0	12	93	130	83	117	1.3	1.5
	Conception %	74	65	81	59	-	83	75	65	84	73		
80-99 days	No. of cows	83	50	25	38	5	18	113	106	107	94	1.1	1.4
	Conception %	88	66	92	76	60	89	88	74	93	83		
>99 days	No. of cows	11	14	15	30	6	181	32	62	30	61	1.1	1.1
	Conception %	82	86	87	97	83	100	81	95	89	97		
Target	Conception %	74		74		74		74		72		1.7	

-D= Day
-Ser= service
- Ser/conc= service per conception

Table 3.8: Frequency of oestrus detector strip (ODS) loss during recording of oestrus.

	Number	Percentage [B]
Oestruses detected	389(341[A])	88
ODS missing	245	72
Reading 0% before loss	136	40
Reading 1-5% before loss	157	46
Reading 5-10% before loss	48	14

[A] 341 oestruses detected by CSD and confirmed by PD results.
[B] percentage of oestruses detected and confirmed by PD of those detected by CSD and ODS.

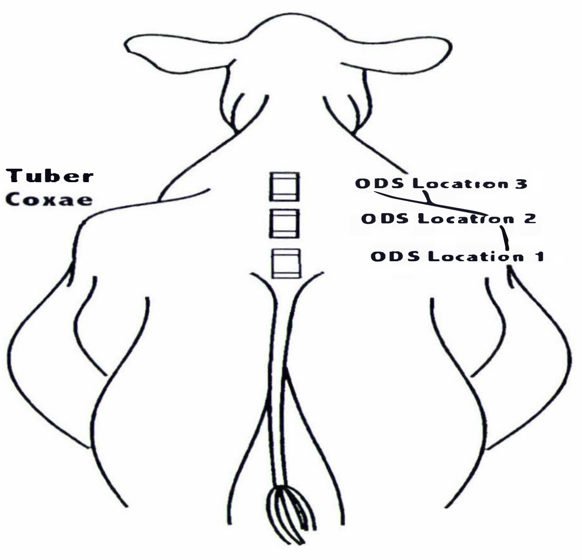


Figure 3.5: The oestrus detection strip locations on the trial cow's lumbo-sacral area (location 2), the lumbar area (location 3) and the sacro-coccygeal area (location 1).

3.4. Discussion

The objective of this study was to test the accuracy and efficacy of ODS, in association with a CSD, in detecting oestrus and to compare this with detection using normal farm management of visual observation and tail-painting conducted by farm workers. The presence of three concurrent detection systems created a dilemma of deciding which oestrus observation was correct, as they varied in their sensitivity of detection. One system indicated oestrus one milking before, or 24 hours before another system indicated the event. Occasionally one method indicated an event 2 or three 3 days before another method indicated it.

The use of diagnosed pregnancy as a standard to confirm the occurrence of oestrus enabled comparison of detection aids. If cows were found not to be pregnant at the time of PD, their prior oestrus history could not be confirmed, so they were excluded from the study. The use of pregnancy to confirm oestrus created a bias in the data in favour of the control group, as the number of cows found pregnant in the control group (n=183) was considerably lower than that in the CSD group (n=213), which excluded from consideration data from the control group that was likely to over-represent the non-detected cows. The study design enabled logging true negative results on daily basis, thus amplifying the specificity and NPV results. The 98% farmer–control group specificity of detection is relatively high but still it equates to 2% of false positive observations. This is equivalent to 202 false positive detections from total oestrus detections in the farmer-control group of 395. This is 51% of farmer-control group detections of oestrus.

The sensitivity and specificity of detection of oestrus in the mating period, using the CSD was significantly higher than was obtained in the control group and by the farmer in the CSD group ($p<0.05$). The false-positive detections of oestrus were slightly higher in the control group (2%) and in farmer detections in the CSD group (1.9%) than for CSD-detected oestruses (0.4%; $p<0.001$). This indicated that the CSD had a positive effect on farm management resulting in increased and more accurate detection of oestrus and thus more cows being submitted for AI that were truly in oestrus than occurred in the control group.

The PPV increased when the sensitivity of detection of oestrus increased. The NPV also increased when the number of true-negative observations of non-oestrus in a herd increased (Cordoba et al., 2001). In both cases the CSD had a higher PPV ($p<0.0001$) and numerically higher NPV ($p=0.28$), indicating that the CSD had a higher probability of detecting cows in oestrus and excluding cows that were not in oestrus.

The positive effect of the CSD on farmer detection of true oestrus, is demonstrated by the higher PPV ($p<0.0001$) for the CSD. The high detection of true oestrus in the CSD group, including among cows which had already been submitted for AI, appeared to lead to a high proportion of short cycles of generally 2–18 days in the CSD group. These would be explained as a consequence of the farmer being responsible for AI decisions which resulted in his submitting cows too early. Thus, a failure of proper identification of oestrus in cows by the farmer at a true oestrus that was then successfully picked up by the CSD would lead to a higher conception rate in the CSD group than in the control group. The detection of genuine short cycles by the CSD in the CSD group may also have led to the increased number of short returns to oestrus (Macmillan, Segwagwe and Pino, 2003) (Figure 3.3).

The CSD and ODS gave false positive readings when the strips were extremely dirty and the system could not detect them so they were recorded as missing or intact (0% reading). If an ODS was wet, it occasionally gave false readings because of the reflection caused by the water on the ODS. The association of high true positives (85%) and low false negatives means that the CSD had better detection efficiency than the farmer (Tables 3.3; 3.4).

Despite brushing cows before applying the glue and ODS, the time allowed when applying ODS may have been inadequate to satisfactorily brush and apply the glue over the cow's rumps, as the ODS were applied while the cows were on the rotary milking platform during milking. That arrangement did not allow sufficient time for the glue to dry on the cow and ODS before application. Intense mounting activity (Williamson et al., 1972) appeared to be a strong factor in ODS loss. Factors such as winter coat shedding (Macmillan and Curnow, 1977), licking of the device (ODS) (Foote, 1975; Kerr and McCaughey, 1984), accidental rubbing (Foote, 1975; Mai et al., 2002) could also be a contributing to ODS loss, as these factors were implicated in the loss of detection aids in previous trials.

High loss rates of rump-mounted devices ranging from 40-60% (Gwazdauskas et al., 1990; Phatak and Touchberry, 1988) have been reported in previous trials. The high loss of ODS (72%) made the determination of a suitable cut off point for the proportion of paint removed unable to be achieved, since many strips were lost before paint removal was detected and recorded. From data that was recorded, the majority of ODS showed a reading between 1-5% of paint removed before being lost. Thus it appears that intense standing to be mounted occurred after that (Table 3.8).

Relocation of the ODS cranially to a region over the lumber spine (ODS location 3) was found to reduce efficacy and could lead to significant data loss due to very low reaction of ODS to mounting activity (mainly in large sized cows), even with a very intense mounting behaviour (Figure 3.5). The relocation over the lumbo-sacral area (ODS location 2) showed much better results in detecting mounting activity than ODS in location 3 and slightly lower losses of ODS (due to mounting intensity) than strips in location 1. ODS in location 2 incurred less movement caused by tail movement and achieved more contact as the ODS lay flatter than in location1.

Tables 3.6 and 3.7 record key indices in the reproductive performance of the farm, this performance is generally outside target levels but within the range of the New Zealand national database records. The reproductive performance and progesterone profiles are in agreement with results reported by Theingtham, Parkinson and Holmes, (2002) and Xu and Burton, (2000b). However these detection errors indicate that improper oestrus detection, mainly through errors of omission originating from either errors of diagnosis or errors of animal identification (Macmillan, 1980) is occurring, which provides a great potential for future improvement in this herd if a more accurate system is employed for oestrus detection.

3.5. Conclusion

The sensitivity, specificity, PPV, NPV and accuracy in the CSD group were higher than in the control group resulting in a higher overall accuracy in the

CSD group. The ODS detected more cows in oestrus that were confirmed by PD and also had fewer false positive detections. This means that the ODS and CSD provided a superior oestrus detection method.

The ODS accurately identified oestrus in some cows in the CSD group that previously had been falsely detected by the farmer and resulted in their being rebred, which led to a higher proportion of short returns, a lower proportion of 20-21 day and 39–45 day returns but resulted in higher 4- and 8-week pregnancy rates than those achieved by the control group. With the positive influence that the CSD had on this farm's performance in the CSD group, it appears that the CSD offers a potential to increase conception rates in some herds if AI is timed using CSD oestrus detection.

3.5.6. Improvements required in the CSD oestrus detection system

3.5.6.1. CSD camera and software

Refinement of the software and its use with a fully compatible camera that is robust to working conditions in the milking shed, plus coupling the software with automatic cow identification could markedly decrease the time spent on herd monitoring for oestrus detection whilst maintaining or potentially improving the accuracy of oestrus detection.

3.5.6.2. Oestrus detector strip adhesive

More research is required into the best adhesive for ODS. In this research, insight was gained about the number of missing ODS. Missing ODS meant that there was a loss of data for the current research since the proportion of paint removed from missing ODS was unable to be recorded.

3.5.6.3. Location of the camera

Placing the camera directly over the ODS to minimise light distortion and false readings was difficult to achieve in this project because a ring bar that holds the vacuum and milk pipes of the rotary shed was situated directly over the cows' rumps on the milking platform. A specially designed structure that avoids the obstruction of the cows' rumps by the ring bar would improve the operation of the detection system considerably.

Chapter Four

Evaluation of a camera-software device to each of four different percentages of tail paint amount removal for detection of oestrus when used on dairy cattle at pasture.

