NOVEL APPROACHES TO EXPRESSION AND DETECTION OF OESTRUS IN DAIRY COWS

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DECLARATION

I hereby declare that this thesis is my own work and that it has not been submitted anywhere for any other degree or award. The work presented herein is my own work and where other sources of information have been used, they have been duly acknowledged.

Elizabeth Homer

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ABSTRACT

Detection of oestrus is a key determinant of profitability of dairy herds, but is increasingly difficult to observe in the modern dairy cow, with shorter duration and less intense oestrus. Current trends in the dairy industry also exacerbate the problem of poor oestrous detection as herd sizes are increasing, yet there is less labour on the farm. As a consequence fewer cows are seen standing to be mounted, the definite sign that a cow is in oestrus. Concurrent with the unfavourable correlation between milk yield and fertility, oestrous detection rates have declined to less than 50%. Although visual detection of oestrus is accurate, it can be time consuming and inefficient. In response to these constraints and poor oestrous detection rates automated methods of detection are currently employed although they are lacking in accuracy and efficiency. The current work investigated possible risk factors among the herd for decreased oestrous expression, measured by activity monitors (Lely-HR Tags), with emphasis on individual cow factors affecting the activity increase at oestrus (n=205 cows). A novel approach was also tested, Ultra-wide band (UWB) technology (Thales Research Technology, UK) for proof of concept that oestrus, mounting and standing to be mounted, could be detected in dairy cows (initial validation studies plus 2 week long trials, n=16 cows; 8 in each).

Several parameters were investigated for their association with maximum activity increase at oestrus using generalised linear mixed models. Activity increases at oestrus between 2 and 4 fold. Various influential factors that affect the activity increase were reported in this study: parity, successive oestrous number post partum and milk yield are inversely related to the activity increase at oestrus and activity increases were affected by time of year for each oestrus event ($P<0.05$). In addition, larger activity increases at oestrus were not related to an increased probability of conception.

The three dimensional position of 12 cows, with their oestrous cycles synchronized, and 4 pregnant control cows were monitored continuously, using UWB mobile units (MU) operating within a base unit (BU) network for a period of 7 days. Cow position was reported twice per second in real-time with this system. In the complete study 10 cows came into oestrus as confirmed by simultaneous visual observation & CCTV recording, activity monitoring (Lely-HR Tags) and by analysis of milk progesterone concentration. Raw data taken from the UWB system were then analysed post trial to determine whether oestrus could be detected; including elevations in cow height and cow interactions.

Furthermore, automated software was developed and script analysis (MatLab R2012b, The MathWorks, Inc., US) was carried out to detect cows in oestrus, reporting the time of oestrus onset in real-time.

UWB accurately confirmed oestrus in 9 out of 10 cows in oestrus as confirmed by real-time video recording and continuous visual observation of activity. Although due to the constraints of the script 1 cow could not be detected in oestrus by UWB as she was the only cow in oestrus at the time equipped with a MU. Further confirmation of oestrus was carried out by physiological measurements; increases in activity on the day of oestrus and low progesterone concentrations <1ng/ml. In addition, UWB accurately confirmed 6 out of 6 cows as not being in oestrus. In conclusion UWB accurately detected cows in oestrus. Furthermore, automated detection by UWB enables the identification of the onset of oestrus, mounting, and when cows are in oestrus and first stood to be mounted, in real-time. Therefore UWB is advantageous because knowledge of onset of oestrus allows for accurately timed artificial insemination (AI) coinciding with ovulation, in order to increase conception rates.

In summary, variables that affect expression of oestrus have been identified by this work. This would allow for identification of cows prone to decreased oestrous expression. In addition UWB accurately detected oestrus when cows displayed mounting and standing to be mounted behaviour. This work has shown 'proof of concept' that with further development UWB could be used as a novel automated method of oestrous detection. Therefore the current work has provided knowledge on factors that influence oestrous expression and possible solutions to the permanent improvement of detection. The work also provides evidence of a novel technology that can be developed in order to increase oestrous detection rates.

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LIST OF ABBREVIATIONS

°	Degree
°C	Degrees Celsius
µM	Micromolar
2D	Two dimensions
3D	Three dimensions
ACTH	Adrenocorticotrophic hormone
ACTRIIB	Activin receptor type II B
AI	Artificial insemination
BCS	Body condition score
BU	Base Unit
CCTV	Close circuit television
CIDR	Controlled internal drug release
CL	Corpus luteum
cm	Centimetre
CU	Control Unit
dm	decimetre
DNA	Deoxyribonucleic acid
E2	Oestradiol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ERα	Oestrogen receptor alpha
ERβ	Oestrogen receptor beta
FGF	Fibroblast growth factor
FH	Frequency hopped
FSH	Follicle stimulating hormone
FSHR	Follicle stimulating hormone receptor
g	Grams
gDNA	Genomic DNA
GH	Growth hormone
GLMM	Generalised linear mixed model
GnRH	Gonadotrophin releasing hormone
GnRHR	Gonadotrophin releasing hormone receptor
GNSS	Global navigation satellite systems
GPS	Global positioning satellites
h^2	Heritability
HCl	Hydrochloric acid

HVDOP	Horizontal and vertical dilution of precision
Hz	Hertz
IFNт	Interferon tau
IGF	Insulin growth factor
kg	Kilogram
l	Litres
LH	Luteinising hormone
LHR	Luteinising hormone receptor
m	Metres
MGA	Melengestrol acetate
MJ/d	Megajoules per day
ml	Millilitre
mm	Millimetre
mM	Millimolar
MU	Mobile Unit
NEBAL	Negative energy balance
ng	Nanogram
P	Probability
P4	Progesterone
PCR	Polymerase chain reaction
pg	Picogram
PGE	Prostaglandin E
PGF	Prostaglandin F
POC	Proof of concept
PRID	Progesterone releasing intravaginal device
PRLR	Prolactin receptor
REML	Restricted maximum likelihood
RFID	Radio frequency identification
rpm	Revolutions per minute
SAG	Sexually active group
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
TAE	Tris-acetate-EDTA buffer
TDOA	Time difference of arrival
UWB	Ultra-wide Band
V	Volts
VTA	Ventral tegmental area

CHAPTER 1 – Introduction & Literature Review

1.1 INTRODUCTION

Dairy cow fertility has been declining rapidly for the past 2 decades and despite being widely reported and internationally recognised (Royal *et al.*, 2000a;Lucy, 2001;Pryce *et al.*, 2004) is a still a major problem that persists to the present. Fertility is reported to be declining at a rate of 1% per annum in the UK (Royal *et al.*, 2000a), with similar patterns in the US, declining at 0.45% per annum (Butler and Smith, 1989). The decline in fertility has been associated with a rapid increase in milk yield per cow (Pryce *et al.*, 2004), whilst genetic selection has focussed on higher milk yields, selection for fertility has been ignored, facilitating the current problem. This negative correlation between yield and fertility and health has also been reported across Europe (Veerkamp *et al.*, 2003;Barbat *et al.*, 2010). In Scandinavian countries fertility and health traits have since been incorporating into breeding programs to provide a total merit index (TMI) for each bull inclusive of yield (Philipsson and Lindhé, 2003). However the negative trend for dairy cow fertility is still been reported (Rodriguez-Martinez *et al.*, 2008).

This challenge will not be aided by current trends in the UK dairy industry over the past decade (which are documented in Table 1.1) where the total number of dairy farms and the total number of dairy cows in the UK has declined but milk yield remains high as the total milk yield per cow has increased. The average herd size is also larger (DairyCo, 2012b). Therefore there are fewer cows producing more milk and more cows on farm coupled with fewer staff. In conclusion infertility in the dairy herd still persists and is linked to the current trends.

Table 1.1 Trends in the UK dairy industry

	2001	**2010**	**2011**
Number of dairy farms	26556	15300	14793
Dairy cow numbers (thousand head)	2251	1857	1814
Average herd size	83	121	123
Milk yield (litres per cow per annum)	6346	7273	7533

Source: Dairy Statistics - An insider's guide 2012 (DairyCo, 2012b)

Fertility in dairy cows is defined as 'the ability of the animal to conceive and maintain pregnancy if served at the appropriate time in relation to ovulation' (Darwash *et al.*, 1997a). Infertility can be caused by failure to initiate oestrous cycles, failure to express oestrus, poor detection of oestrus, failure to ovulate, inadequate corpus luteum function and poor support of embryo development. However, the cause of infertility can be multifactorial (Roche, 2006); associated with the inclusion of Holstein genetics for increased milk production (Royal *et al.*, 2002), influenced by nutrition, production diseases and management (Lucy, 2001). Because of the broad nature of infertility, it is difficult to treat, but since subfertility is the highest economic cost to the dairy industry (Royal *et al.*, 2000a) and erodes the efficiency and profitability of the industry through increased calving intervals and missed reproductive targets there are many gains to be had. Culling for infertility costs the industry through the need for more replacement cows, extra labour for oestrous detection, more inseminations to get cows in calf, extra semen straws and technicians to artificially inseminate and veterinary costs all reducing the net profit per cow and decreasing herd profitability (Roche, 2006). Involuntary culling means more cows are required for the same units of milk to be produced and more non-productive cows are needed as replacements. These extra animals, for no increase in output, require more resources, feed, fertiliser and fossil fuels, at extra cost, and result in greater pollutant emissions; methane, ammonia, nitrate and nitrous oxide which have negative effects on the environment (Garnsworthy *et al.*, 2008). Therefore the cost of infertility is diverse and of major importance. A significant part of the fertility problem is detection of oestrus which results in one of the biggest economic losses (Peralta *et al.*, 2005).

Oestrous detection rates have declined, associated with the decline in fertility with average herd detection rates currently 50% according to the latest DairyCo figures (DairyCo, 2009). Less than 50% of cows in the herd are detected in standing oestrus (Van Eerdenburg *et al.*, 2002), which is the definitive and most accurate sign that a cow is in oestrus (Orihuela, 2000). This correlates strongly with timing of ovulation as oestrus is the overt expression for the physiological, internal mechanism of ovulation (Roelofs *et al.*, 2010). Furthermore, the number of silent heats, especially associated with high producing cows, has increased (Harrison *et al.*, 1990) and hence it is not surprising that fewer cows are detected in oestrus. The number of cows standing to be mounted has declined from 80 to 50% over the past 50 years and duration of oestrus has declined from 15 to 5 hours (Dobson *et al.*, 2008). Intensity and duration of oestrus have also declined, to 8.5 standing events on average and Holsteins are reported to average only 7 hours duration of oestrus (Dransfield *et al.*, 1998). It is also reported that the duration of secondary symptoms decreases with reduced standing time (Yoshida and Nakao, 2005). Therefore, coupled with increasing herd sizes (see Table 1.1) and less labour, it is becoming much harder to detect oestrus.

Visual observation has been previously used for detection of oestrus, although accurate this method is time consuming and impractical (Lehrer *et al.*, 1992). Current focus is centred on automated technologies which vary in detection rate from 80 to 90% but are coupled with high error rates between 17 and 55% (Firk *et al.*, 2002). There are many methods of oestrous detection with different detection efficiencies and accuracies reviewed by Firk *et al*., (2002) and Roelofs *et al*., (2010), yet none have succeeded in increasing oestrous detection rates. Undetected and falsely detected oestrus is costly due to missed and untimely inseminations, caused by extended calving intervals, reduced milk and calf production potential, replacement heifers and semen costs for infertile inseminations (Lehrer *et al.*, 1992). Therefore, emphasis in research needs to focus on efficient and accurate detection of oestrous behaviour and detection of oestrus in relation to the timing of insemination, relative to ovulation.

Expression of oestrus and effective methods of oestrous detection are of great importance to the efficiency and profitability of the dairy industry, because conception rate is strongly influenced by oestrous detection rate (Roelofs *et al.*, 2010). Detection of oestrus allows for insemination at an optimal time coinciding with ovulation, increasing probability of fertilisation with viable sperm and oocyte. Currently since a 40% conception rate is achieved (Royal *et al.*, 2000a), combined with only 50% oestrous detection rates (DairyCo, 2009), only 20% of all ovulations result in pregnancy. Strategies to improve oestrous detection rate can help arrest the fertility decline and increase pregnancy rates contributing to the sustainability of the dairy industry.

1.2 REPRODUCTIVE PHYSIOLOGY

1.2.1 The Bovine Oestrous Cycle

Heifers reach puberty at 6-12 months of age, at a weight of approximately 200-250kg, at which they commence their oestrous cycles (Forde *et al.*, 2011), throughout the whole of the adult cow's life, at intervals of approximately 21 days. Oestrus marks the beginning of the oestrous cycle which is followed by first ovulation, ending at the next episode of oestrus. This occurs every 18-24 days as the cow is polyoestrous (as opposed to the ewe and mare which can only reproduce at certain times of the year), and continues indefinitely (Forde *et al.*, 2011), only interrupted by pregnancy or periods of anoestrus, caused by inadequate nutrition or other factors affecting the delicate hormonal balance (Peters and Lamming, 1983).

The oestrous cycle is the result of cyclical changes in the ovaries controlled by hormone interactions (see Figure 1.1) of the hypothalamus (GnRH; gonadotrophin releasing hormone), the anterior pituitary gland (FSH; follicle stimulating hormone, LH; luteinising hormone), the ovaries (progesterone, oestradiol, inhibin) and the uterus (prostaglandins). These exert their actions by negative and positive feedback mechanisms (Webb *et al.*, 1992;Forde *et al.*, 2011). Figure 1.1 shows the hormonal changes that occur throughout the bovine oestrous cycle and key structures. FSH stimulates follicle recruitment in waves of 2 to 4 per cycle. LH pulses continue to stimulate the growth and development of the dominant follicle, influencing oestradiol secretion by the dominant follicle. Positive feedback of increasing oestradiol levels results in oestrus and increasing LH pulse frequency to a peak which results in ovulation. Progesterone is produced by the corpus luteum (CL) during the luteal phase of the oestrous cycle and increases post ovulation. In the non-pregnant cow prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus causes corpus luteum regression and decreasing progesterone concentration, which allows increasing oestradiol concentrations, due to increased basal LH, resulting in oestrus prior to the LH peak for ovulation (Webb *et al.*, 1992;Webb and Campbell, 2007;Garnsworthy *et al.*, 2008). The oestrous cycle of the cow is best described in 2 phases; the follicular phase and the luteal phase, describing the key structures present during that time. During the shorter follicular phase (4-6 days) oestradiol is the dominant hormone, produced by the growing follicle (Staigmiller *et al.*, 1982), before entering the longer luteal phase (14-18 days), dominated by the CL secreting progesterone (Aerts and Bols, 2010). Oestrus is the period between the follicular and luteal phases, in which the cow is sexually receptive, known as day 0, followed by ovulation at day 1 (see Figure 1.1; Peters and Lamming, 1983).

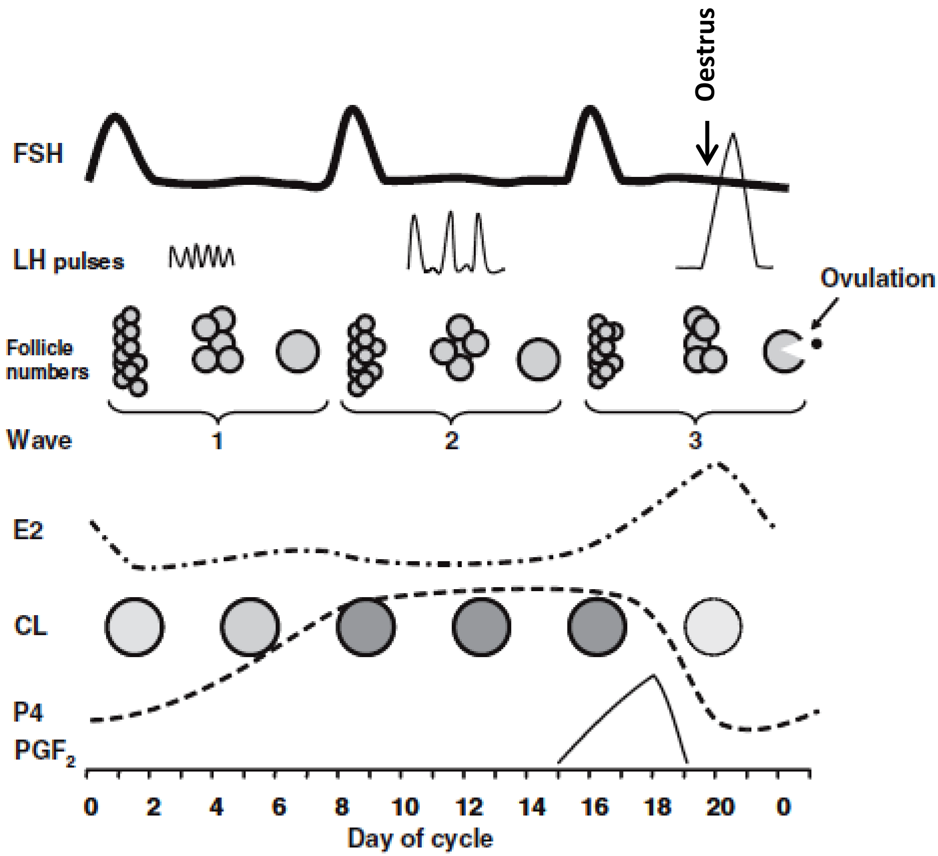


Figure 1.1 *Hormonal Control of the Bovine Oestrous Cycle.* Follicle stimulating hormone (FSH) stimulates follicle recruitment in waves of approximately 2 to 4 per cycle. Luteinising hormone (LH) pulses stimulate the growth and development of the dominant follicle, influencing oestradiol (E2) secretion by the dominant follicle. Positive feedback of increasing E2 levels results in oestrus and increasing LH pulse amplitude to the LH surge which stimulates ovulation. Progesterone (P4) is produced by the corpus luteum (CL) during the luteal phase of the oestrous cycle and increases post ovulation. In the non-pregnant cow prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus causes CL regression and decreasing P4 concentration, which allows increasing E2 concentrations to result in oestrus prior to the LH peak for ovulation (Adapted from Garnsworthy *et al.,* 2008)

1.2.1.1 Follicular Phase

There are a fixed number of primordial follicles established during foetal development (Webb *et al.*, 2004), which deplete thereafter as many follicles become atretic because follicle growth occurs continuously throughout the cow's reproductive life (Fortune, 1993). Cattle are monovular therefore as primordial follicles are recruited most become atretic and do not progress through to selection of the single dominant follicle, and few follicles (<0.1%) from the original store will ovulate (Webb *et al.*, 2003). The duration of this process from primordial to ovulatory follicle is estimated at approximately 4-6 months in ruminants (Webb *et al.*, 2004) with most time (3-4 months) spent in the pre-antral stages of development (Campbell *et al.*, 2000).

Later stages of follicular development occurs in 3 processes; recruitment of pre-antral follicles to maintain growth by gonadotrophic stimulation, selection in which follicles are selected to continue growth and thus escape atresia, and dominance in which one follicle continues to grow and ovulates (Lucy *et al.*, 1992). Waves of sequential growth and atresia occur, caused by increases in the concentrations of FSH. There are usually 2 or 3 waves (Savio *et al.*, 1990) per oestrous cycle, however some cows can show 1 or even 4 waves (De Rensis and Peters, 1999), with the final wave resulting in ovulation (Aerts and Bols, 2010). Each wave recruits approximately 3-5 follicles that grow to >4mm in diameter, until follicles reach 6-8mm diameter, when one follicle is selected for continued growth and becomes dominant and will either ovulate or undergo atresia (Webb *et al.*, 2003).

Follicular development is governed by a period of gonadotrophin independence followed by a period of dependence (Webb *et al.*, 2004). During recruitment, each wave is preceded by increases of FSH secretion lasting 1-2 days, stimulating growth of smaller follicles, <2mm diameter (Webb *et al.*, 2003). FSH dependence occurs with cohorts of 5-20 follicles greater than or equal to 5mm (Forde *et al.*, 2011;Webb and Campbell, 2007). The recruited follicles then begin secretion of oestradiol and inhibin A which negatively feedback to inhibit FSH production, which remains at basal levels for growth and initiation of new follicular waves (Gibbons *et al.*, 1999). The dominant follicle then begins to emerge from the group, when it reaches an average diameter of 8.5mm, and then continues to increase in size. Differentiation between this follicle and the subordinate follicles occurs, although all are eligible for dominance, when the follicle becomes the main inhibitor of FSH and dependency switches to LH (Ginther *et al.*, 1997). Growth of the dominant follicle continues, and the increase in oestradiol causes increased GnRH pulse frequencies, promoting LH secretion. This in turn stimulates oestradiol production by the granulosa cells (Fortune, 1994). This has a local effect on follicle development, but also a systemic effect, acting upon the hypothalamic-pituitary axis to increase LH production further, by positive feedback (Aerts and Bols, 2010). This ultimately results in oestrus, controlled by oestradiol, and the LH surge resulting in ovulation.

1.2.1.2 Oestrus & Ovulation

It is this rise in oestradiol; enhanced by LH, stimulating production of androgen in the theca cells (Garverick *et al.*, 2002) and the subsequent androgens being converted into oestradiol by aromatase enzyme from granulosa cells, which causes oestrus (Fortune, 1994). The positive feedback mechanism between oestradiol and LH, causes LH pulse frequency to increase to about 1 pulse per hour (Roche, 2006). The increase in LH concentration causes a cascade of events that induce the release of the oocyte into the oviduct, by an inflammatory response; involving prostaglandins, particularly prostaglandin E (PGE), produced by the follicle (Aerts and Bols, 2010). Prostaglandins stimulate the proliferation of cells and production of proteolytic enzymes to disrupt the follicle wall, releasing the oocyte (Espey, 1980). This process is ultimately under the control of the follicle itself, timing when it is appropriate to trigger the LH surge for ovulation by production of oestradiol (Roelofs *et al.*, 2010), usually about 10-14 hours after oestrus (Forde *et al.*, 2011).

1.2.1.3 Luteal Phase

LH is the key hormone stimulating luteinisation of the theca and granulosa cells post ovulation, forming the CL from the cells of the ruptured cavity (Alila and Hansel, 1984). The CL consists of small and large luteal cells, which have steroidogenic properties (Smith *et al.*, 1994), which secrete progesterone, along with a range of other cell types. The function of the CL is to produce progesterone, in order to maintain pregnancy if a conceptus is present (Forde *et al.*, 2011). Sustained production of progesterone suppresses GnRH pulse frequency and hence LH secretion to prevent ovulation, but does allow enough LH for the continuation of follicular waves and dominant follicle growth (Savio *et al.*, 1990).

In the non-pregnant cow the corpus luteum undergoes luteolysis around day 17. Oxytocin produced by the corpus luteum binds to oxytocin receptors on the endometrial membrane, stimulating pulsatile release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Flint and Sheldrick, 1983). Oxytocin receptor concentration in the uterus increases throughout the cycle, especially from day 15 to 17 (Robinson *et al.*, 1999), binding oxytocin and inducing the episodic secretion of $PGF_{2\alpha}$ from the uterine endometrium. $PGF_{2\alpha}$ controls CL breakdown, causing decreased progesterone concentrations, reducing the inhibitory effect of high progesterone concentrations (Robinson *et al.*, 2001). This removes the negative inhibition of GnRH secretion resulting in increased LH secretion stimulating increased oestradiol concentrations observed during the follicular phase.

In the pregnant cow the conceptus must signal its presence in order to prevent luteolysis. Progesterone inhibits oxytocin receptor expression in the early to mid luteal phase (Robinson *et al.*, 2001) but the conceptus must signal its presence by producing the maternal recognition of pregnancy signal, interferon tau (IFNτ). The antiluteolytic effects of IFNτ physiologically signal the presence of the conceptus (Demmers *et al.*, 2001). This occurs between days 16-18 when the trophectoderm has produced sufficient quantities of IFNτ and thus prevents the increase in oxytocin receptors. However for IFNτ to be produced the trophoblast must have begun to elongate from spherical to filamentous; no IFNτ production occurs if the embryo is still spherical, regardless of day of the oestrous cycle (Robinson *et al.*, 2006). Pulsatile release of $PGF_{2\alpha}$ from the uterus is blocked because IFNτ inhibits oxytocin receptor expression on the endometrium. This occurs because oestrogen (which induces oxytocin receptor expression) is reduced by pathways decreasing oestrogen receptor concentrations (Demmers *et al.*, 2001), and progesterone concentrations remain high which are necessary to maintain pregnancy.

1.2.2 Management of Reproduction

Management of reproduction is important as improved reproductive efficiency is essential for efficient milk production and strongly influences the profitability of the herd. Strict management parameters must be adhered to in order to produce one calf per cow per year, to keep within an optimal calving interval of approximately 365 days; including a gestation period of approximately 270 days. Therefore to keep within the desired calving interval, depending on the management scenario, cows must be in calf at 40-50 days post partum; involving the steps of uterine involution, resumption of oestrous cycles, expression and detection of oestrus and insemination (Royal *et al.*, 2000a). This is more important when producers operate to a block calving regime when cows have to be in calf at strict intervals, usually within 60-90 days post partum or they risk being culled for infertility. Although it is still important to try and adhere to a strict calving interval when employing all year round calving, as it is not always desirable to have extended calving intervals, due to fluctuation in yield with stage of the lactation curve.

One of the largest contributors to extended calving intervals however, is the percentage of cows ovulating that are not detected in oestrus, causing the biggest losses to the dairy industry (Van Vliet and Van Eerdenburg, 1996;Peralta *et al.*, 2005). Cows must be detected in oestrus so that artificially insemination (AI) coincides with ovulation at the optimal time for conception to occur. Bulls are more accurate at determining true oestrus, but there are major benefits associated with the use of AI, which explains the popularity of AI, and highlights further the importance of oestrous expression and detection.

1.2.2.1 AI vs. Natural Service

One of the main reasons for reproductive technologies, namely AI, are to increase reproductive potential and control breeding for genetic gain so that desirable characteristics are inherited. This approach involves the need for accurate oestrous detection, whereas bulls can detect pheromones and oestrus much more accurately (Lopez-Gatius *et al.*, 2005). However, although bulls are advantageous in this context, and can ‘clean up’ fertilising cows when AI has failed (Lima *et al.*, 2009) they can be dangerous on farm, and diseases are more readily transmitted (Dobson *et al.*, 2008).

AI allows for smaller quantities of semen to be used than is the case during natural mating, therefore genetically superior bulls can fertilise a greater number of cows by using AI. Predicted transmitting abilities (PTAs) are readily available and included in the Fertility Index, for selection of desirable daughter traits when choosing a suitable bull for AI thus controlling heritable traits (Flint, 2002;Wall, 2003). Another advantage of using AI in the dairy industry is the ability to use sexed semen. Heifer calves are required to expand the herd and increase progress with herd genetics, therefore using sexed semen to produce a greater number of heifer calves is advantageous for herd development (Olynk and Wolf, 2007). AI also reduces the need to move livestock, improves biosecurity, and eliminates the transmission of disease (Nicholas, 1996). Therefore, AI enhances the dairy industry, but the main challenge in using AI is insemination at the optimal time for conception to occur in order to achieve high conception rates.

1.2.2.2 AI at the Optimum Time for Conception

AI must occur relative to the time of ovulation in order to result in successful conception. Standing oestrus is the most accurate symptom of oestrus in relation to ovulation, although the exact timing of onset is rarely known (Dransfield *et al.*, 1998). Oestradiol, the hormone responsible for oestrous behaviour, stimulates the surge in LH which results in ovulation and which usually occurs after the display of oestrus, Figure 1.1. Oestrous behaviour is the overt signal for ovulation, which is the internal, physiological mechanism. Therefore insemination must occur relative to the timing of overt oestrus; important for the viability of the oocyte in the female reproductive tract and fertile lifespan of the spermatozoa (Roelofs *et al.*, 2006). Figure 1.2 shows the window of opportunity for AI at the optimal time for maximising conception post behavioural oestrus, ensuring the likelihood of viable sperm and ova coming together in the female reproductive tract. Ovulation occurs 28-32 hours post oestrus (Walker *et al.*, 1996), whereas the fertile lifespan of the oocyte is only 6-12 hours (Brackett *et al.*, 1980). The viable life of the sperm capable of fertilising the egg in the female reproductive tract is 24-30 hours (Hunter and Wilmut, 1983). Therefore the optimal time to inseminate post oestrus is 4-12 hours (Dransfield *et al.*, 1998) in order to ensure the sperm reach the oocyte whilst viable and whilst still capable of fertilisation.

Spermatozoa require approximately 8 hours in the female reproductive tract to undergo capacitation and become capable of fertilising the oocyte. If insemination occurs to close to ovulation then sperm are not mature enough to fertilise the egg (Hunter and Wilmut, 1983). However, if insemination occurs too early before ovulation then the sperm have to reside in the female reproductive tract for too long which could conversely affect their fertilising ability. Roelofs *et al*. (2006) reported that early insemination (approximately 36 hours prior to ovulation) does not affect either the number or the fertilization capabilities of the sperm (Roelofs *et* *al.*, 2006). However, aged sperm have been reported to incur damage to their DNA if they are in the reproductive tract too long. This is reported to affect the oocyte's development after fertilisation which could result in early pregnancy loss due to impaired embryo development (Ahmadi and Ng, 1999). AI after ovulation can also compromise fertility rates because the oocyte becomes aged, impacting upon fertility and development (Roelofs *et al.*, 2006). Instability in the nuclear and cytoplasmic organelles, disruption of cortical granules and the zona block can increase the chances of polyspermy in an aged oocyte, increased further by late insemination as there are a larger number of active sperm (Hunter and Greve, 1997). A further problem of late insemination is that the reproductive tract conditions alter post ovulation, therefore the environment becomes hostile to sperm compromising the chance of conception (Hunter and Greve, 1997).

There are conflicts in reports concerning the optimal time to inseminate post-standing to be mounted (see Figure 1.2). Early studies established the a.m.-p.m. guideline; cows in oestrus during the a.m. should be submitted for AI during the next p.m., and cows in oestrus during the p.m. should be submitted for AI during the next a.m. (Pursley *et al.*, 1998). It has been reported that the pregnancy rates of cows inseminated at the earliest; 0 hours and latest times; 32 hours, post-standing oestrus had significantly lower pregnancy rates per AI. Considerably higher pregnancy rates were achieved in cows bred at 16 hours (middle time period) after the onset of standing oestrus (Pursley *et al.*, 1998). Dransfield *et al*., 1998, reported similarly, that conception rates were increased 4-12 hours after the onset of standing activity. Therefore, oestrous detection is imperative for the correct timing of AI and getting cows in calf for the efficiency of dairy production.

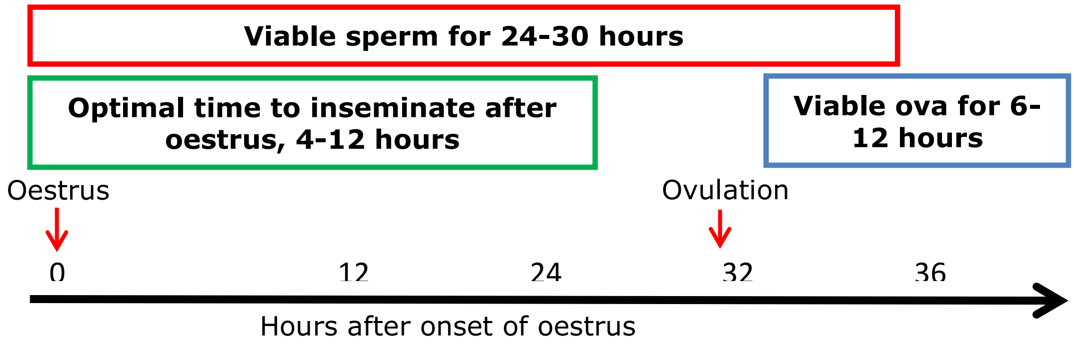


Figure 1.2 *Window of opportunity for artificial insemination post oestrus.* The window of opportunity for AI in order to maximise conception rates. Sperm undergo capacitation and development in the female reproductive tract and are viable for 24-30 hours post insemination, therefore insemination must occur at 4-12 hours post onset of oestrus in order to coincide with ovulation around 28-32 hours post oestrus, and reach the ova which remains fertile for a short time, 6-12 hours, post ovulation.

1.2.2.3 Oestrous Synchronisation

Oestrus can be synchronized and controlled by administration of exogenous hormones by precisely controlling the CL lifespan and follicular waves (Wiltbank *et al.*, 1971;Thatcher *et al.*, 1989;Pursley *et al.*, 1995). This aids reproductive efficiency by controlling the oestrous cycle, acting as an aid to detection of oestrus. Exogenous oestrogens were found to control luteolysis in the early part of the oestrous cycle, but the onset of oestrus was not precise, although pregnancy rates were increased, compared with using progestagens (Wiltbank *et al.*, 1971). Use of exogenous oestrogens is however now banned in the EU. However, luteolysis can be controlled by use of $PGF_{2\alpha}$ since its discovery as a luteolytic agent, or one of its synthetic analogues (Lauderdale *et al.*, 1974). 5 days after injection of $PGF_{2\alpha}$ immediate regression of the CL occurs. The concentration of progesterone rapidly drops to basal levels within 24 hours, which allows LH pulse frequency to increase, causing significant increases in oestradiol concentration. Oestrous behaviour then occurs followed shortly by ovulation. However, CL regression is not always immediate depending on the stage of the follicular wave and the interval to onset of oestrus can be quite variable. If a dominant follicle is present then the onset of oestrus can occur within 2-3 days, but if there is no dominant follicle this could take longer (Diskin *et al.*, 2002).

Conception rates in heifers undergoing synchronisation treatment and in heifers inseminated at natural oestrus have been reported to be unimpaired by treatment with no difference between the two groups (Macmillan and Day, 1982). However conception rates in lactating dairy cows can differ. Some workers reporting lower conception rates in cows inseminated at natural oestrus (Macmillan and Day, 1982) while others reported higher conception rate at detected oestrus compared to synchronized cows (Xu and Burton, 2000). Furthermore, pregnancy rate was often greater in cows synchronised due to increased detection and submission rates (Xu and Burton, 2000;Ryan *et al.*, 1995).

Emergence of new follicular waves can be controlled by exogenous GnRH administration to initiate a new follicular wave which aids the synchronisation of oestrous cycles. GnRH administration will cause ovulation if a dominant follicle is present and a new follicular wave will occur within 3-4 days (Webb *et al.*, 1992;Twagiramungu *et al.*, 1995). GnRH analogues have been implemented into a follicular and luteal phase synchronisation treatment, entitled the Ovsynch protocol, which controls emerging follicle waves and CL regression (Thatcher *et al.*, 1989). This protocol uses timed injections; GnRH administered on day 0 results in ovulation followed by the initiation of a new follicular wave. $PGF_{2\alpha}$ treatment is administered 7 days later causing CL luteolysis, with a second GnRH dose 36-48 hours later inducing ovulation. AI is then carried out at a fixed time (Pursley *et al.*, 1995;Stevenson *et al.*, 1999b;Stevenson *et al.*, 1999a;Thatcher *et al.*, 1989). This protocol is advantageous because fixed timed AI eliminates the need for heat detection, which is cost effective, but heat detection is required if the first insemination fails (Diskin *et al.*, 2002). However, as indicated previously with luteolytic inducing agents, GnRH + $PGF_{2\alpha}$ methods of synchronization do not increase conception rates when compared to AI at observed oestrus (Stevenson *et al.*, 1999a).

The Ovsynch protocol can also be accompanied by the 'Presynch' protocol involving two injections of $PGF_{2\alpha}$ 14 days apart followed by commencement of the Ovysnch protocol 14 days after the second $PGF_{2\alpha}$ injection (Akoz *et al.*, 2008). Another variation on the Ovsynch protocol is Heatsynch; substituting the final GnRH injection 48 hours after $PGF_{2\alpha}$ treatment with an injection of oestradiol cyprionate intramuscularly at 24 hours after $PGF_{2\alpha}$

treatment, followed by AI 48 hours later. However, pregnancy rates did not differ between Ovsynch and Heatsynch protocols (Pancarci *et al.*, 2002).

Progestagens can also be used to synchronise oestrus in cattle by use of internal releasing devices; PRID (progesterone-releasing intravaginal device) and CIDR (controlled-internal drug release) are progesterone releasing devices inserted into the vagina. Other devices are implanted into the ear, such as Norgestomet or are feed additives such as MGA (Melengestrol acetate; Yavas and Walton, 2000). Progesterone supplementation maintains progesterone concentration above 1ng/ml which suppresses oestrus and the LH surge, blocking ovulation (Lucy *et al.*, 2004). Progesterone treatments are particularly useful for treating anoestrus; by decreasing LH initially then increasing LH pulsatility by priming hypothalamic centres to induce oestrus and then ovulation (Yavas and Walton, 2000). Progesterone can also overcome cystic ovaries in cows with a persistent dominant follicle that will not ovulate because of continuous exposure to high frequency LH pulses (Garverick, 1997). Administration of GnRH will can also remove the cystic structure by luteinisation (Garverick, 1997). Progesterone devices were initially used for 14-21 days and upon removal oestrus occurred within 3 days incurring a high oestrus response (Macmillan and Peterson, 1993), although conception rates in cows treated for long periods with progesterone are 10-15% lower than those using short term protocols such as 7-9 days (Lucy *et al.*, 2004). Here short progesterone treatments are now used with a luteolytic dose of $PGF_{2\alpha}$ 0-2 days before device removal (Lucy *et al.*, 2004). If $PGF_{2\alpha}$ is administered before progesterone withdrawal there is a higher degree of synchrony (Macmillan and Peterson, 1993) due to removal of any functioning CL. Overall reproductive efficiency is still reportedly low in animals treated with both $PGF_{2\alpha}$ analogues and progesterone; calving rate was no different between treated (52%) and control (43%) cows (Roche, 1976). Furthermore, cows bred at a detected oestrus compared to fixed timed AI after prior progesterone synchronisation treatments have similar calving rates (Roche *et al.*, 1977).

Oestrous synchronisation has merit for synchronising whole herds in block calving systems, removing the need for oestrous detection as fixed time AI can be used (Lucy *et al.*, 2004) and increasing the intensity and duration of oestrous symptoms as more cows are in oestrous at the same time (Hurnik *et al.*, 1975;Van Vliet and Van Eerdenburg, 1996). Synchronisation also reduces the incidence of problem cows not showing oestrus as anoestrus and cystic cows can be induced into oestrus (Lucy *et al.*, 2004). Therefore the advantages are that the submission rate of cows can be increased, and although conception rate is not necessarily higher, pregnancy rates may be increased through better detection of oestrus or removing the need for oestrus (Lucy *et al.*, 2004).

The availability of hormones and veterinary treatments can vary widely in cost and between countries due to local legislation and regulations. For example, the use of oestradiol is banned in the EU and US, but actively used in Australia and New Zealand. One major consideration in use of synchronisation to remove the need for oestrous detection is whether the benefits outweigh the cost. Profit from return of increased pregnancy rates and shortening the calving interval must outweigh the cost of hormones, labour and potential veterinary treatments for oestrous synchronisation to be widely used. This questions whether an accurate and efficient method of oestrous detection may be more beneficial over use of hormonal treatments as one major negative aspect of synchronisation is the consumer attitude to use of hormones in food production. Oestrous synchronisation is not a tool to improve fertility; the main benefit is improved oestrous detection because of more consistent results in detection.

1.3 OESTROUS DETECTION

1.3.1 General Overview

Oestrus is the period of the oestrous cycle which is accompanied by overt behavioural characteristics caused by changing hormonal profiles, which is the signal for the physiological mechanism of ovulation (Roelofs *et al.*, 2010). It has been reported that part of the reproductive decline is associated with the failure to observe oestrus and failure to interpret the signs of oestrus correctly (Reimers *et al.*, 1985). More recently, Van Eerdenburg *et al*. (2002) reported oestrous detection rates on farm at less than 50%, but showed they could be up to 100% in cows displaying oestrous behaviour, if monitored continuously. Expression of oestrus is a problem as the intensity of oestrus has declined, averaging only 8.5 standing events per cow, and duration of oestrus (time between first and last sign of behavioural oestrus or episode of standing to be mounted) has shortened from 15 to 5 hours (Dobson *et al.*, 2008), lasting only 7 hours on average in Holsteins (Dransfield *et al.*, 1998). The percentage of cows actually standing to be mounted has also declined from 80% to 50% (Dobson *et al.*, 2008). This results in only 50% of cows being observed in oestrus (Van Eerdenburg *et al.*, 2002). Hence detection of oestrus is increasingly more difficult and to improve the decline in fertility oestrous detection rates must be improved.

1.3.1.1 Endocrine, Neural and Genomic Changes Associated with Oestrous Behaviour

Oestradiol is the key regulator that synchronizes the endocrinological and behavioural events to drive oestrus; resulting from the action of ovarian steroids on behavioural centres in the brain (Roelofs *et al.*, 2010). The production of oestradiol from the ovary synchronises mating and ovulation; rising above its threshold in an all or nothing response (Allrich, 1994). During follicle development increasing concentrations of oestradiol are produced and secreted mainly from the dominant follicle (Staigmiller *et al.*, 1982). This increases follicular oestradiol 3-4 days before oestrus, causes circulating concentrations of oestradiol to increase (Roelofs *et al.*, 2010), acting at the level of the hypothalamus to trigger a series of programmed neurological events that result in behavioural oestrus (Reames *et al.*, 2010). Other centres in the brain also trigger the closely related LH surge which is required for ovulation and occurs 28-32 hours after oestrus (Walker *et al.*, 1996). In contrast progesterone from the CL also controls oestrus by inhibiting GnRH and LH pulses which reduces oestradiol concentration (Smith and Jennes, 2001).

Often in the post partum period there is a silent oestrus which involves ovulation without overt oestrous expression. This is thought to be caused by high oestradiol levels following gestation inducing a refractory period (Allrich, 1994). Oestrus can be affected through the duration of progesterone and progesterone amplitude during the luteal phase which can influence the increase in levels of oestradiol. It is suggested that this occurs by the influence of progesterone on the neural mechanisms controlling release of GnRH, influencing the elements targetted by oestradiol to induce the preovulatory LH surge in the ewe (Skinner *et al.*, 2000). It has also been reported in the ewe that previous progesterone exposure can affect the intensity of oestrous expression (Fabre-Nys and Martin, 1991). However, in the cow these mechanisms differ and oestrus can occur without progesterone exposure. Even with low levels of oestradiol, the LH surge and ovulation can still occur; demonstrating that hypothalamic sensitivity to LH and oestradiol differs and could also differ between individual cows (Reames *et al.*, 2010).

Oestradiol is thought to alter neuronal networks, including dendritic connections between cells and receptors, and neurotransmitter release in order to facilitate oestrous expression (Boer *et al.*, 2009). The shift to oestradiol stimulation causes elevated GnRH receptor gene expression in gonadotroph cells which can result in increasing LH pulses stimulated by synthesis and secretion of GnRH (Boer *et al.*, 2009). The increased oestradiol has a self amplifying effect, stimulating the expression of oestrogen receptors in the brain (Pfaff, 2005). Therefore oestradiol indirectly synchronises mating and ovulation.