Abstract

The sensitivity, specificity, predictive values and accuracy of oestrus detection using camera-software device (CSD) or tail paint were compared in a New Zealand spring calving herd. Four hundred and eighty cows grazing on pasture were randomly allocated to two groups (n=240). Cows in the group monitored by CSD were fitted with oestrus detection strips (ODS) that signalled previous mounting activity associated with oestrus. Cows in the control group (n=240) were monitored by a strip of paint applied over the tail head and paint responses were classified into four categories being 1-25%, 26-50%, 51-75%, 76-100% of tail paint removed. Visual observation was conducted on the two groups for 30-45 minutes before morning and afternoon milking and at other random times. The confirmed pregnancy diagnosis results and artificial insemination records were used as a standard to allow comparison of these oestrus detection methods. Parity, body condition score and days in milk at PSM were excluded from the analysis ($p>0.05$) as no confounding was found. The test sensitivity, specificity, PPV, NPV and overall accuracy were higher for the CSD group (85%, 99.6%, 88%, 99.4% and 99% respectively) than for the control group ($p<0.01$). Sensitivity for detection of oestrus in the tail paint removed categories decreased as the required amount of paint removed in a category increased (78%, 73%, 64%, 55% respectively) and this was associated with an increasing false negative percentage (respectively 22%, 27%, 36% and 45%). The specificity of oestrus detection increased (95.3%, 96.5%; 98.4%; and 98.9 % respectively) with increasing amount of paint required to be removed. The farm management practices relying on tail paint findings lead to the drafting of cows for AI and earlier breeding than should have occurred, due to errors in tail paint interpretation. The study further showed that a CSD system can be satisfactory for oestrus detection in seasonally calving dairy herds grazing on pasture.

KEY WORDS: Oestrus detection, camera-software device, visual observation, tail paint, dairy cattle.

4.1. Introduction

Accurate detection of oestrus is a requirement for high reproductive performance when artificial insemination (AI) is used in dairy herds. Failure to detect oestrus caused loss of milk production, long calving intervals and the birth of fewer calves (Reimens, Smith and Newman, 1985; Liu and Spahr, 1993).

Several aids have been investigated and are being developed to improve the detection of oestrus. These aids include pedometry, chin-ball markers, oestrus mount detectors, devices that measure vaginal electrical conductivity and milk temperature (Farris, 1954; Foote, 1975; Gartland et al., 1976; Kiddy, 1977; Macmillan and Curnow, 1977; Eradus et al., 1992). These devices can increase the efficacy of oestrus detection when combined with visual observations, but their efficacy and accuracy may be less effective when used alone (Lehrer et al., 1992).

Mount detectors have the broadest application to beef and dairy cattle. HeatWatch® (DDX, Inc, Denver, CO.) is the only real time radio-telemetric system available. Despite its efficiency and low labour requirement relative to other systems like HeatWatch Express® (DDX, Inc, Denver, CO.) and ShowHeat® (I.M.V. International, Minneapolis,MN), it is associated with high costs per animal. Less expensive but more labour intensive stand alone monitors are ShowHeat® and MountCount® (DDX, Inc, Denver, CO.) (Xu et al., 1998)).

The New Zealand dairy production system is a low cost efficient system, because it utilizes the seasonality of pasture growth to produce milk, which necessitates a 365 day calving interval with a condensed calving pattern (Burke, 2003).

Visual observations, aided by tail painting, are the main methods used for oestrus detection in New Zealand (Macmillan et al., 1988). This technique utilises a strip of paint applied over the tail head and regular observation through the day with inspection of paint strips, particularly at milking times, for evidence of oestrus. The disappearance or disruption of a paint strip is taken to indicate the occurrence of prior mounting activity. Using this oestrus detection aid and visual observation under New Zealand condition, a conception rate of around 65% can be achieved during the first 21 days of a mating season (Xu and Burton, 1996; Xu and Burton, 1997; Xu et al., 1998).

A camera-software device (CSD) was recently developed to automatically detect oestrus based on this tail painting technique with the addition of optical identification as described in Chapter Two. This device provides a means of automatic monitoring of mounting activity that could potentially reduce or eliminate the need for visual observations. As reported in Chapter Three, where diagnosed pregnancy was used to confirm the occurrence of oestrus, it was shown that the use of CSD and oestrus detector strips (ODS) could significantly increase the efficiency and accuracy of oestrus detection when compared with visual observations and tail painting (traditional farmer detection) and that this lead to improved reproductive performance.

The objective of this chapter is:

To compare the efficacy and accuracy of the ODS in association with the use of the CSD in detecting oestrus at each of four different percentages of tail paint removal used under farm conditions.

4.2. Materials and methods

The experiment was conducted in the spring breeding season of 2003 at the Massey University number 4 dairy unit, which operates commercially in the Manawatu district of New Zealand. The experiment took place between the 6th of October and the 4th of December of 2003.

Four hundred and four cows of mixed breed (Friesian, Holstein, Jersey and their crossbreeds) were used in the trial. Cows ages ranged from 24 months to 13 years old (Table 3.1). Before the trial started, animals' reproductive histories were checked for any abnormalities. All animals selected were reproductively sound animals with a body condition score of not less than 3 (scale 1-8; Table 3.2).

Selected animals were randomly allocated to one of two groups using the randomisation function of Microsoft Excel 2000 (Microsoft Excel® for Windows 1989-2001). The randomisation was stratified by age, condition score and days in milk for the animals. The outcome was then analyzed using the one way analysis of variance (ANOVA) and cross-tabulation functions of the SPSS© program (Apache software foundation, SPSS Inc, 12.01 for Windows, 2001, USA) and showed no significant difference between the groups selected (p= 0.259) in these measures. All animals were found eligible for the trial and were randomly allocated to the groups. Seventy-six further suitable cows were randomly added later to the trial, comprising 40 late calving cows and 27 new cows that were added by alternate allocation to the groups as they arrived (see chapter three materials and methods section for detailed information).

4.2.1. Tail paint and oestrus detector strips

At day one, commercially available tail paint (Tell tail oil-based tail paint, fluorescent oestrus detection tail paint, FiL New Zealand Ltd; Mount Maunganui, New Zealand) was applied in strips 18–21 cm long and 5–6 cm wide from the sacral area, posteriorly covering the midline, on the rump of each animal in the control group.

The paint was applied using a brush, initially against the direction of the hair and then, with the direction of the hair to achieve a smooth service (Kerr and McCaughey, 1984). The percentage of tail paint removed from the drafted cows was independently scored and categorised for all cows twice daily during milking times into four categories as follows:

- 1% to 25% of tail paint removal (TP25)
- 26% to 50% of tail paint removal (TP50)
- 51% to 75% of tail paint removal (TP75)
- 76% to 100% of tail paint removal (TP100)

The farmer used three colours of paint that were maintained weekly. Blue was used when a cow calved and was being watched for the first postpartum oestrus, red when a cow was confirmed cycling then green when artificially inseminated. If a cow returned to oestrus after insemination within the artificial breeding period, it was re-inseminated and repainted with green paint.

A cow was considered by the farmer to be in oestrus based on tail paint removal of more than 75%. Cows suspected to be in oestrus were checked for mounting marks such as skin abrasion and hair removal over the sacrum before being drafted. If the paint was believed by the farm manager and workers to be removed for any other reason, the cow had her strip repainted and was sent back with the herd. If a cow was visually observed to be in oestrus it was considered as being in oestrus regardless of its' tail paint status.

ODS comprised Scotchlite (3M 9920, 3M New Zealand Ltd, Auckland, New Zealand) reflective strips of 150 mm x 50 mm, painted with Zylone sheen paint (water based flat acrylic, Resene Paint Limited, Palmerston North, NZ), glued to cows using Ados F2 glue (CRC industries New Zealand Ltd, Auckland , New Zealand). ODS were placed initially on the sacral area of CSD group animal's rump (ODS location; 1 Figure 3.2), after adequate brushing followed by glue applied to the area to ensure maximum adhesion (see chapter two).

The camera for the CSD readings was mounted in an area of the milking shed with relatively low light variation to minimise distortion and at approximately a right angle with the ODS within a range of 150 centimetres to make the measurements of the strips and paint removed more reliable (see chapter two).

The CSD measured and reported the amount of paint removed as a percentage. A cow was considered to be in oestrus if it had a reading of 10% or greater or if the ODS was missing (which assumed that removal occurred from the intensive mounting behaviour).

Images were captured at three second intervals allowing three frames per cow per ODS to be obtained. In the absence of electronic identification, manual collation and recording of the animals and reading for the proportion of paint removed was required. The ODS were maintained weekly, which involved cleaning the very dirty ones and replacing the missing ones during an afternoon milking. ODS that showed a positive reading were removed from cows and replaced after four days.

4.2.2. Inseminations

Inseminations occurred after the morning milking on cows identified the evening before and the same morning (see chapter three material and methods section for detailed information).

4.2.3. Pregnancy diagnosis results

Pregnancy diagnosis by rectal palpation and/or ultrasound examination was calculated on the 27th and 28th of January 2004, which was 51 and 52 days after the end of the breeding season. PD results were used as a standard to allow comparison of the ability of the different oestrus detection methods used to correctly identify oestrus and the non-oestrus periods (when cows were not in oestrus; Figure 3.1; see chapter three materials and methods section for detailed information).

4.2.4. Contingency 2 x 2 tables

Contingency 2 x 2 tables were constructed for each group to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for that method based on the oestruses confirmed by pregnancy diagnosis.

4.2.4.1. Data analysis for contingency tables

The full data set had the two groups designated as the CSD group (group one) and the control group (group two). Within the control group, each tail paint category was considered as an independent method for oestrus detection, applied on the same cows and running in the same time period. Thus four datasets were created to run the analysis between the different oestrus detection methods applied and to compare it to CSD detection in the other group. a matched analysis was run that included method of detection nested within cow as a repeated effect. After running each model, the covariate effects of parity, body condition score and days in milk at PSM were removed stepwise and the relative changes in model coefficients were calculated. A relative change of >15% was interpreted as a confounding effect for that variable on sensitivity, specificity or predictive values (Dohoo, 2003). Statistical significance was declared at $p<0.05$.

4.3. Results

Fifty-seven cows from the control group including 7 cows that were sold or died and 27 cows from the CSD group including 7 cows were sold or died were excluded from this analysis because they were detected by the oestrus detection methods applied but they either showed no evidence of pregnancy or their status could not be determined.

In the control group, TP25 had the highest number of correctly identified oestruses (201 oestruses) but missed 57 true oestruses. As the required amount of paint removed to indicate oestrus increased, the number of correctly detected oestruses decreased with an increase in the number of missed oestruses. TP75 correctly identified 166 oestruses and missed 92 oestruses. TP100 detected 142 oestruses but had 116 missed oestruses. The CSD correctly detected 341 oestruses and missed 65 oestruses (Table 4.1).

The test sensitivity (with 95% confidence intervals (CI)) for the tail paint categories decreased as the required amount of paint rubbed off increased (TP25= 78% CI= 72 to 82%; TP50= 73% CI= 67 to 78%; TP75= 64% CI= 58 to 70%; TP100= 55% CI= 49 to 61%) which equates to an increasing percentage of false negative findings (respectively 22%, 27%, 36% and 45%). Specificity increased with a requirement for an increased amount of tail paint rubbed off (respectively 95.3% CI= 94.9 to 95.7%; 96.5% CI= 96.2 to 96.9%; 98.4% CI= 98.2 to 98.7; and 98.9% CI= 98.7 to 99.1) which equates to a decreasing proportion of false positives (4.7%, 3.5%, 1.6% and 1.1% respectively) as shown in Table 4.2.

In the CSD group, the sensitivity of detection was 85% (CI= 80 to 87%) and this differed significantly from the tail paint groups (TP 25 and TP 50 $p<0.0001$; TP 75 $p=0.0002$ and TP 100 $p=0.0047$) indicated a low (15%) proportion of false negative results. Test specificity was also higher ($p<0.0001$) for the CSD group (99.6% CI= 99.4 to 99.7%) indicating low false-positive results (0.4%) as shown in Table 4.2. The CSD had a higher PPV (88% CI= 84 to 91%; (TP 25 and 50 $p<0.0001$, TP 75 $p=0.0005$ and TP 100 $p=0.03$), NPV (99.4% CI= 99.3 to 99.6 $p<0.0001$). Table 4.3 shows overall accuracy results (with 95% CI) for the two groups and the CSD group had the highest ($p<0.0001$) overall accuracy (99% CI= 98.8 to 99.2).

Table 4.1: Oestrus detection aid results for eligible cows in the control group (n=183) and camera-software device (CSD) group (n=213) over the artificial breeding period of 55 days and confirmed by pregnancy and artificial insemination records.

Group*	Oestrus detection aid	Method results	Oestrus status confirmed by pregnancy	
			Oestrus	Not in oestrus
Control	TP 25	Oestrus (+)	201	459
		Not in oestrus (-)	57	9400
	TP 50	Oestrus (+)	189	341
		Not in oestrus (-)	59	9518
	TP 75	Oestrus (+)	166	153
		Not in oestrus (-)	92	9706
	TP 100	Oestrus (+)	142	105
		Not in oestrus (-)	116	9754
CSD	CSD	Oestrus (+)	341	48
		Not in oestrus (-)	65	11209

- Data for both groups was collected twice daily in the morning and evening and was then pooled into one daily observation for each cow. Cows that were not confirmed pregnant were excluded from the analysis. . Data loss resulted from cows occasionally failed to attend at milking time
- TP25= tail paint removed 0-25%; TP50= tail paint removed 26-50%; TP75= tail paint removed 51-75%; TP100= tail paint removed 76-100%

Table 4.2: Test sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the camera-software device (CSD) group and tail paint categories with 95% confidence intervals (CI) for detecting oestrus.

Group [a]	Oestrus detection method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Control	TP 25	78	95.3	30	99.4
	95% CI	72 to 82	94.9 to 95.7	27 to 34	99.2 to 99.5
	TP 50	73	96.5	36	99.3
	95% CI	67 to 78	96.2 to 96.9	32 to 40	99.1 to 99.4
	TP 75	64	98.4	52	99.1
	95% CI	58 to 70	98.2 to 98.7	47 to 57	98.8 to 99.2
	TP 100	55	98.9	57	98.8
	95% CI	49 to 61	98.7 to 99.1	51 to 63	98.6 to 99.0
CSD	CSD	85 [b]	99.6 [c]	88 [d]	99.4 [e]
	95% CI	80 to 87	99.4 to 99.7	84 to 91	99.3 to 99.6

Sensitivity= (number of true-positive results/ (number of true-positive results + number of false-negative results))
Specificity= (number of true-negative results/(number of true-negative results + number of false-positive results))
PPV= (number of true-positive results / (number of true-positive results + number of false-positive results)).
NPV= (number of true-negative results / (number of true-negative results + number of false-negative results))
TP25= tail paint removed 0-25%; TP50= tail paint removed 26-50%; TP75= tail paint removed 51-75%; TP100= tail paint removed 76-100%
CSD= camera-software device
[a] Data for both groups were collected on a day-by-day basis, twice a day in the morning and evening and then pooled into 1-day observations for each cow. Cows that did not show evidence of pregnancy were excluded from the
[b] TP 25 and TP 50=$p<0.0001$; TP 75 $p= 0.0002$ and TP 100 $p=0.0047$
[c] $p<0.0001$
[d] TP 25 and 50 $p<0.0001$, TP 75 $p=0.0005$ and TP 100 $p=0.03$
[e] $p<0.0001$

Table 4.3: Overall accuracy of tail paint categories and the camera-software device (CSD) groups in oestrus detection with 95% confidence intervals (CI).

Group	Oestrus Detection Method	Overall Accuracy [a]%
Control	TP 25	95
	95% CI	94.4 to 95.3
	TP 50	96
	95% CI	95.6 to 96.3
	TP 75	97
	95% CI	97.3 to 98.0
	TP 100	98
	95% CI	97.5 to 98.1
CSD	CSD	99 [b]
	95% CI	98.8 to 99.2

[a] Overall accuracy = ((True positives + true negatives "all cases truly identified by a test") / (true positives + false positives + true negatives + false negatives i.e. "total population"))
TP25= tail paint removed 0-25%; TP50= tail paint removed 26-50%; TP75= tail paint removed 51-75%; TP100= tail paint removed 76-100%
CSD= camera-software device
[b] $p<0.0001$ to farmer-control and farmer–CSD

4.4. Discussion

The objective of the current study was to compare oestrus detector strips in association with a camera-software device in detecting oestrus to tail painting under normal farm management.

The use of pregnancy as a standard to confirm the occurrence of oestrus, in order to compare the oestrus detection aids and its use for estimating the true oestruses previous to that of the successful AI increased the ability to confirm the occurrence of oestrus. If cows were found not to be pregnant at the time of pregnancy diagnosis, their prior oestrus history could not be confirmed. Reasons to confound the allocation of oestruses are anoestrus inseminations and the presence of non-cycling cows in the herd.

The reported results obtained by the farm management are in agreement with results reported by Fulkerson et al. (1983) where a detection sensitivity of 66% was reported for tail paint use alone in large commercial dairy herds. The farm detection results are in agreement with the results reported by Williamson et al 1972 (56%) and Esslemont, 1973 (67%) where cows were only observed at morning and evening milkings. This may indicate that sufficient time was not allowed for the detection of oestrus by the farm workers in this study and a full reliance was placed on tail paint as a method for detection of oestrus. Kerr and

McCaughey (1984) reported 88.1% of oestruses were accompanied by tail paint removal. Mai et al. (2002) reported 82.6% detection of oestrus by tail paint, whilst Cavalieri et al. (2003a) reported 91.3%. Thus the results reported for tail paint based detections are lower than those reported in the literature.

The potential value of the CSD system can be assessed by comparing its sensitivity, specificity, PPV, NPV and accuracy with those obtained using the tail paint categories. The CSD group had a higher sensitivity, specificity and accuracy than any of the tail paint categories. This is an indication that the CSD would have a positive effect on the farm management of this farm and would result in more detected oestruses if it was the system employed for oestrus detection (see chapter three).

CSD had a higher PPV and NPV than the tail paint groups. The CSD PPV lies within the range of the results reported by Cavalieri et al. (2003a) for the different oestrus detection aids studied (91.7%), but tail paint PPV findings in this study are lower than what was reported for tail paint (91.3%). The PPV reported for tail paint is also lower than the 83% that Fulkerson et al. (1983) reported. The higher predictive values for the CSD indicate that it had a higher probability of detecting cows in oestrus and excluding cows that are not in oestrus than did the tail paint categories. This clearly shows the superiority of CSD over tail paint in this study but it could also imply a lack of thoroughness and attention to oestrus detection when interpreting tail paint findings. As discussed in chapter three, the high PPV for the CSD positively affected the farmers performance (farmer detection comprising tail paint and visual observation; see chapter three) and thus lead to higher overall detection and conception rates.

The reproductive analysis was carried out as reported in chapter three and detailed results are shown. Those results indicate that the CSD oestrus detector strips' reactions to mounting activity are more sensitive than the responses of tail paint as: A) it requires a considerable amount of mounting before the paint on the ODS starts to be worn off or for the ODS to be detached from its location (chapter two); B) the intensity of mounting (oestrus) is lower at the end of the mating season compared to the start of the season, thus affecting the interpretation of tail paint removal (lack of experience in interpreting oestrus behaviour when detected by tail paint) combined with improper identification of cows that were in oestrus, which could explain the high 20-21 day returns and could have lead to decreased submission and conception rates. The association of high true positives (85%) and low false negatives means that CSD had better detection efficiency than farmers using paint (Tables 4.3; 4.4; see chapter three discussion section).