Oestradiol affects certain areas in the brain in order to regulate female sexual behaviour. Specific areas reported to be involved in behavioural oestrus are the arcuate nucleus, ventromedial nucleus, the preoptic area of the hypothalamus and in particular the hippocampus and amygdala are related to behavioural oestrus (Molenda-Figueira *et al.*, 2006). Oestradiol and other hormones, for example IGF-1 and GnRH, can cause up and down regulation of a number of genes in these brain areas known to be involved in oestrous behaviour (reviewed by Boer *et al*., 2009).

Some preliminary work carried out investigating gene expression in the brain at oestrus compared to luteal phase Holstein Friesian heifers has found that oestrous behaviour may be linked to different patterns of gene expression in the pituitary gland, hypothalamus, amygdala and ventral tegmental area (Beerda *et al.*, 2008). The majority of the research into the genomic control of oestrus has been carried out in rodents where increased oestrogen receptor expression in hypothalamic areas at oestrus have been reported (Pfaff *et al.*, 2008) resulting in expression of genes to facilitate oestrous behaviour, stimulating behavioural oestrus and mediating neurotransmission resulting in oestrus (reviewed by Boer *et al*., 2009). However, parallels can be drawn between the brain areas involved in behavioural oestrus between rodents and ruminants (Stormshak and Bishop, 2008). Increased oestrogen receptor expression has also been linked to an increase in locomotion (Smith and Jennes, 2001), which is a similar oestrus response to that seen in cattle (Kiddy, 1977).

1.3.2 Primary Sign of Oestrus

Standing to be mounted (see Figure 1.3) is the primary and definitive sign that a cow is in oestrus and indicates the period when the cow is in a preovulatory and sexually receptive state. As depicted in Figure 1.3 the cow underneath is in oestrus as it is allowing the other cow to mount it, remaining stationary without resistance. This is indicative of 'true oestrus' (Orihuela, 2000).

Standing to be mounted

Figure 1.3 The primary sign of oestrus; standing to be mounted, the underneath cow remaining stationary allowing the above cow to mount (adapted from Van Der Merwe, 2005)

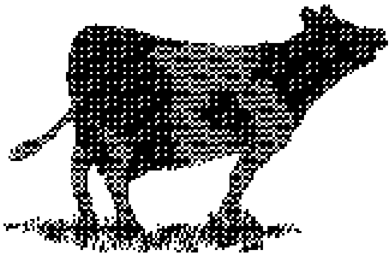
Traditionally cows were visually observed for standing oestrus at periods throughout the day as an accurate form of detection for submitting cows for AI at the correct time. Although duration and intensity of oestrous behaviour has decreased (Dransfield *et al.*, 1998), and not all cows display overt oestrus (Harrison *et al.*, 1990) and only 50% stand to be mounted (Van Eerdenburg *et al.*, 2002). Therefore not all animals that ovulate stand to be mounted, which makes accurate oestrous detection increasingly difficult. However, there are many secondary symptoms of oestrous behaviour that cows display when they are sexually receptive.

1.3.3 Secondary Signs of Oestrus

There are many secondary signs of oestrus that facilitate detection (see Figure 1.4a-f) which can be useful to supplement the diagnosis of oestrus as the duration is often longer than the period of standing to be mounted (Ranasinghe *et al.*, 2009), which is only 1% of the whole oestrous period (Senger, 1994). However, this poses problems in carrying out accurate insemination as these signs and their duration are variable, unlike the primary sign of oestrus, so they do not give an accurate time relative to ovulation (Orihuela, 2000;Ranasinghe *et al.*, 2009). The secondary behaviours may be more useful in the modern dairy cow because she expresses fewer signs of oestrus, with reduced standing behaviour (Dobson *et al.*, 2008). Although it has been reported that the duration of secondary symptoms also decrease with reduced standing time (Yoshida and Nakao, 2005).

The secondary signs of oestrus can be categorised into sexual, social and agnostic interactions which facilitate the change in behaviour of the cow. Sexual interactions are mounting or trying to mount other cows at the head or tail, chin resting and sniffing/ licking the ano-genital (vulva) region of a cow. Social interactions are licking another cow (flank, head, neck) and agnostic interactions such as aggression and butting others (Kerbrat and Disenhaus, 2004). However, some of these symptoms are related to other behavioural characteristics and do not always mean that a cow is coming into oestrus. Observations have been made which show that if a cow is to mount the head of another then she is nearly always (88.5%) in heat, as this is the secondary oestrus sign with the highest degree of accuracy (Britt *et al.*, 1986).

(a) Mounting head to head



(b) Bellowing and restless

(c) Scuffed tail head, dirty flanks and sweating

(d) Sniffing the vulva of other cows

(e) Chin resting

(f) Head butting each other, aggression

Figure 1.4 Secondary signs of oestrous behaviour with the shaded cow (black and grey) exhibiting oestrous behaviour towards the other (black and white). Where there are 2 shaded cows both could be showing secondary signs of oestrus (adapted from Van Der Merwe, 2005)

There are also changes in normal behaviours that are associated with oestrus behaviour; restlessness and frequent bellowing, cajoling, 'soliciting' another cow to mount, reductions in feed intake, Flehmen lip curl (pheromonal response), raising and twitching of the tail and general changes differing from the normal routine (Phillips and Schofield, 1990;Van Vliet and Van Eerdenburg, 1996;Yoshida and Nakao, 2005;Diskin, 2008). Activity is also reported to increase at the time of oestrus by 2 to 4 fold (Kiddy, 1977;Farris, 1954), as cow interactions increase. Further signs associated with the change in behaviour to look for are; a scuffed tail, dirty flanks, patches of hair loss or saliva on the hindquarters, resulting from being mounted (Diskin, 2008). These symptoms are not exclusive to oestrus and can be the result of other everyday activities such as rubbing or cleaning themselves.

Further to the behavioural changes described that occur at oestrus, there are also physiological changes that occur at the time of oestrus, brought about by the hormone oestradiol, which can be used to detect oestrus. Oestradiol can affect the reproductive tract by making it tonic, causing oedema, increasing blood flow and causing it to become highly secretory, whereby excess mucus is produced and presents itself as clear mucus or a 'bulling string' protruding from the vagina. Oedema and increased blood flow cause swelling and redness of the vulva, differing from the normal state, which can also be used to determine oestrus. Accumulation of white blood cells in the uterus, occurring due to the increase in oestradiol concentration, digest bacteria, old sperm and general cell debris and can manifest as another symptom of oestrus as blood protrudes from the vagina similar to the mucus string (Roelofs *et al.*, 2010). Temperature also fluctuates at oestrus; decreasing 2 days before and increasing at oestrus (Firk *et al.*, 2002), but could be caused by many factors such as increased activity also related to oestrus (Kiddy, 1977). Milk yield has also been reported to decrease at the time oestrus (Schofield *et al.*, 1991). However, many of these physiological changes can be the result of other factors, not exclusively related to oestrus. For example routine management tasks or animals being reintroduced into the herd post calving and reestablishing hierarchies can cause increases in activity, and temperature fluctuations and decreases in milk yield could be related to illness or time of year.

1.4 FACTORS AFFECTING OESTROUS EXPRESSION

Poor oestrous detection arises due to lack of oestrous expression; decreased duration and intensity and decreased standing behaviour. Factors related to poor expression can be cow related factors; health, nutrition and milk yield, or environmental factors; housing, flooring and stocking density for example.

1.4.1 Environmental Factors

1.4.1.1 Housing

Housing design affects oestrous expression. If cows are loose housed in barns then they have the freedom to exhibit oestrous behaviours and the chance of these being observed is improved (Phillips and Schofield, 1990). However, if cows are housed in tie stalls with little cow to cow interaction then they cannot exhibit standing behaviour, so detection is based solely on secondary signs of oestrus, which result in a high incidence of detection error and consequently low conception rates (Ranasinghe *et al.*, 2009). Mounts in cubicle houses have been reported to be less frequent than in open barn housing, 7 mounts per hour compared to 11 mounts per hour respectively. At pasture the number of mounts has been reported to be even lower, 5 mounts per hour (De Silva *et al.*, 1981), possibly due to less frequent contact when in an open space and feeding taking priority (Phillips and Schofield, 1990).

1.4.1.2 Floor Type

Floor type has a dramatic effect on oestrous expression. It is documented that cows do not like to be mounted on concrete, particularly when wet, preferring softer surfaces underfoot such as grass, dirt or straw bedding (Britt *et al.*, 1986). When observed on dirt compared to concrete the duration of oestrus was longer, with more total mounts and stands. The duration of oestrus on dirt averaged more than 12 hours for 11 out of 13 cows, whereas only 2 cows displayed on average more than 12 hours on concrete (Britt *et al.*, 1986). The duration of behaviour and number of mounts on rubber covered slats, pasture and straw were all similar, but significantly increased compared to duration and number of mounts on concrete (Boyle *et al.*, 2007). Cows walking on rubber have also been reported to move with a more natural gait, have less bruising of the corium and a lower incidence of lameness. Therefore they are more likely to engage in more natural behaviours including expression of oestrus. However rubber flooring can also be slippery when either wet or worn. If the floor surface is slippery or coarse then mounting behaviour is deterred as cows are hesitant, especially if they have existing foot problems or have previously sustained a fall caused by slippery surfaces (Blowey, 2005). Inclusion of rubber covered slats, with grooves and raised ridges, can improve friction and prevent slipping as well as providing additional cushioning, facilitating increased mounting behaviour (Boyle *et al.*, 2007). Although, Boyle *et al*., (2007) concluded that flooring surface had little effect on standing oestrus their data suggested that the use of rubber flooring reduced foot injuries, which can also affect oestrus expression (see section 1.4.2.1).

1.4.1.3 Stocking Density

Stocking density is the number of cows per unit of space and can affect oestrous behaviour both positively and negatively. Increasing the stocking density can increase the frequency of cows in oestrus meeting and interacting (Orihuela, 2000) as cows in free stall barns display more mounting behaviour than grazing cattle through closeness (De Silva *et al.*, 1981). Conversely, in overcrowded situations cows may not have enough space to display oestrous behaviour and any oestrous expression could be undetected because observation is more difficult with larger numbers of cows in close proximity (Diskin, 2008). If there is insufficient area per cow mounting activity can be indiscriminate, directed towards any cows or nearest herd mates because of the close confinement (Metz and Mekking, 1984). In contrast in spacious areas cows in oestrus can choose who they mount and non-oestrus cows can resist mounting (Diskin, 2008). Close confinement has adverse effects on oestrous detection because animals can be wrongly identified as being in oestrus. Misidentification can occur because of increased number of buttings and aggression which can be mistaken for signs of secondary oestrous behaviour, combined with increased interactions in general (Metz and Mekking, 1984). High stocking densities also can affect expression through related factors; aggression (Metz and Mekking, 1984), overcrowding decreasing the lying time of cattle leading to higher incidence of lameness (Blowey, 2005) and decreases in feed intake because of an increased number of displacements from the feeding area (DeVries *et al.*, 2004). These all have adverse effects on oestrous expression for a number of reasons including stress (see section 1.4.2.3).

1.4.1.4 Temperature and Season

Effects of the environment can influence oestrous expression. Weather, day length, temperature, photoperiod and even lunar cycle have all been reported to have an effect on sexual receptivity and reproductive efficiency; however management systems that eliminate or reduce fluctuations can prevent the effects of seasonality (Orihuela, 2000).

When temperatures increase or decrease the oestrous cycle can become disrupted and oestrous expression becomes affected. Heat stress can be caused by hotter temperatures; duration of oestrus reported as 11 hours in hot climatic conditions, compared to 20 hours in cooler climatic conditions in Holsteins (Gangwar *et al.*, 1965), and in summer months Holsteins averaged 4.5 mounts compared to 8.6 mounts in the winter (Nebel *et al.*, 1997). However there are contradictory reports concerning the effects of temperature on oestrous expression. In one study increased number of standing events was reported in hotter months (Peralta *et al.*, 2005). A cause of reduced expression in hotter months can be due to heat stress, affecting the steroidogenic properties of the developing follicle as the components of the reproductive system become susceptible to extreme temperatures (Wolfenson *et al.*, 2000). Heat stress affecting peripheral concentrations of oestradiol at oestrus (Wilson *et al.*, 1998) coupled with changes in hypothalamic-pituitary-stress axes which evoke a stress response can impact upon behavioural oestrus. It has also been suggested that decreased expression of oestrus in colder climates is due to the carry over effects of heat stress into the cooler months (Wolfenson *et al.*, 2000). The introduction of cooling systems, aiding thermoregulatory mechanisms was increased, as farmers are more aware of the effects of heat (Peralta *et al.*, 2005). However, it is important to note that the reproductive effects of heat are less applicable to UK dairy cows compared to countries where there are extreme changes in climate.

Seasonal variation can also affect the reproductive capability and influence oestrous expression, as cows were originally seasonal breeders. Return to cyclicity is longer if calving is in the winter compared to the summer (Hansen, 1985). Season of birth and season in which puberty is reached can influence the age at which puberty occurs in heifers (Schillo *et al.*, 1983). Exact explanations and mechanisms for these effects are unknown, but it could be due to the influence of oestrogens on LH depending on season. For example, in sheep reproduction can only occur at a certain times of the year due to photoperiod and the effect on reproductive hormones (Legan *et al.*, 1977). Seasonal changes in concentrations of gonadotrophins have also been reported in cattle (Critser *et al.*, 1987), and increases in LH have been reported to be larger in summer compared to winter (Hansen *et al.*, 1982), perhaps due to the inhibitory effects of oestrogens thus affecting oestrous expression.

1.4.2 Health

1.4.2.1 Lameness

Lameness is a one of the most important diseases affecting the dairy industry around the world at present. It is estimated to cost the UK dairy industry on average £240 per case due to veterinary and treatment costs, loss of production and major effects on fertility (Kossaibati and Esslemont, 1997).

Lame cows are 3.5 times more likely to suffer from delayed cyclicity than healthy herd mates (Garbarino *et al.*, 2004). The difference in frequency of standing to be mounted between lame and sound herd mates was reported as 2.4 vs 8.0 events per oestrus, respectively (Sood and Nanda, 2006). However these workers reported that mounting and other secondary oestrous behaviours were similar between groups. Lame cows were also reported to resist mounting on more occasions and also had a shorter duration of oestrus. Furthermore, duration and intensity of oestrus in lame cows are reduced by approximately 50%. Non ovulating lame cows were reported to have reduced LH pulse frequency and lower (0.53 vs 0.76) pulses per hour compared to healthy herd mates, respectively. Thus it is suggested that the stress of lameness reduces the LH pulsatility which drives oestradiol production, therefore low oestradiol production results in reduced oestrous behaviour (Dobson *et al.*, 2008). Progesterone concentration prior to oestrus was lower in lame cows than in non-lame cows (Walker *et al.*, 2008a) where previous progesterone exposure has been reported to affect the intensity of oestrous expression in ewes (Fabre-Nys and Martin, 1991).

Lameness can affect the expression of oestrus via a number of ways. Lameness can alter normal behaviour due to a reduction in dry matter intake and subsequent loss of condition because of more time spent lying down rather than feeding (Blowey, 2005;Walker *et al.*, 2008b). Lameness can affect oestrous expression due to a lower level of activity and thus less interaction with other cows; affecting pedometry and activity monitoring methods of oestrous detection systems and also less primary and secondary displays of behaviour as the cows come into contact less often. Pain and discomfort caused by lameness can also explain the pattern of reduced oestrous expression; 90% of lameness in cows is reported to affect the hind limbs, and as these bear most weight during mounting, result in a decreased submission rate (Sood and Nanda, 2006). Lame cows are also less likely to stand to be mounted, as herd mates can sense diseased and stressed cows and avoid them (Walker *et al.*, 2008a). Conversely, it may be too painful for cows to avoid mounts from other cows and therefore may be falsely identified as showing standing behaviour (Diskin, 2008).

1.4.2.2 Production Related Diseases

Other clinical production diseases associated with poor fertility include; high body condition score (BCS) at calving, low BCS immediately after calving, hypocalcaemia, ketosis, mastitis, retained foetal membranes and endometritis. Production diseases can delay the return to cyclicity following calving increasing the number of days open and extending the calving interval (Roche, 2006;Dobson *et al.*, 2008).

Inflammation from disease can cause prostaglandin production to switch from $PGF_{2\alpha}$ to $PGE_{2,}$ resulting in delayed luteolysis, prolonged progesterone dominance and extended periods of anoestrous (Sheldon *et al.*, 2009). Uterine bacterial infections can impair hypothalamic and pituitary gland function, affecting steroidogenesis in the granulosa cells in response to lipopolysaccharide (LPS) a component of microbes. Therefore oestradiol production can be diminished and hence no expression of oestrus (Sheldon *et al.*, 2009).

Metabolic disorders and gynaecological diseases can be affected by nutrition (Roche, 2006). Interruption of the reproductive processes can be affected by changes in metabolic hormones, e.g. IGF-1 and insulin, which can ultimately affect oestradiol production and oestrous expression (Roche,

2006) and disruption of LH secretion (Dobson *et al.*, 2008). Those cows with production diseases post partum may also be susceptible to lameness and mastitis (Roche, 2006). Hence oestrous expression may be affected due to the stress caused by production diseases.

1.4.2.3 Stress

Many factors particularly related to environment and health can evoke a stress response in cows (see Section 1.4). In general, stress causes disruption to the hormonal equilibrium, reducing the LH surge and affecting oestradiol production and period of exposure, thus decreasing the expression of oestrus (Dobson *et al.*, 2008). Cows are vulnerable to the effects of stress as the oestrous cycle is controlled by positive feedback mechanisms and so hormonal balance and control is very sensitive. Therefore dramatic shifts in hormone levels can have large responses (Liptrap, 1993).

Activation of the hypothalamic-pituitary-adrenal axis occurs in response to stressful situations (Allrich, 1994), which can cause a cortisol response. Cortisol produced in response to stress blocks the LH surge and prevents ovarian steroidogenesis. Therefore oestradiol production for oestrus is inhibited (Liptrap, 1993). To our knowledge there have been no experimental studies investigating long term activation of the hypothalamic-pituitary-adrenal axis. However, short term administration of synthetic corticoids has been shown to prevent the increase in oestradiol that induces oestrous behaviour. On administration of adrenocorticotrophic hormone (ACTH) LH pulse frequency, oestradiol production and the LH surge are decreased (Dobson *et al.*, 2008). Another study has reported that when cortisol is administered, to mimic the stress response, together with physiological concentrations of oestradiol the time of standing oestrus is shortened. Standing oestrus was either delayed or inhibited entirely (Allrich, 1994). It was also reported that low progesterone levels as a result of stress, result in lower intensity of expression (Walker *et al.*, 2008b).

1.4.3 Milk Yield, Nutrition and Genetics

Links between the reproductive and somatotropic axes have been associated with subfertility in the modern dairy cow through nutritional and metabolic interactions. These communicate through the liver, pancreas and adipose tissue to centres in the brain to regulate feed intake, energy balance and metabolism during milk production (Chagas *et al.*, 2007). Circulating concentrations of metabolites and hormones associated with these processes are controlled by the balance between milk production levels, nutrient intake and body tissue reserves. This is also affected by the genetic potential of the animal (Garnsworthy *et al.*, 2008). Therefore milk yield, nutrition and genetics are all interlinked and can influence oestrous expression.

1.4.3.1 High Milk Yield

Milk production has been increasing since the 1950s to the present, with a more dramatic increase in recent years (Lucy, 2001). There has been a simultaneous decrease in reproductive parameters and a marked association with the decreased duration of oestrus (Lopez *et al.*, 2004), which still persists. Milk production in the UK has increased from 7375 litres per cow in 2010/11 and now stands at 7617 litres for 2011/12 (provisional data for 2012; DairyCo, 2012a). This has been associated with low oestrous detection in the UK which has declined to only 50% (DairyCo, 2009).

Oestrous expression has been reported to decrease with increased milk production; Lopez *et al*., (2004) reported that a significant number of low producers had more periods of high intensity and longer duration of oestrus than high producers. Significantly more high producers also had oestrus events of shorter duration and lower intensity compared to low yielding cows. The duration of oestrus was reported to be longer in low yielders than high yielders (10.9 vs 6.2 hours), total number of standing events increased (8.8 vs 6.3) and total standing time increased (28.2 vs 21.7 seconds; Lopez *et al*., 2004). Lactation yield over 305 days has been confirmed to influence oestrous expression. High yielders producing 10814kg compared to average yielders producing 6912kg showed decreased expression of oestrus and a higher incidence of silent heats (Harrison *et al.*, 1990). At first ovulation post partum none of the high yielding group displayed overt oestrus compared to 50% of average yielding cows. At the second ovulation post partum 50% of the high yielding group displayed oestrus compared to 100% of the average yielding group (Harrison *et al.*, 1990).

1.4.3.2 Milk Yield and Nutrition

Dietary improvements to cope with high levels of milk production can result in changes in the pattern of energy metabolism (Gutierrez *et al.*, 2006), which can alter endocrine signalling (Roche, 2006), therefore can affect expression of oestrus. High milk production requires a high plane of nutrition as there is a close correlation, r=0.88, between milk production and dry matter intake (Harrison *et al.*, 1990). Hence lactating cows require higher energy from concentrates to meet the requirements for high milk production (Lopez *et al.*, 2004). This causes an increase in liver blood flow and results in rapid metabolic clearance of steroids, progesterone and oestradiol, by the liver from the blood stream (Sangsritavong *et al.*, 2002). This can result in shorter duration of oestrus due to lower oestradiol concentrations in the circulation (Sangsritavong *et al.*, 2002). Particularly as oestradiol concentrations on the day of oestrus are significantly correlated with duration (r=0.57; Lopez *et al*., 2004). Therefore metabolic clearance of steroids provides a possible mechanism for the effect of high yield reducing the expression of oestrus; high yielders displaying shorter and less intense oestruses (Lopez *et al.*, 2004).

1.4.3.3 NEBAL and BCS

Nutrition can also affect oestrous expression through its effect on the length of NEBAL at the early stage of lactation. Energy balance is the balance between energy intake and output for maintenance and milk production. However additional pressures during late gestation and early lactation for foetal growth and milk synthesis can also put strain on the energy requirements (Wathes *et al.*, 2007b). As output (milk yield) exceeds intake the cow can't meet the energy it requires just through feed intake. Body fat and protein are mobilized from the cow's reserves for production, shifting nutritional requirements and altering metabolic status and energy partitioning (Garnsworthy, 2007). This induces a period of NEBAL, which is often unavoidable and can cause weight loss (up to 50-75kg in weight; Roche, 2006), combined with a loss of body condition (Garnsworthy *et al.*, 2008). However because of selection for higher milk production and an increased ability to mobilize fat and muscle to support production, NEBAL is exacerbated and loss of body condition is more prevalent and prolonged in high producers. Many factors can affect the extent of NEBAL including genetic merit for milk production where the condition is worsened through selection for angularity and lower subcutaneous fat (Veerkamp and Beerda, 2007).

NEBAL can extend for up to 10-12 weeks post partum which can affect the interval to first ovulation (Garnsworthy *et al.*, 2008). Oestrous expression is affected both by the level of NEBAL and body condition of cows. Heat detection rates have been reported to be significantly increased to 84.2% from 58.7% for cows that had a lower BCS loss over the first 100 days of lactation (0.3 vs 0.6 BCS; Mayne *et al*., 2002). Consistent with this, high yielding cows with more severe NEBAL (9.9MJ/d compared to 2.6MJ/d) showed decreased oestrous expression (Mayne *et al.*, 2002). Significant NEBAL has also been associated with low oestradiol concentrations during the periovulatory period (Mackey *et al.*, 1999) explaining the associated decrease in oestrous expression.

Changes in the metabolic status of the cow cause the mobilization of body tissues that affect the levels of hormones involved in fertility. NEBAL attenuates LH pulse frequency which inhibits oestradiol secretion which in turn prevents ovulation. Low energy status coupled with suppressing LH pulses also seems to reduce the responsiveness of the ovary to LH, again inhibiting the production of oestradiol (Butler, 2003) with subsequent impacts upon oestrous expression. Furthermore NEBAL is strongly associated with low levels of blood glucose, insulin and IGF-1 post partum which can limit oestradiol production by the dominant follicle. Metabolic demand causes a reduction in levels of glucose, insulin and IGF-1. Glucose and insulin are associated with the up regulation of LH receptors in the ovary. Indeed insulin and IGF-1 are linked as IGF-1 production is affected by circulating insulin concentrations. IGF-1 levels are also directly related to energy levels are correlate with oestradiol concentrations. This results in an alteration of the sensitivity of the response of the pituitary gland to GnRH, affecting LH pulses, influencing ovarian follicular development and the capability of the follicles to produce oestradiol (Butler, 2003). Therefore factors affecting feed intake and appetite in the periparturient period can affect the linked reproductive and somatotropic axes (Chagas *et al.*, 2007) and thus where possible the amount of body fat and protein mobilization should be minimised to reduce the extent of NEBAL and BC loss (Garnsworthy *et al.*, 2008).

In conclusion dietary intake can impact on the concentration of many hormones associated with reproduction. For example levels of nutrition can influence the ability of the ovary to produce oestradiol, thus affecting oestrous expression.

1.4.3.4 Genetic Factors

The over focus of genetic selection for greater milk yield with the associated changes in nutrition and management that accompany this has resulted in neglect for other production traits in selection. This has resulted in a significant negative impact on fertility. Cows selected for high milk yield are genetically more susceptible to NEBAL (Boer *et al.*, 2009).

Furthermore the reduction in fertility associated with increased milk yield is supported by increasing evidence suggesting that changes in genotype have a significant role in reducing fertility (Chagas *et al.*, 2007). Increased use of Holstein genetics is thought to be the root cause of reduction in reproductive performance because of the over focus on milk yield. However it is possible to maintain production and fertility as demonstrated from evidence in red (Ayrshire) type breeds (Berglund, 2008) and ensuring the appropriate nutritional management (Garnsworthy *et al.*, 2008).

BCS is also a result of genetic selection because modern cows are genetically thinner according to Garnsworthy *et al*., (2008). This is associated with selection for angularity and reduced subcutaneous fat (Veerkamp and Beerda, 2007). In comparing data from 1980-1993 with data from 2000-2006 BCS was shown to decrease from 2.5 to 2.1, respectively (Garnsworthy, 2007). This may therefore predispose the modern dairy cow to lower LH pulses and the associated oestradiol decrease, with reduced oestrous expression as discussed previously.

1.4.4 Herd Factors

The intensity and duration of oestrus varies between individual, cows but there are several factors within the herd which can also influence oestrus expression. The degree of calving spread has been shown to affect oestrous expression due to the number of cows in oestrus at any one time (Ball and Peters, 2004). If the calving spread is 365 days it is more likely that fewer cows will be in oestrus together (also depending on herd size). Oestrous expression could be reduced compared with block calving systems, or following oestrous synchronization, in which a group of cows will be in oestrus together (Ball and Peters, 2004). The number of mounts per oestrus can range from 11 per cow, increasing to 36 mounts with 2 cows in oestrus and 53 with 3 cows in oestrus simultaneously (Hurnik *et al.*, 1975). Duration can also be increased from 7.5 to 10.0 hours when more cows are in oestrus together (Hurnik *et al.*, 1975). Van Vliet and Van Eerdenburg (1996) reported similar results that oestrous intensity and duration increased with increasing number of cows in oestrus.

Cows in oestrus at the same time form a sexually active group (SAG) which changes as additional cows come into oestrus. As discussed it is important for other cows in the herd to be in oestrus to enhance expression because the greater accessibility of sexual partners and the increased stimulation provided by the SAG encourages interaction and standing behaviour (Orihuela, 2000), including the expression of secondary oestrous behaviours (Phillips and Schofield, 1990). Cows in the SAG share similar behaviours and act as good heat detectors for others coming into oestrus or having recently been in oestrus (Diskin, 2008). Mounting is also influenced positively by the familiarity of cows as more oestrous behaviour is displayed in stable groups (Castellanos *et al.*, 1997). Social dominance can have a negative impact on oestrous expression. Dominant herd mates can influence the number of cows in oestrus and have been shown to inhibit standing and mounting behaviour of smaller herd mates (Orihuela, 2000). It has been reported that most mounts are carried out by larger, heavier cows compared to smaller herd mates, however other studies have reported no relationship between dominance and expression (Orihuela, 2000).

1.4.5 Cow Factors

1.4.5.1 Puberty

Puberty is the time when oestrous cycles begin and the first oestrus occurs followed by ovulation when a heifer can conceive. This usually occurs at about 12 months of age in cattle and it is vital for first oestrus to be detected particularly if the heifers are to calve at 24 months of age.

Oestrous expression at puberty is affected by nutritional status. Liveweight gain influences the time of the onset of oestrous cycles. Heifers fed on a high plane of nutrition can reach puberty as early as 5-6 months in the Holstein breed. However heifers will not be ready to support a pregnancy at this age because of the lack of development, for example pelvic and poor mammary gland growth (Sejrsen and Purup, 1997). The onset of cyclicity can be delayed if dietary energy and growth are restricted. Restricted nutrition inhibits LH pulses through the heightened negative feedback of oestradiol and an inadequate GnRH signal (Williams *et al.*, 2002). Onset of cyclicity is also mediated by insulin and IGF-1, which are influenced by diet (as discussed in Section 1.4.3). Therefore it is important to manage nutritional intake heifers for oestrous expression to occur in order for them to calve at 24 months of age, but development can also be affected by genotype, season when pubertal age is attained, social cues and treatment with exogenous progestins (Ball and Peters, 2004).

1.4.5.2 Species and Breed

Breed and species can influence oestrous behaviour. Studies have reported the differences in oestrous behaviour between *Bos taurus* and *Bos indicus* cattle. The duration of sexual receptivity in *Bos taurus* cattle averages between 13.6 and 19.3 hours compared to *Bos indicus* cows with a shorter mean of 6.7 hours (Plasse *et al.*, 1970). The intensity of oestrus is reported to be reduced in tropical breeds (Plasse *et al.*, 1970) and behaviour of *Bos indicus* cattle differs as fewer cows are detected in oestrus by standing to be mounted (Llewelyn *et al.*, 1987). Differences between breeds are also highly prevalent such as comparing the oestrous behaviour of Angus (*Bos taurus*), Brahman (*Bos indicus*) and Senepol (*Bos taurus*) breeds. Angus and Brahman cows exhibited longer periods of oestrus than Senepol but total number of mounts differed; Angus received less than Brahman and Senepol cows, with no reported differences in intensity (Landaeta-Hernandez *et al.*, 2004). It has also been reported that some cows only show inclinination to engage in oestrus with members of the same breed (Galina *et al.*, 1982). Furthermore activity increases at oestrus are also affected by breed; Jersey cows are most active compared to Holstein and Red Dane cattle (Lovendahl and Chagunda, 2006).

Milk yield can also affect expression of oestrus (see Section 1.4.3) which has been largely associated with the decline in fertility through selection for increased yield (Royal *et al.*, 2000a) and introduction of Holstein genetics (Berglund, 2008). Detection efficiency is greater in Norwegian dairy cattle (92.6%) compared to Holstein Friesian cattle (80.3%; Mayne *et al*., 2002). When comparing Holstein and Ayrshire cattle, Ayrshire cattle showed increased mounting activity and more standing heats (Hackett and Mcallister, 1984). However Ayrshire cattle manage to maintain high milk production and good reproductive performance (Berglund, 2008).

1.4.5.3 Parity

Reports generally suggest that oestrous expression decreases with age and increasing parity. It has been reported that mean standing events are significantly lower in third parity cows compared to second and first parity, 5.6, 6.2 and 9.2, respectively (under heat stressed conditions; Peralta *et al*., 2005) and activity increases at oestrus are higher in younger, first parity cows than older cows (Lovendahl and Chagunda, 2009). It could also be that in the heifer SAG there is less stability and familiarity therefore expression is increased and more aggressive (Castellanos *et al.*, 1997). However, conflicting results report that intensity is greater in multiparous cows compared to primiparous as these have greater total scores for oestrus (see Table 1.2; Van Vliet and Van Eerdenburg, 1996). However it has also been recorded that there is no difference in heat detection rate between parity 1 and 2 cows (47.5 and 50.6%) but there are significant increases in heat detection rate when compared with parity 3 and 4 cows (54.7 and 60.5%; Rocha *et al*., 2001).

Explanation for the differences in oestrous expression associated with parity could be attributed to the difference in milk yield. Cows produce increasing milk yields with each successive lactation (Garnsworthy, 2007) and as cows undergo more lactations their metabolic status changes which has been associated with poorer fertility. Relationships between fertility, metabolic and endocrine traits have all been demonstrated to vary with lactation number (Wathes *et al.*, 2007a) and thus may explain the effect of parity on oestrous expression.

1.5 METHODS OF OESTROUS DETECTION

Ideal requirements concerning oestrous detection are; continuous surveillance of the herd, accurate identification of the particular cow in oestrus, minimized labour requirements, high accuracy to detect the physiological and/or behavioural events correlating with time of ovulation, for successful AI, and continuous operation for the entire productive life of the cow (Senger, 1994).

1.5.1 Visual Detection

Visual observation of oestrus is the most accurate method of oestrous detection. Observation of standing behaviour is the most accurate sign that a cow is in oestrus (Orihuela, 2000) correlates with ovulation giving an indication for the optimal time to AI, approximately 12 hours post onset of standing heat (Dransfield *et al.*, 1998). Identifying the primary sign of oestrus is extremely accurate, resulting in only 2% error in wrongly identifying oestrus (Kiddy, 1977). Secondary signs can also be used to identify cows in heat, but are not as reliable an indicator of true oestrus as standing to be mounted, however, these behaviours are useful to identify a recent heat or cows coming into heat, therefore should be more closely observed for the next 48 hours or 17-20 days later (Diskin, 2008).

Traditionally the only method of oestrous detection was to perform visual observations and although accurate this method is time consuming and requires attentive observation so that specific oestrous behaviour is not missed (Firk *et al.*, 2002). Current issues are that too little time is spent observing the herd, observation occurs at the wrong time and in the wrong place; during feeding time or at milking and is infrequent (Diskin, 2008). With intensification, the modern dairy herd is much larger therefore efficiency of detection is compromised. Observations 2 or 3 times a day for 30 minutes, which is typical of commercial farms, yielded 70% efficiency (Van Vliet and Van Eerdenburg, 1996) but in another study only 53% of standing oestruses were observed using the same observation method (Lyimo *et al.*, 2000). Another problem with visual observation is continuity; mounting activity is displayed more frequently early morning and late evening (Hackett and Mcallister, 1984) and 65% of all oestrus activity has been reported to occur between 1800 and 0600 hours (Hurnik *et al.*, 1975) when the herdsman is not present. Therefore, efficiency of visual detection is compromised by modern practice.

A major problem hindering visual detection is poor expression of oestrus in the modern dairy cow. Standing behaviour is not observed in over 50% of cows in oestrus (Van Eerdenburg *et al.*, 2002), and the number of silent heats has increased (Harrison *et al.*, 1990). Reduced intensity and duration (Dransfield *et al.*, 1998) also exacerbate the problem of observation at set times. Standing to be mounted is reported in less than 37% of oestruses when observed for 30 minutes 12 times per day are used. This is reduced to 12% when only 3 observations of 30 minutes (Van Eerdenburg *et al.*, 1996) which is a more practical time frame. Therefore a continuous method of oestrous detection is required to improve the efficiency of visual detection.

1.5.1.1 Behavioural Scoring

Van Eerdenburg *et al*., (1996) developed a scoring system to aid visual detection (Table 1.2). Standing to be mounted was included as the top score that a cow was in oestrus but behavioural scoring also takes into account secondary signs of oestrus (see Section 1.3). Using primary and secondary signs of oestrus allowed all aspects of oestrus to be monitored and the associated scores accumulated a total for oestrous behaviour (Van Vliet and Van Eerdenburg, 1996). When a score of over 100 points was achieved within a 24 hour period the cow was deemed in oestrus. Using this scale and 12 observations for 30 minutes per day in a 6 week study a detection rate of 100% was achieved for all normally cycling cows (Van Eerdenburg *et al.*, 1996). However 12 observations per day is impractical and time consuming. Therefore a more practical method of 3 observations for 30 minutes per day and a threshold of 50 points was developed which achieved 74% detection rate (Van Eerdenburg *et al.*, 1996). Behavioural scoring is a reliable method of visual observation because it includes all behaviour that is associated with oestrus. Yet this method does not give an indication of the onset of oestrus and therefore does not relate to the optimal time for AI.

Table 1.2 Table of behavioural scores relating to particular oestrous behaviours. When a total of 100 points is reached within a 24 hour period the cow is judged to be in oestrus (Van Eerdenburg *et al.*, 1996)

Oestrus Symptoms	**Scoring Scale**
Other Symptoms	
Mucus vaginal discharge	3
Cajoling	3
Restlessness	5
Sniffing/licking of the ano-genital region	10
Chin resting/ rubbing	15
Mounting Symptoms	
Mounted by other cow but resisting mount	10
Mounting (or attempting to mount) other cows	35
Mounting head of other cows	45
Standing heat	100

1.5.1.2 Fertility Records

Recording all heats is essential for good breeding management and in cases of infertility is the first thing to refer to. Animals must be clearly identifiable by ear tag, freeze mark or other method which should be detectable from a distance when observing. Breeding records should include: 1) animal I.D., 2) calving date (plus information relevant to calving), 3) pre-breeding heat dates, 4) service dates, sire and inseminator, 5) date of pregnancy and 6) expected calving date and any previous problems such as post partum diseases (Diskin and Sreenan, 2000). Records allow improved efficiency allowing farmers to work out the cycles of particular cows calculating approximate dates of oestrus and identifying any cows that have not shown oestrus for further attention, although do not directly aid identification of cows in oestrus.

1.5.1.3 Synchronization

To aid oestrous detection and improve submission rates synchronisation protocols (discussed in Section 1.2.2.3) can be used to synchronise the oestrous cycle. Synchronisation allows for a predetermined time period in which cows should display oestrus which aids detection as the herdsmen knows when and which cows to observe (Diskin and Sreenan, 2000). Furthermore some protocols involved fixed timed AI which removes the need for detection of oestrus altogether (Thatcher *et al.*, 1989). Detection is also enhanced by synchronising the oestrous cycles of a group of animals; therefore the intensity of oestrus is increased making detection easier (Hurnik *et al.*, 1975).

1.5.1.4 Teaser Animals

A bull among the herd can aid visual detection by detecting subtle signs of oestrus that humans cannot, identifying cows in oestrus. Bulls are often more accurate at detecting oestrus than humans. Vasectomised bulls known as teasers are a useful aid to detecting oestrus as they still allow for controlled breeding (Holmann *et al.*, 1987) and have been reported to marginally improve submission rates, 69% vs 61%, on 5 herds (Gordon, 2006). Androgenised (treated with testosterone or oestradiol) cows or steers, or cows with follicular cysts (with elevated oestradiol production) have increased expression of oestrus (Van Eerdenburg *et al.*, 2002) and can act in the same way as a teaser bull to encourage displays of oestrus and identify cows in oestrus. However use of bulls can incur problems as bulls may develop a preference for certain cows mounting these more frequently and ignoring others. This can complicate detection. Preference may also be given to the SAG ignoring any cows in oestrus outside of this group (Foote, 1975). The presence of a bull can also upset the herd dynamics and decrease female-female mounting. This can make detection of cows in oestrus more difficult as responsibility for oestrous detection is placed solely upon one bull (Orihuela, 2000). Use of teaser animals is a useful aid to visual detection but still requires labour for frequent observations of bull and cow behaviour.

1.5.1.5 Heat Mount Detectors

Further aids to oestrous detection are the use of tail paint, teaser animals with chin markers and pressure activated heat mount detectors, to determine when an animal has stood to be mounted. Applying tail paint to the head of the cow's tail indicates standing activity has occurred because the paint rubs off or becomes obviously smudged after mounting. Addition of chin markers to teaser animals also indicates when standing has occurred because the standing cow becomes marked with paint whilst being mounted by the teaser (Foote, 1975). Pressure activated heat mount detectors such as Kamar, Bovine Beacon, Check Mate, and Estrotect are also used as evidence that mounting has occurred. Heat mount detectors are fixed onto the sacrum (tail head) and when pressure is applied through mounting the colour changes to indicate that standing oestrus has occurred (Diskin, 2008). Most colour changes are to red or a noticeable colour from a distance when mounting has occurred for a period of 2-4 seconds, but can also occur gradually by rubbing off the silver surface, revealing more colour as mounts progress with the progression of oestrus.

Heat mount detectors are useful for oestrous detection relieving the pressure and time needed for visual observation, although this is still required to determine a positive result. The efficiency of detection is enhanced with heat detection aids, although this does not take into account cows that do not stand to be mounted (Firk *et al.*, 2002). Kamar detectors were reported to detect 98% of cycling cows in an early study; 31% triggered the day before oestrus and 33% on the day of oestrus, however, 30% of detectors were lost and 6% did not detect oestrus at all (Foote, 1975). Therefore, heat mount detectors prove useful when they remained fixed to the rump, but the incidence of error is very high associated with chin rubbing activating receptors, lost receptors caused by coat changes and general activation from the general environment, which also applies to tail paint (Firk *et al.*, 2002).

1.5.2 Physiological Changes

Physiological changes occur during oestrus caused by the rise in oestrogens and can be used as signs to detect oestrus.

1.5.2.1 Hormone Concentrations

It is widely known that at the time of oestrus progesterone concentrations are basal and oestradiol rises to a peak on the day of oestrus, before declining thereafter and allowing progesterone to increase (see Figure 1.1). These hormone concentrations can be detected in milk or in blood plasma and used to determine oestrus.

Progesterone concentrations remain high up to approximately 4 days before oestrus where they begin to decrease with regression of the CL. Concentrations of progesterone are reported to be lowest 3 days before oestrus, at oestrus and for a 3 days post oestrus before increasing again (Friggens and Chagunda, 2005). The concentration of progesterone in milk has been shown to decline from >10 to <3ng/ml at oestrus (Firk *et al.*, 2002) and in plasma decrease from >6ng/ml to <0.1ng/ml at oestrus (Claycomb and Delwiche, 1998).

Conversely oestradiol increases at oestrus for a short period in the absence of progesterone (Roelofs *et al.*, 2010). The concentration of oestradiol in plasma and milk are reported to correlate although are higher in plasma (Monk *et al.*, 1975). Oestradiol has been reported to average 1.3pg/ml in the milk of non pregnant cows, ranging from undetectable to 22.9pg/ml, including pregnant cows (Pape-Zambito *et al.*, 2007). At oestrus the concentration of oestradiol increases yet is present in small concentrations for a short period reported as only 8.7pg/ml on the day before and 7.4pg/ml on the day of oestrus (Lopez *et al.*, 2004).