4.5. Conclusion

The present study showed that the CSD system had a higher sensitivity and accuracy than the tail painting technique for oestrus detection. This resulted in a higher overall accuracy for the CSD than that of tail painting. There was a significant difference between detection by the CSD and all tail paint removal categories showing that it can be a satisfactory means of oestrus detection in seasonal dairy herds grazing on pasture. With the positive influence that the CSD had on the farm performance, as shown in chapter three, it appears that the CSD offers the potential to increase conception rate if AI is conducted based on using the results of CSD oestrus detection.

The normal farm management practices (comprising tail painting and visual observation for oestrus detection as was described in chapter three) leading to the drafting cows for AI, resulted in cows being drafted and bred earlier than they should have been. The ODS then accurately identified oestrus due to its higher PPV in these cows in the CSD group which resulted in their being rebred. Thus there was a higher proportion of short returns, a lower proportion of 20-21 day and 39--45 day returns and higher 4-week and 8-week non-return rates than occurred in the tail paint detected groups.

Chapter Five

Evaluation of a camera software device as an aid to oestrus detection for dairy cattle at pasture using progesterone analysis and pregnancy outcome to confirm oestrus.

Abstract

The combined use of serial progesterone analysis and pregnancy outcome as confirmation of oestrus occurrence were used to estimate the sensitivity, specificity, predictive values and accuracy of oestrus detection using a camera-software device (CSD) compared with on farm management (Visual observation and tail paint) in a New Zealand spring calving herd. Four hundred and eighty cows grazing on pasture were randomly allocated in to two groups (n=240). Cows in the group monitored by CSD were fitted by oestrus detector strips that signalled mounting activity associated with oestrus. Cows in the control group (n=240) monitored by farm management (comprising tail paint use and visual observation). Visual observation of the two groups was for 30-45 minutes before morning and afternoon milking and at other random times. Milk samples were taken three times per week over the mating period for progesterone analysis. Confirmed pregnancy, artificial insemination and milk progesterone results were used as outcomes to allow comparison of the different oestrus detection methods (κ=0.74). Parity, body condition score and days in milk at PSM were excluded from the analysis ($p>0.05$) as no confounding was found. The test sensitivity ($p<0.039$), specificity ($p<0.001$; $p<0.012$ for farmer-CSD/-control respectively), PPV ($p<0.0001$), NPV ($p=0.55$; $p=0.01$ farmer-CSD/-control respectively) and overall accuracy ($p<0.05$) for the CSD group were higher than for the control groups. The normal farm management practices leading to the drafting cows for AI resulted in cows being drafted and bred earlier than they should have been. The CSD system can be satisfactory for oestrus detection in seasonally calving dairy herds grazing on pasture where detection by farm workers is poor.

KEY WORDS: Oestrus detection, camera-software device, visual observation, milk progesterone, tail paint, dairy cattle.

5.1. Introduction

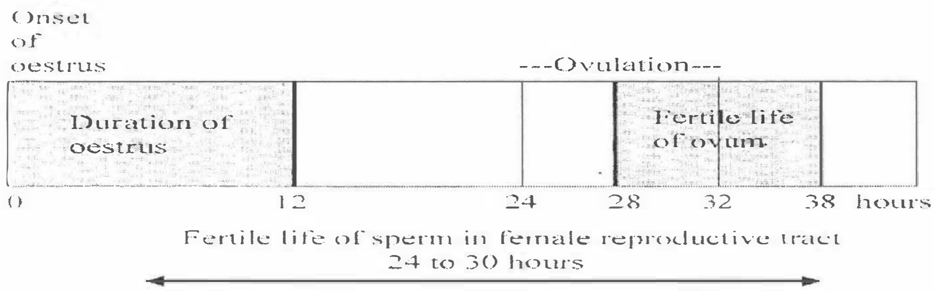
Detection of oestrus is essential in any planned breeding program if efficient reproduction is to be achieved. In herds with seasonal calving patterns, identification of cows that are cycling before the mating season starts is important as is the correct identification and submission of cows in oestrus. In controlled mating programs, oestrus detection is undertaken by stockmen, with the most common method being observation of cow mounting and standing behaviour at milking time. This may be sufficient in small herds but it is generally not sufficient in larger herds (Williamson et al., 1972).

The onset of oestrus is evenly distributed during the day (Walker, Nebel and McGilliard, 1996). Dransfield et al. (1998) showed that 24.1% of all oestrus periods had low intensity and short duration (<1.5 standing events per hour, <7 hours duration). This influences oestrus detection efficiency and accuracy (Walker et al., 1996). Trimberger (1943) suggested that for artificial insemination (AI) to be successful a cow should be inseminated at approximately 12 hours after initial observation of standing oestrus.

For an AI program to be successful, efficient and accurate oestrus detection must be applied together with proper semen handling technique and the correct time of AI relative to ovulation. AI success is affected by the length of the functional viable life of the sperm and ova, sperm transfer time from the insemination site to the fertilisation site and the timing of ovulation (Trimberger and Davis, 1943; Thibault, 1973; Wilmut and Hunter, 1984; Hawk, 1987) (Figure 5.1). Inefficient detection of oestrus can result in lost milk yield, a decreased number of calves born per life time, increased days open and an increase in reproductive culling (Chenault, Thatcher, Kalra, Abrams and Wilcox, 1975; Brackett, Oh, Evans and Donawick, 1980; Walker et al., 1996).

The objective of this chapter is:

To evaluate a camera software device (CSD) used with oestrus detector strips (ODS) to normal on farm management (comprising tail paint use and visual observation) first, in identifying the onset of oestrus and the accurate *timing of artificial insemination using milk progesterone results and second, an economic evaluation of CSD and ODS system.*



Copied from Nebel et al. 2000, p25.

Figure 5.1: Average time relationships among reproductive events associated with fertilization in the bovine.

5.2. Materials and methods

The experiment was conducted in the spring breeding season of 2003 at Massey University number 4 dairy, which operates commercially in the Manawatu district of New Zealand. Information about animals' histories selection and group analysis were detailed in chapter three materials and methods.

The trial was conducted from 6th October to 4th December of 2003. Cows were milked twice daily at 5:30 am and 3:00 pm using a rotary platform milking parlour. Milk samplings were collected on Monday, Wednesday and Friday mornings for six weeks to monitor the levels of milk progesterone (P4). Foremilk samples (10 ml) were collected into plastic containers before the milking cups were attached. One tablet of potassium dichromate (0.032 mg/tablet, MERCK, Merck KGaA, Germany) was added to the collected milk samples as a preservative then samples were kept in cool chests containing ice until refrigerated at -20°C pending progesterone analysis. Milk progesterone concentrations were measured using radioimmunoassay. Milk samples of the period 20th October 2004 to 11th November 2004 (23 days) were used for milk progesterone analysis.

Some animals had a reproductive treatment because they were not cycling. In total, 59 cows were treated between the14th to 23rd of November. All treated cows were included in the analysis as they were found to be distributed more or less equally (31 CSD group and 28 control group; see chapter three material and methods section for details about treatments specifications and animals) between the two groups and the treatments occurred outside the 23 day interval selected for analysis.

5.2.1. Tail painting, ODS and visual observations

Commercially available tail paint (Tell tail oil-based tail paints, fluorescent oestrus detection tail paint, FiL New Zealand Ltd; Mount Maunganui, New Zealand) was reapplied at day one of the trial (see chapter three). A cow was considered by the farmer to be in oestrus based on only tail paint findings if a removal of more than 75% of tail paint occurred. If a cow was visually observed in oestrus, tail paint findings were then neglected by the farmer. Cows suspected to be in oestrus were closely checked for mounting marks such as skin abrasion and hair removal over the sacrum before being drafted. If the paint was believed by the farm manager and workers to be removed for any other reason than oestrus, the cow was then repainted and sent back with the herd (chapter three materials and methods).

ODS were glued on the sacro-coccygeal area of CSD group animals' rumps (ODS location 1, Figure 3.5), after adequate brushing and with glue applied to the area to insure adhesion as described in chapter two. The camera for the CSD readings was mounted in a reasonably low light variation area to minimise distortion and at approximately a right angle with ODS within a range of 120–150 centimetres to make the measurements of ODS and removed paint more reliable as reported above in chapters two and three.

5.2.2. Inseminations and pregnancy diagnosis results

Insemination occurred around 19 hours after detection of oestrus for cows detected in the afternoon milking and 4 hours after oestrus detection for those detected in the morning.

Pregnancy diagnosis by rectal palpation and/or ultrasound examination was conducted on the 27^{th} and 28^{th} of January 2004, which was 51 and 52 days after the end of the breeding season. The pregnancy diagnosis (PD) results as detailed in chapter three were used in conjunction with milk progesterone results as a standard to allow comparison of the ability of the different oestrus detection methods to correctly identify periods of oestrus in this chapter.

5.2.3. Milk progesterone results and radioimmunoassay

Milk progesterone was assayed using a double-antibody radioimmunoassay using described methodology (Nara and First, 1981; Larson, Butler and Currie, 1997). Each milk sample was thawed then an aliquot was transferred to another tube and diluted by a factor of 11 in Phosphate Buffer Saline (PBS) (Kit diluent, MP Biomedicals, USA).

Kit standards (prepared in a human serum matrix containing sodium azide and gentamycin sulphate, MP Biomedicals, USA) were diluted in PBS by adding the same volume as the milk samples.

Progesterone concentrations in milk were measured by radioimmunoassay. Samples were assayed in duplicate. Ten μl of diluted milk in PBS was incubated with 20 μl of iodinated progesterone and 50 μl of antiserum (^{125}I- progesterone and antiserum ImmuChem^{TM} Double Antibody Progesterone ^{125}I RIA kit for in vitro diagnostic use, MP Biomedicals, USA; 4 000 cpm) for 1 hour at 37°C. 50 μl of precipitant solution (MP Biomedicals, USA) was added and each sample was vortexed thoroughly, then centrifuged for 15 minutes at 2,000 g at 4°C. Twenty μl starch (50 g/l starch (Sigma) plus 0.1 g/l neutral red (BDH) in PBSG) was added to increase adhesion of the pellet to the tube, then the samples were centrifuged for a further 15 minutes at 2,000 g at 4°C and the supernatant aspirated off. The pellets were counted on a LKB Wallac 1261 Multigamma gamma counter for 2 minutes each.

The cross-reactivity of the progesterone antibody with other steroids was tested by MP Biomedicals. Cross-reactions are as follows: 20α-dihydroprogesterone (5.41%), desoxycorticosterone (3.80%), corticosterone (0.70%), 17α-hydroxyprogesterone (0.67%), pregnenolone (0.41%), androstenedione (0.23%), testosterone (0.16%) and 11-desoxycortisol, pregnenolone sulphate, cholesterol, dehydroepiandrosterone, ethiocholanolone, oestradiol-17α, oestradiol-17β, oestrone, oestriol, andosterone, aldosterone, cortisol and DHEA-S (<0.1%).

A serial dilution of milk in PBSG was parallel to the progesterone standard curve. The quantitative recovery of progesterone in milk was measured by adding different amounts of standard progesterone to three milk samples. The recoveries of added progesterone were 98.6 $\pm$ 7.0%, 97.8 $\pm$ 6.7% and 94.0 $\pm$ 8.5% (Anon, 1994).

The sensitivity of the progesterone assay was the minimum hormone concentration that could be consistently distinguished from zero. It was determined as the hormone concentration at the mean - 2 standard deviations from the zero hormone point on the standard curves. The assay sensitivity, expressed as ng steroid/ml milk, was 1.30 ng/ml.

Solutions of progesterone in milk diluted by a factor of 11 in PBS, were used as low, medium and high level controls in every assay. The mean concentrations of progesterone in these solutions were 4.29 $\pm$ 0.17, 19.40 $\pm$ 1.80 and 72.30 $\pm$ 4.30 ng/ml respectively. The intra-assay coefficient of variation for each solution was determined by conducting an assay with 25 duplicates of each solution. The intra-assay coefficients of variation for progesterone were 8.0%, 8.8% and 5.2% for low, medium and high solutions respectively. Inter-assay coefficients of variation were calculated from duplicates of the solutions included at the beginning and end of each assay. The inter-assay coefficients of variation for 20 assays were 9.7%, 10.7% and 6.4% for low, medium and high solutions respectively.

Progesterone (P4) profiles were constructed for each cow against each day of the selected period (23 days). Values of P4 concentration for the days on which milk collections did not occur were interpolated between the values of P4 on previous and subsequent collections. A value of 1.7ng/ml was set as a cut off point between values of progesterone regarded as high and low. Previously it was claimed that this rule maximises the run length of low P4 and minimises the run length of rising P4 during a period (Ducker et al., 1986).

A normal ovulatory pattern was considered to have occurred when at least three days of low P4 values were followed within seven days by at least six days of high P4 values (Ball et al., 1983; Kerr and McCaughey, 1984; Ducker et al., 1986; Lamming and Darwash, 1998; Saumande, 2002). Milk P4 concentrations greater than 1.7ng were considered to indicate an active Corpus Luteum (CL) (Lamming, Darwash, Wathes and Ball, 1998). Luteal phases of less than or equal to 10 days were considered as short cycles (Bulman and Lamming, 1979; Riley, Peters and Lamming, 1981; Lamming and Darwash, 1998; Royal et al., 2000).

The following atypical ovarian activities were investigated using progesterone profiles, delayed ovulation type II (DOII; P4 concentration <1.7ng for ≥ 12 days), Persistent CL type II (PCLII; P4 concentration ≥ 1.7ng for ≥ 19 days during postpartum cycles subsequent to the first) and anoestrus (persistent low levels of P4) (Bulman and Lamming, 1979; Lamming and Darwash, 1998; Royal et al., 2000).

A Kappa test (κ) was used to measure the agreement between the oestrus results derived from pregnancy information and the P4 results with each used as independent standards to confirming oestrus. The kappa value is used to estimate the level of agreement between the two standards used for the same cows. A kappa value of 0 indicates no agreement and a value of 1 indicates a perfect agreement. For the current research, a kappa value between 0 and 0.4 would indicate a low level of agreement, a kappa value between 0.4 and 0.6 a moderate level of agreement and a value higher than 0.6 a high level of agreement.

5.2.4. The use of milk progesterone and pregnancy information with calving dates to confirm the occurrence of oestrus

Progesterone and pregnancy data were merged for pregnant cows (PD positive and calved) and also for non-pregnant cows. Each of the oestruses confirmed by pregnancy was accompanied by low P4 (<1.7ng) values for those pregnant cows that subsequently calved. Therefore low P4 concentration was used only to confirm the occurrence of oestrus for non-pregnant cows that were otherwise excluded from the analysis. For all but the last fertile breeding where all pregnant cows had low P4 values, P4 concentration was considered as the true indicator of occurrence of oestrus when a discrepancy was found between pregnancy diagnoses confirmed oestrus outcomes (see chapter three) and P4 results.

The following rules were applied for the oestrus detection methods used in this research (Appleyard and Cook, 1976; Heckman et al., 1979; Ball et al., 1983; Ducker et al., 1983; Kerr and McCaughey, 1984; Ducker et al., 1986; Lamming et al., 1998; Saumande, 2002; Cavalieri et al., 2003a) when PD and P4 results were merged:

i. Oestrus and detection (with the corresponding detection method) were considered to be correctly timed if they occurred when the P4 value on the day of the event was less than 1.7ng.
ii. A true detection by any of the methods used was credited if detection occurred within an identified oestrus period with a clear ovulatory pattern as described above in section 5.2.3.
iii. Detection on the day before, or the day after a defined oestrus period was credited for the corresponding detection method as a true detection of oestrus.

5.2.5. Data analysis and economic evaluation of CSD system

Contingency 2 x 2 tables were constructed for each group to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy based on the oestruses confirmed by both pregnancy data and P4 results. The methodology of constructing the contingency tables and data analysis was as detailed in the materials and methods section of chapter three, with the difference of using the combined PD confirmed oestrus and P4 confirmed oestrus as a reference to confirm the occurrence of oestrus for each cow. Milk progesterone concentrations and pattern outcomes were analysed using Chi-square testing. The statistical analyses employed to assess the oestrus detection methods used were descriptive statistics, cross-tabulation, analysis of variance (ANOVA), Chi-square, Kappa test through SPSS© (Apache software foundation, SPSS© SPSS Inc. 12.01 for windows, 2001, USA) and logistic regression using SAS® (SAS Institute Inc 8.02. 1999-2001, Cary NC, USA).

Economic returns (estimated as additional to that from control cows) were calculated (Rougoor and Williamson, 1991) based on DairyWin reproductive analysis outcomes (Chapter three result section). The main cost factors considered for the economic evaluation were AI costs NZ$25 per dose, rearing heifers NZ$750, price of claves NZ$130, culling NZ$400, milk solid price/Kg NZ$4.20, costs for induction NZ$ 15, vet visits NZ$50 and variable costs of feed for extra milk solids NZ$0.35/Kg ms (Appendix I).

5.3. Results

Forty-two cows from the control group and 17 cows of the CSD group were excluded from this analysis because they did not have a traceable progesterone profile due to lack of interpretable progesterone data results from samples being lost in laboratory, or misidentification of samples at the time of analysis.

The merged progesterone and pregnancy based results showed strong agreement (κ=0.74). Cross-tabulation results and oestrus detection aids are shown in Table 5.1. The CSD had a significantly higher sensitivity (73% {205 oestruses}, 95%CI: 68%-78%; $p<0.039$), specificity (99.4%, 95%CI: 98.4%-99.2%; $p<0.001$, $p<0.012$ for farmer-CSD/-control respectively), PPV (86%, 95%CI 81.0%-90.0%; $p<0.0001$) and overall accuracy (98%, 95%CI 98.7%-99.5%; $p<0.05$) than the farmer in the control group and in the CSD group). The CSD had significantly higher NPV than farmer in the control group ($p=0.01$) and higher than farmer in the CSD group ($p=0.55$; Tables 5.2-3).

The sensitivity of oestrus detection ($p=0.4$), Specificity ($p=0.05$), PPV ($p=0.4$), NPV ($p=0.05$) and overall accuracy ($p=0.13$) did not differ between farmer in the control group and CSD group. Parity, body condition score and days in milk at the PSM were excluded from the analysis, as they did not have a significant effect on the sensitivity or specificity of the test ($p>0.05$).

Data on the progesterone studies and oestrus observations were obtained from 421 cows (CSD=223, control=198). The progesterone profiles indicated that 217 possible oestruses occurred for the CSD group and 193 oestruses for the control group. The length of each oestrus event as indicated by low P4 values for the two groups was 7.12 $\pm$ 0.043 (S.E.) days that occurred during the selected period of 23 days. Representative patterns of milk progesterone concentration for control and CSD group cows are shown in Figure 5.2 (A-E). Milk progesterone concentrations and patterns were similar between the two groups ($p>0.05$; Table 5.4).