Both of these physiological changes have the potential to be used as a method of oestrous detection. Milk samples are easier to collect than plasma for sampling daily, although with both sampling methods it is time consuming to process the samples. Progesterone concentrations are easier to measure as it is present in much higher concentrations, but there is no determinant for the exact timing of oestrus or relationship with ovulation (Lovendahl and Friggens, 2008). In a study by Starbuck *et al*., (2006) timing of follicular phase events in relation to progesterone concentration was investigated. It was found that luteolysis and thus time of progesterone decrease gave the least precise indication for oestrus and ovulation. Time from luteolysis to oestrus ranged between 24 to 40 hours and from luteolysis to ovulation, 64-136 hours (Starbuck *et al.*, 2006). Furthermore, cows with long follicular phases were reported to have longer intervals from luteolysis to the LH surge, oestrus and ovulation (Starbuck *et al.*, 2006) highlighting the variability of the timing of follicular events when using progesterone concentration for oestrous detection. However progesterone monitoring is an efficient method to monitor the overall cycle of the cow, for pregnancy detection and to determine any ovarian problems (Opsomer *et al.*, 1998). Oestradiol on the other hand correlates more precisely with ovulation (Lopez *et al.*, 2002) but because of the low concentration is harder to detect. Furthermore peak oestradiol concentration at oestrus differs between cows as each has a different physiological threshold (Lopez *et al.*, 2002) therefore difficulties will arise determining a universal threshold to define oestrus. However, sampling and further processing reduces the efficiency of hormone monitoring for the purpose of oestrous detection (Friggens and Chagunda, 2005), although this can be overcome by use of automated biosensors (Delwiche *et al.*, 2001a;Lovendahl and Friggens, 2008).

1.5.2.2 Milk Yield

Milk yield has been reported to significantly decrease at the time of oestrus by 2-6% and it is reported that cows housed in free stall barns exhibit an 8.2% decrease on the day of oestrus (reviewed by Firk *et al*., 2002). It has also been reported that cows presented with an oestrogen challenge to mimic oestrus have a 2kg reduction in milk yield (Britt *et al.*, 1986). The drop in milk yield at oestrus has been explained by the increased restlessness in cows at oestrus and decreased feed intake, and upon resumption of normal behaviour yield has been seen to increase at the following milking post oestrus (Britt *et al.*, 1986). However, yield is an unreliable method for determining oestrus due to the variation between cows and the effects of disease and environmental factors that can cause milk yield to decrease. It has been reported that some do not show a decrease in yield at oestrus and in one herd only 33% of cows showed a significant reduction in milk yield (Schofield *et al.*, 1991).

1.5.2.3 Body and Milk Temperature

The normal body temperature of a cow is approximately 38.6°C. This has been reported to decrease a few days prior to oestrus, with minimum temperatures recorded 2 days before, increasing at oestrus by 0.1 to 0.5°C (reviewed by Firk *et al*., 2002). Body temperature at oestrus has also been reported to increase by 1.3°C every 21 days to identify time of oestrus, however this was also reported to fluctuate with changing seasons (Piccione *et al.*, 2003). Measurements of body temperature can be taken via implants inserted into the rectum, vagina or ear of the cow or manually. Temperature measurements are useful although manual measurements would be time consuming and unpractical. However temperature can be affected by environmental conditions, level of physical activity which increases at oestrus and local inflammatory responses (reviewed by Firk *et al*., 2002).Temperatures of 38.0 to 39.3°C were recorded from milk in 95% of cows showing oestrus; with a small increase of 0.2°C for the preceding 3 days, however these results brought about a high incidence of false positives (Mcarthur *et al.*, 1992). Milk temperatures are also still influenced by housing systems and the environment.

1.5.2.4 Vaginal Mucus Resistance

An increase in mucus production during oestrus caused by oestradiol secretion (discussed in Section 1.3.3) can be used as a predictor of cows in oestrus. The same hormonal changes also affect the electrical resistance of the reproductive tract (Firk *et al.*, 2002) alongside swelling from tissue hydration which alters resistance (Ezov *et al.*, 1990). It is reported that electrical resistance is highest in the luteal phase and decreases during the follicular phase, lowest coinciding with the onset of oestrus and surge of LH (Leidl and Stolla, 1976). Vaginal resistance is reported to correlate with milk progesterone concentration during the oestrous cycle, r=0.22 (Gartland *et al.*, 1976). There is a high degree of variation in electrical resistance at oestrus between cows (Gartland *et al.*, 1976) which makes this method unreliable for a universal method of detecting oestrus. This variability was confirmed by Rorie *et al.* (2002) who reported that not all animals have low resistance during oestrus causing difficulty for accurate detection. Generally, low readings between 30 and 40 ohms indicate oestrus and pregnancy rates of 82% have been achieved for 874 cows using resistance below 30 ohms as a detector of oestrus and basis for insemination (Leidl and Stolla, 1976). However, vaginal resistance can be influenced by other factors such as cysts and ulcerous inflammations and the continuous sampling needed to determine the changes in resistance, which can cause irritation of the reproductive tract resulting in inflammation which will both hinder results and cause discomfort (Firk *et al.*, 2002). The animal's temperament, positioning of the probe and nutritional (mineral) imbalance can also alter resistance (Rorie *et al.*, 2002). Vaginal mucus resistance as a measure of oestrous detection is impractical but could be overcome by implants continuously recording resistance and plotting the results to observe changes over time.

1.5.2.5 Rectal Palpation and Ultrasonography

Rectal palpation and ultrasonography are used to evaluate ovarian activity and diagnose the stage of the oestrous cycle. Rectal palpation is used to detect the presence of the corpus luteum. Ultrasound is used to determine which ovary the growing follicle is situated on by measuring the diameter, then once a preovulatory follicle is obvious the ovary can be monitored at intervals to determine the time of ovulation, which is when the follicle disappears, for AI (Roelofs *et al.*, 2005). These techniques can also be used to detect silent heats, rectal palpation with 69.7% accuracy and ultrasonography 89.0% accuracy (Zdunczyk *et al.*, 2009). However these techniques can be time consuming and impractical as a method of oestrous detection, and must be carried out by either a veterinarian, which is costly, or a skilled stockperson (Foote, 1975).

1.5.3 Automated Technologies

Automated technologies are advantageous for oestrous detection because they monitor cows for oestrus 24 hours a day continuously requiring little input from the herdsman. Most electronic technologies fulfil the criteria set out by Senger (1994), for the 'ideal' system of oestrous detection. Although automated methods of detection are efficient they lack accuracy at detecting oestrus when compared to frequent visual observations and error rates are often increased. Automated methods must also be accurate at detecting signs of oestrus that are related to ovulation in order to correctly time AI to improve conception rates.

1.5.3.1 Pedometers and Activity Monitors

Pedometry and activity monitoring detect changes in the physical activity of the cow through motion sensing analysis. The relationship between physical activity and stage of the oestrous cycle was first documented by Farris (1954), before confirmation by Kiddy (1976) reporting that cows are approximately 2 to 4 times more active when in oestrus compared to when they were not.

Activity is measured by a mercury switch in the pedometer/ activity monitor which is turned on or off by cow movement. The device is fixed around the neck or leg of the cow and data downloaded at milking on entry to the parlour or by infrared sensors around the barn and analysed automatically. When activity increases above a threshold level or significantly from the baseline a cow is said to be in oestrus and this is flagged up automatically for further attention and submission for AI (Firk *et al.*, 2002). There are significant activity increases on the day of oestrus than on any other day (Schofield *et al.*, 1991), although it has been said that activity increases linearly and gradually from 72 to 16 hours before oestrus and from 16 hours to oestrus activity increases rapidly (Arney *et al.*, 1994). It is also reported that a marked increase in activity occurs 4 hours before peak oestrus allowing for optimal timing of AI (Nebel *et al.*, 2000). Activity is an accurate method of oestrous detection as At-Taras and Spahr (2001) reported activity corresponded most closely to standing behaviour. Secchiari *et al*., (1998) also confirmed the relationship between activity increase, time of insemination and successful conception. Yet this relies upon the frequency of activity records per day.

The efficiency of recordings determined by activity are in the range of 60 to 100% combined from several studies (Senger, 1994), mostly averaging 80-90% accuracy (Firk *et al.*, 2002;Lehrer *et al.*, 1992). Some studies even achieved 100% accuracy (Arney *et al.*, 1994;Schofield *et al.*, 1991). Low values for accuracy arise from false positives; technical faults and loss of devices, but also the severity of activity increase. When the increase in activity is bigger (4 fold compared to 2 fold) and duration of sustained activity is longer (approximately 4 hours), detection becomes more efficient and accurate (Rorie *et al.*, 2002), as a clear distinction between non oestrus and oestrus can be made. Error rate has been reported between 17 and 55% in a range of studies (Firk *et al.*, 2002) due to routine management tasks causing an increase in activity. However, activity monitoring is an efficient method of oestrous detection, more so than visual detection because continuous monitoring of data provides a reliable signal for the onset of oestrus. It is also economical, although not applicable to every production system such as tie stall cows; detection rate of only 14-20% (Firk *et al.*, 2002), or when cows do not display any activity increase for various reasons; ill health, lameness or when the threshold for increased activity is not reached (Roelofs *et al.*, 2005).

1.5.3.2 Electronic Heat Mount Detectors

Electronic heat mount detectors such as HeatWatch are similar to pressure activated heat mount detectors (see Section 1.5.1.5) without the need for visual observation. These radio telemetric devices measure the pressure from standing activity; which is defined as 3 standing events in 4 hours. When activated a radio signal is emitted which is picked up by a receiver/ repeater, relayed to a buffer then ultimately to a computer where the important information for the herdsman is stored. The identity of the cow, time of mount, date and duration are all recorded, and from this timing of heat onset and time for AI can be estimated. Different lists are also generated: oestrus, suspected oestrus, non return (no mounts in 25 days), brief cycle (13 day cycles) and inactive list (At-Taras and Spahr, 2001).

Efficiency of detection using electronic heat mount detectors is reported as 86.8%, which was similar to recordings from activity monitors when compared (At-Taras and Spahr, 2001) with a low error rate of 2% (Rorie *et al.*, 2002). However when compared to visual detection electronic heat mount detectors were more efficient, 91% compared to 51%, respectively, due to continuous monitoring and quick identification of cows not showing oestrus. Peralta *et al.* (2005), however, reported different efficiencies when comparing detection methods under compromised conditions. Visual detection and HeatWatch had similar efficiency; 49.3% and 48% of oestrus periods detected, respectively, with lower efficiency for activity monitors; 37.2%. Moreover, in this study the conception rate for cows detected by HeatWatch was much greater than that of visual detection, 17.3 vs 6.2% respectively.

Therefore, electronic heat mount detectors are efficient at detecting oestrus as they monitor continuously. However they only detect standing behaviour and do not take into account secondary signs of oestrus which are important as not all cows stand to be mounted (Dobson *et al.*, 2008). Although their accuracy is compromised by generating a number of false negative diagnoses thought to be caused by uncoordinated mounts not triggering the pressure sensitive device and through several false positives caused by the general environment triggering devices, also incurring error through loss of the transmitter from the tail head (At-Taras and Spahr, 2001), which has been reported by many groups (Firk *et al.*, 2002).

1.5.3.3 Milk Progesterone Biosensors

Milk progesterone concentration drops below 3ng/ml of milk at oestrus and can be used as a detection aid for pin pointing the drop in progesterone, allowing oestradiol to increase (Friggens and Chagunda, 2005). Monitoring progesterone concentration is a useful tool for monitoring the reproductive status of the cow throughout the oestrous cycle (discussed in Section 1.5.2.1) and can be automated using an in-line approach monitoring hormone concentration at each milking by biosensors (Delwiche *et al.*, 2001a). The specificity of the sensors can detect subtle changes in concentration and provide details on ovarian function, metabolic status and disease such as mastitis. Numerical data can then be downloaded and compared online and used to determine the health and fertility of cows and most importantly the timing of ovulation (Mottram *et al.*, 2002). These biosensors successfully detected all 19 ovulatory events but the variation in progesterone concentration resulted in a 26% error rate due to variability between cows (Delwiche *et al.*, 2001a). This group made improvements to the sensitivity of the immunoassay and developed usable data available in real-time within 10 minutes of sampling (Delwiche *et al.*, 2001b) yet this method of detection is not fully validated and is not available commercially. HerdNavigator, on the other hand, is a commercial method of online progesterone monitoring which was developed with elements to detect health, fertility and metabolic status (Lovendahl and Friggens, 2008). The model as described by Friggens and Chagunda (2005) has reported 99.2% in the model of confirmed oestrus and 93.7% using ratified oestrus and progesterone curves (Friggens *et al.*, 2008). Monitoring of progesterone concentration does have advantages over other methods of detection because silent ovulations can be detected; 55.2% of first ovulations post partum were silent and detected by milk progesterone concentration but not activity (Ranasinghe *et al.*, 2010). However the accuracy of this technique declines as timing of decrease in progesterone concentration has a weak relationship with timing of ovulation (Lovendahl and Friggens, 2008).

1.5.3.4 4sight

4sight is an optical electronic method of oestrus detection using digitised surveillance monitoring combined with optical electronic sensors, to recognise mounting behaviour and distinguish between primary and secondary mounting displays. Mounting and specific behaviour trigger a photosensitive beam which identifies the individual cows in oestrus through a preloaded database containing images of the 4 sides of each cow. A list formulates each day of cows that have been mounted, those that have mounted others, the time at which this behaviour occurred, an optimal time for AI and any cows not seen in oestrus for 21 days. In 2 trials 4sight has been reported to detect 100% of oestruses and correctly identify the 19 cows not cycling, and when used commercially heat detection was 90%, however conception rates were reportedly poor (Esslemont, 2006).

4sight overcomes the difficulties of visual detection, but does not account for cows not displaying overt oestrus but this system is advantageous because it will identify those cows which are eligible but have not shown oestrus. This method is efficient for oestrous detection and is accurate at detecting oestrus, although more trials on a number of herds will be needed to confirm the efficacy of this method. Esslemont (2006) calculated the benefits of 4sight and reported that the increase in profit/cow/year would be £86 and set to increase with the length of time using 4sight and improving detection. However, this does not take into account the initial cost of installation.

1.5.4 Genetic Selection

The decline in dairy cow fertility resulting from genetic selection for milk yield ignoring other selection traits is well known; therefore genetic selection for fertility traits should also be possible. This, however, is not as easy, because fertility traits are widespread and largely influenced by external factors such as environment and management. By improving oestrous expression oestrous detection rates should also increase because it will be easier to identify cows in oestrus.

Breeders can select for fertility traits from breeding values in the UK Fertility Index in which sires can be selected for breeding based on their daughter's fertility. However, low heritability (h^2=<0.05; Berglund, 2008) and the slow rate of genetic gain hinders any improvements in fertility that may be gained. This is because data from daughters can only be recorded at the earliest 2 years following their first calving and further data collected at the 2nd calving, making the bull 4 years of age before any breeding values are in place, when it will have subsequently bred with other cows (Flint *et al.*, 2008).

However, genetic selection for oestrous behaviour is not well defined and is often bypassed in relation to selection, except in Sweden where heat detection scores for oestrous symptoms are recorded as selection indices (Berglund, 2008). It has however, been reported that a link can be made between oestrous behaviour and genetic selection, reporting that days to first activity increase post partum, verified by pedometer data, was heritable h^2=0.18, with repeatability of 0.18 indicating this trait is predominantly determined by genetics (Lovendahl and Chagunda, 2009) which is advantageous when selecting for oestrous behaviour. The heritability of duration and strength of oestrus were also determined, yet they were of low heritability: duration; 0.02 and strength; 0.04 (Lovendahl and Chagunda, 2009).

Genetic selection for oestrous detection has also been reported in relation to hormonal factors associated with fertility; milk progesterone levels, GnRH response and metabolic hormone levels, which can all affect hormones in the oestrous cycle and oestradiol production to influence oestrous detection. These endocrine traits are less affected by management making heritability estimates more reliable. Milk progesterone measurements throughout the milk progesterone curve have been reported as heritable in defining the time to 1st ovulation and commencement of luteal activity, h^2=0.17 (Royal *et al.*, 2002). The heritability of progesterone is similar to activity estimates of days to 1st activity increase. In response to GnRH, LH and FSH are produced, which have a direct relationship with the production of oestradiol. By using the GnRH response of a bull's progeny, fertility and oestrus activity can be predicted at an earlier age. The GnRH response, measured as concentration of LH has high heritability, h^2=0.51 and data is available at 4-5 months in bulls, with the inclusion of heifers also in the Fertility Index (Royal *et al.*, 2000b). Metabolic hormones; insulin, IGF-1 and GH (growth hormone) control the metabolites; FFA (free fatty acids) and glucose, and are all linked to ovarian function, thus can affect oestrous expression through GnRH pulses and steroidogenesis. The heritabilities of FFA, glucose and insulin are all moderate h^2=0.11 to 0.30, largely due to the influence diet has on these variables (Hayhurst *et al.*, 2007). Inclusion of this data for genetic selection is relevant because of the relationship of these hormones and metabolites to NEBAL and BCS. Circulating levels of these hormones can be included in breeding values and can predict susceptibility to NEBAL and low BCS at calving, early in the animals life, which can impact upon fertility and oestrous expression (Flint *et al.*, 2008).

There has been little genetic selection for oestrous expression but several factors seem promising. Milk progesterone, GnRH response and metabolic hormone levels have all been reported to influence oestrous expression and are reported to be heritable. Inclusion of heritable estimates for indicators of strong oestrous expression could aid oestrous detection.

1.6 AIMS & OBJECTIVES

Many existing methods of oestrous detection; visual, physiological and automated, have flaws in either their accuracy, efficiency or both, and do not meet the ideal requirements described by Senger (1994). Poor oestrous expression is also a hindrance to effective oestrous detection; less intense, shorter duration (Dransfield *et al.*, 1998) and less than 50% seen in standing oestrus (Dobson *et al.*, 2008). However, due to the multifactorial nature of the control of expression of oestrus it is difficult to identify methods to improve expression. The work described within this thesis focuses on the individual cow variation of oestrous expression. The aim is to improve expression permanently through genetics therefore to improve oestrous detection rates.

Ideally 24 hour continuous automated surveillance is required to minimise labour requirements and cost. However a method that can accurately detect reliable signs of oestrus to increase detection rates from 50% to the current target of above 70% is required (DairyCo, 2009). Importantly to improve herd fertility the ideal system for identifying cows in oestrus must detect cows standing to be mounted, the definitive sign of oestrus (Orihuela, 2000) and the period which is most significantly correlated with the time of ovulation (Roelofs *et al.*, 2005) resulting in improved conception rates. Hence there is a need to develop a robust system to identify both cows approaching oestrus and cows in oestrus (standing to be mounted), in real-time to overcome the limitations of earlier systems, in order to maximise pregnancy rates and thus profitability.

In summary the objective was to formulate solutions to improve oestrous detection by enhancing expression of oestrus and by developing a novel technology for precise, real-time monitoring to detect cows in oestrus.

Aims of this work were to:

- Investigate cow factors that affect expression of oestrus measured by a current automated method of oestrous detection.

- Investigate individual cow factors such as genetic variation that may affect the expression of oestrus.

- Develop novel positioning technology to detect oestrus. The aim was to monitor 3 dimensional cows positioning to detect cows approaching oestrus and cows in oestrus.

CHAPTER 2 – Effect of Cow Factors on Oestrous Expression

2.1 INTRODUCTION

A main contributory factor to poor fertility in the dairy cow (Royal *et al.*, 2000a;Butler, 2003) is poor oestrous expression; only 50% of cows are reported to show signs of standing to be mounted, the definite sign that a cow is in oestrus (Van Eerdenburg *et al.*, 2002). Other measures of oestrus suggest that duration and intensity of oestrus has also decreased; only 8.5 standing events at oestrus and duration only lasting on average 7 hours in Holsteins (Dransfield *et al.*, 1998).

Associations have been found between parity (Macmillan *et al.*, 1996) (Garnsworthy *et al.*, 2008), seasonal variations (Critser *et al.*, 1987) and milk yield (Royal *et al.*, 2000a) and their effects on fertility. Previous work has recognised that oestrous expression can be influenced by cow factors, focussing on duration and intensity, and standing events (Van Eerdenburg *et al.*, 1996;Peralta *et al.*, 2005), but there have been few investigations into the effects of cow factors and their associations with activity at oestrus, measured by activity monitors.

Emphasis should be placed on identifying cows at risk of poor oestrous expression. It is important to identify factors that affect oestrous expression in order to implement management systems for improvement of oestrous detection. This study investigated the effects of parity, time period of oestrus, oestrous number, days post partum and milk yield for their effects on activity. Associations between the activity increase on day of oestrus and probability of conception were also investigated. The aims of this study were to identify the effects of measurable cow factors on activity increases at oestrus.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Animals used in this study were 205 Holstein Friesian dairy cows housed at Nottingham University Dairy Centre. The lactating cows were kept indoors in groups of approximately 40. Housing consisted of a purpose built shed with 4 pens, which was well ventilated, with rubber matting, cubicles and shavings for comfort whilst lying. All cows were fed the same silage based diet, with concentrates at milking. Cows were milked by 4 robotic milkers (Lely Astronaut A3 AMS units) voluntarily; visiting from 2 to 6 times per day. Oestrous detection was measured by activity monitors with data downloaded by infrared sensors, and by visual observation. Three full time staff were employed to work at the dairy unit, of which 2 were working at any one time. Cows were checked for signs of oestrus by the herdsperson first thing in the morning, at approximately 6am, and again in the evening, between 8pm and 10pm. However, this left a long period of time in which the cows were not monitored and could exhibit oestrous behaviour which could go undetected, if visual observation was the only method of detection.

2.2.2 Data Collection and Analyses

Activity monitors were worn around the cows' necks (Figure 2.1), measuring daily activity and identified increases which signalled that a cow was in oestrus. The cows wore Lely Qwes-HR Activity Tags which measured cow movement and movement intensity through a 3 dimensional accelerometer sensor which was expressed as a general activity index. Activity was expressed as counts from an instrument specific algorithm within the activity monitor, which is patented and undisclosed by the manufacturers. Data was recorded by a microprocessor and stored in the memory. Activity data were downloaded at milking from the cows' transponder, which was read on entry to the robotic milker, but could also be downloaded from infrared identification units which were mounted in the barn. Activity data were downloaded at intervals throughout the day and split into activity units per 2 hour intervals.

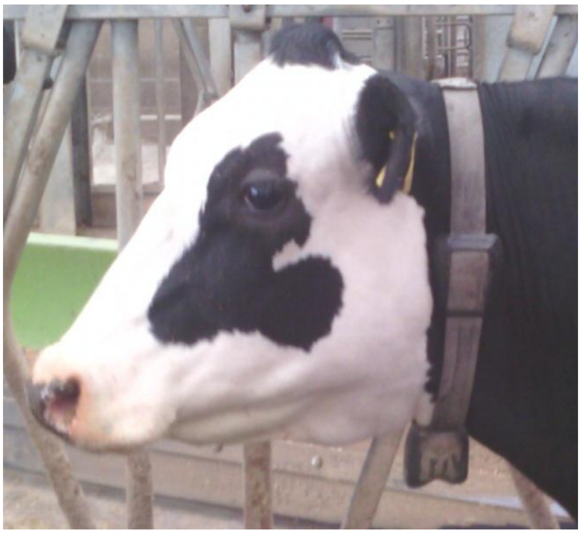


Figure 2.1 Example of a cow wearing a Lely-HR Tag activity monitor around her neck

Activity data spanning 2 years (16.03.2008-11.02.2010) were collated from 205 cows, including 930 individual oestruses across different lactations and different stages of lactation. Activity data was analysed by plotting activity against date and time. This resulted in 12 activity readings daily, with peaks denoting oestrus (Figure 2.2).

From the activity data oestrus could be determined by peaks on the graph (Figure 2.2) and numbered for each oestrus during the lactation. These were determined either by increases in activity, visual detection or both. All peaks after 25 days post partum were identified as oestrus since increases in activity before this could be due to cows re-entering the herd after solitary calving and establishing hierarchies, mixing with new herd mates and entering a new environment. Oestrus was identified as either recorded or not recorded. Recorded was defined as oestrus which was confirmed by the herdsmen and where mounting and standing behaviour had been observed. Any peaks that did not correspond to recorded oestruses from the farm database were included in analysis as unrecorded oestrus events. Unrecorded oestrus events as seen by an increase in activity, were used in the data analysis, and identified as showing no overt signs as recognised by the herdsmen. Any recurring oestrous cycles within 10 days were discounted from analysis as these may be due to management practices or general errors and hence not a true representation of the cyclical activity.

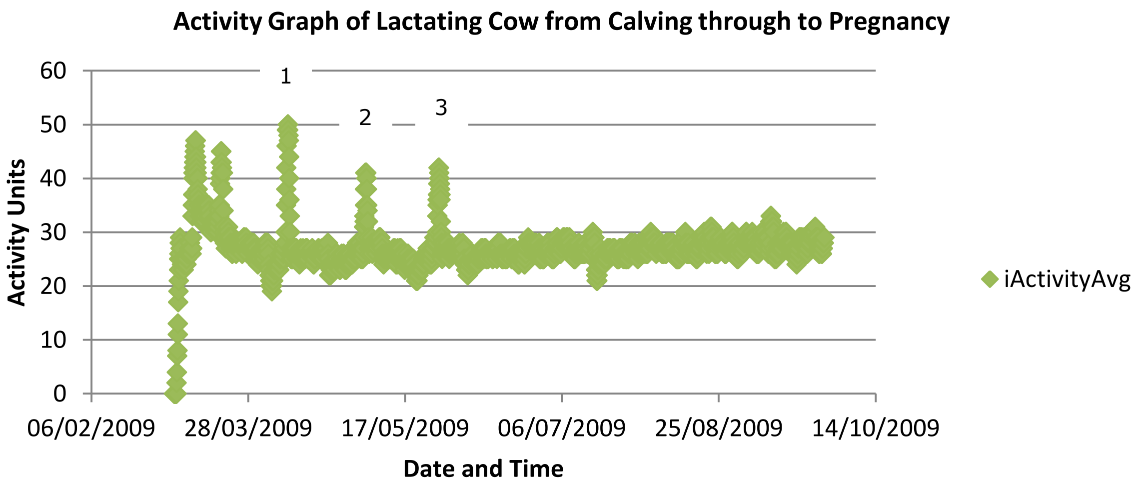


Figure 2.2 Graph of activity units plotted against date and time; initial peaks are due to entry into the herd post calving and the initial trough is due to calibration. Peaks 1, 2 & 3 correspond to oestrus, compared to the baseline average activity

Two oestrous parameters per cow were determined to analyse for associations with cow factors. Strength was calculated as a percentage increase from average baseline activity (baseline calculated over a 4 day rolling average) at oestrus and the maximum activity at oestrus was determined to use as measurements of activity. An increase in activity at oestrus was defined as 3 consecutive periods of increased activity compared with the baseline before the onset of increased activity. The smallest threshold for activity increase was 30% from baseline.

Herd fertility records provided information on each oestrus, insemination and whether this resulted in pregnancy. General information about each cow, such as milk yield, was downloaded to the system during milking.

All data were compiled into a database to identify associations between activity data for oestrous expression and cow factors such as age, stage of lactation, milk production and time of year when oestrus occurred, and whether conception was successful after AI. The data collected are presented in Table 2.1.

2.2.3 Statistical Analyses

Statistical analysis was carried out using Genstat 15th edition (VSN International Ltd, Hemel Hempstead, UK). Activity data were analysed as generalized linear mixed models (GLMM) using the residual maximum likelihood (REML) procedure, with Poisson distribution and logarithmic link function. The model fitted fixed effects for days in milk (DIM), milk yield (all definitions; Table 2.1), parity (classified according to lactation number as 1, 2 and ≥3), oestrous number and time of year (classified as Jan-Mar, 1; Apr-Jun, 2; Jul-Sept, 3; Oct-Dec, 4), individually. For the random effects of the model, individual cows represented subjects to allow for multiple oestruses per cow. The significance of fixed effects was assessed by Wald tests. The resulting model was:

$$Y_{ij} = \mu + V_i + C_j + \varepsilon_{ij}$$

where Y_{ij} is activity at oestrus,

the fixed part of the model consists of

μ the overall mean,

V_i the effect of the individual variable (Table 2.1),

C_j the random effect of Cow, and

ε_{ij} the residual error.

Table 2.1 Definitions of cow factors analysed for their effects on the activity increase at oestrus

Variable	Definition
Days in Milk (DIM)	Number of days post partum until each individual oestrus event were investigated for the association between DIM and activity at oestrus.
Milk Yield at Oestrus	The average milk yield was calculated over each oestrus period to investigate the effect of level of production at the time of oestrus on the activity increase. Milk yield at oestrus was calculated as the average of 5 days before, the day of oestrus and 5 days post oestrus. This accounted for daily variation and decrease in yield that is reported at oestrus.
Average Daily Milk Yield	The total milk yield to each oestrus event was divided by the number of DIM to calculate a representative daily average. This variable was used to assess whether the overall level of daily production within each lactation had an effect on oestrous expression.
Cumulative Lactation Yield	The total milk yield produced per lactation up to each individual oestrus event was used to assess the effect of overall level of production on activity.
Parity	Cows were grouped into parity 1, 2 and 3 corresponding to cows in their 1st parity, 2nd parity and cows in their 3rd parity and above. These were analysed for the effect of age and lactation number on oestrous expression.
Oestrous Number	Successive oestruses per lactation were analysed for the effect of multiple oestrous cycles on the expression of oestrus. The whole data set was analysed with oestrus events per lactation included in statistical analysis as variates, n=930. However this analysis may include poor cows or cows that were later culled for infertility. The average number of oestrous cycles per lactation in the total data set was 3.12, therefore cows that conceived at their 3rd oestrous cycle were analysed separately for the effect of oestrous number on expression. Further investigation then looked at the difference between the 1st and 2nd oestrus event.
Time of Year	This variable takes into account the time of year in which each oestrus event occurred; 1 - Jan-Mar, 2 – Apr-June, 3 – July-Sept and 4 – Oct-Dec. This was to account for environmental variables such as day length which could physiologically affect the expression of oestrus.
Successful Conception	Activity increase at oestrus was analysed for its effect on whether successful conception was related to oestrous expression.

The interaction between parity and milk yield were then analysed for the combined effect on activity at oestrus using GLMM. The resulting model was:

$$Y_{ijk} = \mu + MY_i + P_j + C_k + \varepsilon_{ijk}$$

where Y_{ijk} is activity at oestrus,

the fixed part of the model consists of

μ the overall mean,

MY_i the effect of milk yield; at oestrus, daily average and cumulative yield,

P_j the effect of parity

C_k the random effect of Cow, and

ε_{ijk} the residual error.

2.2.3.1 Statistical Analysis of Successful Conception

The effect of activity on the probability of conception was analysed using a Binomial model with logit link function. Activity was analysed as part of the fixed model, with cow number included in the random model. Pregnancy (classified as 1 = pregnant and 0 = not pregnant) was analysed as the response variate with insemination (1 = yes inseminated) included as the binomial totals. All inseminated cows were included in analysis.

2.3 RESULTS

The results reported in this section are the significant associations between activity and the cow factors discussed in Table 2.1. Maximum activity at oestrus ranged from 26 to 150, averaging 68 activity units. Percentage increase in activity at oestrus ranged from 31 to 200%, averaging 87%. However, the maximum increase in activity at oestrus was not the biggest percentage increase from baseline. This was also the same for the minimum activity increase at oestrus, where this was not the smallest percentage increase from baseline. The correlation between percentage increase from baseline and maximum activity at oestrus was r=0.57, showing a moderate positive correlation.

2.3.1 Effect of Days in Milk, Parity, Oestrous Number and Time of Year of Oestrus on Increase in Activity

The effect of parity, oestrous number and time of year on activity at oestrus are reported in Table 2.2. Maximum activity at oestrus was significantly associated with parity, oestrous number and time of year. DIM was not significantly associated with the activity increase at oestrus. As parity increased the activity increase at oestrus became smaller. First parity cows had an average activity count 10 units higher at oestrus than those in parity 3 and above. Percentage increase in activity at oestrus was also significantly related to parity, P=0.044, where 2nd parity cows had larger increases in activity from baseline compared to 1st parity and 3rd parity or greater cows.

When analysing all cows with multiple oestrous cycles per lactation it was reported that oestrous number was significantly related to the activity increase at oestrus, P=0.009. Activity increases at oestrus were smaller further through the lactation as the number of oestrus events without conception occurred. However, this may be inclusive of poor cows that were later culled for infertility. The average number of oestrous cycles per lactation from the complete data set was 3.12. When analysing cows with 3 consecutive oestrous cycles to conception, n=151, activity increase at oestrus was significantly associated with oestrous number, P=0.01. The activity increase at oestrus was greater for the 2nd oestrus post partum compared to the 1st and 3rd. Further investigation into the 1st and 2nd oestrus post partum, n=215, revealed no significant difference in the activity increase.

Time of year of oestrus was significantly associated with activity at oestrus, P=0.004. Activity increases were greater in periods 2 and 3, coinciding with longer day length, compared to periods 1 and 4.

2.3.2 Effect of Milk Yield on Increase in Activity

Three different measurements of milk yield are reported to investigate their relationship with activity at oestrus (Table 2.1). The significance of these parameters are reported in Table 2.3. They all showed the same pattern that as yield increased activity at oestrus decreased. Milk yield at oestrus (P=0.002) and average daily yield to oestrus (P=0.002) significantly affect the increase in activity at oestrus. Cumulative lactation yield to each individual oestrus was not significantly related to activity at oestrus although followed the same inverse relationship of increasing yield and decreasing oestrous expression.

2.3.3 Effect of the Interaction between Milk Yield and Parity on Activity at Oestrus

The interaction between milk yield (at oestrus, daily average to oestrus and cumulative lactation yield) and parity was analysed for the combined effect on the increase in activity at oestrus. Results are reported in Table 2.4. When correcting for the effect of milk yield at oestrus on activity, and then including parity in the model, the result was significant ($P<0.05$) for both individual fixed effects. Milk yield negatively affects the increase in activity at oestrus, as does increasing parity. This was the same for the effect of average daily milk yield to oestrus ($P<0.05$). However in both of these analyses the interaction between the 2 variables on activity at oestrus was not significant. The same trend was seen when analysing cumulative lactation yield to oestrus although the result was not significant.

2.3.4 Activity Increase and Conception Rate

Activity was not significantly associated with the probability of conception ($P=0.064$). However, results indicate a trend that the greater the activity increase at oestrus, the higher the probability that a cow would conceive to an insemination.

Table 2.2 Effects of parity, oestrous number and time period of year on activity at oestrus

Variable	*P* value	Effect[a]	Oestrus Events, n	Standard Error	Mean Prediction of Activity[b]
		Parity			
Parity	<0.001	1	321	0.03	71
		2	314		68
		>2	295		61
		Oestrous Number Post Partum			
Oestrous Number	0.01	1	151	0.02	65
		2	151		68
		3	151		64
		Time Period			
Time Period of	0.004	1	230	0.02	65
Year		2	180		68
		3	263		69
		4	257		66

n = total number of oestrous events for each parameter
[a]Effect of each variable on activity at oestrus
[b]Mean prediction of activity generated by the statistical model

Table 2.3 Effect of milk yield on the activity increase at oestrus

Variable	*P* value		Effect	Standard Error
Milk Yield at Oestrus	0.002	Constant	4.206	0.02
		Effect	-0.004	0.001
Average Daily Milk Yield to Oestrus	0.002	Constant	4.204	0.02
		Effect	-0.004	0.001
Cumulative Lactation Yield to Oestrus	0.077	Constant	4.203	0.02
		Effect	-0.0000047	0.0000026

Table 2.4 The effects of the interaction between milk yield and parity on the activity increase at oestrus

Variable	*P* value		Effect	Standard Error
Milk Yield at Oestrus	0.002	Constant	4.299	0.03
		Effect	-0.006	0.003
Parity	0.001	Constant Parity 1	4.229	0.04
		Effect at Parity 2	-0.00788	0.04
		Effect at Parity >2	-0.11654	0.04
Milk Yield at Oestrus x Parity	0.160			
Average Daily Milk Yield to Oestrus	0.002	Constant	4.221	0.03
		Effect	-0.006	0.003
Parity	<0.001	Constant Parity 1	4.221	0.03
		Effect at Parity 2	-0.00467	0.03
		Effect at Parity >2	-0.11041	0.03
Average Daily Milk Yield to Oestrus x Parity	0.111			
Cumulative Lactation Yield to Oestrus	0.074	Constant	4.251	0.02
		Effect	-0.0000097	0.0000056
Parity	<0.001	Constant Parity 1	4.251	0.03
		Effect at Parity 2	-0.03592	0.03
		Effect at Parity >2	-0.13872	0.03
Cumulative Lactation Yield to Oestrus x Parity	0.376			

2.4 DISCUSSION

The objective of this study was to identify factors associated with the activity increase at oestrus. Activity increases at the time of oestrus between 2 and 4 fold (Kiddy, 1977). In this study the increases in activity were reported expressed as maximum activity at oestrus and the percentage increase in activity from baseline. There was a moderate positive correlation between percentage increase and maximum activity. This could be explained because some cows may have a high average baseline activity but a small maximum activity and thus their oestrous expression is decreased, or vice versa where cows are generally inactive yet at oestrus become very active. Several cow factors were found to have a significant effect on maximum activity at oestrus; parity, oestrous number, time of year and milk yield ($P<0.05$).

2.4.1 Effect of Parity, Oestrous Number and Time of Year on the Increase in Activity

2.4.1.1 Parity

Parity was negatively associated with activity; as parity increased, activity at oestrus decreased. These results are consistent with previous reports of the effect of lactation number and parity on activity; activity increases at oestrus, recorded by electronic activity monitors, were higher in younger, first parity cattle compared to older, later parity cattle (Lovendahl and Chagunda, 2009). Furthermore as lactation number increased the number of steps, recorded by pedometer, has been reported decrease at oestrus (Yániz *et al.*, 2006). It is reported that with each additional lactation number cows walking activity at oestrus was less by 21.4% (Lopez-Gatius *et al.*, 2005). Other reports on the effect of lactation and parity on oestrous expression also concur with this work; mean standing events were lower for 3^{rd} parity cows (5.6) compared to 2^{nd} (6.2) and 1^{st} parity (9.2) cows (Peralta *et al.*, 2005), although this was carried out under heat stressed conditions.

It has been reported, however, that heat detection rate was not different between parity 1 and 2 cows (47.5% and 50.6%), but heat detection rate for parity 3 and 4 cows was significantly increased (54.7% and 60.5%; Rocha *et al*., 2001). Other conflicting reports suggest that intensity of oestrous expression differs between primiparous and multiparous cows

(361 vs. 578 points, respectively, from the table of behavioural scores, determined by the authors' scoring system), with multiparous cows displaying more intense oestrus (Van Eerdenburg *et al.*, 1996). In another study oestrus activity was lower in heifers, 5.5 mounts per hour, increasing to 7.9 mounts per hour for cows in the 4th lactation or above (Gwazdauskas *et al.*, 1983), indicating increased expression with increasing lactation number. This could be due to conditioning and sexual experience, but when heifers were removed from the analysis the results became insignificant and there was less of a pattern (Gwazdauskas *et al.*, 1983).

In the current study it was possible that the reduction in activity, with increasing parity, was influenced by differences in milk yield. However when analysing the effect of both milk yield and parity (Table 2.4) on activity the interaction was not significant. It could be explained however that cows in later lactations have greater milk yields per lactation, with maximum yield around the 4th lactation (Garnsworthy *et al.*, 2008). Higher milk yields could negatively influence oestrous expression (Lopez *et al.*, 2004). Changes in energy balance with each successive lactation (Coffey *et al.*, 2002) and altering metabolic profiles between 1st lactation cows and older (Wathes *et al.*, 2007a), can affect oestrus expression through metabolite effects on hormones controlling the oestrous cycle. Cows in their first lactation use nutritional ingredients for growth as well as for lactation and reproduction i.e. for conception and for pregnancy (Sheldon *et al.*, 2006). This may result in a proportionately more severe NEBAL and hence cows have difficultly recovering (Meikle *et al.*, 2004). This may continue and affect expression of oestrus in subsequent lactations; therefore activity may also be decreased in later lactations.

Furthermore because cows in later lactations are more likely to produce greater milk yields (Garnsworthy *et al.*, 2008) they may suffer increased NEBAL (Macmillan *et al.*, 1996). The extent of the effect of lactation on fertility, and oestrus, is more severe in cows fed on concentrates and conserved forages (>20%) compared to cows fed at pasture (<10%). Therefore there appears to be an association with nutrition and milk yield affecting severity of NEBAL (Macmillan *et al.*, 1996), and thus affecting activity at oestrus. Hence high yielders are more susceptible to NEBAL and low BCS, because they cannot consume enough energy to meet the demands of their high level of production (Wathes *et al.*, 2007b;Garnsworthy, 2007). High yielders experience a more pronounced loss of body condition, due to energy balance regulation, which has been related to poor oestrous expression (Mayne *et al.*, 2002). NEBAL and loss of body condition has been related to attenuation of LH pulse frequency and low levels of blood glucose, insulin and IGF-1 which together impair the production of oestradiol by the dominant follicle (Butler, 2003). However, Macmillan *et al.,* (1996) also found that the effect of milk yield and stage of lactation was not an absolute indicator of NEBAL. Lower yielding cows could also have lower feed intake and thus have a more severe energy deficit which could affect oestrus. However NEBAL is highly correlated with genetic improvement for milk yield (Veerkamp and Beerda, 2007).

A further possibility is that as cows get older their activity decreases due to age (Lovendahl and Chagunda, 2009). It is also possible that cows are culled for infertility and only fertile cows survive to the next lactation; so a larger proportion of the cows in later lactations display oestrous behaviour and oestrus is more easily detected.

2.4.1.2 Oestrous Number

As oestrous cycles progressed without conception the activity at each oestrus decreased post partum. Typically the first oestrus post partum is silent (Ferguson, 1996) and it is possible that the first oestrus was removed from analysis in this study. All oestruses before 25 days post partum were removed, due to increased activity levels because of re-entry of cows into the herd after solitary calving, due to increased activity associated with the establishment of hierarchies. The current results demonstrate that activity is greater at the 2nd oestrus, although there was no significant difference between activity at 1st and 2nd oestrus. Therefore activity at oestrus decreased from the 3rd oestrus onwards. Previous reports have suggested a similar pattern, that oestrous expression increases up to the third oestrous cycle post partum (Ferguson, 1996;Thatcher and Wilcox, 1973).

Peralta *et al*., (2005) reported a significant increase in number of standing events in cows less than 79 days in milk, compared to those more than 80 days in milk, where there was also a larger proportion of problem cows (Peralta *et al.*, 2005). This is in agreement with results of the current study and provides an explanation for increased activity in the first oestrous cycles after calving. Another possible explanation for the decrease in oestrous expression with increasing lactation is that a greater cumulative yield of milk has been produced. However these cows are moving into positive EBAL so it is less likely that the effects of lactation yield are seen on oestrous expression at this stage. Lower activity levels in later lactation could be related to the larger proportion of problem cows more than 80 days in milk (Peralta *et al.*, 2005). In conclusion there may be many underlying causes for decreased oestrous expression and it is possible that problem cows do not survive to get in calf due to culling for infertility.