Overall, extra AI costs (62.3%) and costs for raising extra heifers (34.1%) were the major cost factors. Veterinary costs (examination, drugs, travel and treatment) accounted for 0.4% of the total costs. Extra returns from milk production due to the predicted concentrated calving pattern and fewer inductions are the major return factors (62%), supplemented by extra returns expected from calf sales at 24.2%. The anticipated changes in the reproductive performance when the CSD system is used for oestrus detection could increase farm profitability by NZ$82.70 per cow (Appendix I).

Table 5.1: Oestrus detection aid results for eligible cows in the control group (n=198) and camera-software device (CSD) group (n=223) in the selected breeding period of 23 days that was confirmed by milk progesterone analysis and pregnancy to artificial insemination.

Group*	Oestrus detection aid	Method results	Number with oestrus status confirmed by these methods	
			Oestrus	Not in oestrus
Control	Visual observation and tail paint (Farmer)	Oestrus (+)	152	95
		Not in oestrus (-)	70	4237
CSD	CSD	Oestrus (+)	205	33
		Not in oestrus (-)	74	4817
	Visual observation and tail paint (Farmer)	Oestrus (+)	191	86
		Not in oestrus (-)	88	4764

* Data for both groups was collected twice a day AM and PM and then pooled into one daily observation for each cow. Cows with untraceable progesterone collections were excluded from the analysis.

Table 5.2: Test sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for camera-software device (farmer, CSD) and control (farmer) groups with 95% confidence interval (CI) for detecting oestrus.

Group	Oestrus detection method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Control	Farmer	68	98.6	62	98
	95% CI	62 to 74	97.7 to 98.4	55 to 67	98.2 to 99.8
CSD	CSD	73 [a]	99.4 [b]	86 [c]	98.7 [d]
	95% CI	68 to78	98.4 to 99.2	81 to 90	98.3 to 98.9
	Farmer	68	98.5	69	98.4
	95% CI	63 to 74	98.1 to 98.7	63 to 74	98.1to 98.7

Sensitivity= (number of true-positive results/ (number of true-positive results + number of false-negative results))
Specificity = (number of true-negative results/(number of true-negative results + number of false-positive results))
PPV= (number of true-positive results / (number of true-positive results + number of false-positive results))
NPV= (number of true-negative results / (number of true-negative results + number of false- negative results))
[a] $p<0.039$ for farmer-CSD/-control
[b] $p<0.001$ for farmer-CSD; $p<0.012$ for farmer-control
[c] $p<0.0001$ for farmer-CSD/-control
[d] $p=0.01$ for farmer-control; $p=0.55$ for farmer-CSD

Table 5.3: Overall accuracy of camera-software device (farmer and CSD; n = 223) and control (farmer; n = 198) in oestrus detection with 95% confidence interval (CI) in the selected period of 23 days.

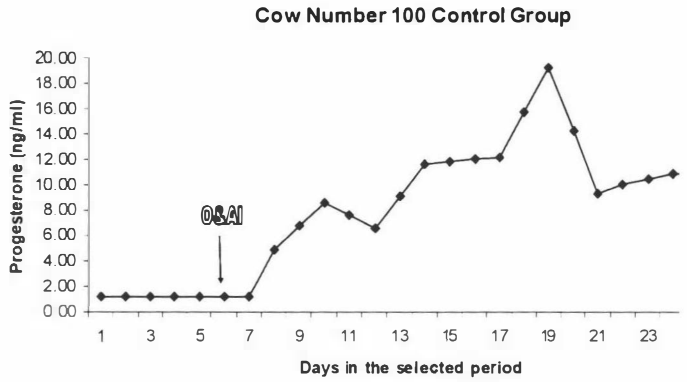
Group	Oestrus Detection Method	Overall Accuracy [a]%
Control	Farmer	97
	95% CI	96.3 to 97.2
CSD	CSD	98 [b]
	95% CI	98.7 to 99.5
	Farmer	97
	95% CI	96.5 to 97.4

[a] Overall Accuracy = ((True positives + true negatives "all cases truly identified by a test") / (true positives + false positives + true negatives + false negatives "total population"))
[b] $p<0.05$ for farmer-control/-CSD

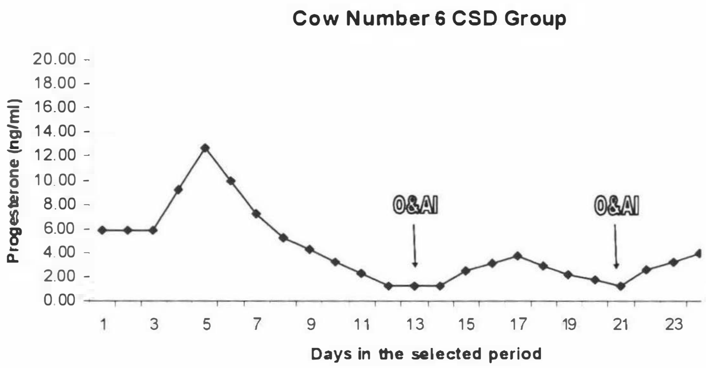
Table 5.4: The incidence of ovarian patterns (%) in the control (n = 198) and camera-software device (CSD) group (n = 223) trial cows during the selected period of 23 days.

Ovarian pattern (%)	Control Group	CSD Group	P value [a]
Normal [b] (%)	61	64	>0.05
DO II [c] (%)	5	7	>0.05
Anoestrus [d] (%)	17	16	>0.05
Irregular [e] (short cycle) (%)	7	5	>0.05

[a] Chi square test generated P-Value
[b] Normal = was considered to be occurring when a period (at least three days were set) of low P4 values were followed within seven days by a period (at least six days) of high P4 values
[c] DOII = Delayed ovulation type II occurs after recommencement of ovarian activity
[d] Anoestrus = Progesterone profile indicating consistence low level of progesterone or negligible events of progesterone rise.
[e] Irregular = Luteal phase <10 days



A



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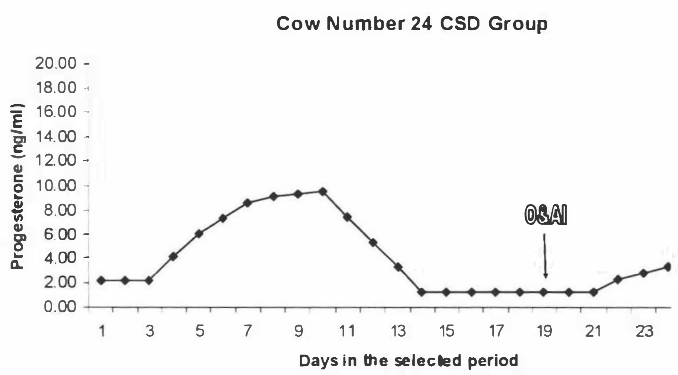
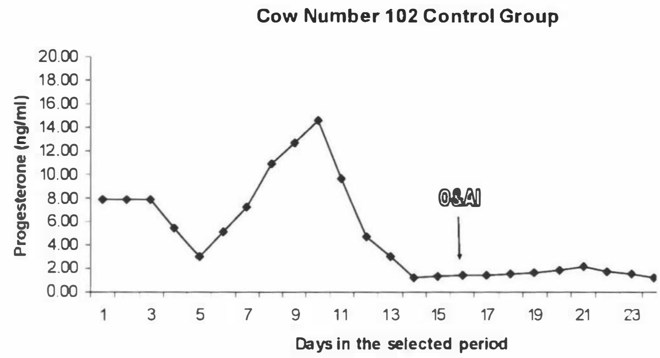
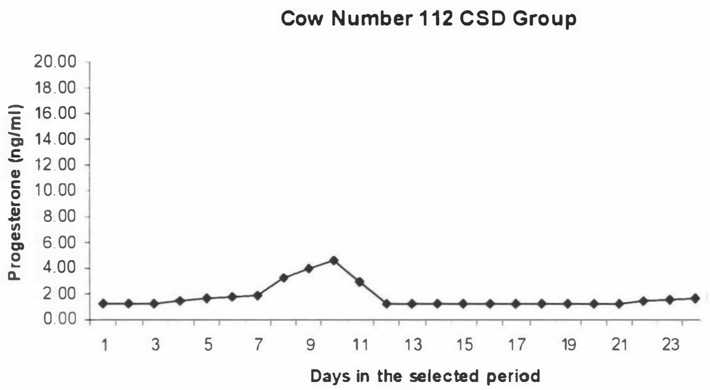


C

Figure 5.2: Representative milk progesterone profiles of trial cows showing a normal (A) pattern and abnormal patterns (B-E) of progesterone profiles. O and AI mark behavioural oestrus followed by artificial insemination.



D



E

Figure 5.2: Representative milk progesterone profiles of trial cows showing a normal (A) pattern and abnormal patterns (B-E) of progesterone profiles. O and AI mark behavioural oestrus followed by artificial insemination.

5.4. Discussion

A 95% sensitivity and positive predictive value are suggested for an ideal oestrus detection system (Senger, 1994). In previous trials worldwide this has not been achieved. For example, in the USA approximately 50% of oestrus cycles are undetected. A sensitivity of 36.8% was reported in Uruguay for lactating dairy cattle (Barr, 1975). In Northern Ireland the average rate of detection was 71% (Sawyer, Russell-Brown and Silcock, 1986; Koelsch, Aneshansley and Butler, 1994; Shipka, 2000; McCoy et al., 2002).

Detection sensitivity and PPV for the CSD and farm management differed from that reported in chapter three (section 3.3) as different cows from those used in the chapter three analysis were used in the analysis in this chapter and also different time interval (shorter interval and the start of breeding season that could have affected the sensitivity of detection of oestrus and intensity of oestrus expression). Detection sensitivity for both CSD and farm management also differed from that reported in the literature for other oestrus detection aids, with accuracy being within the reported range (Table 1.6). The difference may be due to variations in assessing the efficacy of the respective aids.

A study occurred to evaluate the effectiveness of oestrus detection using KaMaR™ (K) and/or Hot Flash (HF) rump-mounted detectors and to assess the effectiveness of androgenised cows (AC) for oestrus detection. Standing oestrus was found in 95.4 plus or minus 1.1% of controls, 84.3 plus or minus 3.5% of cows with K, 79.1 plus or minus 3.9% of cows with HF and 70.4 plus or minus 6.3% cows with K and HF. Accuracy of standing oestrus based on low milk P4 was 97.4% for controls, 87.9% for K and 81.1% for HF (Gwazdauskas et al., 1990). Another study determined the efficacy of detecting oestrus in 72 synchronised Bunaji cows using unaided visual observation (52.2%), tail painting (82.6%), KaMaR™ (82.6%) and CMD (76.8%) (Mai et al., 2002). Using the KaMaR™ oestrus detector with a teaser bull, the efficiency of oestrus detection averaged 100% vs. 71.4% using the bull alone (Huang et al., 1987).

The sensitivity results of this trial are superior to those reported for the DEC® (Showheat; IMV Technologies, France) in France where a detection efficiency of 35.4% was reported (Saumande, 2002). However these results are poorer than results from previous trials performed on smaller and synchronised herds under field conditions using pedometers, radio-telemetric transmitters (HeatWatch®), tail-paint and oestrus mount detectors as oestrus detection aids, where the mean sensitivity of detection of oestrus was found to be 92.5% (Cavalieri, Flinker, Anderson and Macmillan, 2003).

As a consequence of the high detection of true oestrus in the CSD group including in cows which already had been submitted for AI, a high frequency of apparently short cycles (generally 2–18 day returns) occurred in the CSD group cows. This and the variation in detection between the CSD and controls in this trial and when compared to previous results could be explained as:

A) The farmer was responsible for AI decisions according to what he believed was true or false oestrus. This resulted in his submitting cows too early in oestrus. One hundred and thirty cows from the total herd were detected and inseminated at least once with P4 values >1.7 ng/ml; (range 1.71-24.9 ng/ml). The farmer misidentified 28 cows with P4 >2.11 ng/ml; (range 2.11-8.5ng/ml) that were not in oestrus that were later successfully detected in oestrus by the CSD. In the control group, 102/130 cows were detected and were inseminated with high progesterone values (P4 values >1.78 ng/ml; (range 1.78-26.7 ng/ml). The lower proportion of non-oestrus inseminations in the CSD group presumably was a factor leading to the higher conception rate observed, than was observed in the control group.

CSD had a higher PPV ($p<0.0001$) and higher NPV (farmer-CSD $p=0.55$, farmer-control $p=0.01$) indicating that the CSD has a higher probability of detecting cows in oestrus and excluding cows that are not in oestrus. The trial also indicated that the CSD may positively influence a farmers detection of true oestrus, since detection by the farmer in the CSD group had a higher PPV ($p=0.4$) than farmer detection in the control group and also a higher NPV ($p=0.05$). Farmer oestrus detection in the study herds requires more attention when cows come into oestrus as is shown by the low PPV for the farmer in the control and the CSD groups, compared to CSD detection results.

B) The lower sensitivity, PPV and thus detection rate by the farm management reported in this study confirms the superiority of the CSD over farm management in the study herd and documents an apparent lack of accuracy in interpreting tail paint results. Lower detection rates have been reported previously as due to insufficient frequency of observations and skills for detection of oestrus (Williamson et al., 1972; Esslemont, 1974; Cavalieri and Fitzpatrick, 1995; Cavalieri, Eagles, Ryan and Macmillan, 2003b).

C) Detection of genuine short cycles due to a high accuracy in the CSD group would lead to an increased frequency of short returns (Fagan and Roche, 1988; Tegegne, Geleto and Kassa, 1993; Burke, Mihm, Macmillan and Roche, 1994; Ghosh, Agarwal, Shankar and Yadav, 1996; Yavas and Walton, 2000; Taponen et al., 2002). Many of the oestruses detected by the CSD system were inseminated and led to pregnancy.

The high incidence of short returns in New Zealand dairy herds was previously found to be associated with errors of diagnosis and errors of identification (Macmillan, 1998) and thus reflect the effect of management over the oestrus detection aid employed (Foote, 1975). For the detection of oestrus to be highly effective, sufficient time must be allowed to catch animals with a short period of oestrus behaviour, proper animal identification and the herdsman's experience in interpreting all signs of oestrus or impending oestrus (Fulkerson, Sawer and Crothers, 1983). Those and other factors should be investigated if detection rates are to be improved (Cavalieri et al., 2003a).

The CSD ODS gave false positive readings when they were extremely dirty (Tables 3.3-3.4; as discussed in chapter three discussion section). The percentage of false positives results in this study (farmer-control/-CSD groups 1.4,1.5% vs. CSD group 0.6%) were lower than those reported by (Gwazdauskas et al., 1990) which ranged between 3.8-18.5% and the results reported by (Cavalieri et al., 2003a) that ranged between 5 and 10%. This variation may be due to a variation in the efficacy of the aids. The percentage of false negative results in this study (Farmer in control and CSD groups 32% vs. CSD group 27%) agree with the results reported by (Mai et al., 2002) where false negative results ranged from 24.6 - 47.8%. Tables 3.6 and 3.7 record key indices in the reproductive performance of the farm and were discussed chapter three.

5.5. Conclusion

The sensitivity, specificity, PPV, NPV and accuracy in the CSD group were higher than in the control group. The ODS detected more cows that had oestruses that were confirmed by the combined PD and P4 confirmations and had fewer false positive detections. A significant difference was shown between the two methods by logistic regression analysis. This means that in this trial the ODS and CSD were superior as a method for the detection of oestrus than the farm management in this study using tail paint combined with visual observation.

Progesterone profile analysis was found to be similar between the two groups and cows in both groups were found to be similar in their ovarian patterns (normal and atypical patterns). This indicates that it was the effect of the method of oestrus detection that influenced detection of the occurrence of oestrus, rather than a difference in oestrus cycling between the groups.

As was concluded in chapter three, it appears that normal farm management practices leading to the drafting of cows for AI, resulted in cows being drafted and bred earlier than they should have been. Subsequently the present study confirmed that the CSD system can be satisfactory for oestrus detection in seasonally calving dairy herds grazing on pasture. The sensitivity and the accuracy of oestrus detection with the CSD were higher than those achieved by the farm management using tail painting and visual observations. With the positive influence that the CSD had on the farm's performance it appears that the CSD offers the potential to increase conception rate if AI is timed using the results of CSD oestrus detection.

General Discussion.

6.1. Introduction

The objectives of this thesis were specified as follows:

To develop a system that can efficiently detect previous mounting activity using an enhanced tail-paint technique and digital image analysis.

To develop a digital image analysis system that reliably detects a tail-paint based mounting detector device and accurately measures paint removal.

- *To describe and discuss key components of the camera-software device (CSD) system and the mechanisms of its function and data processing.*
- *To obtain insights about the oestrus detector strips (ODS) and the paint used as a part of them. Also to test the prototype image analysis software (VIPS), designed to automatically detect, read and interpret painted ODS. Further aims were to identify the location of the strips on the cows' rumps that provided the most reliable results and to test the efficacy of the glue used to attach strips under environmental conditions.*
- *To test the accuracy of CSD in measuring known and controlled paint removal from the ODS and to set limits to assist in their interpretation.*

To automate the reading of the tail-paint oestrus detection technique using an imaging system and a computer to assess and interpret the image the system must accurately identify the oestrus detector strips then detect oestrus automatically sense the amount of paint removed from the reflective strip placed over the sacral region of cows during the breeding season. The goals are to increase the efficacy of oestrus detection and minimise cow wastage in New Zealand.

The CSD described in this thesis is based on the previously successful use of tail paint, with modification and technological extension to allow automatic reading and interpretation. The automation is based on the optical detection of the ODS and an assessment of their condition. There are potentially two places where errors can occur when using this technique in oestrus detection. Firstly, the percentage of paint removed from the ODS when an oestrous cow is mounted may not accurately reflect the degree of oestrus behaviour (chapter two). Secondly, the measurement of the paint removed and interpretation applied by the software used in the process may have inherent errors (chapter three).

Chapter two, details investigations extending the previous work on the reflective strip (ODS) and paint (testing durability and suitability for the intended purpose under field conditions). The chapter also details an investigation into the format of the reflective strip to enable it to be detected and measured accurately using colour digital video cameras.

The main conclusions of the field research were stated and considered in Chapters three, four and five. The performance of ODS and CSD in the trials were satisfactory but they still require further development to achieve a commercially acceptable performance. The effectiveness of the system as an alternative management strategy for oestrus detection was demonstrated in Chapters three and five. This discussion will highlight the main outcomes of the work reported in this thesis and will identify needs for further research.

The statistical analysis used to analyse this research resulted from an extensive search through the literature and a series of trial analyses using approaches recommended by different statisticians. The nature of the data (collected twice a daily on consecutive days for the detection of oestrus for several methods) made the comparison between the different groups and interpretation of the findings complex, given the size of the data set, value of any one observation of the parameters used particularly to test specificity, NPV and overall accuracy. There was a very large number of correct null observations of the non occurrence of oestrus.

The difficulty of the data analysis and need for a valid data analysis approach, caused the data to be analysed several times using different approaches. Approaches used included methods estimating the efficiency of detection of oestrus (Number of detected oestrus/ total oestrus expected over the interval), using measures of agreement (Kappa), measures of disagreement (McNemar test), accuracy using contingency tables, survival analysis and the statistical parameters currently used.