2.4.1.3 Time of Year

There was a larger increase in activity at oestrus, in periods 2 and 3 compared to periods 1 and 4. Periods 2 and 3 are normally associated with hotter temperatures, and it is suggested that heat stress and factors associated with heat stress, affecting follicle development and steroidogenesis, can influence oestrous expression (Roche, 2006). Reports in this area are inconsistent; some authors report increased expression of oestrus in the hotter, summer months (Peralta *et al.*, 2005); others report the opposite, with increased expression in the colder, winter months (Nebel *et al.*, 1997). However, the patterns associated with temperature are mostly from outside the UK, and few reports from studies in the UK have found any association between temperature and oestrous expression. This relationship applies more to countries with hot climates with large fluctuations in temperature. Some components of the reproductive system are susceptible to extreme temperatures compromising the steroidogenic capabilities of the theca and granulosa cells (Wolfenson *et al.*, 2000;De Rensis and Scaramuzzi, 2003).

The results of the current study, however were recorded in the UK temperate climate and therefore could be affected by other causal effects of seasonal variation on reproduction; day length, photoperiod, humidity, level of nutrition, management or combinations of these factors (Critser *et al.*, 1987). Cattle are not seasonal breeders in the strictest sense, as they now breed and cycle all year round, but seasonal influences can have an effect. This is more subtle than in the sheep, in which reproduction can only occur at certain times of year and is strictly controlled by photoperiod influencing the ability of oestrogens to inhibit LH (Legan *et al.*, 1977). The influence of season on cattle reproduction has been linked to a number of events associated with reproduction; return to cyclicity is longer if calving is in winter compared to summer (Hansen, 1985) and season of birth and season of attainment of puberty can influence age at puberty in heifers (Schillo *et al.*, 1983).

Seasonal changes in peripheral concentrations of gonadotrophins have been reported (Critser *et al.*, 1987), which could explain the influence of season and photoperiodic variations in activity levels at oestrus. An increase in LH release in cows has been reported in summer compared to winter (Hansen et al 1982), perhaps related to the effects of oestradiol, as described for the sheep (Legan *et al.*, 1977). This might explain how photoperiod can affect reproductive behaviour, especially oestrous expression.

2.4.2 Effect of Milk Yield on Increase in Activity

There was an association between increasing milk yield and declining activity and oestrous expression. This trend follows the widely recognised change in cattle reproductive physiology recorded over the past 50+ years, coupled with rapidly increasing milk production since the 1950s (Lucy, 2001). The average increase in yield from 2010/11 to 2011/12 is 241 litres per cow with the average yield at 7617 litres per cow per annum (provisional 2012 data; DairyCo, 2012a). However poor conception rates still persist (Royal *et al.*, 2000a;Butler, 2003).

High milk yields have been reported to affect oestrous expression. Harrison *et al*., (1990) reported that low yielders showed stronger oestrous expression than high yielders. When comparing the duration of oestrus in high and low yielders, low yielders had a longer duration of oestrus, 10.9 vs. 6.2 hours. Total standing events was also increased, 8.8 vs. 6.3, as was total standing time, 28.2 vs. 21.7 seconds (Lopez *et al.*, 2004). Studies of activity also concur with the results of the current study where there was a clear pattern between high milk production and lower activity at oestrus (Yániz *et al.*, 2006). Lopez-Gatius *et al*. (2005) also reported that for each 1kg increase in milk yield walking activity at oestrus decreased by 1.6%.

Possible explanation for the effects of milk yield on reduced activity at oestrus is through the interlinking reproductive and somatotropic axes which can be influenced by metabolite levels and influence hormone production (Chagas *et al.*, 2007). High yielding animals require a high plane of nutrition, which increases the rate of metabolic clearance by the liver, rapidly removing steroid hormones, oestradiol and progesterone from the blood (Sangsritavong *et al.*, 2002). This results in lower circulating oestradiol and a reduced duration in the system (Lopez *et al.*, 2004), which could impact on oestrous expression.

Furthermore NEBAL related to high producing cows (discussed in Section 2.4.1.1) can affect the level of oestrous expression. NEBAL attenuates LH pulse frequency which inhibits oestradiol secretion which in turn prevents ovulation. Low energy status coupled with supressing LH pulses also seems to reduce the responsiveness of the ovary to LH, again inhibiting the production of oestradiol (Butler, 2003) with subsequent impacts upon oestrous expression. Furthermore NEBAL is strongly associated with low levels of blood glucose, insulin and IGF-1 post partum which can limit oestradiol production by the dominant follicle. Metabolic demand causes a reduction in levels of glucose, insulin ang IGF-1. Glucose and insulin are associated with the upregulation of LH receptors in the ovary. Indeed insulin and IGF-1 are linked as IGF-1 production is affected by circulating insulin concentrations. IGF-1 levels are also directly related to energy levels are correlate with oestradiol concentrations. This results in an alteration of the sensitivity of the response of the pituitary gland to GnRH, affecting LH pulses, influencing ovarian follicular development and the capability of the follicles to produce oestradiol (Butler, 2003).

Milk yield is also affected by season and photoperiod thought to be associated with the effects of increasing IGF-1 related to long day photoperiod increasing milk yield (Dahl *et al.*, 2000). Therefore oestrous expression may be affected through increased yield and the effects of IGF-1 influencing oestradiol production.

2.4.3 Activity Increase and Conception Rate

In the current study when activity was increased, the probability of conception occurring increased although this results was not significant ($P=0.064$). Out of 773 inseminations at observed oestrus only 243 resulted in pregnancy. It has been reported that the probability of conception occurring increases with increasing oestrous number (Darwash *et al.*, 1997b). Also the chance of a cow conceiving is increased with greater oestradiol levels (Lopes *et al.*, 2007;Perry *et al.*, 1991). Pre-ovulatory follicle size has been directly related to oestradiol concentration on the day of AI ($P<0.05$) (Lopes *et al.*, 2007). This indicates that follicle steroid biosynthesis can affect the outcome of AI (Lopes *et al.*, 2007) as oestradiol produced by the ovulatory follicle can influence oestrous expression. Lopes *et al.,* (2007) concluded that larger pre-ovulatory follicles had greater oestradiol concentrations and were associated with pregnancy. Therefore increased activity at oestrus could be associated with increased oestradiol concentrations and increased probability of conception.

From a management point of view, greater activity at oestrus can influence the probability of pregnancy because oestrous detection is more likely and the timing of AI relative to ovulation will be more precise. However a larger data set is needed in order to draw meaningful conclusions about increased oestrous expression and the probability of conception from these results.

2.5 CONCLUSION

In conclusion, important factors affecting expression of oestrus have been identified in this study. Parity, oestrous number post partum and milk yield were inversely related to the activity increase at oestrus. Time of year of oestrus also influenced the activity increase possibly related to photoperiod or day length. These results have largely confirmed the results of other studies into factors affecting oestrous expression. Herein they largely agree with the general consensus of opinion on factors that affect oestrous expression, although few studies into the expression of oestrus have been carried out using activity monitoring, highlighting the novelty of this study. Therefore cow factors have been identified that can affect oestrous expression. Further work is required to investigate variation in oestrous expression, between individual cows to investigate if there is genetic variation in the activity increase at oestrus.

CHAPTER 3 – Single Nucleotide Polymorphisms and Their Association with Oestrous Expression

3.1 INTRODUCTION

Oestrous detection is becoming increasingly difficult in the modern dairy cow as oestrous expression is diminished. Cows display a shorter and less intense oestrus (Van Eerdenburg *et al.*, 1996;Dransfield *et al.*, 1998), with fewer cows standing to be mounted (Dobson *et al.*, 2008). Therefore poor expression of oestrus leads to more difficult detection of oestrus (Lucy, 2001). It is possible that genomics might provide a novel solution to the problem.

Using a genomic approach to improve oestrous expression involves investigating the possible associations of single nucleotide polymorphisms (SNPs) with phenotypic traits that denote oestrus. A SNP is a single base pair change in the sequence of DNA which causes variation in the genotype. A SNP can take on two allelic forms, interacting with other SNPs at different loci, causing common variants among the population, and thus differences in the phenotype of animals in many traits (Berglund, 2008). These polymorphisms in DNA can affect gene expression, translation and transcription which in turn can affect protein function. With rapid development of genomics dense SNP arrays were invented working on the principle of 1000s of SNPs approximately 1cm apart in the genome. It is expected that there will always be a SNP in close proximity to a gene or DNA fragment of interest inherited by linkage disequilibrium (Meuwissen *et al.*, 2001). The BovineSNP50 provides a low cost, high density, genome wide genotyping in cattle to enhance selection (Illumina, 2011). However, use of SNP chips is based on associations of sequence variation and not on understanding the biological information to make more informed decisions based on phenotypic information. There are many effects of SNPs and so a relationship must be determined between each SNP and a functional trait (Ibeagha-Awemu *et al.*, 2008). Studying genomic information allows links to be made between SNP variants and physiological data (Berglund, 2008), which is beneficial for selection as gains in fertility can be achieved using genomic selection whilst sustaining high milk production (Veerkamp *et al.*, 2000).

A number of SNPs have been reported for their association with reproductive traits and associations with fertility. SNPs in FGF2 and STAT5A have both been associated with embryo survival and fertilisation rate (Khatib *et al.*, 2008a;Khatib *et al.*, 2008b). SNPs in the LH receptor gene have also been reported for their association with days to first service and calving interval (Hastings *et al.*, 2006). However, as yet there is no report of relationships between SNPs and their effects on oestrous expression. Therefore SNPs previously reported both for their involvement in the oestrous cycle and for their effects on fertility were investigated using a candidate gene approach to see if they were linked to activity increases at the time of oestrus.

Identifying SNPs encoding for higher levels of oestrous expression would allow the development of breeding programs to improve oestrous detection rates, both cumulatively and permanently. By improving oestrous expression more cows would be detected in oestrus, thus increasing submission rates for AI and at a more optimal time coinciding with ovulation. This would lead to improved conception rates. Therefore, the objective of this work was to identify DNA polymorphisms that would provide a means of identifying those cows that exhibit oestrus more strongly, in order to improve productivity through improved oestrous detection rates.

3.2 MATERIALS AND METHODS

3.2.1 Animals & Phenotypic Data

Animals used in this study were 205 Holstein Friesian dairy cows housed at Nottingham University Dairy Centre, as described in Chapter 2. Oestrous detection was measured by activity monitors and activity data analysed, as described in Chapter 2, to determine a measurement of oestrous expression calculated as maximum activity at each oestrus.

3.2.2 Blood Sampling, DNA Extraction and Genotyping

Blood samples were collected from the coccygeal vein of each cow under ethically approved Home Office License regulations. Oestrus, insemination and pregnancy, and activity data were known for all animals. Blood samples were then sent off to be extracted and genotyped commercially by KBiosciences Ltd (Herts, UK), using primer extension. DNA was genotyped at 41 loci, in 18 genes as listed in Table 3.1. SNP results were given levels; 0 as the most common genotype within this sample of cows (although these cows may not be a true representation of a general wildtype population owing to previous selection within the herd), 1 as the heterozygote and 2 as the mutant homozygote, for inclusion in analysis.

3.2.3 Gene Selection

SNPs were chosen for analysis that had previously been identified on the basis of their involvement in reproductive processes. Genes were chosen for association with i) hypothalamic/ ovarian/ uterine function and ii) a role in central nervous pathways controlling oestrous behaviour and iii) association with production traits such as milk yield, energy balance and feed intake and metabolic influences which can all impact upon fertility and oestrous expression. The genes, positions of SNPs and variations in the DNA, and the previously reported effects on certain traits, are reported in Table 3.1. Many genes could be associated with oestrous behaviour as they encode for or influence key hormones that regulate oestrus. Genes that encode transcription factors and signalling molecules can influence oestrus because they in turn control gene expression of receptors and important molecules. These in turn affect the production and concentrations of oestrus inducing hormones, mainly oestradiol, and related pathways that control behaviour induced by elevated oestradiol. Therefore genes selected for study were those that have been linked to certain observed phenotypes associated with reproduction and could be associated with the expression of oestrus.

Table 3.1 SNPs on genes investigated for their associations with an increase in activity at oestrus

Gene Name	**Gene Symbol/Variant** (used in this study)	**SNP and Position**	**Effects on Traits; Fertility and Production**	**Reference**
Activin Receptor Type IIB	ACT_IIB_45	Highly polymorphic within intron	Association with reproduction	(Flavin *et al.*, 1996)
	ACT_IIB_46			
	ACT_IIB_503			
	ACT_IIB_86_END			
	ACT_IIB_95			
Oestrogen Receptor-α	bERA_prom_SNP173	Promoter region, position 173	Oestrogens play a main role in reproduction	(Szreder and Zwierzchowski, 2004)
	ESR1 ex1 A503C	Exon 1 A503C		(Szreder and Zwierzchowski, 2007)
	ESR1	Exon 8		
Oestrogen Receptor-β	bERB_ex4	Exon 4		
	bERB_ex7	Exon 7		
Gonadotrophin Releasing Hormone Receptor	bGNRHE_ex1_SNP_340	Exon 1, position 340	Associations with fertility	(Derecka *et al.*, 2009)
	bGNRHR_ex1_SNP_286	Exon 1, position 286		
	bGNRHR_ex1_SNP_421	Exon 1, position 421		
	bGNRHR_ex1_SNP_490	Exon 1, position 490		
	bGNRHR_prom_SNP_1189	Promoter region, position 1189		
	bGNRHR_prom_SNP_966	Promoter region, position 966		
Luteinizing Hormone-β	bLHB SNP1588	SNP1588		
Fatty Acid Synthase	FASN 16009a/g	BTA19, 16009A -> G in exon 34	Milk fat content in Holstein Friesians	(Roy *et al.*, 2006)
	FASN 763g/c	BTA19, 763G -> C in exon 1		
	FASN 17924 a/g Thr/Ala	BTA19, g.17924A> to G (Thr -> Ala)	Fatty acid composition of milk fat	(Morris *et al.*, 2007)
	FASN 18663t/c	BTA19, g.18663T>C	Fatty acid compositions	(Zhang *et al.*, 2008)

Table 3.1 Cont.

Follicle Stimulating Hormone Receptor	FSHR_L502L FSHR_N669N FSHR_S596S FSHR_T658S FSHR_T685T	Leu502Leu Asn669Asn Ser596Ser Thr658Ser Thr685Thr		
Growth Hormone Receptor	GHR Phe279Tyr	Phe279Tyr in trans membrane domain	Effect on yield and protein and fat percentage and protein and fat yields Affects feed intake, feed conversion and body energy	(Blott *et al.*, 2003;Banos *et al.*, 2008)
	GHRA257G ex10	Exon 10 A857G	Associated with milk fat and protein yields	(Kaminski *et al.*, 2006)
Leptin Promoter	leptin_promoter -963 leptin_promoter_1 -1457	C963T A1457G	Milk yield, feed and dry matter intake Association with fertility, energy balance and protein yield	(Liefers *et al.*, 2005;Banos *et al.*, 2008)
Luteinising Hormone Receptor	LHR_L490L LHR_Q527H LHR_W467C	Exon 11, Leu490Leu Exon 11, Gln527His Exon 11, Trp467Cys	Associations with fertility and production; affecting calving interval, days to first service and production index	(Hastings *et al.*, 2006)
Neuropeptide Y	npy_ex1	Exon 1	Associations to average daily gain, body weight and feed conversion ratio	(Sherman *et al.*, 2008a;Bahar and Sweeney, 2008)
Neuropeptide Y Receptor Y2	NPYRY2			

Table 3.1 Cont.

Peroxysome proliferator-activated receptor-γ coactivator-1α	PPARGC1A C1892t/c intron9	c.1892 + T>C in intron 9	Association with milk fat yield	(Weikard *et al.*, 2005)
Prolactin	PRL 89398 g/a R	G8398A	Milk yield and fat percentage in 1st lactation	(Brym *et al.*, 2005)
Prolactin Receptor	PRLR Ser18Asn	Ser18Asn in signal peptide	Associated with milk protein and fat yields	(Viitala *et al.*, 2006)
Ribosomal Protein S6 Kinase	rs29019569CT	BTA2, C>T, Base pair position 316 880	Effects on feed efficiency	(Sherman *et al.*, 2008b)
Signal Transducer and Activator of Transcription 1	STAT1 c3141t	C3141T in 3' UTR	Allele C associated with increases in milk fat and protein percentages	(Cobanoglu *et al.*, 2006)
Signal Transducer and Activator of Transcription 5A	STAT5A g12195c	G12195C in exon 8	Associated with decreases in milk protein and fat percentage Associated with embryonic survival rate	(Khatib *et al.*, 2008b;Khatib *et al.*, 2009)

3.2.4 Sequencing of DNA in the Laboratory

Four blood samples were sequenced in the laboratory undergoing DNA extraction, polymerase chain reaction (PCR) amplification, gel extraction for purification and then sequencing of the gondaotrophin releasing hormone receptor (GnRH-R) gene. The GnRH-R gene was chosen for sequencing in the laboratory for a learning exercise because primers for this gene had been previously optimised in the laboratory.

3.2.4.1 DNA Purification

Frozen samples were incubated at 37°C (Mini 18L CLAD Incubator) and defrosted rapidly to aid red blood cell lysis. 3ml of blood was then purified using the Gentra Puregene Blood Kit (Qiagen, Sussex, UK). The of blood was added to 50ml tubes containing 9ml of Red Blood Cell (RBC) Lysis Solution, supplied with the kit, vortexed and centrifuged (DuPont Sorvall RC5C) at 10000rpm for 15 minutes. The supernatant was removed, the pellet resuspended in 9ml RBC solution and the process repeated twice more, to ensure ample lysis of cells. After final centrifugation, the supernatant was discarded, the pellet resuspended in the remaining residual and 3ml of Cell Lysis Solution (supplied with the kit) added to dissolve all remaining structures into solution, aided by incubation at 37°C (Mini 18L CLAD Incubator) for 1 hour.

Post incubation samples were cooled on ice to aid precipitation of the proteins. 50ml chloroform and isoamylalcohol (both sourced from Sigma-Aldrich, Dorset, UK) was prepared (49:1) and 3ml added to 50ml phase lock gel tubes (Eppendorf, Stevenage, UK), along with 3ml phenol (10mM Tris HCl, pH 8.0, 1mM EDTA; sourced from Sigma-Aldrich, Dorset, UK). DNA in the Cell Lysis Solution (from previous step) was transferred to the phase lock gel tubes, vortexed and centrifuged (DuPont Sorvall RC5C) at 10000rpm for 15 minutes. Using the phase separation technique allowed differentiation between the DNA and the protein. DNA in the upper aqueous phase was separated from the protein fraction in the lower organic phase. The DNA sample was transferred to clean 50ml phase lock gel tubes and phase separation carried out again using 5ml chloroform isoamylalcohol. After the second centrifugation the DNA aqueous layer was transferred to a new 50ml tube containing 1ml of Protein Precipitation Solution (supplied with the kit) and centrifuged again for 15 minutes.

The supernatant, following centrifugation with Protein Precipitation Solution, was removed into a 15ml tube containing 3ml isopropanol 100% and inverted approximately 50 times. The DNA was precipitated out of solution using isopropanol. After centrifuging (DuPont Sorvall RC5C) for 10 minutes a small white pellet remained. If no pellet was present then the sample was stored at -21°C overnight and re-centrifuged. The supernatant was pipetted off carefully avoiding the pellet and surrounding area. The supernatant was then washed with 2ml 70% ethanol (Sigma-Aldrich, Dorset, UK), inverted several times and centrifuged for 10 minutes. After centrifugation the DNA pellet was air dried and rehydrated with 250µl DNA Hydration Solution (supplied with the kit) and incubated at 65°C for 1 hour (Techne DRI-BLOCK DB.3A). Concentrations were measured by nanodrop prior to PCR.

3.2.4.2 PCR

100ng genomic DNA (gDNA) was then amplified by PCR using primers specific for the GnRH-R. Primers were designed using the DNA sequence obtained from the NCBI Genbank for locus AF034950 *Bos taurus* GnRH-R. The sequence for the forward primer was 5′ GGTTTTTTTTTTAGAAAAC 3′ and the sequence for the reverse primer was 5′ GAACAGTGGTTTTCATTCTG 3′. Purified primers were obtained (HPSF; high purity salt free) from Sigma (Sigma-Aldrich, Dorset, UK). PCR reactions were assembled as described in Table 3.2 and performed using the Eppendorf Mastercycler (Eppendorf, Stevenage, UK), with the following thermal cycling conditions: 95°C for 30 seconds, 30 cycles of 95°C for 30 seconds, 59°C for 30 seconds, 68°C for 30 seconds and final extension of 68°C for 5 minutes.

Table 3.2 PCR reaction reagents

Reagent	Volume (µl)
Quick-Load *Taq* 2X Master Mix	25
$MgCl_2$ (25mM)	1
Forward Primer (10µM)	2
Reverse Primer (10µM)	2
Water	To top up to 50µl

3.2.4.3 DNA Clean Up

PCR products were mixed with loading dye and run on 1% agarose gel in TAE buffer with ethidium bromide slowly at 65V until sufficient separation was achieved. PCR products were removed from the gel using the QIAquick Gel Extraction Kit (Qiagen, Sussex, UK). The DNA band was excised using a scalpel under a UV light machine and dissolved at 50˚C on a heat block (Techne DRI-BLOCK DB.3A) in 3 volumes of Buffer QG (supplied with the kit) to bind the DNA. Samples in solution were added to a QIAquick spin column in a 2ml collection tube (both supplied with the kit), and samples centrifuged (MiniSpin, Eppendorf, Stevenage, UK) at 10000rpm for 1 minute. 700µl of Buffer PE (supplied with the kit) was then added to the column, centrifuged at 10000rpm for 1 minute, and the process repeated to wash the DNA. The empty column and collecting tube were then centrifuged for 1 minute to remove the residual alcohol, before eluting the DNA with 30µl of 2mM Tris solution, pH 7.0-8.5 and centrifuging for 1 minute to collect the purified PCR product.

3.2.4.4 DNA Sequencing

Samples were sent for sequencing (Beckman CEQ8000 Sequencer) with the PCR primers detailed in section 3.2.4.2 for the promoter region of the bovine GnRH receptor gene, which identified the SNPs at positions 966 and 1189.

3.2.5 Statistical Analyses

Statistical analysis was carried out using Genstat 14th edition (VSN International Ltd, Hemel Hempstead, UK). Activity data were analysed as generalized linear mixed models (GLMM) using the residual maximum likelihood (REML) procedure, with Poisson distribution and logarithmic link function. The model fitted fixed effects for SNPs (variates; wildtype homozygote, 0; heterozygote, 1; mutant homozygote, 2). For the random effects of the model, individual cows represented subjects to allow for multiple oestruses per cow but only 1 SNP per cow. The significance of fixed effects was assessed by Wald tests. The resulting model was:

$$Y_{il} = \mu + S_i + C_l + \varepsilon_{il}$$

Other variables were then included into the statistical model fitted as fixed effects for SNPs (variates; wildtype homozygote, 0; heterozygote, 1; mutant homozygote, 2), parity (classified according to lactation number as 1, 2 and ≥3) and oestrus time period (classified as Jan-Mar, 1; Apr-Jun, 2; Jul-Sept, 3; Oct-Dec, 4). This was to account for the combined effects on activity, eliminating external cow factors and seasonal effects to concentrate on the association between SNP and activity. The resulting model was:

$$Y_{ijkl} = \mu + S_i + P_j + O_k + C_l + \varepsilon_{ijkl}$$

where Y_{ijkl} is activity at oestrus,

the fixed part of the model consists of

μ the overall mean,

S the effect of SNP,

P the effect of parity,

O the effect of oestrous season,

C_l the random effect of Cow, and

ε_{ijkl} the residual error.

3.3 RESULTS

Results are reported for the association between SNPs at 41 loci on 18 genes and activity at oestrus. None of the genes studied (Table 3.1) were found to hold a true significant association with the activity increase at oestrus. 205 cows were blood sampled although due to the availability of genotyping at each locus and activity data at oestrus the number of cows in each analysis varied slightly and was often less. This highlights the seriously limited animal numbers used in this study.

Two SNPs on 2 genes were found to be significantly associated with activity at oestrus ($P<0.05$); STAT5A g12195c on the signal transducer and activator of transcription 5A (STAT5A) gene and ACT_IIB_95 on the activin receptor type II B (ACTRIIB) gene. Activity increases at oestrus were greater in cows with the mutant genotype (GG) compared to the wildtype genotype (CC) for the STAT5A gene ($P=0.028$). Activity increases at oestrus were associated with smaller increases in cows with the mutant genotype (GG) in the ACTRIIB gene, compared to the wildtype genotype (AA; $P=0.048$). These SNPs were also reported significant when including parity and time period of oestrus in the statistical analysis. However, in a study of 41 loci it would be expected to randomly find 2 false positives at a significance level of $P<0.05$, which is reflected in these results.

3.4 DISCUSSION

The objectives of this study were to identify SNPs that are associated with increased oestrous expression. It is widely known that physical activity increases at the time of oestrus (Farris, 1954), with activity increasing from two- to four-fold (Kiddy, 1977). The genes investigated in this study were not found to have a true significant association with the activity increase at oestrus.

SNPs on the ACTRIIB and STAT5A genes were found to be significant ($P<0.05$) although this study investigated a large number of loci where it would be expected to randomly find 2 false positive associations at a significance level of $P<0.05$. Polymorphisms in the STAT5A gene have been previously reported to affect fertility; affecting fertilisation rate and embryo survival (Khatib *et al.*, 2009). STAT proteins are involved in cytokine signalling pathways converting signals in the cytoplasm and acting as transcription factors in the nucleus, to regulate gene transcription (Kisseleva *et al.*, 2002). STAT5A is also activated by more than 35 polypeptide ligands and the resulting gene transcription has involvement in a broad range of physiological responses (Darnell, 1997), such as mediating peptide hormones and cytokines (Selvaggi *et al.*, 2009). This could explain the significant result found in this study, by the association of STAT5A and hormones of the oestrous cycle to influence oestrus. However due to the many roles of STAT5A and its indirect involvement in the expression of oestrus the significance of this result is decreased. Similarly the same decrease in significance can be applied to the association between the ACTRIIB gene and oestrous expression. The ACTRIIB gene has a role in ovarian folliculogenesis and is present on theca cells, granulosa cells and oocytes (Knight and Glister, 2003). Inhibin and activin are the two main ligands for the ACTR, and have been demonstrated as intrafollicular regulators in ruminants controlling folliculogenesis and steroidogenesis (Hutchinson *et al.*, 1987;Shukovski *et al.*, 1991). Therefore this gene could influence the production of oestradiol which causes oestrous expression. However, the significant effect of this SNP on activity at oestrus is decreased as one moves further from a direct cause and effect relationship.

Upon investigation of the direct involvement of these genes in the expression of oestrus it becomes clear that these results are both false positives. Therefore it is likely that in reality this study found no true associations using this experimental approach.

Furthermore the animal numbers used in this study were seriously limited and a substantial number of cows (at least 20000) would be required to achieve meaningful results.

3.4.1 Limitations of Study

Limitations of this study are that the animal numbers used in the study were small and thus seriously limited the study. Although these results indicate an association between activity and 2 SNPs, the true significance is questionable. Therefore a much larger population of at least 20000 cows, but ideally approximately 200000 cows, would be required to assess the true effects of each SNP and achieve meaningful results. Furthermore, much larger datasets are needed to make associations between SNPs and traits of low heritability (Berry *et al.*, 2012). In the current study one particular limitation is that the effects of certain SNPs (although not significant) seem greater because of the low frequency of alleles. To improve this it would be advantageous to have representative cohorts of cows for each SNP allele. To extend this work it would also be advantageous to include pedigree information and investigate oestrous expression of many daughters from a few select sires.

Considerable challenges arise from this type of research relating to the quality of data collected; the observed effects may be attributable to closely linked genes, variations in a gene, or result directly from the experimental design (Veerkamp and Beerda, 2007) thereby occurring by chance or due to artefacts. Another approach to improve upon the current study would be to carry out a genome wide association study to assess if any common genetic variants are associated with a particular trait of interest. Using this approach to investigate SNPs simultaneously for their effects on oestrous expression would control the rate of false positives (Berry *et al.*, 2012) and would avoid random significant associations as seen in this study. This would allow genes to be identified near the SNP and allow for further investigation into the causal effects of these genes (Hoglund *et al.*, 2012) without speculation about the physiological mechanisms involved. Careful interpretation of the data must also be carried out to ensure that the association between genotype and phenotype is plausible, and that the SNP of significance is related to fertility and oestrous expression. However success of this type of study depends on the heritability of the trait, where heritability of oestrous expression is low, but also the number of phenotypic records of that trait (Berry *et al.*, 2012). To improve upon this study multiple measurements for oestrous expression could be used such as maximum activity at oestrus, percentage increase in activity at oestrus, days to first episode of high activity and days to commencement of luteal activity. Furthermore, using physiological measurements of oestrus gives a more accurate interpretation of oestrus that is less affected by management factors and the environment.

3.4.2 Implications of a Genomic Approach

Implications of using genomic selection over traditional genetic selection are that the improvements in genetic gain are realised quicker than using traditional selection methods. Therefore identification of SNPs for improved oestrous expression could lead to inclusion of SNPs in breeding programmes and thus aid reversal of the decline in oestrous detection rates. Genomic selection is a revolutionary technology enhancing dairy cattle breeding, with the amount of information on a molecular level rapidly increasing due to sequencing of the bovine genome in 2003 (http://www.hgsc.bcm.tmc.edu/projects/bovine). Studying SNPs and their variation and making links between physiological data allows this information to be used in selection programs and incorporation into the fertility index (Berglund, 2008). Because of the low heritability of reproductive traits, h2=<0.05 (Berglund, 2008) genomic selection provides a method of rapid and cumulative genetic gain with a possible doubling the rate of genetic gain (Hayes *et al.*, 2009). The rate of genetic gain can be accelerated by genomic selection because the need for progeny testing is removed as the animal's genotype is fixed at birth (Schaeffer, 2006). Potential problems can arise from unproven progeny breeding, however, such as inheritance of negative undesirable traits (Berglund, 2008;Hayes *et al.*, 2009). Also, it is reported that many SNPs have large residual variations when used to predict traits of future generations, which vary widely at an individual level, affected by many non genetic factors (Veerkamp and Beerda, 2007).

Although the gains achieved by SNP selection are cumulative through generations, the estimated genotype effects are reported to change over time therefore the information may only be useable for 7-8 generations

(Berglund, 2008) and SNP reliability may decrease (Hayes *et al.*, 2009). However, the accuracy of SNP selection has been reported to be increased between 10 and 30% even for traits with low heritability (h2<0.10) (Muir, 2007). Furthermore, accuracy of genomic estimated breeding values will persist for more generations if the reference population consists of data from multiple generations (Hayes *et al.*, 2009). Therefore effective interpretation and integration of genomic information into breeding programs should assist the optimal selection of animals for increased oestrous expression, but with potential for milk yield to be maintained (Veerkamp *et al.*, 2000).

3.5 CONCLUSION

In conclusion, this study found no true association between the SNPs on genes investigated and the activity increase at oestrus. However this is attributed to the small population size used in this study and the candidate gene approach used. Future efforts involving investigation of SNPs affecting the expression of oestrus, in order to improve oestrous detection rates, would be to use a much larger cohort of cows (at least 20000) and to include the effects of multiple physiological traits related to oestrus, investigated simultaneously as part of a genome wide association study. Therefore any significant associations between SNPs on important genes and oestrous expression would be true associations and not false positives occurring by chance. Activity monitoring is efficient at detection of oestrus, but not the most accurate method, and therefore problems with measuring oestrus via activity monitoring arise when cows do not show an increase in activity due to lameness or ill health, or when cows display other signs of oestrous behaviour. Improvement of oestrous expression is one sustainable solution to improving poor oestrous detection rates, however accurate and efficient methods of oestrous detection must also be considered in order to increase detection rates to supersede the current target of 70% (DairyCo, 2009). Visual observation of oestrus is time consuming and, although accurate, may be unproductive and uneconomical. Therefore automated behaviour recording or physiological measurements may be better suited to the modern dairy industry.

CHAPTER 4 – Development of a Novel Technology for the Purpose of Oestrous Detection

4.1 INTRODUCTION

Detection of oestrus is a key determinant of profitability of dairy herds (Pecsok *et al.*, 1994). At present oestrous detection rates are only 50% (DairyCo, 2009) but have the potential to be much higher with appropriate methods of oestrous detection, reaching the current herd target of above 70% (DairyCo, 2009). Therefore there is a clear opportunity in the market for development of a novel technology for the purpose of oestrous detection.

Traditionally oestrous detection was performed just by visual observation, although due to increasing herd sizes and decreasing oestrous expression, this method although most accurate, is now less successful and inefficient. Currently oestrous detection is moving towards automated technologies that analyse the traits being measured, with the aim to accurately and efficiently detect oestrus according to the criteria set out by Senger (1994). Many automated technologies have been reviewed (Firk *et al.*, 2002;Rorie *et al.*, 2002;Roelofs *et al.*, 2010), with the most common and successful at present being pedometry/ activity monitoring (At-Taras and Spahr, 2001). However, activity monitoring has its limitations and may only increase oestrous detection rates by 10-12% (DairyCo, 2009).

In order to detect cows that are standing to be mounted and cows that are mounting, to accurately detect oestrus it will be necessary to employ a novel technique such as ubiquitous positioning. Ubiquitous positioning is a technology to locate people, objects or both, anytime, whether indoors or outdoors or moving between the two. The subject must be located at predefined location accuracies which can be aided by the support of one or more location-sensing devices and associated infrastructure to assist definition of coordinates (Meng *et al.*, 2007). Many approaches to precision positioning are able to define the subject's location although they vary in their suitability for use to monitor oestrus in dairy cows.

Ubiquitous positioning mainly focuses on global navigation satellite systems (GNSS), including GPS (US), GALILEO (EU), GLONASS (Russia) and Compass (China) which are capable of 3D positioning. Single receiver GNSS is capable of accuracies ranging from a few metres to tens of metres depending on the technologies and algorithms employed, although it cannot be used for positioning indoors or in obscured environments due to poor satellite visibility and, hence, poor accuracy and reliability. GPS (the Global Positioning System) is the main technology currently providing absolute global positioning, within the above accuracies, although as with all GNSS, GPS signal quality and reliability are severely degraded indoors and in obstructed environments. Therefore although the principle is established, this method is unsuitable for the purpose of oestrous detection; cows are housed indoors and greater accuracy is required to monitor precise cow interactions. A potential solution to the degraded accuracy of GNSS is augmented GNSS to increase the integrity, reliability, accuracy and continuity of position. Horizontal accuracy can be increased from 10-12 metres to 1-2 metres, although this is not useful for the purpose of oestrous detection, and indoor positioning is still a big challenge even with augmented GNSS systems (Meng *et al.*, 2007). High sensitivity GNSS and assisted GPS enhance accuracy, however are still not capable of precise positioning indoors (Meng *et al.*, 2007).

There are location sensing technologies that aim to overcome the limitations of the above systems and aim to address the issues of ubiquitous positioning for use in obstructed environments; ground based pseudolites, Ultra-wide band (UWB) and radio frequency identification (RFID). Pseudolites can supplement GNSS by providing extra ranging signals and improved transmitter geometry to enable precise positioning in restricted areas with the possibility of use indoors. RFID can also be combined with GNSS to provide precise positioning in areas that GNSS cannot reach, although RFID only functions in 2D. Both methods are limited in their accuracy and ability to provide indoor positioning, although showing potential, they are in the early stages of development (Meng *et al.*, 2007). In contrast, UWB technology is capable of monitoring location in 3 dimensions in indoor environments. One UWB system, developed by Thales Research UK (TRT), has reported accuracy calculated to 'a fraction of a metre' in a range of indoor and harsh environments in all 3 dimensions, for example achieving 30cm accuracy in the most difficult dimension of height (Ingram, 2006). UWB also has proven use in harsh environments; monitoring emergency personnel for example in burning buildings, forest fires or during natural disasters (Ingram *et al.*, 2004;Ingram, 2006;Harmer *et al.*, 2008;Dona *et al.*, 2009).

In summary UWB seems a good option to pursue for the purpose of oestrous detection, as location of cows can be monitored and thus we can gain precise knowledge of cow interactions. This will allow the detection of cows which are mounting each other, and most importantly identify which cows are standing to be mounted. The aim was to develop UWB for potential use in proof of concept trials for the detection of oestrus in dairy cows.

4.2 ULTRA-WIDE BAND (UWB)

UWB is defined as any radio signal transmitted within a fractional bandwidth of greater than 25%, above 2GHz, or an absolute bandwidth of greater than 500MHz. This means that because of the wide bandwidth very fine time resolution of signal transmission/ reception can be achieved, allowing for highly accurate positioning. Furthermore, the Thales system technology overcomes positioning in challenging environments by making use of bandwidth and frequencies within a frequency hopped (FH) system, which will enable high accuracy positioning indoors (Challamel *et al.*, 2008). The FH system, uses a direct sequence of spectrum signals spread over 10 to 20MHz bandwidth which hop over around 1GHz at 10 to 100 thousand hops per second, meaning that UWB has greater immunity to interference (Harmer, 2004), and therefore can provide high accuracy positioning inside a building as proven at TRT (Harmer *et al.*, 2008).

UWB has a fixed infrastructure to allow positioning of the roaming mobile units which are mounted on the cows (see Figure 4.1). A typical UWB unit (see Figure 4.2) can be set up as a base unit (BU), mobile unit (MU) or control unit (CU). A reference network is established consisting of BUs which are of known location with exact coordinates for their position. The BU broadcasts its absolute position to all other units, which receive and store this information. This allows the MU to calculate its own position. The MU continually listens to other units' transmissions and calculates the 3D position fix which it transmits to the CU connected to a computer. The UWB units sample at a rate of 2Hz so position is relayed to the CU twice per second. One BU is also nominated the master unit which remains in direct line of sight of all other BUs during communication as a reference point in order to maintain accurate calculation of MU position (Harmer *et al.*, 2008).

The principal of UWB works on using 4 time difference of arrival (TDOA) measurements to determine the 3D position of the MU in real-time. The fixed nodes (BU) transmit to the next fixed node in a ring format where the MU also receives these positions and measures the TDOA to determine position (Ingram *et al.*, 2004). Investigation carried out by the collaborating group at Nottingham Geospatial Institute (NGI) has demonstrated that increasing the number of BU increases the accuracy of UWB (Xiaolin Meng, personal communication).

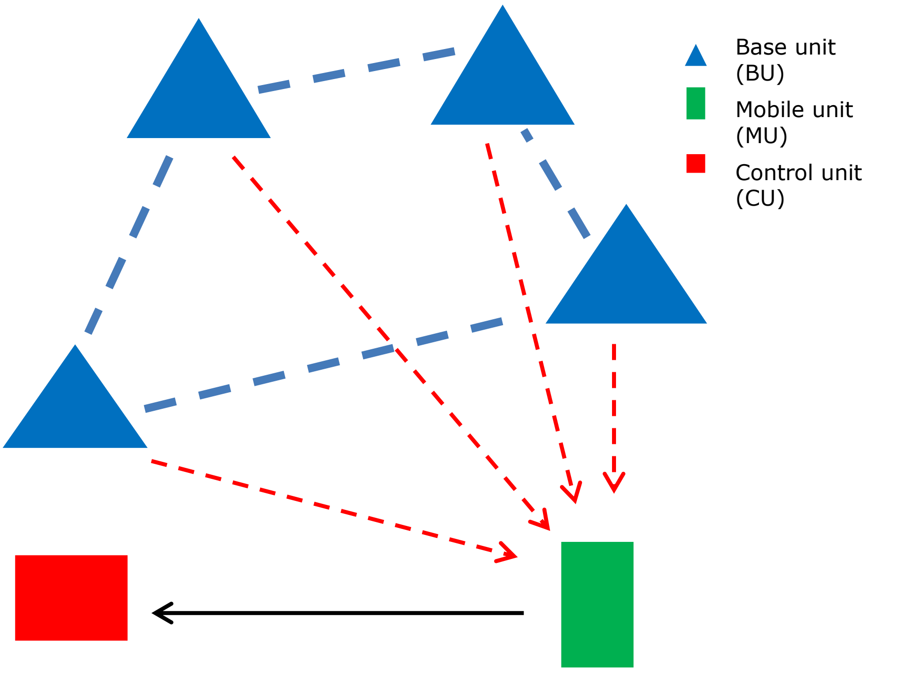


Figure 4.1 BUs form a reference network between themselves in absolute positions which are known by the CU. The MU calculates its own position based on communication with the BU and reports to the CU

Based on this description of UWB, it is clear that this novel technology possesses the basic credentials for an effective method of oestrous detection; accuracy, measuring and relaying position in real-time and for its proven use in harsh, obstructed environments, such as the dairy farm.

4.2.1 Initial Testing of UWB Accuracy at the Dairy Farm

In order to determine the suitability of UWB for use at dairy farms preliminary tests to confirm accuracy in this particular environment were carried out. As the UWB unit was still in prototype format, with no internal battery, it needed to be connected to a battery (12 Volts Maintenance free Sealed Lead-acid Battery, RS Components, Northants, UK) for power (see Figure 4.3) and was positioned on cows in backpacks (Cassidy Covers, Ireland; see Figure 4.4).

Initial tests were carried out to investigate signal strength and accuracy of position within the dairy barn. The dairy farm is an environment with many obstructions; steel girders, heavy machinery and with lots of structural metal work. Furthermore the cows are milked by robots whereby the cows enter into the machine one at a time to be milked, which is an enclosed area, and may cause obstruction to the signal. Within the cow barn in each cow location (one location houses approximately 40 cows each, all milking via 1 robot, with 4 locations in total) there are cubicles for individual cows to stand or lie down in, in comfort on shavings, which are raised by 20cm. Cubicles line the centre of the barn, some may be in ideal positions for good signal quality and some may be in poor positions where there is more obstruction. Opposite the cubicles is the feed passage where cows stand with their heads facing outwards and are fed. This is also an open area where cows stand and mainly interact with one another; therefore the accuracy of UWB in these areas must be determined.

Figure 4.2 UWB unit that can be configured as either base unit, mobile unit or control unit

When handling cows they are taken to the management/AI stalls, which is an area behind the robots, outside of the main cow location, where up to 10 cows can be separated for handling in a controlled environment. The management/ AI stalls are behind the locations where the cows live and was therefore outside of the BU network. Positioning accuracy needs to be tested in all of the potential areas where cattle may interact, particularly areas which may relay a poor signal due to obstruction by structural components of the farm, to determine whether UWB is suitable for use at the dairy farm.

Preliminary static and kinematic tests in the cow barn using small and large networks of BUs to monitor MU position demonstrated accuracy in all 3 dimensions: X and Y horizontal positioning and Z vertical positioning. Static tests are useful to determine the overall accuracy of position recorded by UWB in reference to the BU network when the MU is stationary for a period of time. Kinematic accuracy is testing the accuracy of the MU when the unit is mobile and thus changing position. To test the horizontal positioning accuracy, a 360° prism was used attached to a pole, along with a UWB MU and tracked by a surveyor's total station (TCA2003, Leica Geosystems, Switzerland). The total station is a machine that measures the position of the prism by making angle and distance measurements using reflections of infrared light, to calculate the exact position coordinates of the prism. The position of the prism recorded via the total station, defining a 'ground-truth' position, was compared with the position recorded from the MU in reference to the BU network and relayed back to the computer in order to test the horizontal accuracy of static and kinematic positioning. Figure 4.5 shows the static test results and Figure 4.6 demonstrates the kinematic test results. Due to the different sampling rate between the two measurements (total station and UWB), these points cannot match each other one-to-one, but these figures demonstrate that the position calculated by the total station compares well with the UWB position in the horizontal, X and Y axis. The total station provides millimetre level accuracy which is treated as the true position, therefore when comparing the UWB position against the total station position; UWB achieved 2 to 3cm accuracy in the horizontal dimension.

Figure 4.3 UWB mobile unit set up, connected to a 12 Volt battery

Figure 4.4 Mobile unit and battery set up in backpack monitoring cow movement

Therefore this demonstrates that X and Y positioning is precise with few erroneous signals, and is more precise than Z positioning. Z is the most difficult axis to achieve accuracy due to the geometry of the BU transmitters. Therefore vertical positional accuracy of UWB was tested by equipping cows with backpacks and MUs (see Figure 4.4) and monitoring their behaviour, movement and position within a small network of BUs. Three cows were monitored in a controlled environment in a series of short tests lasting approximately 10 minutes with records made of the cows' behaviour to compare with the UWB results.

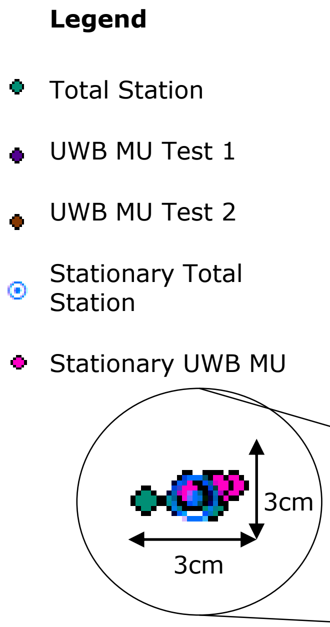


Figure 4.5 Static test to compare UWB precision in horizontal axes

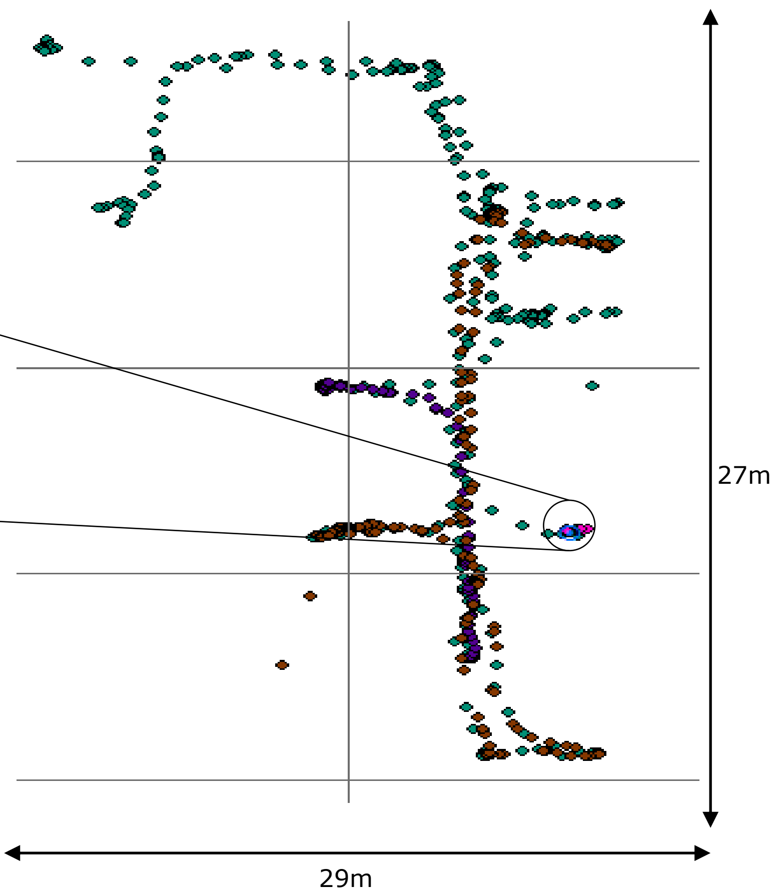


Figure 4.6 Kinematic tests to compare UWB precision in horizontal axes

The results are presented in Figure 4.7: a) stationary cow, b) general cow movement without any changes in height and c) cow displaying changes in height; stepping into the cubicle, lying down and then standing up again before walking off. Results from this series of tests show positive results that cow position can be monitored using UWB. Slight changes in height such as stepping into cubicles (an increase of approximately 20cm) can be detected by UWB as well as major changes in height such as lying down (approximately 50cm height change) which can also be identified by UWB.

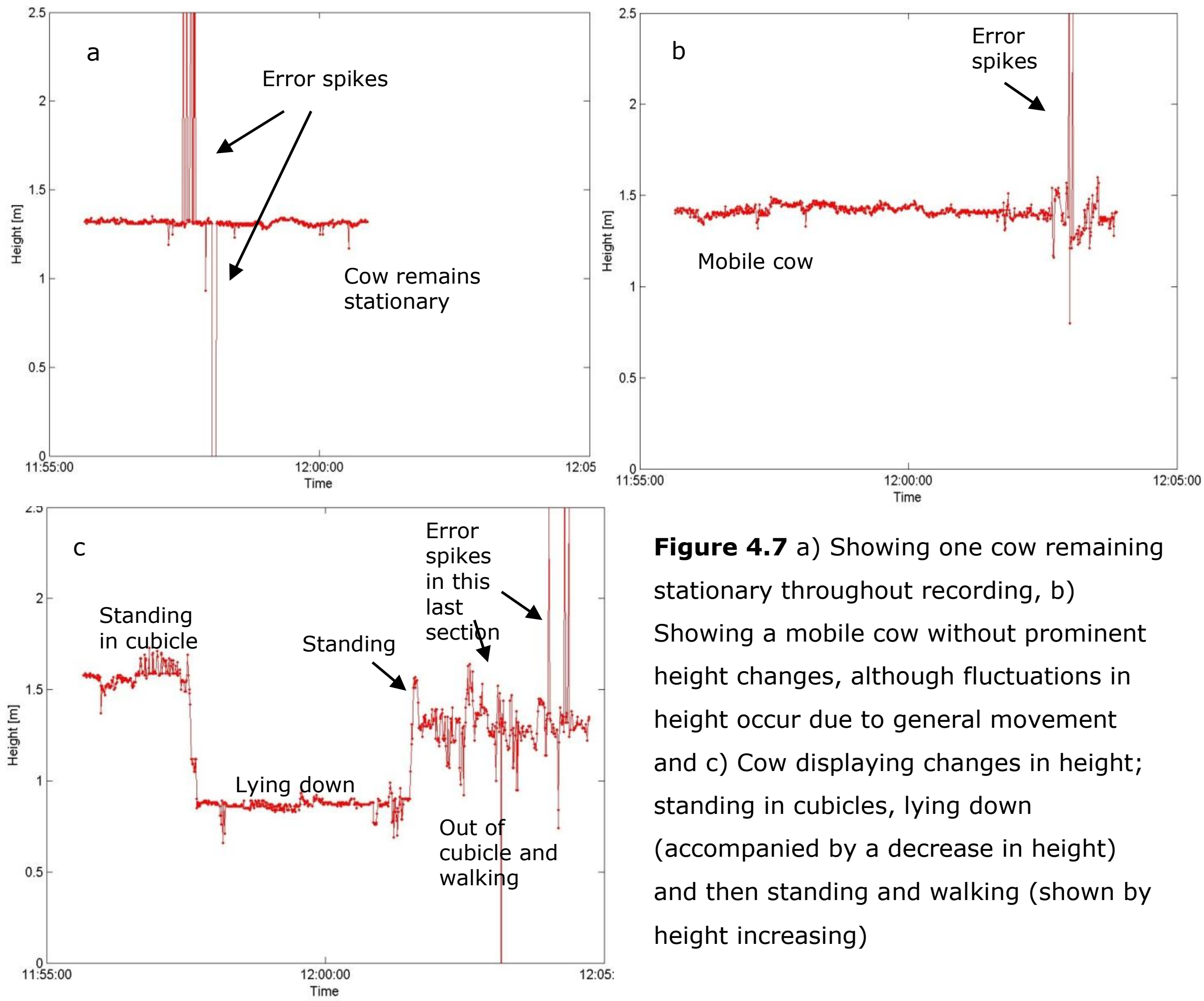


Figure 4.7 a) Showing one cow remaining stationary throughout recording, b) Showing a mobile cow without prominent height changes, although fluctuations in height occur due to general movement and c) Cow displaying changes in height; standing in cubicles, lying down (accompanied by a decrease in height) and then standing and walking (shown by height increasing)

In conclusion, centimetre accuracy can be demonstrated in the horizontal axes, and better than decimetre accuracy can be achieved in the vertical axis. Loss of line of sight has been shown to cause spikes and deterioration in signal quality, although these can be removed when analysing the UWB data. Obstructions; steel structural beams, machinery and robotic milkers are no problem for the signal, when BUs are set up in optimal geometry which is most important for achieving good signal quality and accurate positioning. Spikes and error are attributed to poor geometry.

4.2.2 Constellation Development

To overcome problems of accuracy and signal quality arising from poor geometry of the BUs an optimal network of BUs was installed in permanent positions to span the area covering 2 pens of approximately 40 cows.

Figure 4.8a) and -b) depict the BU set up; a) showing the 6 BUs around the barn perimeter and b) the 2 BUs in the roof, essential for the height component of UWB. Figure 4.8 shows the UWB BU network in their exact, fixed positions which are the optimal positions for best signal coverage in the concerned area. These known coordinates are entered into the computer, and form the basic network in which MUs will then calculate their position. Figure 4.9 complements Figure 4.8 by showing areas of better and worse coverage; blue shows where there is good signal through to red which are areas of poor signal coverage. Accuracy of geometry is measured by the dilution of precision (DOP) in the horizontal (H) and vertical (V) dimensions in the HVDOP diagram (Figure 4.9). The dilution of precision indicates the potential accuracy of positioning observation for the given BU network; the higher the DOP value the worse the accuracy. Figure 4.9 shows that coverage was best in the central position, where there was little interference from the structural components, but signal quality deteriorates closer to the corners, in positions close to steel doors and the building infrastructure. However, the overall coverage that the BU network provided was demonstrated to be of good quality and allowed for accurate communication and positioning by UWB.

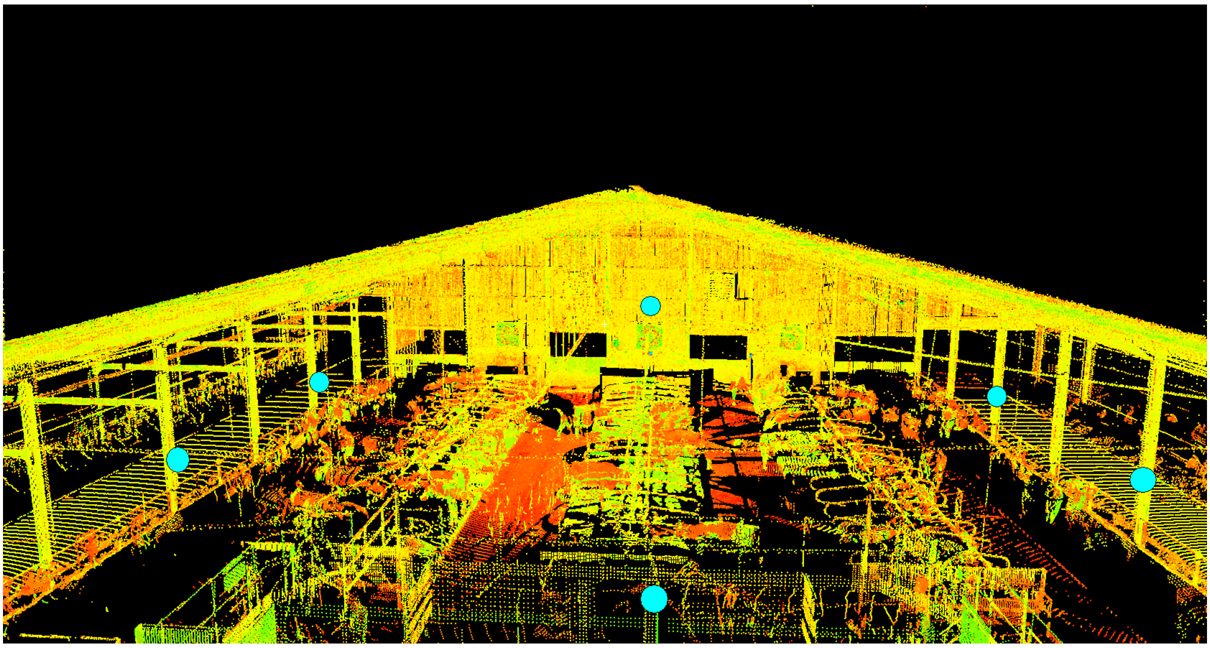


Figure 4.8 a) Infrared picture showing the position of the 6 BUs (blue dots) around the perimeter

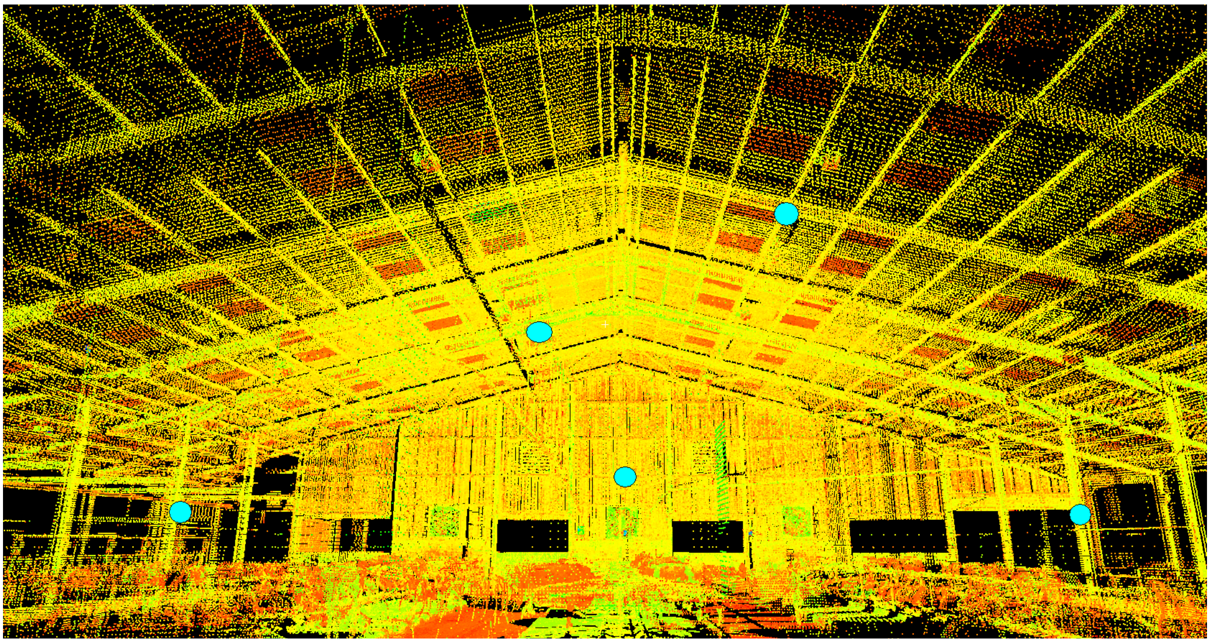


Figure 4.8 b) Infrared picture showing the position of the 2 BUs in the roof (uppermost blue dots)

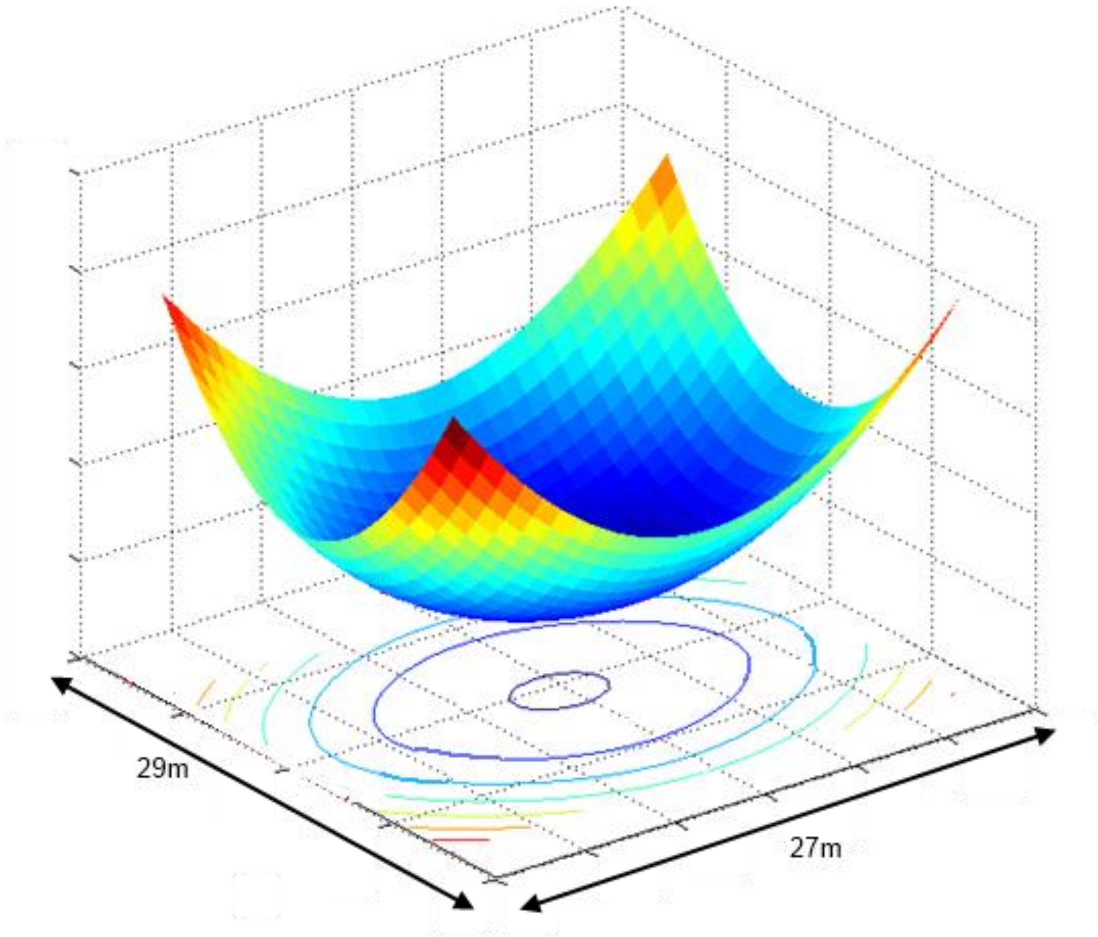


Figure 4.9 Horizontal – Vertical Dilution of Precision (HVDOP) diagram of UWB network signal coverage; blue = good, red = poor

4.3 PRELIMINARY TRIALS

4.3.1 Testing BU Geometry

The BU geometry in the dairy barn was tested in a series of static and kinematic tests, focussing on accuracy in the height component as this was the most difficult to achieve, yet the most important for the purpose of oestrous detection. MU position was tested in areas of good and poor signal quality, according to Figure 4.9, as determined by the HVDOP.

Primary tests were carried out to determine MU orientation and which position, if any, was best, which could influence the backpack design. Two MUs were monitored in a short test for 20 minutes, one with the antenna in vertical orientation and one in the horizontal orientation, both attached to a pole. The MUs were swapped over in orientation and position and the recording repeated. Figure 4.10 shows 2 graphs highlighting the difference in error between unit orientations. It is clear that the vertical antenna positioning has less error, and a constant position, therefore it was concluded to use the MU in the vertical antenna position.

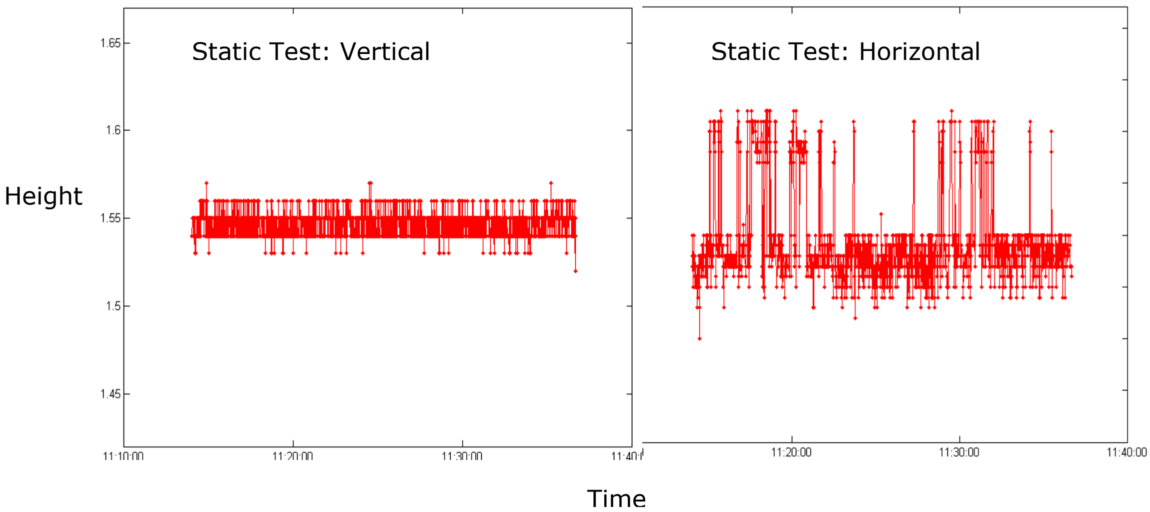


Figure 4.10 Graphs showing the best MU orientation; vertical antenna orientation has less erroneous spiking compared to the horizontal orientation

Kinematic tests with an MU attached to a pole were also carried out by a person walking and moving the pole, to measure the X and Y horizontal positions, using the total station and 360° prism (also attached to pole) to compared to the exact position and UWB recorded position for accuracy. The sampling rate between total station and UWB are different (as described in section 4.2.1), but the results presented in Figure 4.11 show that UWB is matching the 'truth' position of the total station within the optimal BU network.

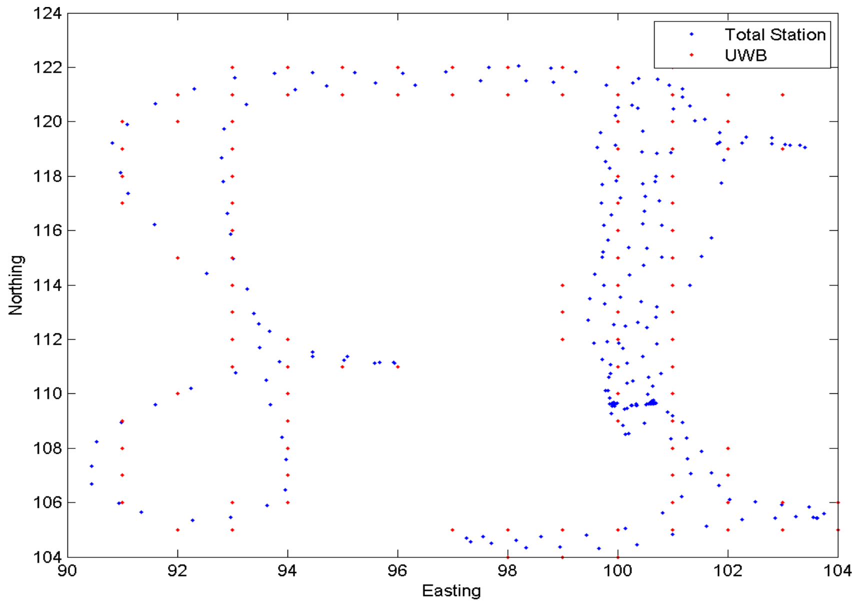
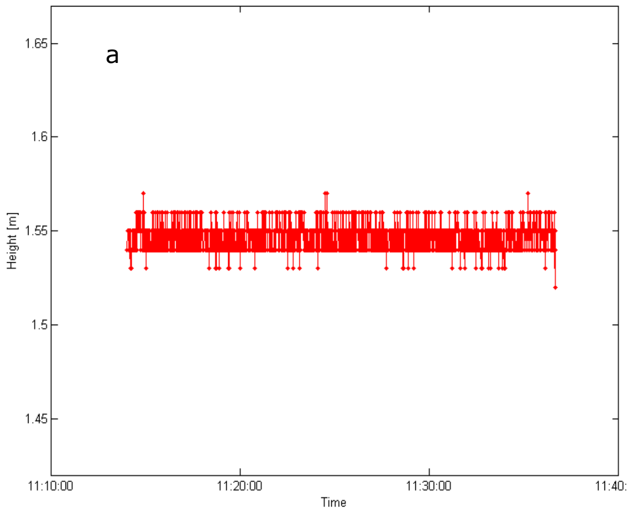
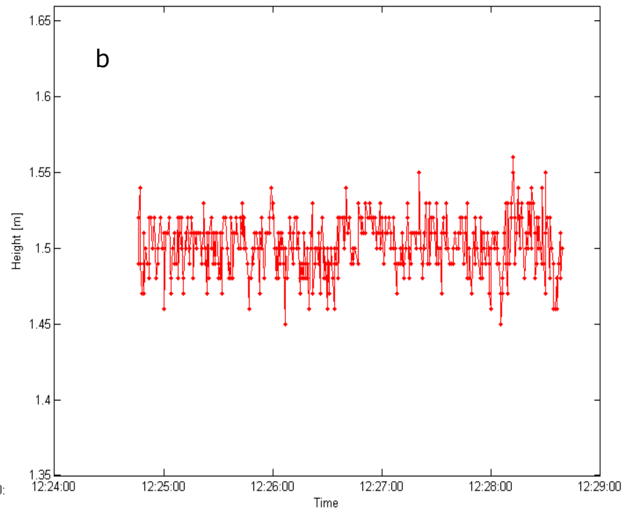


Figure 4.11 Kinematic test in optimal BU network showing X and Y horizontal positions; red = UWB, blue = total station measuring truth coordinates (NB: Due to the different sampling rate between the two measurements, these points cannot match each other one-to-one). Scale in metres

Having developed static accuracy and accuracy of kinematic tests of X and Y positions, height accuracy had to be determined in order to finalise the suitability of UWB for oestrous detection. Primary static tests with a MU mounted on a pole were carried out within the BU network as shown in graphs of Figure 4.12. Positions of good and poor geometry were chosen to test the positional accuracy; in the centre of the barn and BU network, in the far corner which is of poor geometry where there was potential obstruction from steelwork and in the robotic milker which is a very enclosed environment (as discussed in section 4.2.1).





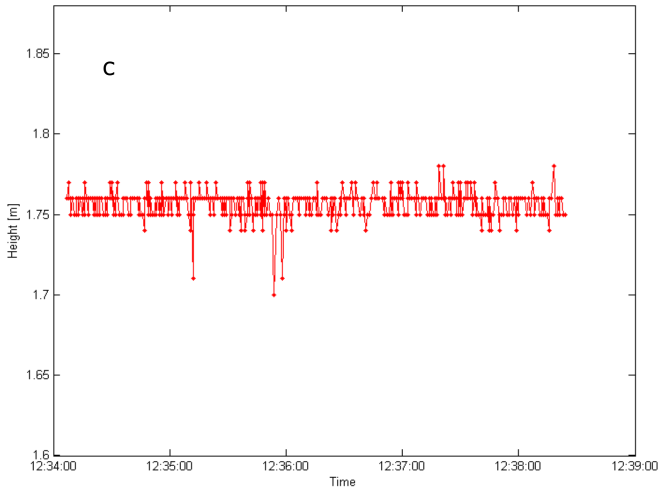
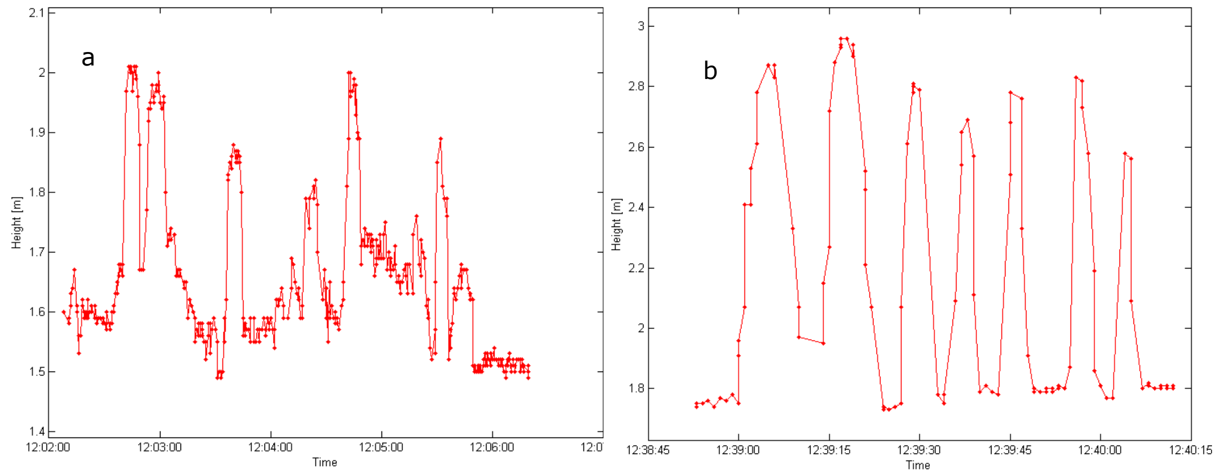


Figure 4.12 Static tests to determine height accuracy of BU geometry in areas of good and poor signal quality according to HVDOP (Figure 4.9). a) static test in area of good signal quality, b) static test in area of poor signal quality in far corners of the location and c) static test in robotic milker, an area of suspected high multipath and poor signal quality as identified by HVDOP

These tests demonstrated that in areas of good quality UWB signal coverage, according to the HVDOP, that height can be determined to centimetre accuracy. Importantly this was still achievable in areas of poor signal quality with the average height being determined with only 10cm error and in the robotic milker it is surprising to see that accuracy is equal to that in areas of 'good positioning' as shown in Figure 4.9. The height is 20cm higher in the robotic milker due to a step up, which is noticeable by the different static heights in Figure 4.12; however it is encouraging that this elevation in height could even be detected. Progression onto kinematic tests to determine height accuracy followed this series of static tests using the same methods; MU attached to a pole and someone walking around, altering the height manually. Results are presented in Figure 4.13, showing that general movement within the network can be monitored as the MU gradually changes height. This finding is consolidated with the knowledge that height changes can still be determined in areas of poor coverage, even when occurring in quick succession so as to simulate the effect of a mounting cow. For example height changes could be determined close to the robotic milker (as mounting cannot occur in the robot).



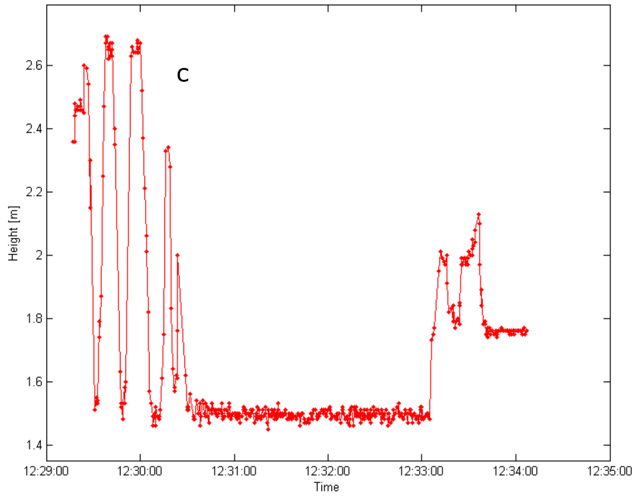


Figure 4.13 Kinematic tests to determine height accuracy of BU geometry in areas of good and poor signal quality according to HVDOP (Figure 4.9) a) kinematic test in central position of good signal quality, involving changes in height, b) kinematic test in area of poor signal quality in far corner of location and c) kinematic test close to the robotic milker, an area of suspected high multipath and poor signal quality as identified by HVDOP

These results concluded that the 8 BU network provided accurate results of MU position, with reduced spiking and error due to the best possible geometry of BU network. In summary, this work led to the validation of UWB accuracy in good and poor positions within the cow barn. The next step was to monitor cows' behaviour to accurately determine their position at a given time.

4.3.2 Short Cow Tests

The purpose of the short tests was to monitor multiple cows' behaviour and achieve the best possible set up of the equipment. In a series of 3 half day tests 4 to 6 cows were monitored on each occasion with each cow wearing backpacks (see Figure 4.4) with an MU attached to a battery (see Figure 4.3). Cows were selected for use in the trial and removed from their location in the cow barn to the management/ AI stalls where the cows were equipped with backpacks. They were then returned to their location with their position being recorded by UWB and signals relayed to a computer where information about the cows' movements was stored for post-trial analysis. Cow behaviour was also monitored by CCTV (PTZ Dome Camera, Pelco-D 2400) and by visual observation, where records were made of cow number, time and position in a location and general behaviour/ activity of each cow.

Results of these short trials allowed for problems with the equipment and set up to be identified and these are outlined and discussed in Table 4.1. Problems encountered due to equipment setup, occurring mainly because UWB is not designed for the purpose of cattle monitoring, were; wire connections, MU antenna breakages, battery power and units 'dropping out' of signal range. Once these issues were resolved cows could be monitored with continuity of data collection and positioning could be accurately identified, see Figure 4.14. Cow behaviour and position were recorded continuously for a period of approximately 2 hours (9.45am to 11.45am) using UWB, CCTV and visual observation. Initially there is some spiking at the beginning of recording which could be due to the cow returning from the management/AI stalls into the location, which is inside the BU network. The graph of absolute height then gives a clear indication of the cows movements; standing at the feed passage, entering the cubicles which is shown by an increase in height, then lying down in the cubicles shown by a marked decrease in height, finally ending with minimal spiking as the cow entered the robot to be milked.

The most important conclusion from this set of experiments was that all cows displayed natural behaviour whilst wearing backpacks (Figure 4.14). In conclusion, the three small trials allowed identification of problems that occurred due to UWB set up and for these problems to be resolved (Table 4.1) which allowed progression to monitoring cows' position and thus their behaviour.

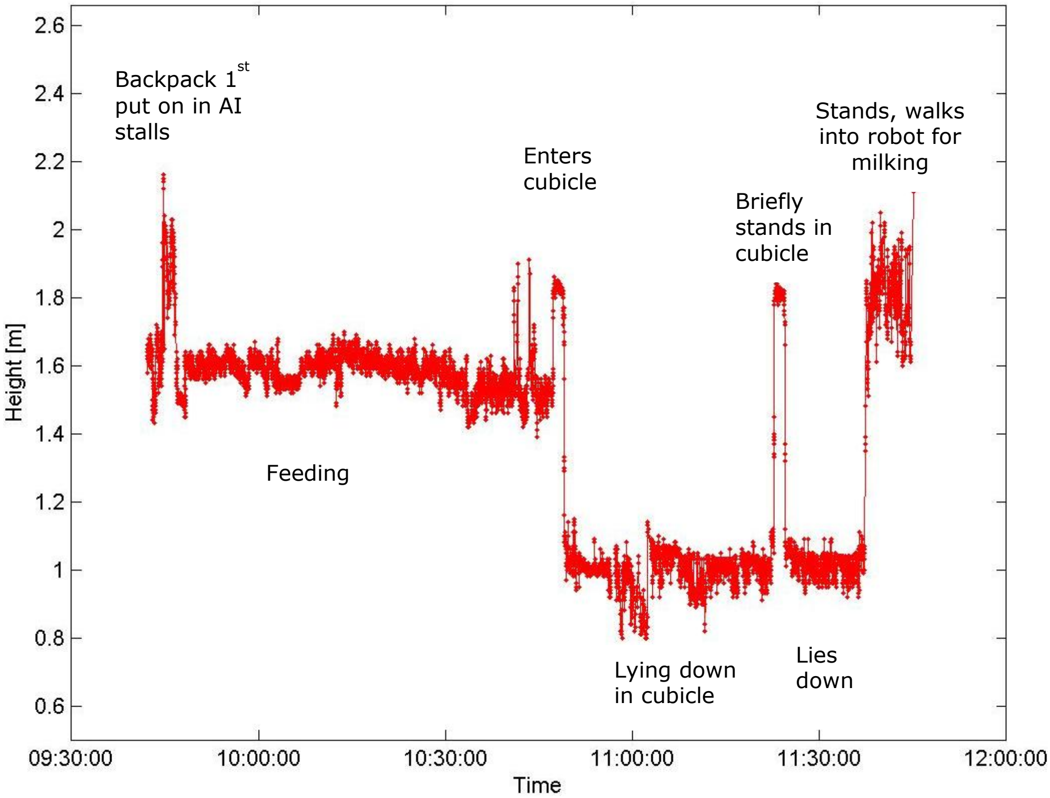


Figure 4.14 Annotated graph of height changes monitoring one cow's behaviour and position for a sustained period of time by UWB, which was verified by CCTV monitoring and visual records.

4.3.3 Longer Cow Trials

After establishment of an optimal BU network and optimal set up of equipment, to test further whether UWB would be suitable for the purpose of oestrous detection, cow behaviour was monitored during oestrus.

4.3.3.1 12 Hour Trial

The purpose of a 12 hour trial was to monitor cows early in the morning, when oestrous behaviour is more commonly displayed, in order to monitor actual oestrus, mounting activity and standing to be mounted by UWB positioning. This would then determine the suitability of UWB for the purpose of oestrous detection.

Table 4.1 Problems and solutions concerning equipment set up for monitoring of dairy cows using UWB

Testing Setup	Problem	Solution
Wire connection	• Short circuiting • Disconnection when using a middle connector between battery and MU • Cows attracted to visible wires, therefore disconnecting from battery connection • Due to prototype format of MU, connection to wire was quite weak, strengthened by cover and fastened with electric tape	1 wire connected from battery to MU secured into place on battery and MU connection with support, and hidden from view covered by electric tape.
MU antenna breakages	Due to prototype format antenna protrudes from unit and is prone to breakage with general cow movement, standing up and lying down etc. and the general environment.	Made protective hard plastic cover that did not interfere with signal quality, confirmed by tests at NGI.
Battery power	In view of the need for long term use battery power was tested to see how long MU would last. 2 batteries to 1 unit lasted double the length of time as 1 battery to 1 unit, which is beneficial for long term testing, although the connection was weaker.	1 battery connected via 1 wire connector, which lasts for 13.5 hours. Therefore long term trials should require changing twice daily.
'Dropping out'	Signal becomes weak and occasionally units are no longer communicating for a short period of time. This was occasionally due to battery power and connection trouble, but was also attributed to poor position in the cow location disrupting line of sight, especially when the cows remained stationary for long periods. Connection was resumed upon movement.	Unit resolved the problem almost instantly by reappearing when reconnection was found.

Cows’ behaviour was assessed at 5am and 2 cows in oestrus were selected for use, alongside 3 control cows not in oestrus. All 5 cows were equipped with batteries and one MU in a backpack (see Figures 4.3 and 4.4) to record position by UWB. Their behaviour was recorded both by CCTV (PTZ Dome Camera, Pelco-D 2400) and visual observation. Problems were encountered when the backpacks were first put on and the cows released into their locations, with wiring connections and backpacks slipping for the 2 cows in oestrus as these were so active. However data collection continued for approximately 12 hours monitoring oestrus by UWB between 05:06:50 and 16:32:24 hours. Other problems were then encountered towards the end of the trial as batteries ran out of power, however, this did not impact on the results as oestrous behaviour had ceased.

Data were then analysed by comparing time of mount, which cows were mounting and standing to be mounted, and duration of mount from CCTV and visual records with UWB raw data. UWB data was then analysed by running a script (MatLab R2012b, The MathWorks, Inc., US) with set, defined limits to define a mount, in order to detect oestrus automatically. Limits were chosen for height, and relationship in the X and Y dimension, to determine oestrus by the cows’ interactions. Duration per mount of 3 seconds was incorporated as a limit in the script; mounts of short duration (less than 3 seconds) were discounted from the analysis because of their similarity with error spikes.

When comparing visually recorded mounts post trial with UWB raw data, all mounts could be identified. Results of automated analysis are reported in Table 4.2, identifying the time when mounting and standing to be mounting occurred, which clearly identified the 2 cows in oestrus. The first initial mounts that occurred were in the management/AI stalls which are outside of the BU network therefore these mounts were discounted from analysis as these could be erroneous. The UWB data collected clearly show that mounting and standing to be mounted can be recorded by UWB and detected by specialist scripts to analyse cow position in relation to oestrus. 31 mounts in total were identified by UWB (see Table 4.2), but 48 mounts were reported from CCTV footage (although this included short mounts of less than 2 seconds), which demonstrates that the majority of mounts, 65%, were detected by UWB.

Table 4.2 Table showing results of 12 hour UWB trial to monitor cows in oestrus reporting the time, duration and description of each oestrus event between 2 cows; A and B

Time, hh:mm:ss	Duration, seconds	Mounting Cow	Standing Cow
08:55:59	15	A	B
09:17:49	10	A	B
09:20:54	9	B	A
09:24:52	12	A	B
09:28:29	8	B	A
09:30:44	3	A	B
09:35:57	7	B	A
09:39:31	7	A	B
09:42:20	4	A	B
09:48:34	8	B	A
09:49:38	8	A	B
10:02:53	3	A	B
10:08:00	4	B	A
10:16:29	8	A	B
10:22:57	7	A	B
10:32:04	11	A	B
10:33:24	3	B	A
10:33:47	3	A	B
10:33:51	12	B	A
10:37:49	4	A	B
10:44:37	6	A	B
10:48:31	5	A	B
10:53:32	9	B	A
10:56:34	5	A	B
11:00:04	4	B	A
11:08:33	4	A	B
11:15:59	7	A	B
11:21:30	17	A	B
11:33:05	4	A	B
12:03:11	4	A	B
13:00:18	3	B	A

An equally important observation from this trial was that no mounting was reported for the 3 control cows which all displayed natural behaviour, feeding, lying and ruminating, which was expected and corresponded with visual observations. However, from script analysis 2 false positive mounts were identified; an oestrus cow mounted a control cow and vice versa. Although this may be possible, CCTV analysis post trial confirmed that the automated UWB record was false. Possible explanations are that the MUs could have been close to one another when actual mounting was occurring, or that the cows were in an area of poor signal strength causing spiking and an erroneous position. However, having only 2 erroneous mounts in total was considered very promising for these preliminary trials, as this did not indicate oestrus and hence cows would not be inseminated on the basis of 1 episode of standing to be mounted or mounting another cow.