6.2. Camera-software device testing and modification of oestrus detector strips

In a previous study (Williamson and Butler, Unpublished Data) a suitable reflective strip and paint combination that was easily detected optically was investigated. Reflective strip durability was tested under field conditions and both Zylone sheen acrylic (Resene low sheen paint, Resene Paint Limited, Palmerston North, New Zealand) and 3M Scotchlite reflective strip (3M Scotchlite reflective strip 9920, 3M Auckland, New Zealand) were selected (chapter two). The reflectivity and measurement of both intact ODS and after being rubbed from cows' mounting behaviour were captured by a video-computer link and analysed using video image processing software (VIPS) (Bailey and Hodgson, 1988). Within VIPS a script was developed to automatically identify the presence of a potential ODS, based on a threshold of light intensity in the captured image being exceeded.

6.2.1. Preliminary testing of automatic detection of oestrus detector strips trial

The preliminary testing of CSD and ODS trial outcome was useful initially to test computer-software (VIPS) proto-type performance in automatically detecting and interpreting ODS and allowed insights to be gained about ODS. The trial demonstrated that ODS with thicker paint layers wear off better and are as a result more sensitive than those with a thin paint layer. The trial also highlighted the importance that the ODS paint layer completely mask the reflective strip surface. Otherwise it could be misinterpreted by the CSD that the ODS already had paint worn off, as the reflective surface of the strip appeared through the paint and mislead the program (chapter two).

The trial has also identified the Sony™ (SX-900) camera a suitable camera to be used in the future trials in preference to the Intel™ web cam camera. The Intel™ web cam appeared not to be able to distinguish removed paint from the enhanced glossiness due to mounting, which lead to errors in reading the paint and the strips. As an outcome of the trial, ODS location was recommended to be over the sacral area (closer to the caudal area of the sacral spine) of the spine (chapter two), this was later proven to be impractical, as it was associated with high loss rate of ODS (chapter three). The experience that was gained in experiment one, redirected the course of the research at that stage to further investigate a suitable paint.

6.2.2. Troubleshooting trial of oestrus detector strips

The suitability of the paint material was successfully reinvestigated in this trial and Zylone sheen acrylic (Resene low sheen paint, Resene Paint Limited, Palmerston North, New Zealand) showed a better paint removal response than the original paint Resene Acrylic low sheen (water based flat acrylic, Resene Paint Limited, Palmerston North, New Zealand) and there was no increase in glossiness when the paint was subjected to rubbing during mounting by cows as measured by VIPS. Thus Zylone sheen is the most suitable of the paints tested to apply on the ODS for oestrus detection (chapter two).

6.2.3. Accuracy of the CSD in measuring paint removal from the ODS

The accuracy of CSD using two different cameras was also investigated using linear regression (chapter two). The slope obtained in the regression in experiment number three means that the CSD overestimated the area of paint removal by 12% when using the Intel™ web cam. However, the Sony™ (SX-900) camera has more powerful features and a lens that eliminated this problem and thus gave a more accurate measurement of ODS status. Despite a slight overestimation of 0.3% resulting from reflection glare of the ODS index area or the area where paint was removed, it was found to be constant for reflective areas ranging from 0% to 100% of the total strip areas.

6.2.4. Camera-software device and normal farm management trial

The objective of this thesis was to automate the reading of the tail-paint oestrus detection technique using an imaging system and a computer to assess and interpret the image and accurately identify the oestrus detector strips, then detect oestrus automatically (chapter two). The goal was then to compare it to normal farm management comprising tail paint and visual observations. Cows detected in oestrus using the CSD system achieved significantly improved reproductive performance, indicating that the CSD system was satisfactory for detection of oestrus in seasonally-calving dairy herds grazing on pasture and did improve oestrus detection performance in the study herd which otherwise had poor detection of oestrus (chapters three-five).

The reproductive analysis of herd reproductive performance for each experimental group conducted using DairyWin™ clearly showed a difference in performance between the designated groups. The results reported for first service conception rate within the CSD group is higher than is generally reported in the literature (ranging from 27.3% (Cordoba and Frick, 2002) to 63% (Smith and Macmillan, 1980)), while for the control group it is within the lower end of what has been reported (Smith and Macmillan, 1980; Kerr and McCaughey, 1984; Phatak and Touchberry, 1988; Kerr, Mcgowan, Carroll and Baldock, 1991; Cavalieri and Fitzpatrick, 1995; Cordoba and Fricke, 2002). The Dairywin™ analysis provides support regarding the positive impact of the strips and CSD on the farm's performance (chapter three).

6.3. General conclusion

In the field trial reported here the use of pregnancy to confirm oestrus created a bias in the data in favour of the control group, as the number of cows found pregnant in the control group was considerably lower than that in the CSD group (chapters three-five), which excluded from consideration more data from the control group and may have caused an over-estimation of control group performance. The study design also utilised a logging of true negative results on a daily basis that led to amplification of the specificity and NPV results, as explained in chapter three. Although 98% farmer–control detection specificity, for example, is relatively high it equates to 2% of false positive detections out of the total observations, which is equivalent to 202 false positive detections (total oestrus detection by farmer-control group was 395) which is 51% of all of the farmer-control group detections of oestrus.

The field trial (chapter three-five) showed that the CSD is accurate in identifying ODS on cows rumps, without confusing them with other objects in the surrounding environment such as metal and wet reflecting surfaces. This was achieved by setting a threshold of light brightness that identified the ODS and the exposed areas (chapter two). Also assessment and reading of the areas of removed paint and exposed reflective areas of the ODS was sufficient to identify almost all occurrences of oestrus. It was however observed that that some patches of manure on the ODS prevented the reflection of the light from parts of the ODS, thus not giving absolutely correct readings. Reflection from water (glinting) was also a problem as light reflection occurred from painted areas as well as exposed areas, occasionally giving false reporting of the ODS status.

Under normal farm management in the control group, more cows were drafted for AI and bred when they were not in oestrus. Cows detected in oestrus using the CSD system achieved improved and phenomenal reproductive performance as shown by DairyWin analysis in chapter three despite the low sensitivity and PPV reported in chapter three and five. Thus indicating that the CSD system was satisfactory for detection of oestrus in seasonally-calving dairy herds grazing on pasture and also that it can improve oestrus detection performance in herds with poor detection of oestrus. In conclusion, the sensitivity, specificity, PPV, NPV and accuracy in the CSD group were higher than in the control group, resulting in a higher overall accuracy in the CSD group. The ODS detected more cows in oestrus that were confirmed by PD and also had fewer false-positive detections. This means that the ODS and CSD provided a superior method of detection of oestrus. We should thus reject the null hypothesis of this study.

In order to further develop image analysis of ODS and correctly report their status more accurately it is proposed to work further on refinement of the software to decrease the image processing time and to identify a more robust camera that is compatible with the working conditions in the milking shed.

Coupling the software with automatic cow identification would decrease the time spent on herd monitoring and oestrus detection. Despite this decreased time input, the accuracy of oestrus detection could be improved.

More research is required to determine the best adhesive for the ODS and to improve the convenience of ODS application. In the current research, insight was gained about the number of missing ODS that occurred with the current system. Missing ODS meant that there was a loss of data in the current research since the proportion of paint removed from missing ODS was not able to be recorded from the strips that were missing.

A major problem in the current research was placing the camera directly over the ODS to minimise light distortion and false readings. This was difficult to achieve because a ring bar that holds the vacuum and milk pipes of the rotary shed was situated over the cows' rumps. Installation of the camera in a position that avoids the obstruction of the ring bar would improve the operation of the detection system considerably.

Appendix 1: Economic evaluation for Massey University, Dairy 4 unit based on field trial observation outcome during the spring breeding season (Before) compared to the proposed outcome if the camera-software device is used for oestrus detection.

HERD CHARACTERISTICS	Before	After
Herd Size	480	480
Planned start of calving (PSC)	38565	38565
Due Calving Pattern: 0 - 4 weeks	211	336
5 - 7 weeks	125	96
8 - 10 weeks	129	48
11 and more	15	0
No. of cows induced	15	0
No. of injections / cow	1.7	1.7
No. of days earlier calving		
due to induction	21	21
Mean calving date (days after PSC)	34.12291667	23.6
Cull cows	32	25
Calf losses (not induced) (%)	10	5
Calf losses after induction (%)	20	20
Services per conception	2.2	1.4
Genetic Prodn Potential (kg ms)	310	310
PRICES		Value NZ $
Semen and insemination costs per dose		25
Cost of rearing Heifers		750
Price of Calves		130
Price of Cull cows		400
Milk solids price / kg		4.2
Variable Cost of feed for extra Milk Solids ($ per kg ms)		0.35
Costs of Induction: Price of Injection		15
Visit fee and traveling vet		50

LIVESTOCK BUDGET		BEFORE		AFTER	
Appendix1 continued					
No. Calves born / year		480		480	
no. died		49.5		24	
Replacements Needed		32		25	
Calves for sale		398.5		431	
BUDGETED RETURNS FROM IMPROVED HEAT DETECTION					
ADDITIONAL RETURNS					
Increased Milk Solids Production (kg)					
	dt Less Induction		186		
	dt Conc Calv Pattern		5758.14		
	dt earlier PSC		0		
	dt avg cow age		0		24965.388
Extra Calves for sale			32.5		4225
TOTAL EXTRA RETURNS					29190.388
COSTS NO LONGER INCURRED					
Veterinary Costs of Induction					549.525
Semen Costs			384		9600
Costs of Raising Extra Heifers			7		5250
TOTAL COSTS NO LONGER INCURRED					15399.525
ADDITIONAL COSTS					
Costs of Herd Reproductive Health Program					
Extra Feed for Extra Production					2080.449
TOTAL ADDED COSTS					2080.449
RETURNS NO LONGER OBTAINED					
Decreased Cull Cow Sales			7		2800
TOTAL RETURNS NO LONGER OBTAINED					**2800**
NET RETURN FROM IMPROVEMENT					**39709.464**
Net Return per Cow					**82.72805**

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