Limitations from this trial were that the UWB, laptop for data collection and CCTV times were not synchronised, therefore no conclusion can be drawn about the real-time characteristics of UWB. However these data did show that UWB could effectively detect oestrus and distinguish between cows in oestrus, eligible for AI, and cows not in oestrus.

In conclusion, these results provided a strong basis for progression with the UWB system in that cows in oestrus could be clearly distinguished from control cows. When cows are in oestrus, mounting and standing to be mounted, the height changes could be identified and relative positions and changing dynamics of cow position could be used to identify oestrus. Also when cows were not in oestrus, mounting and standing behaviour were not identified. Although there were 2 incidences of false positive results, these were only one per cow, which would not be taken as confirmatory evidence that a cow was in oestrus.

4.3.3.2 Increasing MU Number

To further develop UWB for the purpose of oestrous detection it was necessary to monitor several cows at once. These trials involved increasing the number of MUs to test how much UWB positioning data could be collected and recorded at once. MUs were mounted on cows in backpacks as discussed previously. Initially 10 cows were equipped with UWB in backpacks and their position recorded. This meant that 18 UWB units (MU and BU) were communicating at once. The UWB set up worked well and data collection was possible. Trials then progressed into monitoring 15 cows in a 24 hour trial (detailed below), however problems were encountered as units 'dropped out' (as highlighted in Table 4.1), except there was no resolution of this issue as on each occasion 3 units failed to communicate. With 15 MU and 8 BU the total number of units sampling at once equalled 23 UWB units. It was concluded upon reflection that only 20 UWB units were capable of communicating simultaneously (a current Thales system constraint), sampling at a rate of 2Hz, which could be increased to 40 UWB units if sampling rate was decreased to 1Hz. This meant that for future trials the maximum number of cows could only be 12; 12 MU and 8 BU, sampling at 2Hz, relaying precise position at twice per second.

4.3.3.3 24 Hour Trial

The purpose of the 24 hour trial was to ensure natural behaviour occurred in all cows whilst wearing the backpacks, and to test whether recording by UWB could continue for a longer period of time. Initially 15 cows were involved in a 24 hour trial, although due to units 'dropping out' (because of the maximum UWB unit number as discussed above); the positioning data of only 12 cows was recorded over 24 hours. Importantly monitoring of the 12 cows for 24 hours continued without problem, except for battery power failing and the need to change batteries to ensure the MUs continued to communicate position in order to obtain, as much data as possible. Data from this trial confirmed that UWB position of 12 cows can be recorded continuously, and that all cows display natural behaviour when wearing the backpacks.

Data from the 24 hour trial was also analysed via an automated script, and results compared against CCTV records (as detailed in section 4.3.3.1). During this trial no cow showed oestrus. However, 4 incidences of false mounting were reported upon script analysis, involving only 6 cows; therefore maximum number of 2 mounts occurred, according to the script, per cow. This data was therefore not indicative of oestrus due to the insignificant number of mounts occurring in a 24 period, and mounts were confirmed as false positives by post-trial CCTV analysis.

In summary, 12 cows can be monitored accurately using UWB, even in positions where signal quality may be poor. UWB is capable of recording precise cow movements and importantly oestrous behaviour, recording changes in height and the positional relationship between 2 cows engaging in this activity.

4.4 CONCLUSION

The aim of this work was to develop UWB as a novel technology with the potential to monitor oestrous detection in dairy cows. Particular challenges were the environment of the dairy which could affect accuracy of position. Optimising BU positioning led to precise positioning in 3 dimensions, 2 to 3cm accuracy in horizontal axes and approximate 10cm accuracy in vertical plane, but mostly in achieving higher precision. Most importantly these data have demonstrated that oestrus can be detected in dairy cows; identify the mounting cow and the cow standing to be mounted. Limitations of this work arose due to the prototype set up of UWB, although equipment had been tested and problems resolved to ensure the capability for longer–term monitoring of dairy cows. The results from the preliminary tests provide definitive evidence that UWB could be used for the purpose of oestrous detection. Future work would be to develop proof of concept trials to test UWB for the purpose of oestrous detection, monitoring dairy cow behaviour continuously, in real-time for automated detection of oestrus.

Chapter 5 - Proof of Concept of UWB for the Purpose of Oestrous Detection

5.1 INTRODUCTION

Oestrous detection is a major contributor to poor fertility as only 50% of all cows are detected in standing oestrus (Van Eerdenburg *et al.*, 2002), with no current improvements to this figure. Detection of oestrus is an essential prerequisite for AI, so arguably is the central event in bovine reproduction. Therefore increased oestrous detection rates are a necessity to improving dairy cow fertility and profitability of the dairy herd (Pecsok *et al.*, 1994). In order to improve oestrous detection rates a method of oestrous detection must be developed which is both efficient and accurate at detecting oestrus (Senger, 1994); such as UWB, described in Chapter 4, which may provide a novel solution to the problem.

Many attempts to develop automated methods of oestrous detection have not succeeded commercially for a variety of reasons. A possible solution to overcome the limitations of current oestrous detection techniques is to measure cow position rather than just activity. Global positioning system (GPS) is a possible solution to monitor 3D position, although GPS cannot determine a subjects' precise position with accuracy indoors. Ultra-wide band (UWB) technology (as discussed in Chapter 4) has shown potential to accurately determine cows' position with centimetre accuracy in the dairy barn and automatically detect both mounting and cows standing to be mounted. The position of cows and their interactions can be recorded by UWB and inferences made about their behaviour in order to detect oestrus. These behaviours can be detected in real-time, therefore can be used to determine the onset of oestrus and thus allow for accurately timed AI to coincide with ovulation and maximse conception rates. It is evident that UWB has the ability to overcome the limitations of current oestrous detection techniques.

Aims of this study were to develop proof of concept of UWB to detect oestrus by focussing on:

- Developing software and communications to enable real time positioning and identification of cows approaching oestrus (mounting cows) and in standing oestrus (standing to be mounted) by monitoring their behaviour

- Develop techniques for analysis of the real-time positioning information so that these data could be used in a commercial farm situation to detect oestrus, coupled with physiological measurements to confirm stage of the oestrous cycle, to confirm oestrus
- Develop communications to monitor a herd of several hundred cows, in real-time, to detect oestrus automatically

5.2 MATERIALS AND METHODS

Three week long trials were carried out monitoring 3D cow positioning using Ultra-wide band (UWB) technology (as described in Chapter 4) for automatic detection of oestrus. In the first proof of concept trial 1 (POC 1) a UWB mobile unit (MU) was attached to a battery (12 Volts Maintenance free Sealed Lead-acid Battery, RS Components, Northants, UK) and mounted on the cow in a backpack (Cassidy Covers, Ireland; see Figure 4.4). In the second trial, proof of concept trial 2 (POC 2) and third trial, proof of concept trial 3 (POC 3), different batteries (12 Volt 22 Amp hours Tracer Lithium-Polymer Power Pack, Deben Group Industries Ltd., Suffolk, UK) were used as these had more power and required less frequent battery changes.

5.2.1 Animals

Animals used in this study were Holstein Friesian dairy cows housed at Nottingham University Dairy Centre, as described in Chapter 2. In each trial 6 cows oestrous cycles were synchronised for them to come into oestrus during the trial for monitoring by UWB. Control cows were also used in each trial to compare UWB position recording between oestrus and non oestrus cows (negative control). In POC 1 three pregnant cows were monitored and 3 non-pregnant cows that did not come into oestrus as controls. In POC 2 and POC 3 two pregnant control cows were used in each trial.

5.2.2 Training

All cows used in this study were trained with backpacks prior to the beginning of each trial to become familiar with the equipment and extra weight. All animals displayed natural behaviour; feeding, lying in cubicles and ruminating and, as results from preliminary trials show (see Chapter 4), mounting behaviour was not inhibited.

5.2.3 Experimental Design

Figure 5.1 describes the general experimental design of the proof of concept trials. On day 0 six cows were synchronised with CIDRs. Milk sampling and activity monitoring also commenced on day 0, throughout UWB recording and continuing until 5 days after, to ensure collection of data before, during and after oestrus. On the morning of day 6 UWB recording began and the 6 cows to be synchronised were injected with Estrumate. On day 7 CIDRs were removed, and then during days 8, 9 and 10 cows displayed oestrus. UWB recording ended on the morning of day 13 (for POC 2 and 3; ended prematurely at day 11 for POC 1) after recording UWB data for 24 hours for 7 days. Visual observation and CCTV recording occurred simultaneously with UWB data collection (Figure 5.1).

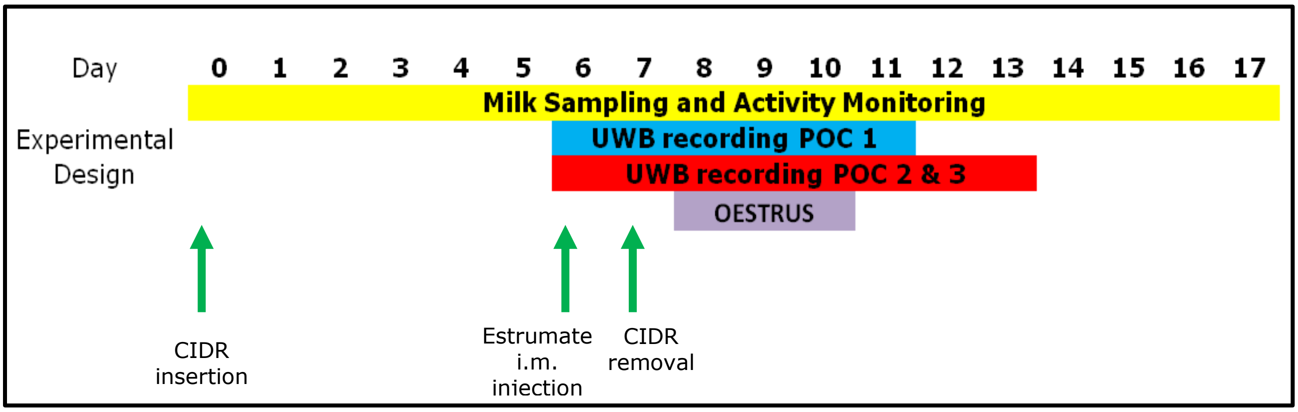


Figure 5.1 *A diagram to show the experimental design of proof of concept trials per day.* Cows were synchronised with CIDRs at day 0. Milk sampling and activity data collection also began at day 0 through to the end of the trials at day 17. Estrumate injection was administered intramuscularly at day 6, with UWB recording also beginning in the morning of day 6. On day 7 CIDRs were removed which allowed for oestrus to take place on the following days. UWB recording ended on the morning of day 13 recording continuously for 24 hours over 7 days (in POC 2 and 3). UWB recording during POC 1 ended prematurely on day 11.

5.2.4 Synchronisation

Six cows were selected for synchronisation and approved for use on trial by the Named Veterinary Surgeon. On day 0 one Eazi-Breed™ CIDR device (InterAg, Hamilton, New Zealand; Cockburn Veterinary Group, Leics, UK) was inserted per vagina of each cow. The CIDR (controlled internal drug releasing) device is an intra-vaginal pessary containing 1.38g progesterone in elastic silicone moulded over a nylon spine, used to synchronise oestrus in ruminants. Insertion was carried out using an applicator disinfected in Savlon solution (Novartis Consumer Health, Horsham, UK; 1:100 dilution). The CIDR was loaded into the applicator and lubricant applied (Vet Lubigel; Dechra Veterinary Practice, Shropshire, UK) to the protruding end of the device. The vagina of the cow was cleaned immediately prior to device insertion and the loaded applicator inserted with a slight upward orientation into the vagina and the CIDR expelled by depressing the end of the applicator. The applicator was withdrawn and disinfected. Following insertion the animal's blood progesterone reached maximum concentration within an hour of insertion and was maintained until removal. On day 6 2.0ml Estrumate (Cloprostenol sodium; Cockburns Veterinary Group, Leics, UK) was administered to each cow intramuscularly. Estrumate is a synthetic prostaglandin analogue structurally related to $PGF_{2\alpha}$, which facilitated regression of the CL if present. At day 7 CIDRs were removed causing progesterone concentration to decline, which allowed oestradiol concentrations to increase due to increased LH pulses (see Chapter 1). Devices were removed by gently pulling on the exposed removal tag. This resulted in oestrus approximately 48 hours later.

5.2.5 Data Collection

5.2.5.1 Ultra-wide Band (UWB) Recording

The UWB network was set up (as described in section 4.2.2) with 8 base units (BUs) forming a network to cover the 2 locations in which cows from these studies were housed. All BUs were in direct line of sight to one BU nominated as the master unit, set up in optimal positions to reflect accurate positioning coordinates of the MUs assigned to each cow. Each cow was equipped with 1 MU, the number of each UWB unit recorded against cow number, and one battery. The UWB MUs were set to sample at 2Hz, twice per second, and send their position to the control unit connected to the computer where data was recorded and stored. UWB data recording began on the morning of day 6 and was scheduled to end on the morning of day 13 thus recording cow position by UWB continually for 7 days. Battery changes were scheduled at intervals throughout the trials in order to maintain continuity of data collection. Unfortunately in POC 1 failing battery power and recharging batteries became too frequent and the decision was made to end the trial prematurely at day 11. During POC 2 three different batteries were used to ensure power failure was avoided, with changes of power occurring once every 24 hours. In both POC 2 and 3 UWB data collection was continuous from day 6 to day 13.

5.2.5.2 Milk Sampling

Milk samples were collected via a Lely Shuttle Milk Sampler from the start of oestrous synchronisation on day 0 until day 17. As cows entered the robot for milking they were identified to be sampled and approximate 20ml milk samples were collected. Samples were preserved with one potassium dichromate tablet (Broad Spectrum Microtabs II; D&F Control Systems, Inc., USA) per sample and refrigerated at 4°C until measurement. Sufficient samples were collected per trial in order to analyse hormone concentrations, before, during and after oestrus. However, because of the automated nature of the sampling method occasionally a sample would be missed. Furthermore during POC 3 a communication error occurred causing robotic system failure which meant that a couple of days had no samples, although these were post oestrus.

5.2.5.3 Activity Recording

The current method of oestrous detection on farm is by activity monitors, worn around the cows' necks, which are detailed in Chapter 2. Activity data collection was continuous over the trial period from day 0 to day 17, before oestrus, during and post oestrus.

5.2.5.4 CCTV & Visual Observation

CCTV video recording and visual observations were used to confirm oestrus and the cows' actual behaviour in order to compare and aid development of UWB data analysis. During POC 1 CCTV (PTZ Dome Camera, Pelco-D 2400) videos continuously recorded over the period of UWB recording. In POC 2 CCTV recording was unavailable due to cow location, therefore continuous visual observation was used to record cow behaviour. Visual observation began 24 hours after CIDR device removal, commencing with hourly observations for periods of 15 minutes as cows began to show early signs of oestrous behaviour and increased interest in other cows, increasing to more frequent observations, eventually resulting in continuous observation as cows began mounting and then standing to be mounted by others. Four hours after the last episode of standing to be mounted occurred, oestrus was deemed to be over if cows were no longer showing any interest in each other. Information recorded was; mounting cow ID, standing cow ID, date and time of mount, duration of mount, any other relevant information relating to position. In POC 3 both CCTV (Smart Witness Wireless System SWC101S; Maplin, Notts, UK) and visual observation of cow behaviour were used to confirm oestrus by UWB recording.

5.2.6 Data Analysis

5.2.6.1 Assay for Milk Progesterone

Milk progesterone concentration was determined using a 96 well microtitre plate-based enzyme-linked immunosorbent assay (ELISA) supplied as a commercially available kit (Ridgeway Science Ltd., Alvingdon, UK). Microtitre plates coated with antibody stored at 4°C were warmed to room temperature (25°C) prior to use. The foil seal was removed, wells emptied and plate blotted dry onto tissue paper. Prior to assay reagents, whole milk standards; 0, 1, 2, 5, 10, 20 and 50ng/ml, and quality controls; 2ng/ml and 5ng/ml, (progesterone in milk from an ovariectomized animal or cow in oestrus) supplied with the kit, and samples were brought to room temperature and thoroughly vortexed to ensure homogeneity of the samples. A volume of 10µl of standards, quality controls and samples were added to the wells in duplicate. 200µl of progesterone enzyme label (supplied) was added to each well and the plate incubated at room temperature for 1 hour 30 minutes. After incubation, wells were emptied and washed 3 times with cold water, tap drying on paper between each wash. The substrate solution supplied was then added to each well at a volume of 200µl and incubated in the dark at 25°C for 20 minutes to allow the colour to develop. Strong colour denoted low levels of progesterone (heat or not pregnant) and weak colour, high levels of progesterone (mid cycle or pregnant). The immunosorbance of each well was recorded at a wavelength of 570nm using an automated plate reader (Labsystems Multiskan Ascent 354) and processed using specific software to read the absorbance and transform into progesterone concentration (Ascent Software Version 2.6, Thermo Labsystems). The assay kit was capable of a sensitivity of 5pg/ml although a sensitivity of this scale was not required for this purpose and therefore the lowest standard was 1ng/ml. The inter-assay coefficient of variation was 12.6%.

5.2.6.2 Activity Data Analysis

Activity data collected over the trial period was analysed by plotting activity against date and time. This resulted in 12 activity readings daily of the mean activity for 2 hourly periods with peaks denoting oestrus. Date and time of activity increase, peaks on the graph, were used to confirm oestrus detected by UWB and to compare methods of detection.

5.2.6.3 Analysis of CCTV and Visual Records

After POC 1 CCTV video footage recorded during the trial was analysed in order to record the mounting behaviour of each cow in oestrus. The quality of data from POC 1 was poor due to lack of continuity of data collection, but selected oestrus data could be determined from the UWB raw data by comparing the time of oestrus events with the CCTV recording.

The visual records of oestrus from POC 2 and POC 3 were compared with UWB data to determine if the specific positions associated with oestrous behaviour were recorded by UWB. The visual records were then compared to UWB data which had been analysed automatically by script algorithms to determine the accuracy of the script at predicting individual episodes of mounting and standing to be mounted.

CCTV footage from POC 3 was also used to confirm or deny any mounts declared by automated script analysis that did not correspond to visual records. These were either due to the UWB system or human error.

5.2.6.4 UWB Analysis

UWB data were analysed to determine if mounting had been recorded by increases in the Z positioning coordinates. The UWB data were then analysed by an automated script (MatLab R2009b, The MathWorks, Inc., US) to determine episodes of oestrous behaviour by the cows relative position in relation to each other. Finally, automated script software was developed to detect cows in oestrus.

5.2.6.4.1 UWB Data

UWB raw data were analysed against the times of mounting events recorded by visual observation to determine whether the elevation in height that occurred during mounting had been recorded by UWB. Mounts were recorded as 'identified mounts' if the events that were visually observed matched with increases in height coordinates present in the UWB data, or not identified mounts if the visual observations did not match with increases in height coordinates in the UWB data. The UWB observations were expressed as a percentage of the total number of mounts visually observed and recorded (minus UWB error; where data was missing because coordinates were not recorded by UWB, signal quality was poor therefore accuracy had deteriorated or when the unit had turned off) to determine the percentage accuracy of UWB.

5.2.6.4.2 Script Analysis

It was clear from analysis of UWB data that cow positions and elevations in height during oestrus were recorded which prompted development of a script to automatically analyse UWB data and declare mounting and standing events occurring by individual cows. Script 1 was developed to take into account the average dimensions of a Holstein Friesian dairy cow and thus the relative position between 2 cows whilst one stood to be mounted by another. Firstly data were filtered to remove any outlying values above and below the set height limits for a mount (minimum; 1.3m and maximum; 2.6m) and to remove data of poor quality (<80% signal strength). Secondly, values were interpolated where epochs of 1 or 2 seconds of height data were missing but could be interpreted from the seconds before and after the period of missing data. These coordinates of abnormal height were removed where the mean height for a prolonged period (5 minutes) of data exceeded 1.8m and then the value of 0 was assigned to epochs of missing data to aid processing. Lastly, to identify individual mounts from the UWB data set, different limits were applied by Script 1 to define a mount, taking into account the position of 2 MUs on the 2 cows during mounting.

Limits were assigned for the relative position of the 2 MUs in the horizontal axes (X and Y) and vertical axis (Z), in relation to each other during mounting. Two different sets of limits were applied to the script in order to detect mounting; 'loose' limits and 'tight' limits. 'Tight' limits were implemented to achieve greater precision and 'loose' limits were used to detect a greater number of events.

When the difference in X coordinates between MU1 and MU2 is less than 1.0m, the difference in Y coordinates of MU1 and MU2 must be between 0.7m and 2.3m ($\Delta X<1.0m$, $0.7m<\Delta Y<2.3m$; this could also be applied vice versa; $\Delta Y<1.0\text{m}$, $0.7\text{m}<\Delta X<2.3\text{m}$). Meanwhile, the difference in height between MU 1 and MU 2 in the vertical, Z, axis must be between 0.4m and 1.0m ($0.4\text{m}<\Delta Z<1.0\text{m}$) of each other, and within the constraints of the X and Y coordinates. The limits described are the 'loose' limits designed to detect maximum number of mounting and standing occurrences. Absolute values were also assigned for the Z coordinate, to eliminate unlikely declarations of height, where the absolute height of the standing cow must be below 1.8m and the absolute height of the mounting cow must be above 1.9m. Furthermore, duration of greater than 3 seconds was assigned to the script, where if 2 MUs are within the set relative and absolute limits for X, Y and Z for longer than 3 seconds the script will declare that mounting has occurred.

'Tight' limits for the difference between 2 MUs during mounting were assigned as $\Delta X<0.8\text{m}$, $0.8\text{m}<\Delta Y<2.1\text{m}$ (or vice versa; $\Delta Y<0.8\text{m}$, $0.8\text{m}<\Delta X<2.1\text{m}$) in the horizontal axes, whilst between 0.4m and 0.85m ($0.4\text{m}<\Delta X<0.85\text{m}$) in the vertical axis. Absolute values remained the same as for the 'loose' limits. The objective of script development was to develop a balance between matching rate (visually observed mounts and mounts declared by the script, minus UWB error) and script accuracy (number of mounts declared by the script as a proportion of total mounts occurring recorded by visual observation). This would allow for optimal detection. The 'loose' limits script achieved better detection of mounting and standing events and shall herein be called the optimised script.

5.2.6.4.3 Script Analysis for Oestrous Detection at Herd Level

After determining individual mounting events, as described in the previous section, a final automated script was applied to detect cows in oestrus, Script 2. Script 2 used the optimised relative limits for mounting. Script 2 first identified an episode of standing to be mounted/ mounting from Script 1. The time (t) was recorded and cow number (n). Script 2 then determined if any further standing to be mounted/ mounting occurred by cow 'n' during the 3 hour period following time 't', using reports of individual mounting declared by Script 1. If the answer was 'yes' then the cow was confirmed in oestrus and the time of onset of mounting and time of onset of standing to be mounted was reported. The cow was thus declared in oestrus. If Script 1 reported isolated cases of individual mounts, not recurring within 3 hours, then the cow was not declared in oestrus.

5.3 RESULTS

Results demonstrate positive proof of concept that UWB can detect cows in oestrus. Furthermore oestrus activity is reported in real-time. This was verified by milk progesterone concentration, activity monitoring and visual observation.

The results from POC 1 were restricted due to several limitations, but when data collection was continuous the 3D positions of cows were recorded by UWB and could detect cow interactions indicative of their behaviour. The sequence of interaction recorded between 2 cows is plotted in Figures 5.2 to 5.5, showing 2 episodes of oestrous behaviour. These data show the 3D coordinates in the X, Y and Z axes changing in real-time as the cows move and interact. UWB detected when one cow mounted (blue line) and another cow stood to be mounted (red line), indicative of oestrus. It is clear that the 2 cows moved closer together followed by mounting, before moving apart (see Figure 5.2 to 5.5). The X and Y horizontal coordinates show the local position and the Z axis depicts the actual height change in centimetres occurring at one second intervals denoted by specific numbered time points. Furthermore, these figures confirm UWB accuracy as the increased height, which changed during mounting, could be detected from 160cm to 220cm.

The 2 episodes of oestrus reported by UWB during POC 1 were true oestrus behaviour as confirmed by visual detection from CCTV footage assessed post trial, milk progesterone concentration <1ng/ml for both cows (Figure 5.6; Cow 533 and 543) and increased activity (Figure 5.9; Cow 533 and 543).

Data from POC 2 and 3 complement the results of POC 1 and showed that cows mounting and standing to be mounted could be detected by UWB. Table 5.1 and 5.2 report analyses of the UWB raw data for events recorded by UWB, compared with mounts identified by visual observation. Percentage accuracy of UWB is the proportion of mounts detected by visual observation and CCTV that were identified in the UWB data, minus any mounts that cannot be identified due to UWB errors, but taking into account any mounts that were not recorded by UWB. Percentage accuracy for POC 2 was 83.3% and for POC 3 was 85.9%, showing that a large proportion of actual mounting can be detected by UWB through changes in height. The error from mounts that were not identified was where UWB had failed to record cow interaction, but these events were in small proportion; 16.7% in POC 2 and 14.1% in POC 3.

Results differed when the automated script was applied to analyse the UWB data (see Table 5.3 and 5.4). The matching rate between UWB declared mounts and visually observed mounts was determined by the optimised script; 76.4% in POC 2 and 67.4% in POC 3. Accuracy of the optimised script was less good at detecting true mounts; 56.07% in POC 2 and 51.24% in POC 3. It was clear from the individual results presented in Tables 5.3 and 5.4 that the total number of mounts declared by script analysis exceeded the number of mounts that were observed visually. The incidence of UWB error and mounts not declared by the script were low when compared to the number of false mounts declared by the script, indicating that specificity of the script for individual mounting and standing events was poor. Approximately half of all actual mounts were detected. In an attempt to increase the accuracy of detection and specificity of the script 'tight limits' were used to analyse UWB data. Here the script accuracy decreased for POC 2, 54.8%, and increased slightly for POC 3, 52.2%. Matching rate however decreased dramatically to 55.7% for POC 2 and 41.9% for POC 3, which was as expected. However, without improvements to the script accuracy this approach did not benefit accurate oestrous detection.

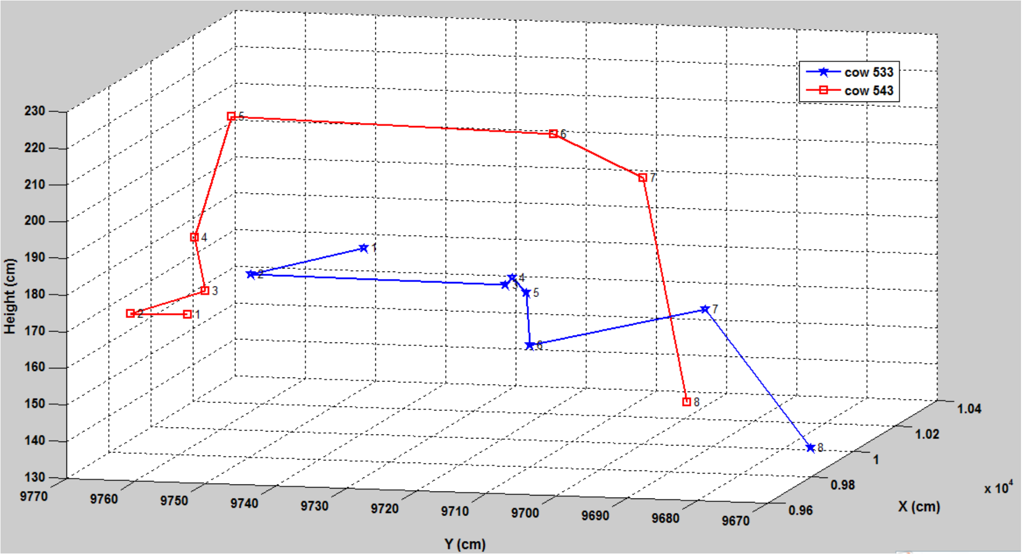


Figure 5.2 Graph showing mounting between 2 cows with 8 numbered points showing the sequence in time as cow 533 (blue line) stood to be mounted by cow 543 (red line). The 3 dimensional positions are shown; horizontal x and y local coordinates and height z position in cm

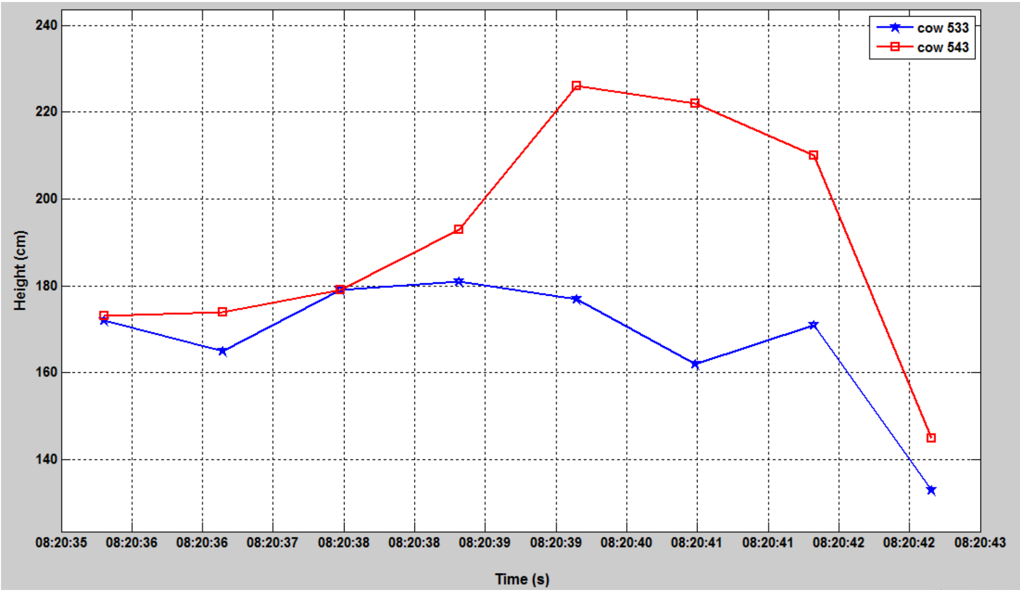


Figure 5.3 Graph complementing Figure 5.2 showing only the height changes in sequence of mounting as cow 533 (blue line) stood to be mounted by cow 543 (red line) in real-time, with time in the x axis and height to the nearest cm on y axis

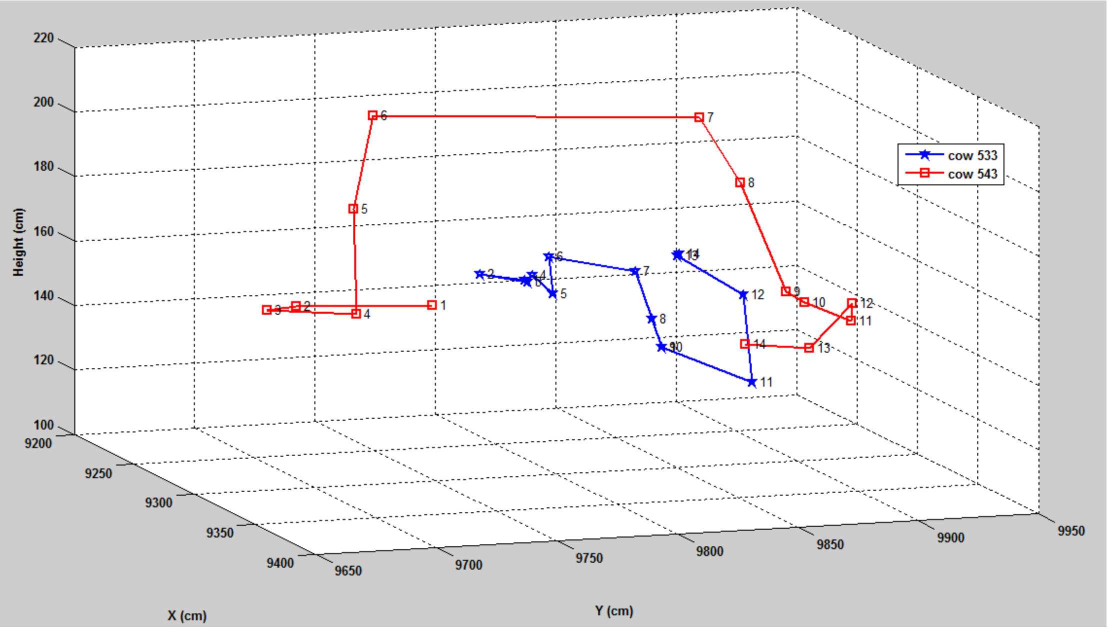


Figure 5.4 Graph showing a second episode of mounting between 2 cows over 14 numbered time points. This demonstrates the sequence in time as cow 533 (blue line) stood to be mounted by cow 543 (red line). The 3 dimensional positions are shown; horizontal x and y local coordinates and height z position in cm

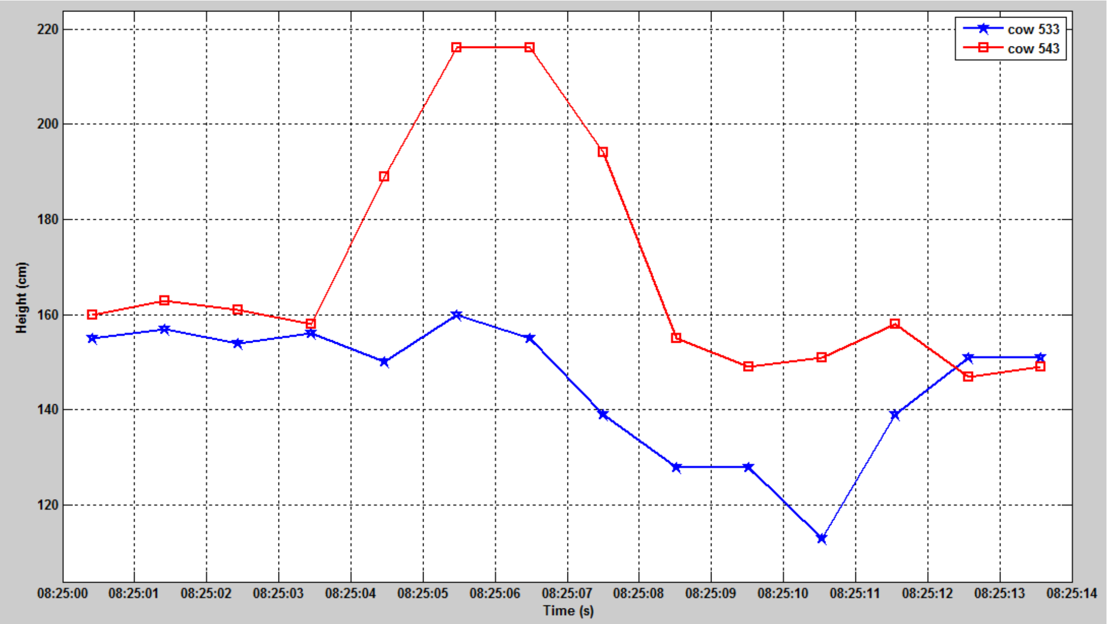


Figure 5.5 Graph complementing Figure 5.4, showing only the height changes in the sequence of mounting as cow 533 (blue line) stood to be mounted by cow 543 (red line) in real-time on x axis and height on y axis, to the nearest cm

Table 5.1 Results from POC 2 showing each cow's mounting behaviour identified from the UWB data, expressed as a percentage of the total mounts by visual observation

Cow Number	Total Number of Mounts	Identified Mounts[a]	Not identified[b]	UWB Error[c]	Percentage Accuracy[d], %
48	20	14	3	3	82.4
539	129	81	20	28	80.2
320	78	65	11	2	85.5
323	15	11	1	3	91.7
611	28	23	4	1	85.2
516	0	0	0	0	100.0
292	0	0	0	0	100.0
620	0	0	0	0	100.0
TOTAL	**270**	**194**	**39**	**37**	**83.3**

[a]Number of visually observed mounts identified in the UWB data
[b]Number of visually observed mounts not identified in the UWB data
[c]Error by UWB, where mounts were undetected; missing data, poor signal quality or UWB unit off
[d]Percentage accuracy = identified mounts/(total number of mounts – UWB error)*100

Table 5.2 Results from POC 3 showing each cow's mounting behaviour identified from the UWB data, expressed as a percentage of the total mounts by visual observation

Cow Number	Total Number of Mounts	Identified Mounts[a]	Not identified[b]	UWB Error[c]	Percentage Accuracy[d], %
1	37	34	1	2	97.1
123	58	39	5	14	88.6
313	17	15	2	0	88.2
623	37	28	8	1	77.8
424	37	27	8	2	77.1
127	1	1	0	0	100.0
38	2	2	0	0	100.0
106	0	0	0	0	100.0
TOTAL	**189**	**146**	**24**	**19**	**85.9**

[a]Number of visually observed mounts identified in the UWB data
[b]Number of visually observed mounts not identified in the UWB data
[c]Error by UWB, where mounts were undetected; missing data, poor signal quality or UWB unit off
[d]Percentage accuracy = identified mounts/(total number of mounts – UWB error)*100

Daily mounting activity determined by the optimised script is reported in Tables 5.5 and 5.6. These results showed that the automated script analysis declared mounting on all days of the trial where mounting only occurred on one day in POC 2 and over 2 days in POC 3. 97 out of 173 declared mounts were visually observed mounts during POC 2 on one day when cows were in oestrus and 62 out of 117 declared mounts were visually observed mounts during POC 3 spanning 2 days when cows exhibited oestrous behaviour. These results again suggested 50% error in the accuracy of the script. In Tables 5.5 and 5.6, however, an important pattern to note is that the level of error was increased on the days when cows were in oestrus compared to days of no oestrus activity. Furthermore the number of erroneously declared mounts by the script was substantially increased for cows in oestrus compared to those not in oestrus.

Results of the automated analysis of UWB data are reported in Table 5.7 to detect cows that are in oestrus rather than individual events of mounting and standing to be mounted. Oestrous data was reported by cow number and in order of date and real-time of onset of mounting. Onset of standing to be mounted was also reported. The results show that 9 out of 10 cows were correctly identified in oestrus by the script; confirmed by visual observation, milk progesterone concentration of <1ng/ml (Figure 5.7 and 5.8) and increased activity at oestrus (Figure 5.10 and 5.11). One cow in oestrus could not be identified by UWB automated analysis however these results were discounted from analysis as this was an effect of the script design not due to UWB error. Therefore 9 out of 9 cows were correctly detected in oestrus. Importantly 4 control pregnant cows were correctly identified as not in oestrus; confirmed by visual observation, high progesterone concentration >10ng/ml (Figure 5.7 and 5.8), and no activity increase from baseline (Figure 5.10 and 5.11). Two cows that were synchronized to come into oestrus but did not show any overt signs of oestrus were also identified not in oestrus by UWB automated analysis. These cows had no increase in activity but progesterone concentration was low, which may indicate a silent oestrus.

Table 5.3 Efficiency and accuracy of the optimised script at identifying mounts from POC 2 on day of oestrus.

Cow Number	539	320	323	611	48	516	292	620	Total
Total number of mounts[a]	41	70	11	25	0	0	0	0	147
Total mounts identified by the script[b]	29	89	18	36	0	0	0	1	173
Identified mounts[c]	20	50	7	20	0	0	0	0	97
Not identified mounts[d]	3	6	0	2	0	0	0	0	11
Out of boundary[e]	6	11	0	2	0	0	0	0	19
UWB error[f]	12	3	4	1	0	0	0	0	20
Matching Rate*, %	**69.0**	**74.6**	**100.0**	**83.3**	**-**	**-**	**-**	**-**	**76.4**
Script Accuracy*, %	**69.0**	**56.2**	**38.9**	**55.6**	**-**	**-**	**-**	**-**	**56.1**

Table 5.4 Efficiency and accuracy of the optimised script at identifying mounts from POC 3 on days of oestrus

Cow Number	1	123	313	424	623	127	38	106	Total
Total number of mounts[a]	24	51	6	7	27	0	2	0	117
Total mounts declared by the script[b]	24	37	14	14	26	0	5	1	121
Identified mounts[c]	15	27	4	1	13	0	2	0	62
Not identified mounts[d]	0	5	1	1	3	0	0	0	10
Out of boundary[e]	6	4	0	4	6	0	0	0	20
UWB error[f]	3	15	1	1	5	0	0	0	25
Matching Rate, %*	**71.4**	**75.0**	**80.0**	**53.9**	**50.0**	**-**	**100.0**	**-**	**67.4**
Script Accuracy, %*	**62.5**	**73.0**	**28.6**	**14.3**	**59.1**	**-**	**40.0**	**-**	**51.2**

[a]By visual observation, the actual number of mounts that occurred
[b]Total number of mounts declared by the algorithm and script from the relative positioning of the cows, noting the increased number of false positives causing erroneous declaration of mounting
[c]Mounts identified by the script that match with mounts identified by visual observations
[d]Mounts observed but not identified by the script
[e]Coordinates for the positions were outside of the boundaries set by the script, but these mounts were determinable from the raw data
[f]Error resulting from the UWB system, resulting in mounts unable to be identified; either through missing data, units turned off or from poor signal quality
*Measure of the efficiency and accuracy a) script matching rate is correctly identified mounts/ total number of visual observations minus UWB error (=c/(a-f)*100) b) script accuracy is calculated as a percentage of the total number of correctly identified mounts/ total number of mounts according to the script (including false positives) (=c/b*100)

Automated analysis of oestrous behaviour provided quantitative information about oestrus (Table 5.8). Average duration of oestrus as determined by UWB was 8.4 hours mounting and 8.9 hours standing to be mounted, with a strong positive correlation (r=0.88) between these behaviours for individual cows. These results also show that mounting began before standing behaviour and ended earlier. Total duration of oestrus differed between cows and ranged from 2.0 hours to 14.0 hours, averaging 10.7 hours. Duration of oestrus reported by UWB is shorter than the average duration of sustained increased activity, 12.6 hours. However the duration of increased activity is more consistent between individual cows ranging from 10 to 16 hours, but maximum activity at oestrus had a wide range from 67 to 133 activity units. Duration of increased activity and maximum activity bore no relationship to each other (r=-0.14), nor did maximum activity at oestrus and duration of total oestrus (r=-0.45).

5.3.1 Milk Progesterone Concentration Profiles

The daily progesterone concentrations in milk throughout the trials are reported in Figures 5.6, 5.7 and 5.8, from POC 1, POC 2 and POC 3, respectively. In all cows synchronised with CIDRs the progesterone concentration increased upon insertion and declined steadily thereafter, declining rapidly upon CIDR removal to <1ng/ml. Progesterone concentration remained basal, <1ng/ml, in all cows that displayed oestrus and began to rise steadily indicating that a CL was present. In synchronised cows that did not display oestrus, progesterone concentration remained low, which could indicate a silent oestrus. All non-pregnant cows eligible for oestrus in POC 1 (Figure 5.6) had increasing progesterone concentrations >3ng/ml suggesting that they were not in oestrus and entering the luteal phase of the oestrous cycle. Pregnant control cows from all 3 trials had consistently high progesterone concentration, above 10ng/ml, which fluctuated as expected.

5.3.2 Activity Data

Activity profiles are reported in Figures 5.9, 5.10 and 5.11 corresponding to the activity of all cows throughout each trial, POC 1, POC 2 and POC 3, respectively. For all cows that did not show oestrus activity remained at basal levels, as shown in the scatter graph, without any obvious peak. Activity levels fluctuated daily in each 2 hour period, but with no deviation from the normal distribution of activity. When comparing the graphs of no oestrus and oestrus it is clear to see that for each cow displaying overt oestrus (standing and mounting behaviour; confirmed by visual observation) there was a definite peak of increased activity above baseline. It is also clear to see that each cow had a different level of activity increase at oestrus ranging from 67 to 133 activity units (Table 5.8). Synchronised cows that did not display oestrus but had low progesterone concentration did not have a peak in activity.

It is also worth noting several points of increased activity that were not associated with oestrus. A number of the small peaks were related to routine management tasks such as foot trimming (Figure 5.11; small peaks noticeable for all 8 cows nearing the end of the trial) and handling of cows at the beginning of trials when backpack training.

Table 5.5 Number of mounts declared by the script per day from POC 2

Cow Number	**48**	**539**	**320**	**323**	**611**	**516**	**292**	**620**	**Total**
Monday	1	1							**2**
Tuesday				1					**1**
Wednesday		1			1				**2**
Thursday		29	89	18	36			1	**173(97)***
Friday	3	2					7		**12**
Saturday				1		1			**2**
Sunday						1	2		**3**

NB Cow 539, 320, 323 and 611 were all in oestrus on Thursday

Table 5.6 Number of mounts declared by the script per day in POC 3

Cow Number	**1**	**123**	**313**	**424**	**623**	**127**	**38**	**106**	**Total**
Monday				1			1		**2**
Tuesday	2	1	1		1	1	4		**10**
Wednesday	1	3		2	1	4	1	3	**15**
Thursday	24	30		14	10		2	1	**81(42)***
Friday		7	14		16		3		**40(20)***
Saturday				5	1		1	2	**9**
Sunday	2			3	2		2		**9**

NB Cow 1, 123, 313, 424 and 623 were all in oestrus on either Thursday or Friday or both days
*Total number of mounts detected, followed by the actual number on that day in brackets

Table 5.7 Cows detected in oestrus in real-time by the automated script in order of date and time of onset of mounting., Onset of standing to be mounted for cows in oestrus is reported in real-time which could be used to identify the time for AI for optimal conception rates.

Cow Number	Date	Time of Onset, mounting	Time of Onset, standing to be mounted
323	19/01/2012	01:31:10	02:46:35
539	19/01/2012	01:38:08	02:51:47
320	19/01/2012	02:46:35	08:04:46
611	19/01/2012	09:14:57	09:34:57
424	22/03/2012	06:20:38	06:29:42
1	22/03/2012	06:29:42	09:56:29
123	22/03/2012	13:58:39	14:05:29
623	22/03/2012	18:47:43	22:38:59
313	23/03/2012	09:57:51	11:12:08

Table 5.8 Comparison of oestrus; maximum activity, duration of increased activity, duration of standing to be mounted, duration of mounting and maximum activity

Cow Number	Maximum Activity at Oestrus	Duration of Sustained Activity Increase, hours*	Duration of Mounting by UWB, hours**	Duration of Standing to be Mounted by UWB, hours**	Total Duration of Oestrus by UWB, hours***
1	67	16	11.5	10.0	13.5
48	85	12	-	-	-
123	-	-	9.0	11.5	11.5
313	90	10	1.5	1.0	2.0
320	86	14	10.5	8.0	13.0
323	87	14	10.5	13.0	14.0
424	83	12	9.5	11.0	11.0
533	133	10	-	-	-
534	119	10	-	-	-
535	102	16	-	-	-
539	85	14	9.5	11.0	13.0
543	69	16	-	-	-
611	83	10	4.5	5.0	5.0
623	67	10	9.0	9.5	13.5
Average	**88.9**	**12.6**	**8.4**	**8.9**	**10.7**

*Duration of sustained increased activity from baseline over the period of oestrus
**Duration rounded to the nearest half hour from 1st mounting/ standing episode to last mounting/ standing episode as recorded by UWB
***Duration of oestrus from first episode of mounting or standing to last episode of standing or mounting, rounded to the nearest half hour

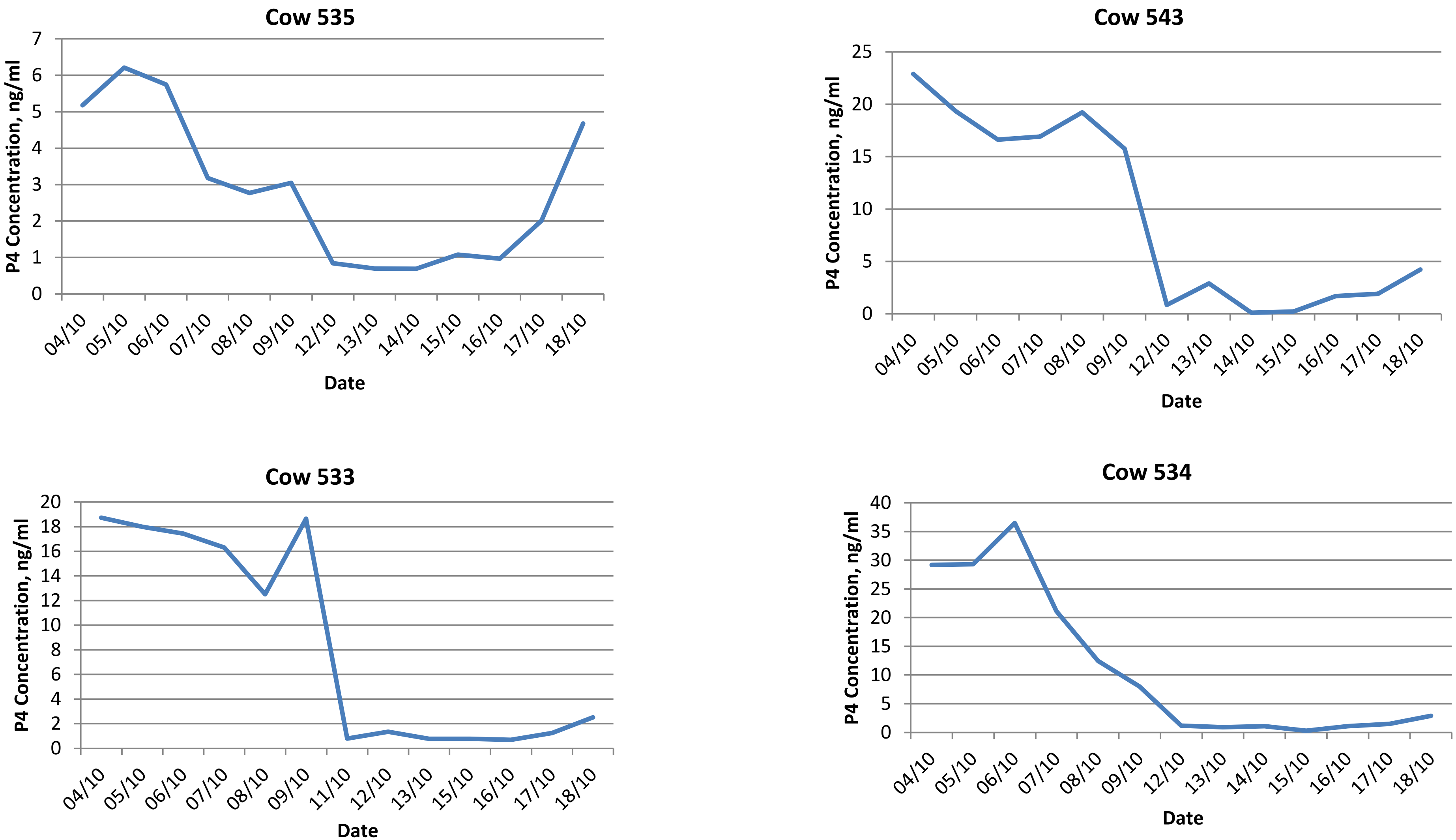


Figure 5.6 Milk progesterone profiles from POC 1 of cows in oestrus. Progesterone concentration (ng/ml) plotted against date; cow 535, 543, 533 and 534 were all synchronised to come into oestrus shown by high progesterone concentration which declines thereafter, showing when the cow is in a physiological state to enter into oestrus

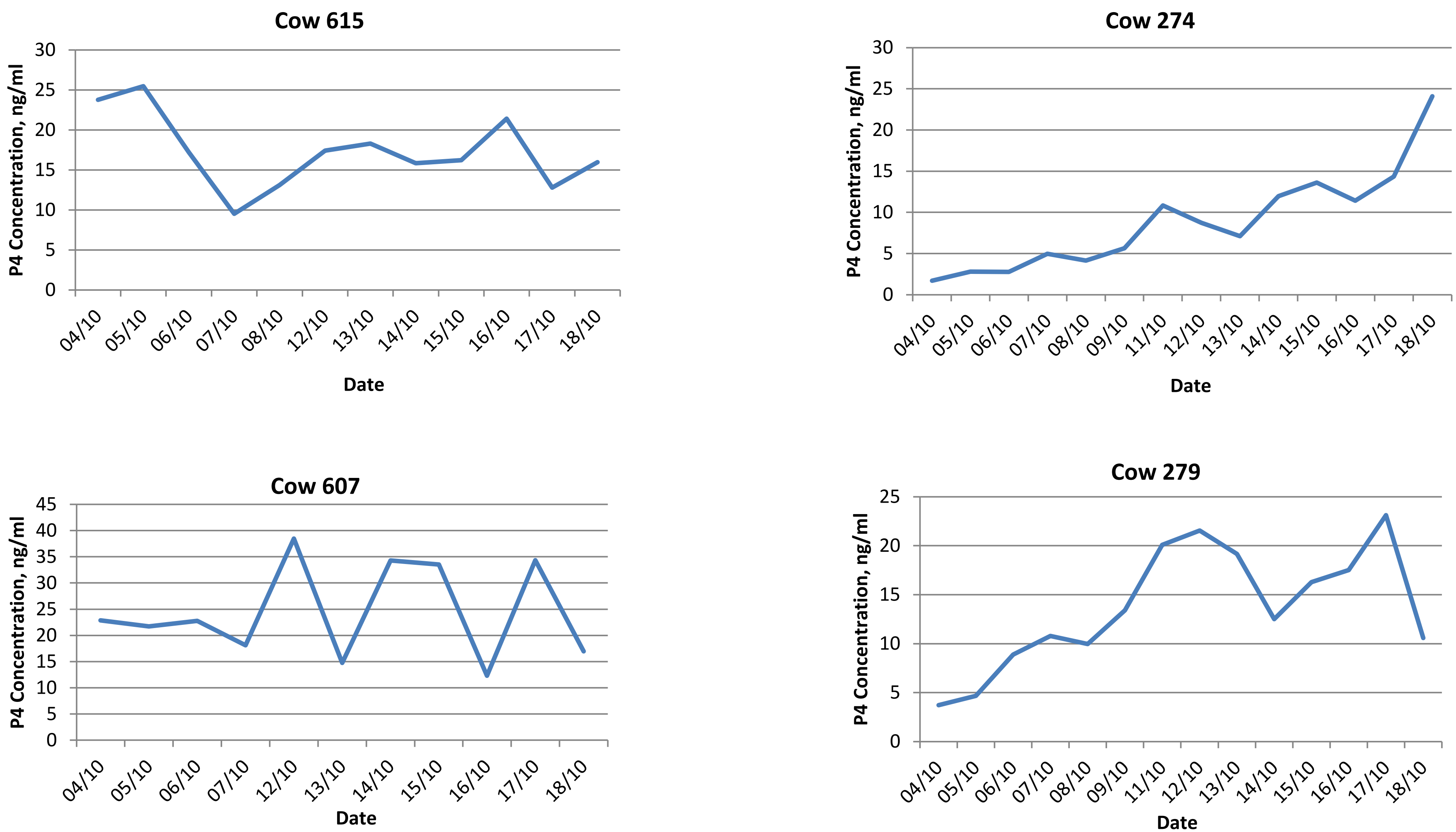


Figure 5.6 (Cont.) Milk progesterone profiles from POC 1 of cows that did not come into oestrus, progesterone concentration (ng/ml) plotted against date; cows 615 and 607 are both pregnant cows with high progesterone cows, and cows 274 and 279 are entering into the luteal phase with increasing progesterone concentration post oestrus known from records prior to the trial)

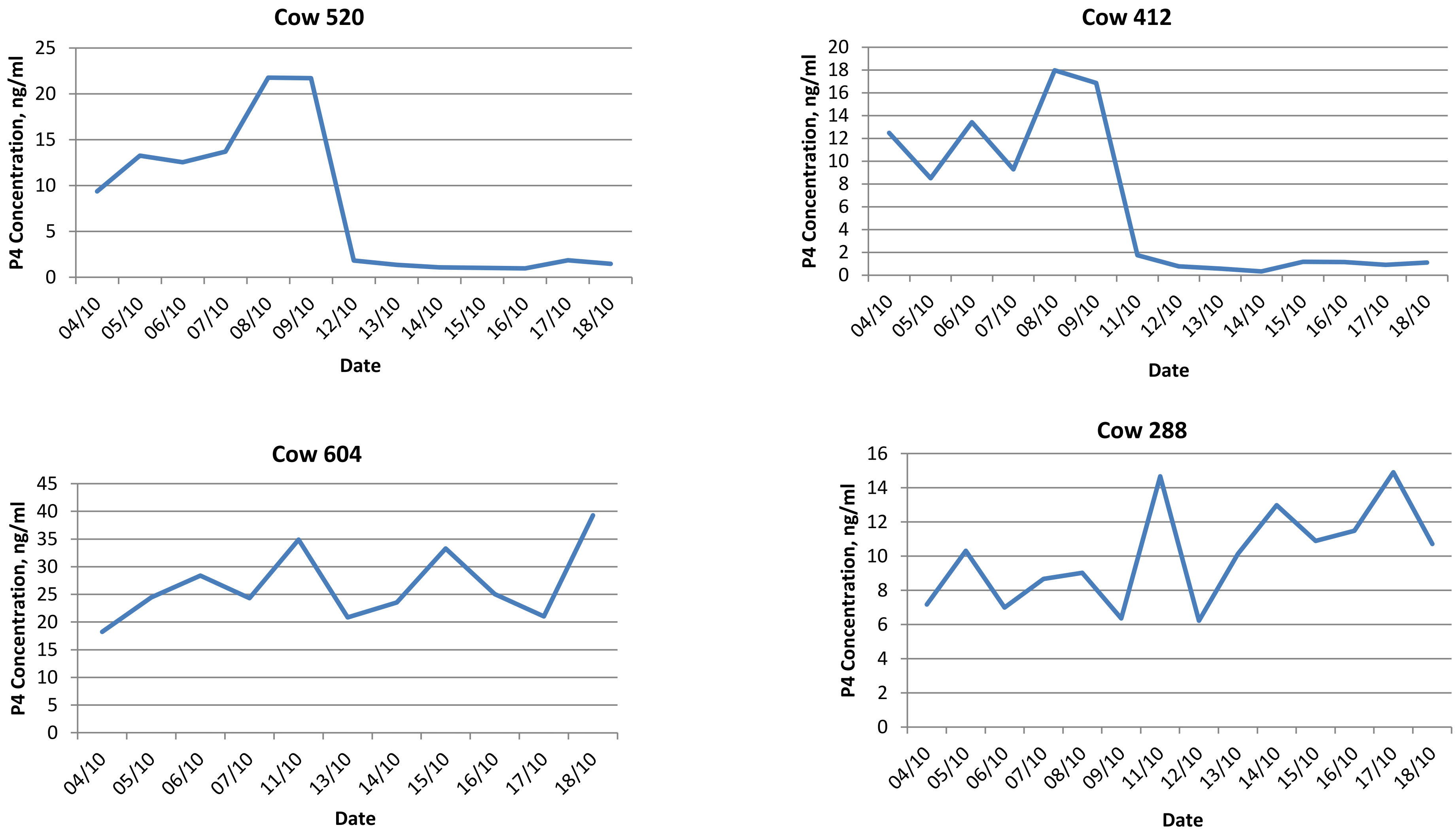


Figure 5.6 (Cont.) Milk progesterone profiles from POC 1 of cows that did not come into oestrus, progesterone concentration (ng/ml) plotted against date; cows 412 and 520 were both synchronised but did not come into oestrus, cow 604 is pregnant with high progesterone and cow 288 is an open cow (not pregnant), but is in the luteal stage of the oestrous cycle as shown by high progesterone concentration and records prior to trial

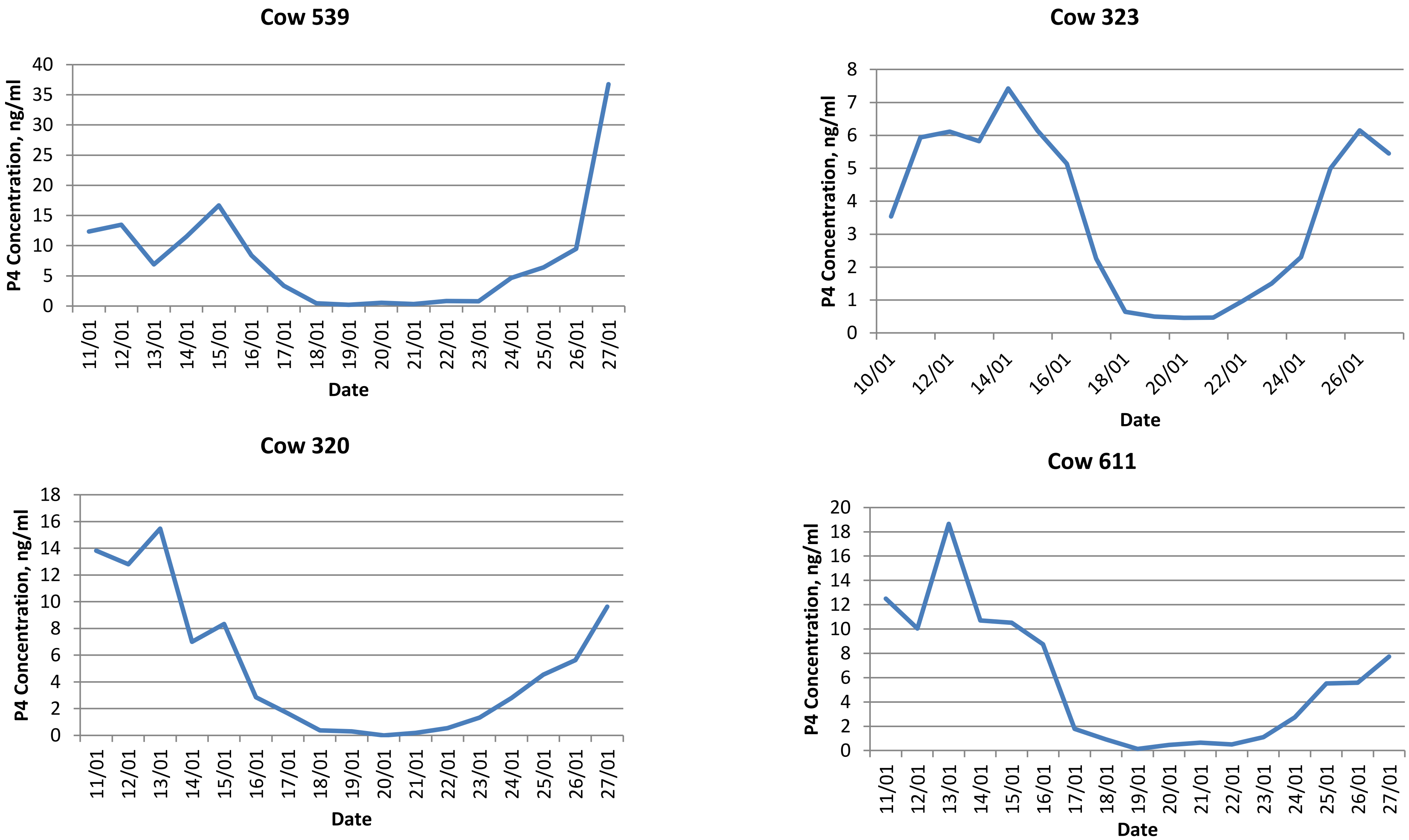


Figure 5.7 Milk progesterone profiles from cows in POC 2, progesterone concentration (ng/ml) plotted against date; cows 539, 323, 320 and 611 were all synchronised and came into oestrus

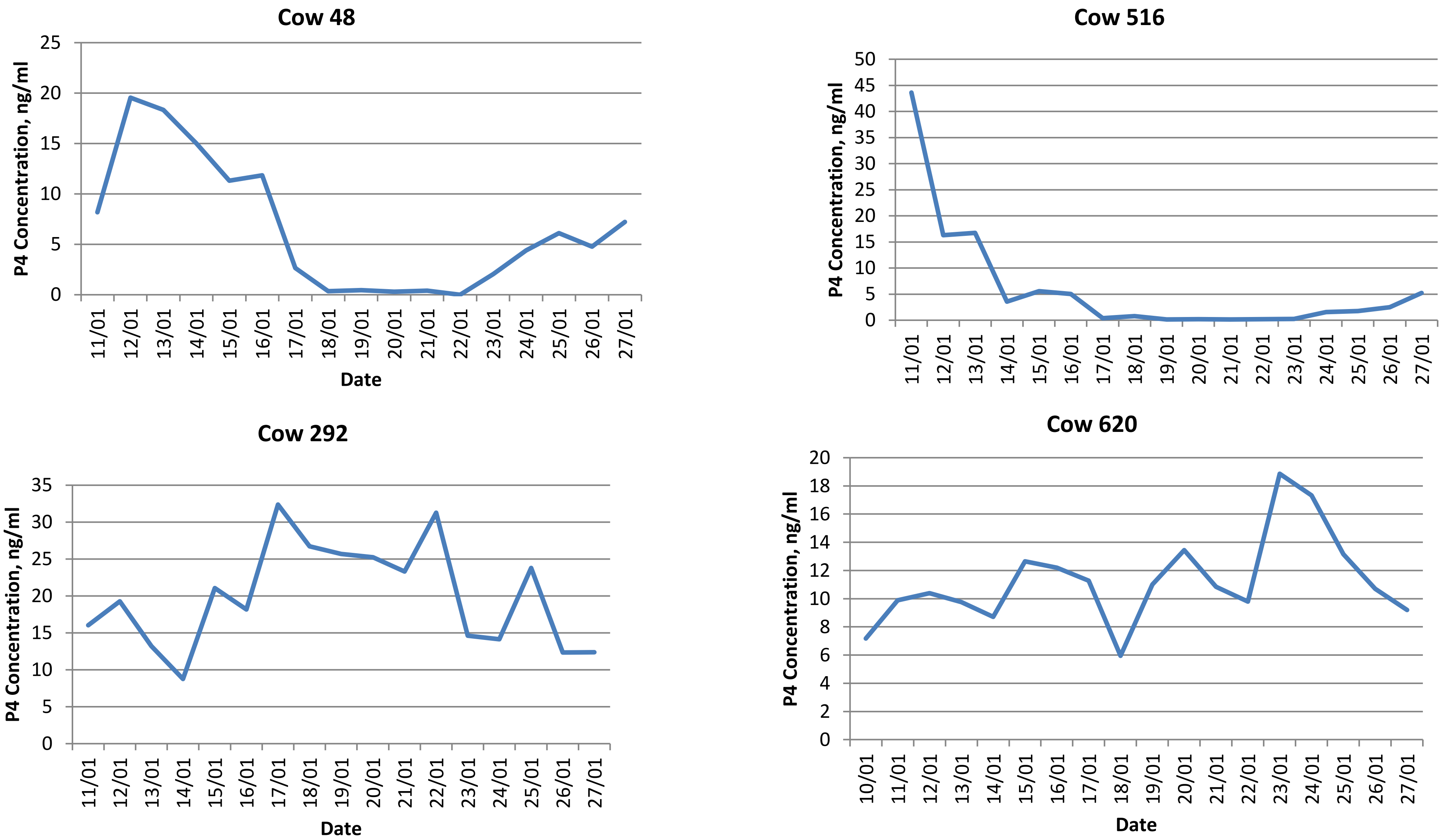


Figure 5.7 (Cont.) Milk progesterone profiles of cows in POC 2, progesterone concentration (ng/ml) plotted against date; cow 48 was synchronised and came into oestrus, cow 516 was synchronised but did not show oestrus, and cows 292 and 620 are pregnant controls with high progesterone concentration.

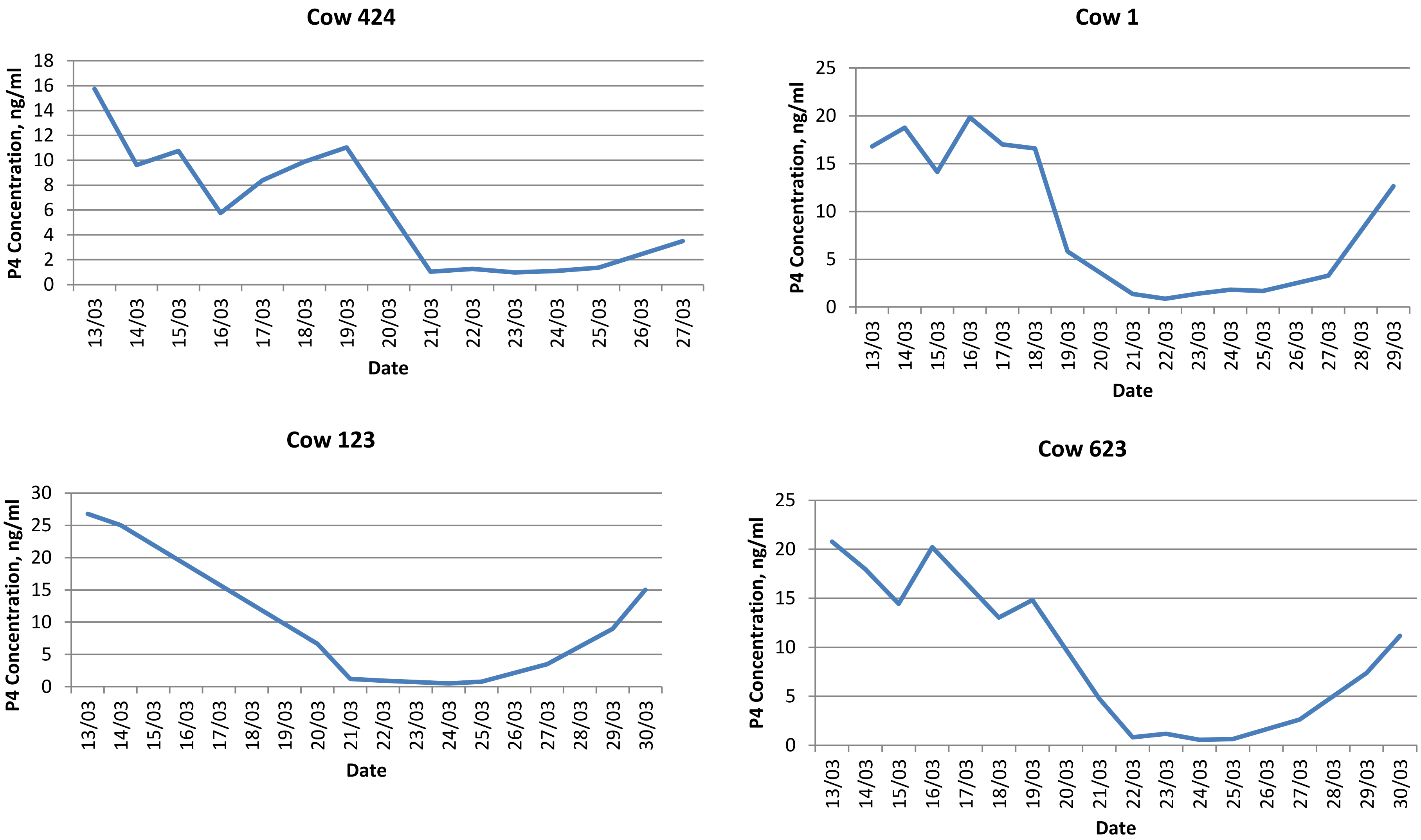


Figure 5.8 Milk progesterone profiles from cows in POC 3, progesterone concentration (ng/ml) plotted against date; cows 424, 1, 123 and 623 were synchronised and came into oestrus.

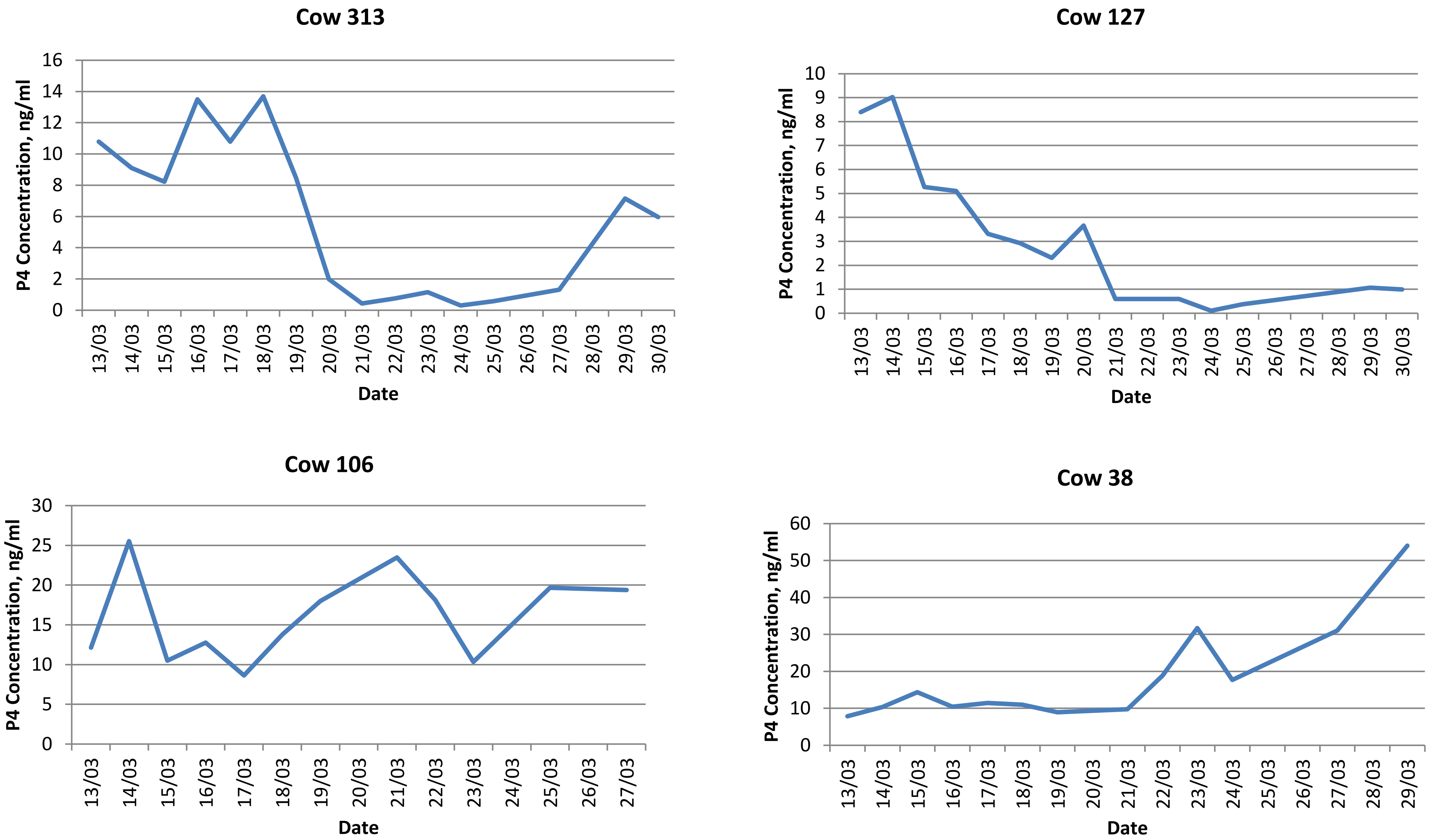


Figure 5.8 (Cont.) Milk progesterone profiles from cows in POC 3, with progesterone concentration (ng/ml) plotted against date; cow 313 was synchronised and came into oestrus, cow 127 was synchronised but did not come into oestrus, and cows 106 and 38 were both pregnant cows with high progesterone concentration throughout.

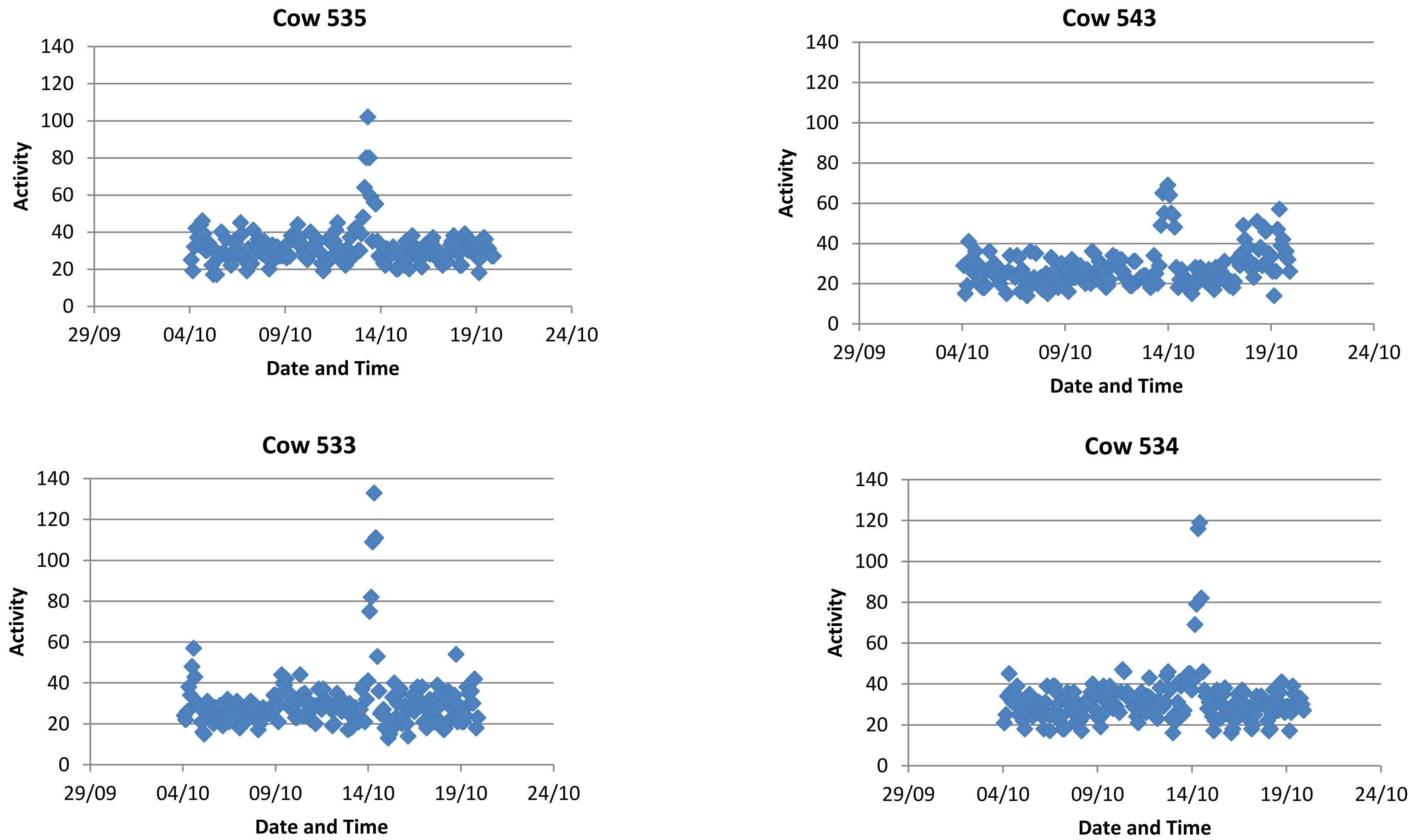


Figure 5.9 Activity graphs from POC 1 showing activity plotted against date and time; cows 535, 543, 533 and 534 came into oestrus during the trial and have a clear peak of activity from baseline activity

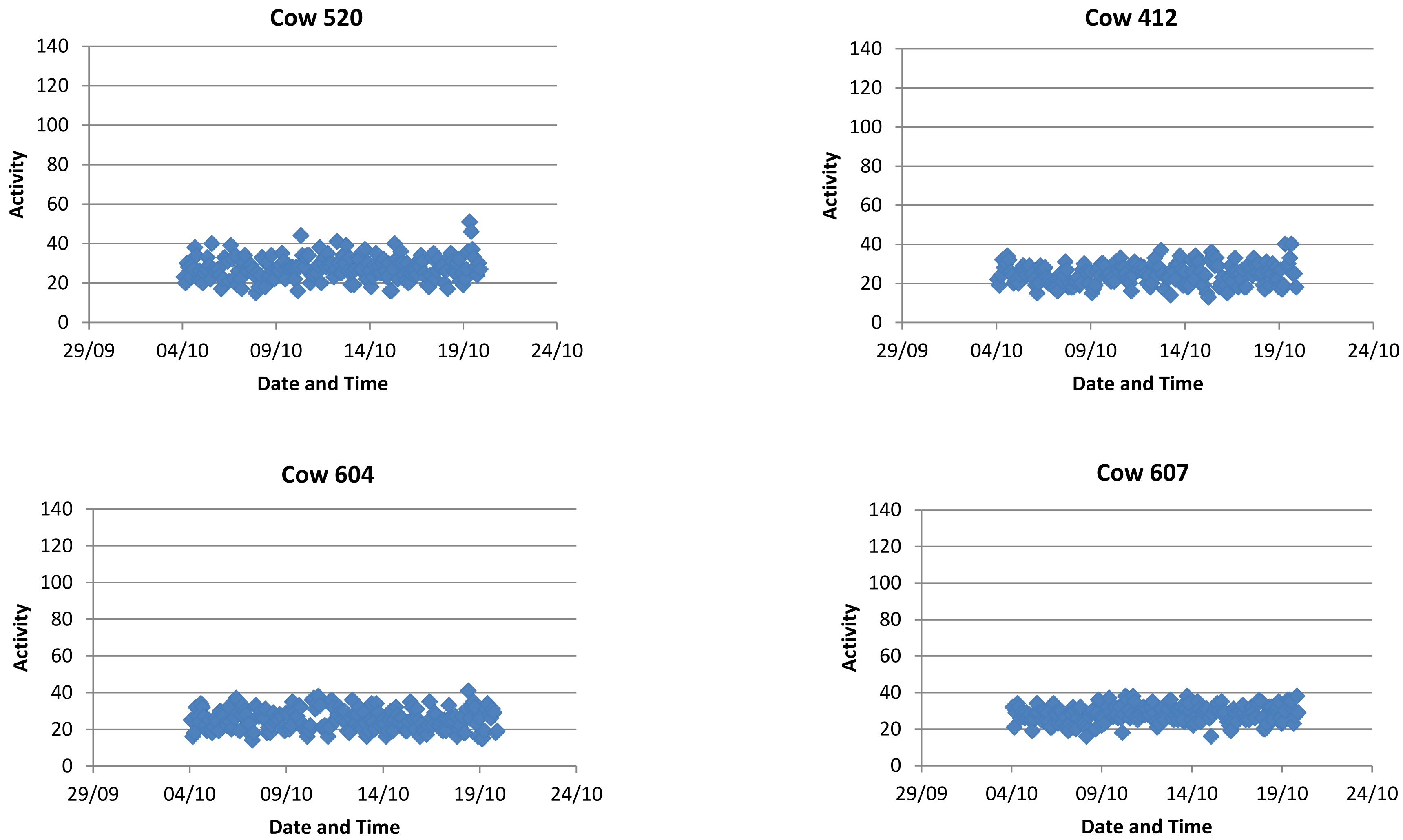


Figure 5.9 (Cont.) Activity graphs from POC 1 showing activity plotted against date and time; cows 520, 412, 604 and 607 did not come into oestrus as shown by no peak in activity from baseline

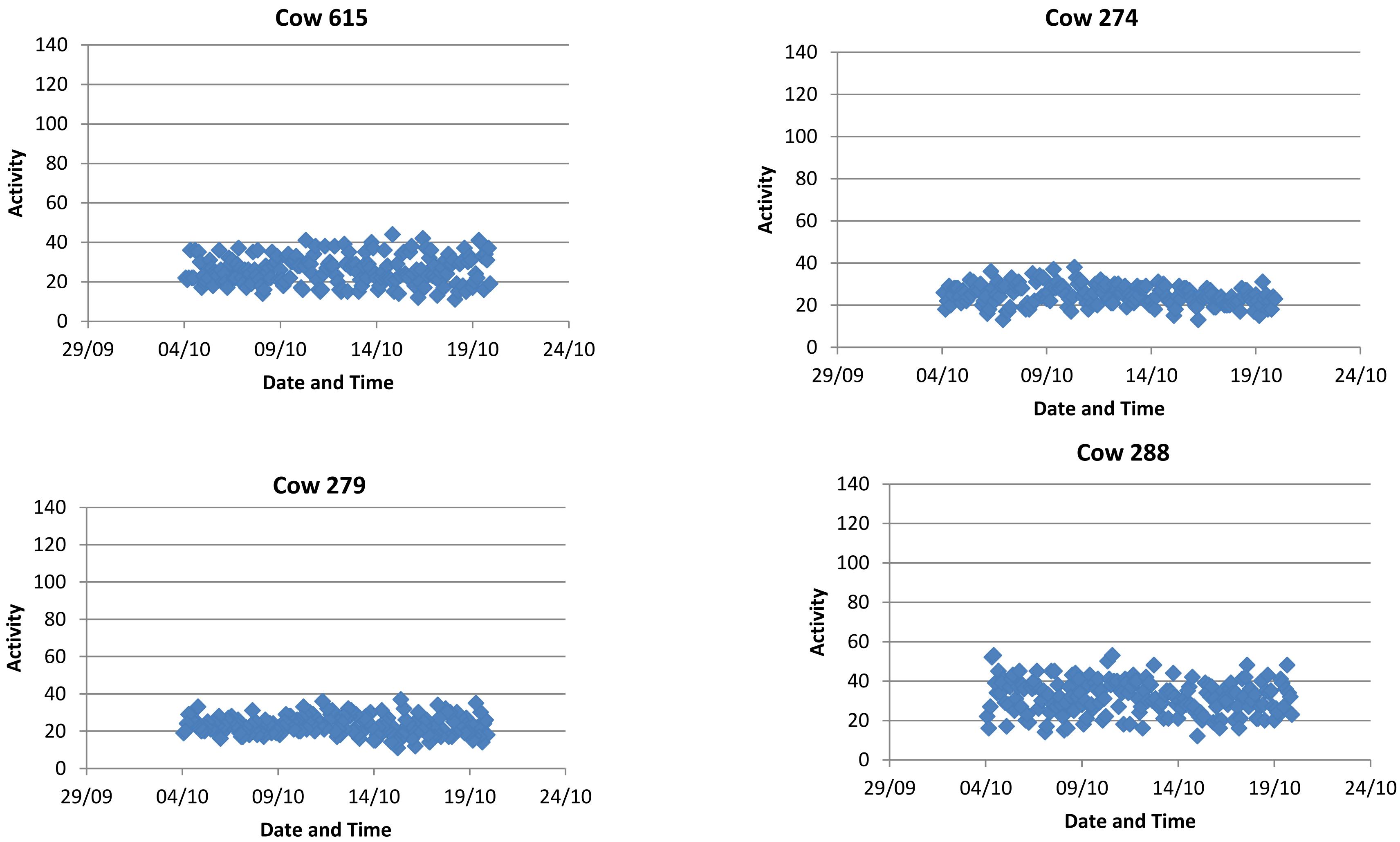


Figure 5.9 (Cont.) Activity graphs from POC 1 showing activity plotted against date and time; cows 615, 274, 279 and 288 did not come into oestrus and have no peak in activity from baseline

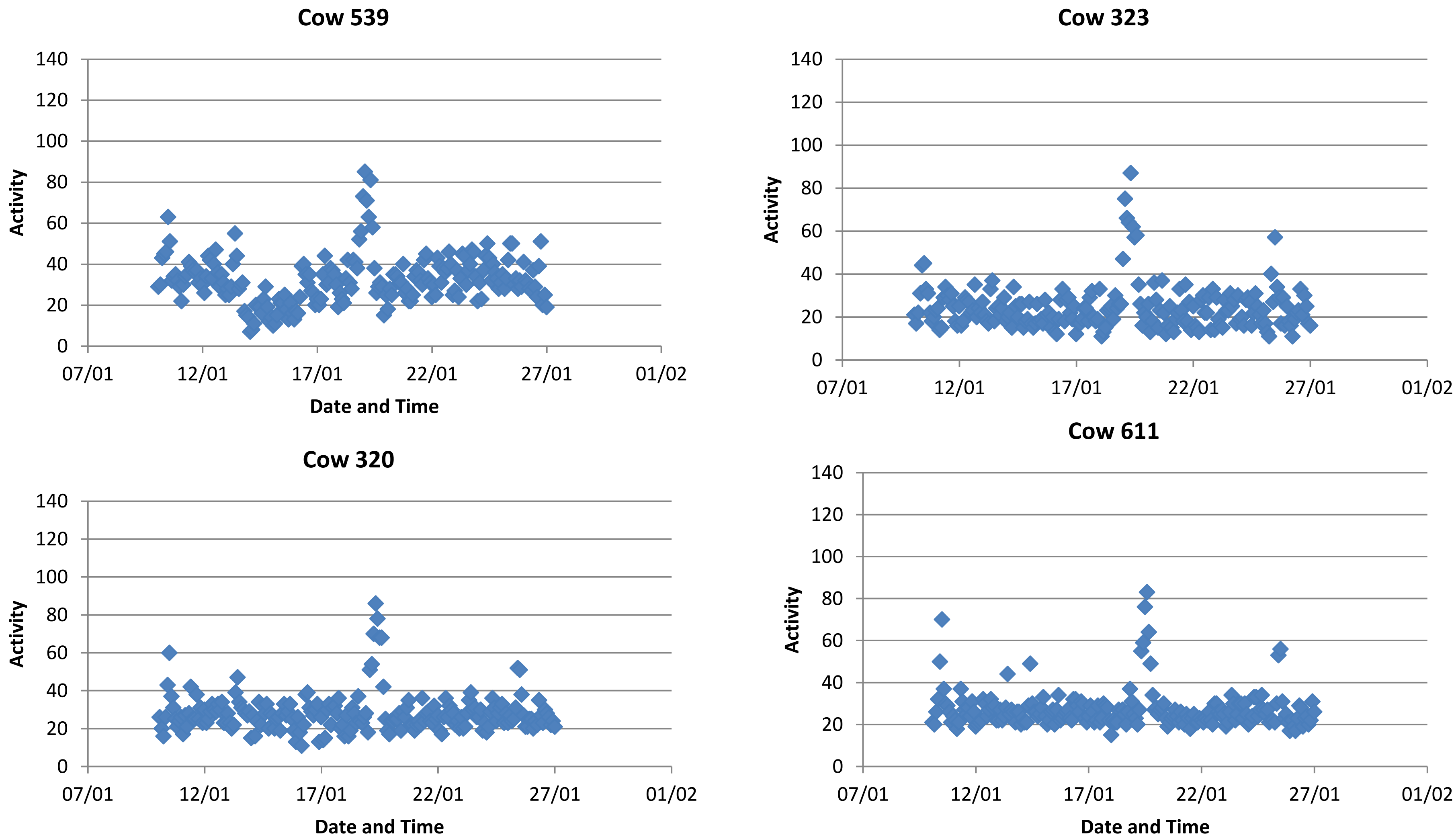


Figure 5.10 Activity graphs from POC 2 showing activity plotted against date and time; cows 539, 323, 320 and 611 all came into oestrus during the trial and have a clear peak compared with baseline activity, several initial peaks correspond with initial backpack training of the cows and smaller peaks towards the end of the trial are due to routine management - foot trimming.

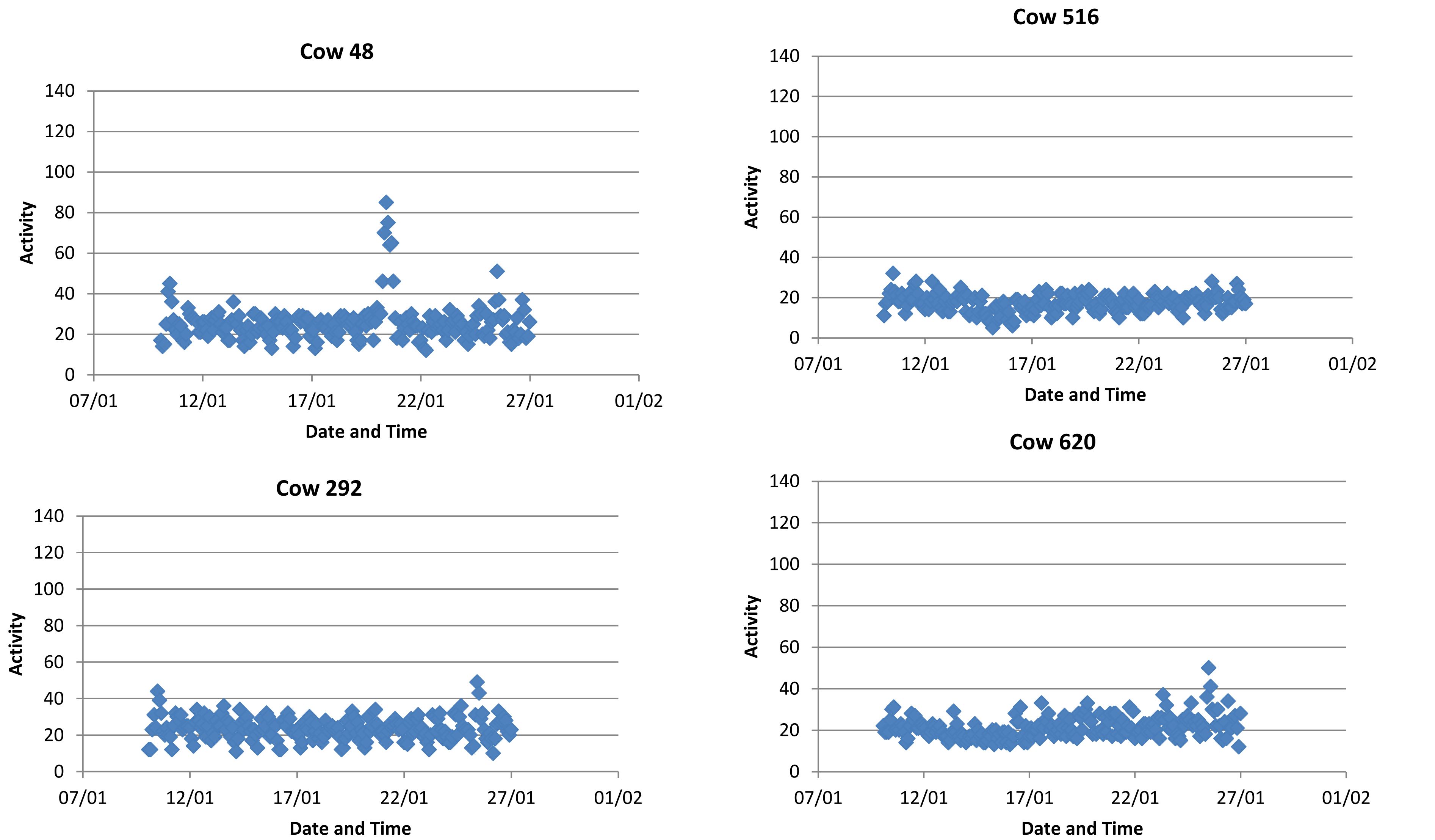


Figure 5.10 (Cont.) Activity graphs from POC 2 showing activity plotted against date and time; cow 48 came into heat as shown by a clear peak and cows 516, 292 and 620 did not come into oestrus, with no definite oestrus peak in activity, several initial peaks correspond with initial backpack training of the cows and smaller peaks towards the end of the trial are due to routine management - foot trimming.

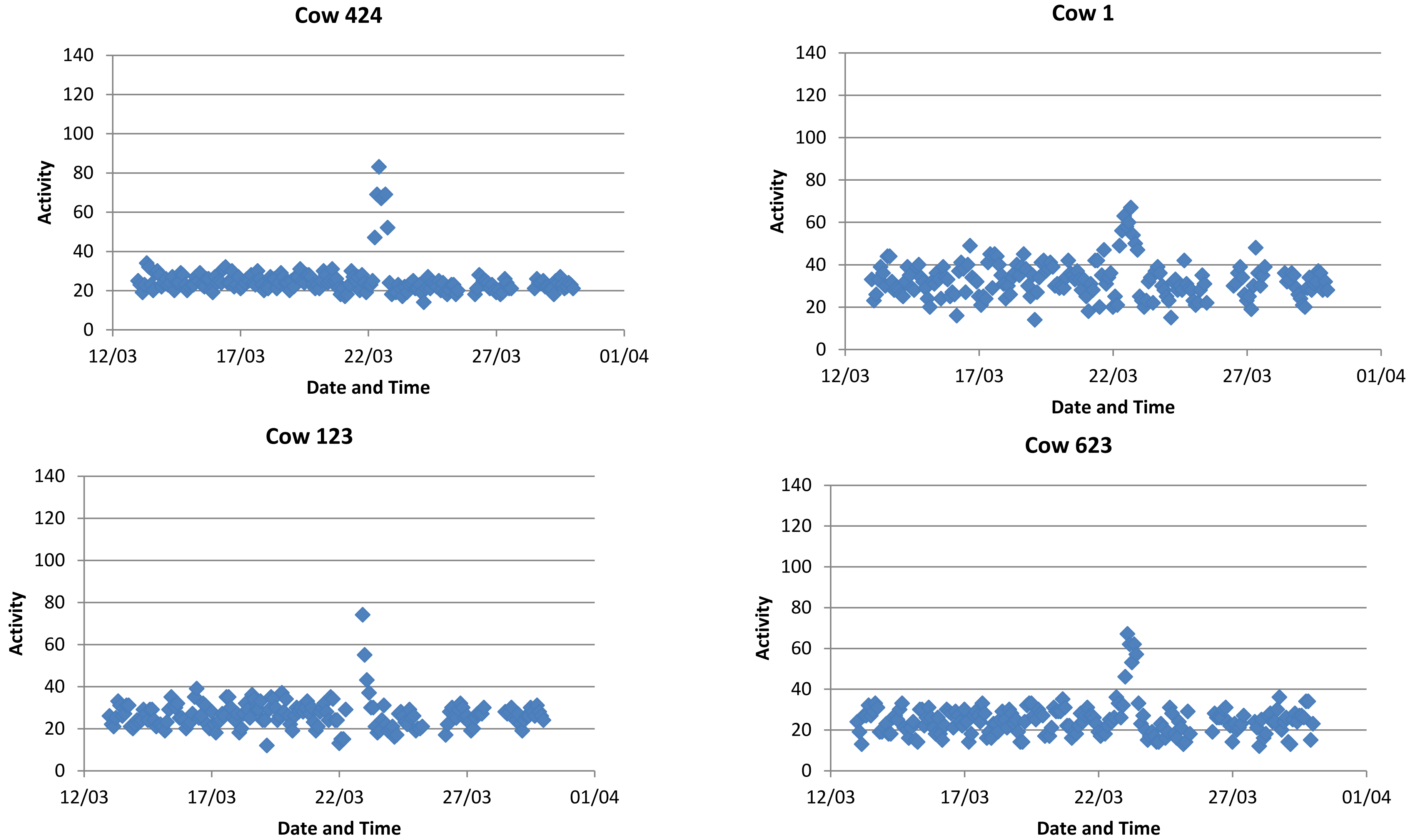


Figure 5.11 Activity graphs from POC 3 showing activity plotted against date and time; cows 424, 1, 123 and 623 all came into oestrus during the trial as shown by a clear peak in activity compared with baseline activity.

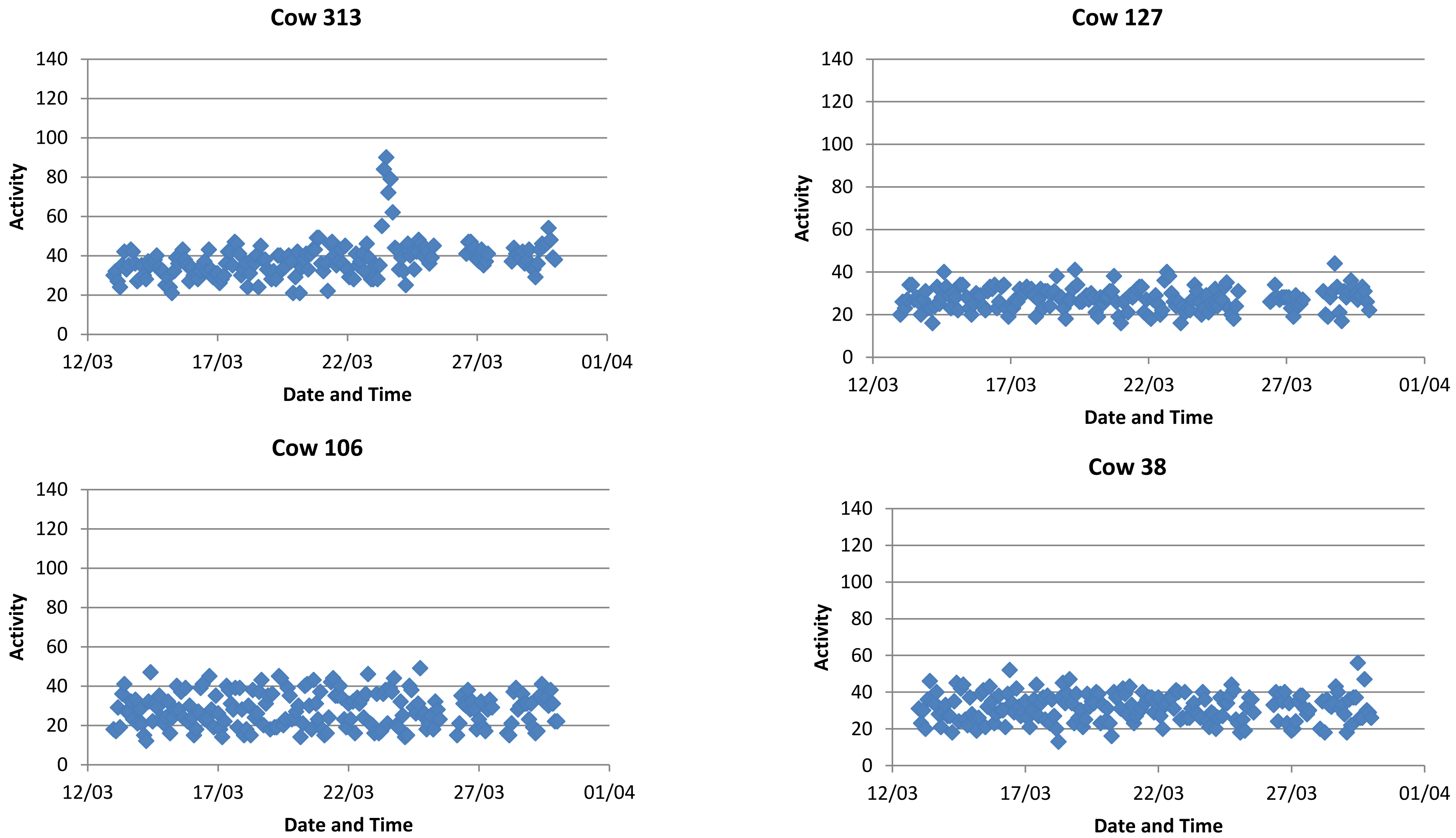


Figure 5.11 (Cont.) Activity graphs from POC 3 showing activity plotted against date and time; cow 313 came into oestrus as shown by a clear peak in activity, but cows 127, 106 and 38 did not show oestrus.

5.4 DISCUSSION

The aim of this work was to develop a novel method of oestrous detection in order to improve oestrous detection rates and hence improve herd productivity. This study investigated using positioning technology to demonstrate proof of concept that UWB could detect oestrus. The objectives were to demonstrate: i) identification of cows approaching oestrus; cows mounting other cows in oestrus; cows standing to be mounted, by monitoring their position in real-time, ii) develop techniques to analyse data such that this information could be used in a commercial situation together with the use of physiological information to determine stage of the oestrous cycle and iii) to develop associated communications technology to be able to monitor a herd of cows and detect cows in oestrus automatically.

The data presented here supports the proof of concept that UWB can be used as a method of oestrous detection. Here it is reported that both cows coming into oestrus and cows in oestrus can be identified in real-time, where oestrous behaviour can be distinguished from the normal behaviour of non-oestrus cows. This was confirmed by visual observation, milk progesterone concentration and activity monitoring, which all serve as methods of oestrous detection for comparison.

5.4.1 UWB

UWB accurately measured 3 dimensional positioning of cows when monitoring was continuous. The interactions been 2 cows in 3 dimensions could be recorded and the height increases when mounting occurs during oestrus could be identified in real-time (Figures 5.2 to 5.5). UWB provides a high degree of horizontal accuracy, where vertical accuracy and changes in height (such as those that occur during mounting) have been difficult to achieve. This is because the physical geometry of BU networks provide many horizontal observations, from one side of the dairy barn to the other in both X and Y dimensions, but the vertical observations are all from overhead with none available from below. Therefore there are not as many observations in the Z dimension and thus it is more difficult to achieve as high accuracy in the vertical dimension (the same principle applies to GPS satellite positioning, with all satellites visible being overhead). Furthermore, vertical signals are thus more at risk of obstruction, and reflection is more common, affecting accuracy of precision. However, this work using UWB has demonstrated centimetre accuracy in the Z axis; averaging 10cm, but often being even more precise (<10cm, see Chapter 4).

Previous reports of UWB accuracy have been reported to a 'fraction of a metre' in indoor and harsh environments, such as the dairy barn, with approximate 30cm accuracy in the height component (Ingram, 2006). This study reports the changes in MU height occurring during mounting from approximately 170cm to 230cm (Figure 5.3) and 160cm to 220cm (Figure 5.5). Therefore the achievable accuracy in the dairy barn far exceeds the precision needed to monitor oestrous behaviour. UWB is capable of recording position related to oestrous behaviour as demonstrated by the results of POC 1. However, during POC 1 there were several limitations with data collection caused mainly by failing battery power. This resulted in discontinuity of data collection, poor quality data and resulted in ending the trial prematurely. The data set for oestrus was thus incomplete and individual mounting information per cow could not be determined. POC 2 and POC 3 complemented the initial findings of POC 1, and with changes to experimental procedures and equipment a complete continuous set of UWB recorded positions was collected over each week long trial period, which could be used for automated analysis.

Analysing the UWB raw data and comparing against visually recorded mounts demonstrated that the matching rate was >80%, which shows that a high proportion of mounts and cow positions were recorded by the UWB network. A small proportion of mounts were undetectable due to UWB error which was a problem with the technology, but this is probably attributed to the prototype stage of development of the equipment (discussed in section 5.4.4). An equally small proportion of mounts were simply not identifiable in the UWB data as changes in height had not been recorded. This could potentially be attributed to mounts of less than 3 seconds duration. This is such a short movement that the position coordinates may not have been recorded. Unidentified mounts may also have been due to poor signal quality arising from areas of poor geometry in the dairy barn and therefore positions were not recorded by UWB, particularly if mounts were of short duration (<3 seconds) and occur in these areas.

Automatic analysis of UWB data by the optimised script was used to detect individual mounting and standing to be mounted events. The matching rate between visually recorded mounts and mounts declared by the script averaged 70% which was lower than the matching rate of visually observed mounts and mounts recorded by UWB. The automated matching rate was perhaps lower because one function of the script was to detect only mounts of >3 seconds. This is because there is a pattern of UWB error of short spiking similar to mounts of short duration. However, this does not pose a problem for the detection of oestrus as most 'true' mounts were detected, demonstrating that for cows in oestrus mounting lasted longer than 2 seconds, ensuring more robust detection of cows in oestrus. Average duration of individual mounting events has been reported as 3.20 to 3.36 seconds (At-Taras and Spahr, 2001) which can be easily identified by UWB. The matching rate between declared and visually observed mounts is also perhaps lower as one cow (Cow 48) was eliminated from the automated analysis due to a function of the script. The script detects oestrus by the positioning relationship between 2 cows, both equipped with UWB; however when Cow 48 was in oestrus it was the only cow with a UWB MU in oestrus at the time.

Script accuracy in identifying actual mounts concurring with visual records was lower (averaging only 53.7%; see Table 5.3 and 5.4). A larger proportion of mounts are declared by the script than actually occurred, and only 50% of the script declared mounts match with visual observation of mounts. This indicates that specificity of the script for correct mounts and standing to be mounted is lower. The number of mounts that are not identified is relatively low which is promising, indicating that most oestrous behaviour can be recorded. As previously mentioned UWB errors occur due to the early stage of development of the technology, and a proportion of mounts are unable to be detected as their limits are out of the boundaries set by the script. Limits were as follows; minimum mounting height at 1.3m and maximum mounting height at 2.6m, or when the relative position of the 2 MUs were outside of the set limits for X and Y: $X(Y)<1.0$m, 0.7m$<Y(X)<2.3$m or Z: 0.4m$<Z<1.0$m. Therefore if the mounting behaviour occurs outside of the set limits then it cannot be detected which results in error as a cause of the script. This reduces the accuracy of detection, not UWB, the detection method. Therefore alterations were made to the script to include 'tight limits' (relative position between the 2 MUs decreased, X and Y: $X(Y)<0.8$m, 0.8m$<Y(X)<2.1$m and Z: 0.4m$<Z<0.85$m); which could potentially decrease the matching rate, but increase specificity for true mounts. This would increase the certainty that a cow was actually in oestrus. However, this was unsuccessful as matching rate decreased dramatically to an average of 53.5%, without any increase in script accuracy; therefore the optimised script resulted in the best oestrous detection.

From the daily analysis of the reports of oestrus, mounting and standing behaviour, it was clear that the incidence of erroneously declared mounting was more prevalent on days when cows were actually in oestrus (Tables 5.5 and 5.6). It was also clear that more error was attributed to cows that came into oestrus during the trial compared to control cows not in oestrus. The increased error during oestrus could be due to the number of cows in oestrus at once; for example increased number of cows in oestrus increases oestrous expression (Hurnik *et al.*, 1975;Van Vliet and Van Eerdenburg, 1996), which may mean that the error is a result of the experimental design because 6 cows were synchronised to come into oestrus simultaneously. In a commercial situation the number of cows in oestrus together may be lower which could result in a more accurate detection rate.

Spikes in the data affect the accuracy of mounting detection because in the UWB data error spikes are recorded in the same format as actual mounts and so could be mistaken. Falsely declared mounts were investigated by looking at the MU position on CCTV cameras. This showed that there was no pattern associated with the error spikes. Potential reasons could be attributed to a cows' position in an area of poor geometry in the dairy barn (Figure 4.9), or due to non-line of sight from the master unit (Harmer *et al.*, 2008). Because the dairy farm is a complex environment with various obstructions, line of sight may become obstructed and thus cause reflections in the signal which give false or less accurate 3D positions. Although, filtering by script analysis can help to eliminate these spikes. Upon further analysis of the data from POC 2 and POC 3 it appears that data quality in POC 2 was better than POC 3, therefore data from POC 3 required further filtering. It is obvious that 2 MUs in POC 3 had higher incidence of error and more false positives were declared by script analysis (Cow 38 and 623; control cow and oestrus cow). These particular MUs were tested against a normally functioning MU in the same BU network in the same positions of good geometry; results revealed no difference in position or error, leading to the conclusion that error could be attributed to a cow's favoured position within the location, which could be an area of poorer geometry and more obstruction. Furthermore it is possible that when the cows lie down, especially in areas of poor geometry, that MUs may lose line of sight with the master unit or become obstructed by other objects or cows, which could contribute to error.

In summary, these results were extremely important in providing the basis for automated detection of oestrus by UWB; because error increased on days of oestrus and was associated with cows in oestrus. Hence a clear distinction could be made between those cows in oestrus, thus eligible for AI, and those that were not showing behavioural oestrus.

5.4.2 UWB for Automated Oestrous Detection

This novel method of oestrous detection accurately detected all the 9 cows in oestrus in this study using automated analysis of UWB data. The herd level script was developed to detect oestrus in a group of cows following analysis of UWB error, which as discussed increased with oestrous behaviour. The herd level script can detect initial mounting behaviour and therefore identify cows coming into oestrus. If mounting and/ or standing to be mounted continue and occur again within 3 hours the cow is deemed in oestrus. In total 10 cows displayed oestrus during trials POC 2 and 3, although one cow was discounted from analysis (as explained above), therefore these results show that 9 out of 9 cows (100%) can be detected in oestrus automatically by continuous monitoring of cows' 3D position to detect mounting and standing to be mounted. In support of these results 6 out of 6 cows were also correctly identified as not being in oestrus.

Time of onset of oestrus, mounting, can also be determined although this requires further investigation. This sign of oestrus is important to monitor because mounting and disorientated mounting are more intense behaviours which are displayed around the time of oestrus (Van Eerdenburg *et al.*, 1996). Therefore in the case that standing to be mounted is not displayed, as fewer cows stand to be mounted (Dobson *et al.*, 2008), mounting can be a useful indicator of oestrus. Importantly standing to be mounted can also be identified, as this is the definitive sign that a cow is in true oestrus (Orihuela, 2000). Standing to be mounted is also the most closely related sign of oestrus to ovulation and therefore if the time of onset is known provides an accurate prediction for ovulation and when to AI. Standing heat occurs 26.4 hours before ovulation (Roelofs *et al.*, 2005), showing how real-time UWB detection of standing to be mounted can be a useful predictor for time of AI which is required in order to maximise conception rates. However, because standing heat is only displayed in a limited number of cows, detection of real-time mounting behaviour is also useful. Ovulation can still be predicted by mounting, occurring 28.7 hours post onset of mounting (Roelofs *et al.*, 2005). This is particularly advantageous especially when only one cow is in oestrus. Here UWB is advantageous because mounting is displayed in 90% of periods of oestrus (Roelofs *et al.*, 2005).

The oestrus data recorded by UWB should be beneficial for increasing conception rates because of the real-time perspective of mounting and standing to be mounted in relation to optimal timing of AI; 4 to 12 hours after the onset of standing activity (Dransfield *et al.*, 1998). Other real-time advantages of UWB have also been proven because the end of oestrus can be determined, which can also provide an indication for optimal timing of AI (see Figure 1.2). UWB has the capability to notify the herdsmen when cows are in oestrus, which allows for visual observation and confirmation and further action that needs to be taken such as submitting the cow for AI if she is eligible.

Further novel aspects of using UWB for oestrous detection are that because both mounting and standing to be mounted can be identified the accuracy at detecting oestrus increases. When monitoring standing heat alone by visual observation heat detection rates of only 12%-30% have been reported (Van Eerdenburg *et al.*, 1996;Roelofs *et al.*, 2005), but when increasing behaviour monitoring to also include mounting behaviour the detection rate has been reported to increase to 61% (Roelofs *et al.*, 2005). Furthermore, when including all behavioural aspects of oestrus as described by Van Eerdenburg *et al.,* (1996) the detection rate has been reported to increase significantly to between 74%-90% (Van Eerdenburg *et al.*, 1996;Roelofs *et al.*, 2005), highlighting future potential for UWB as 3D positioning has many possibilities for monitoring cow interactions.

Oestrus detected by UWB was confirmed principally by visual observation. Due to the experimental design of this study continuous visual observation was used to confirm oestrus accurately. In a more practical situation this would not be possible because it would be too time consuming. Visual and CCTV records confirmed the results reported by UWB; 9 cows were seen mounting and standing to be mounted, and 6 cows did not engage in oestrous behaviour. There was however one exception when a pregnant cow (Cow 38) made two attempts to mount another cow probably because the level of oestrous activity was high. These isolated mounts were recorded by UWB, but this cow was not identified as being in oestrus by herd level script analysis. This behaviour of pregnant cows is not uncommon as it is reported that 6% of pregnant cows become involved in displays of oestrous behaviour (Erb and Morrison, 1958). This cow was also confirmed pregnant by a milk progesterone concentration >10ng/ml throughout the trial period.

Progesterone concentration profiles were plotted post trial for analysis of stage of the oestrous cycle, correctly confirming the physiological status of the other pregnant cows as their concentrations all remained above 10ng/ml. The progesterone concentration of all cows displaying oestrus as identified by UWB confirmed the eligibility for oestrus. All the progesterone concentrations were basal, <1ng/ml, indicating that the negative effects of progesterone on oestradiol (via reduced LH patterns of release) had been removed so that oestradiol could increase resulting in oestrus (Chenault *et al.*, 1975). For the synchronised cows that did not come into oestrus (Cows 412, 520, 516 and 127) progesterone concentration was low and remained so, indicating that these cows were at a different stage of follicular development (Twagiramungu *et al.*, 1995) or the presence of an ovarian cyst (Garverick, 1997). Another possible reason for no oestrus, especially in the case of Cow 516, where progesterone concentration began to rise post synchronisation signifying the luteal phase of the oestrous cycle, is that a silent ovulation could have occurred (Harrison *et al.*, 1990). Silent ovulations are ovulations unaccompanied by behavioural oestrus, perhaps due to the effects of oestradiol on different centres in the hypothalamus (Reames *et al.*, 2010). Silent ovulation is prevalent in high yielding cows (Harrison *et al.*, 1990), as were used in this study. Other possible reasons for not displaying overt oestrus are due to the particular cow’s social status and hierarchy as dominant cows may inhibit oestrus in subdominant cows (Gwazdauskas *et al.*, 1983;Allrich, 1994;Orihuela, 2000). UWB did not detect the potential silent ovulation which highlights the potential advantages of using hormone analysis to detect oestrus (Lovendahl and Friggens, 2008). However, activity monitoring did not identify this potential silent oestrus either. This is typical of silent ovulations as increased activity does not usually precede a silent ovulation (Ranasinghe *et al.*, 2010).

All cows that were not reported in oestrus by UWB had constant baseline activity throughout the trial and were not detected in oestrus by activity monitoring. It is clear from the activity data profiles that all cows reported in oestrus by UWB were confirmed in oestrus by increased activity, with increases in activity from basal levels. The level of maximum activity at oestrus differed per cow ranging from 67-133, but this bears no relationship to duration of oestrus as measured by UWB. This finding relates to the earlier work described in Chapters 2 and 3 because the two measurements of oestrous detection measure different signs of oestrous which could also relate to certain cow factors or SNPs for increased oestrous expression.

UWB satisfies the criteria defined by Senger (1994) for the ideal oestrous detection system. Continuous surveillance of the herd is required 24 hours a day to monitor constantly for oestrus, with minimised labour requirements. The real-time onset of oestrus is reported by UWB, and most incidences of oestrus in these trials occurred during unsociable hours when herdsmen would not be present, and cows may not be identified in oestrus. This is in agreement with previous data stating that most oestrous behaviour occurs during 1800 and 0600 hours (Hurnik *et al.*, 1975). The duration of total oestrus in this study averaged 10.7 hours, which is higher than previous reports suggesting the average time of oestrus lasts only 7 hours (Dransfield *et al.*, 1998). However this could be attributed to the number of cows in oestrus at once in this study; oestrous expression is increased when the number of cows in oestrus increases (Hurnik *et al.*, 1975;Van Eerdenburg *et al.*, 2002). Yet this indicates that no matter what time of day oestrus is expressed or how short the duration of oestrus it can still be detected by UWB, despite the range of total oestrus duration between trials varying from 2.0 to 14.0 hours. Furthermore, UWB accurately identified individual cows in oestrus and was able to detect the behavioural events at oestrus that are correlated with ovulation.

This work has satisfied the aims of this study as cows approaching oestrus and cows in oestrus can be detected in real-time. This was confirmed both by milk progesterone concentrations to identify stage of the oestrous cycle and by other detection methods; visual observation, CCTV records and activity monitoring. Furthermore the work demonstrated that UWB data could be analysed automatically to detect oestrus, accurately detecting 9 out of 9 cows in oestrus, with the potential to extend to a 100 cow herd. However, one must be careful with the 100% success rate described here as sample size is limited. In order to progress further with this novel technology larger herds must be monitored by UWB to achieve a true detection rate.

5.4.3 Comparison of Methods of Oestrous Detection

Oestrous detection in this study was carried out by continuous visual observation and CCTV recording, milk progesterone analysis and activity monitoring, alongside UWB to test proof of concept of a novel technology.

Due to the experimental design this study required continuous visual observation 24 hours a day which was necessary to confirm oestrus accurately; all types of primary and secondary oestrous behaviour could be monitored and recorded, which has been reported to achieve 100% accuracy of detection (Van Vliet and Van Eerdenburg, 1996). In a more practical situation, however, visual observation is carried out at set times only twice or three times per day. Time of day, frequency and duration of observations can affect accuracy and efficiency (Roelofs *et al.*, 2010); detection rate at milking decreases by 30-41% (Cavastany *et al.*, 2008) and number of cows detected in standing oestrus increases by 30% when observations increase from 2 x 30 minutes, to 3 x 30 minutes per day (Roelofs *et al.*, 2005). Due to lack of time and/ or willingness, not enough time is dedicated daily to visual observation in a commercial situation and therefore efficiency is poor and detection rate decreases (Lehrer *et al.*, 1992). Efficiency of oestrous detection by visual observation has been reported as 74% when considering all types of oestrous behaviour (Van Vliet and Van Eerdenburg, 1996), but when focussing solely on detection of standing heat during set intervals efficiency is often less than 50% (Roelofs *et al.*, 2006;Van Eerdenburg *et al.*, 2002;Van Vliet and Van Eerdenburg, 1996).

Hormone analysis has particular advantages over other methods of oestrous detection because the oestrous cycle is regulated by several key hormones which can all indicate reproductive status of the cow, therefore periodic measurement of a key hormones can be used to predict reproductive status (Delwiche *et al.*, 2001a). Progesterone concentrations decrease to basal over the oestrous period (Friggens and Chagunda, 2005) and allow oestradiol to increase through removal of negative feedback in the absence of progesterone (Reames *et al.*, 2010). However measurement of progesterone concentration is inefficient and time consuming, requiring labour for sample collection, and post processing for analysis of results which is more difficult in a commercial situation (Friggens and Chagunda, 2005) to produce usable data for predicting potential eligibility for AI. A more sustainable method of hormone analysis for oestrous detection would be to use an automated in-line approach (Delwiche *et al.*, 2001a). The progesterone biosensor has been reported to correctly identify all 19 ovulatory events occurring in one particular study, although it had a 26% error rate arising from variability and percentage fat in individual milk samples (Delwiche *et al.*, 2001a). A commercially available method of in-line milk progesterone monitoring is HerdNavigator with elements to detect health, fertility and metabolic status (Lovendahl and Friggens, 2008). The method, as described by Friggens and Chagunda (2005), has reported 99.2% detection using the model of confirmed oestruses (where insemination resulted in a confirmed pregnancy, n=121) (Friggens *et al.*, 2008). Furthermore this method detected oestrus in a number of cases (n=16) where progesterone concentration did not decrease below 4ng/ml, the set threshold (Friggens *et al.*, 2008), which is advantageous in situations where cows may not display behavioural oestrus due to high progesterone concentration. Furthermore this model also had sufficiently high detection rate when using ratified oestrus (where the shape of the progesterone profile matches that of the average progesterone profile at confirmed oestrus) as a measure of detection confirmation; 93.7% (Friggens *et al.*, 2008). However despite this method performing as well as other detection methods, progesterone monitoring is still not precise at detecting the correct time for AI (Lovendahl and Friggens, 2008), unlike increases in oestradiol concentration which correlate more precisely with time of ovulation (Lopez *et al.*, 2002). It has been reported that fixed time AI on the basis of progesterone concentration actually decreased pregnancy rate (Eddy and Clark, 1987). Progesterone measurements can however aid detection by determining errors in oestrous detection methods (Nebel, 1988). By accurately predicting physiological status and any irregularities in oestrous cycles (Lovendahl and Friggens, 2008) and through detection of silent oestrus where cows fail to display any overt behaviour (Ranasinghe *et al.*, 2010) as described in the current study where 2 synchronised cows with low progesterone did not demonstrate oestrous behaviour, progesterone monitoring is advantageous. Due to the disadvantages of milk progesterone monitoring Lovendahl and Friggens (2008) decided to combine activity monitoring with milk progesterone measurements (where possible) to overcome the negative aspects. Activity monitoring could also monitor non-lactating heifers, yet in lactating cows aid progesterone measurements to provide a more accurate estimate of time of ovulation and optimal time to AI, as activity measurements are given every 1 or 2 hours (Lovendahl and Friggens, 2008). Combining methods of detection has been reported to increase detection rate (Peralta *et al.*, 2005) where this work is advantageous because future generations of UWB have potential to incorporate activity monitoring as well as mounting and standing to be mounted.

It is well known that activity increases at the time of oestrus as first reported by Farris (1954) and later confirmed by many others. Activity has been reported to increase from 2 to 4 fold at the time of oestrus, and varies widely per cow (Kiddy, 1977). Activity measurements are valuable for detecting oestrus. Detection rates for monitoring oestrus with pedometers and activity monitors average 80%-90% in most investigations (Lehrer *et al.*, 1992;Firk *et al.*, 2002), which is sufficiently high for detection of oestrus, and there have also been reports of 100% detection rate (Schofield *et al.*, 1991;Arney *et al.*, 1994). Although it has been suggested by Van Vliet and Van Eerdenburg (1996) that activity monitoring must be combined with observation of standing heat in order to detect all cows in oestrus. This is because the increases in activity recorded by pedometers in their study were only related to specific types of oestrous behaviour (Van Vliet and Van Eerdenburg, 1996). However in contrast to the high detection rate, error rate is also high, reported between 17-55%, attracting a large number of false positive responses (Firk *et al.*, 2002) which is one of the main problems associated with automated technologies. Peralta *et al*., (2005) compared the efficiency of detection systems and concluded that detection efficiency was only 37.2% by comparing detected periods of oestrus with total number of oestrus periods, although this study was carried out under heat stressed conditions. However, efficiency is largely affected by the threshold used to define the increase in activity as it differs between cows (Table 5.8; Roelofs *et al*., 2005) and can be largely affected by routine management tasks (as seen in this study; Figure 5.9 to 5.11) such as introducing new cows to an established group where new dominance order is reestablished increasing activity, indicating false oestrus events. Therefore this method may still require use of cow calendars to achieve high detection rates. However, increase in activity cannot always accurately predict time of ovulation as it has been reported that there is a reduced relationship between standing to be mounted by another cow, attempting to mount other cows and other sexual activities, with pedometer readings (Van Vliet and Van Eerdenburg, 1996). Hence UWB should be beneficial for the more precise timing of AI and could potentially improve conception rates.

In the modern herd there is limited use for non-automated methods of detection. Progesterone monitoring is accurate, although not precise at detecting oestrus, and does not relate to exact time of ovulation. Activity monitoring is efficient at detecting oestrus, but is coupled with a large error rate, and although providing an estimated window of time for AI, results in low conception rates because of the lack of relationship between activity increase and timing of ovulation. This could perhaps be related to activity only being recorded in 2 hourly intervals and because activity monitoring is retrospective reporting oestrus only at milking therefore could be reported 12 hours post onset of oestrus. Unlike UWB which records position twice per second, in real-time, alerting staff to perform visual observations and take action as oestrus happens. Hence UWB has the advantage of being efficient and yet accurate by detecting standing heat, the true indicator of oestrus (Orihuela, 2000). This method can also detect mounting, as well as standing to be mounted, which are the signs of oestrus most closely related to time of ovulation, in order to maximise conception rates (Roelofs *et al.*, 2005).

5.4.4 Limitations of UWB

Weaknesses of UWB are mainly because it is still in the early stages of development as a prototype system, and because of its prior application (monitoring emergency personnel for example in burning buildings/ forest fires or during natural disasters (Ingram *et al.*, 2004;Ingram, 2006;Harmer *et al.*, 2008;Dona *et al.*, 2009) as it has not been developed for the purpose of monitoring cows. The impractically of the UWB unit size and battery power were major limitations for the purpose of oestrous detection. Having to mount units in backpacks is not ideal and changing batteries at least once every 24 hours is inefficient, requiring excessive labour. However, there is potential to develop the UWB product further, decreasing the size so that it can be worn on the cow's neck collar, like activity monitors. Furthermore, UWB can be run on lower battery power by removing unnecessary functions included in the prototype and installing an internal battery to last for a prolonged period of time, if not the productive life of the cow. The UWB units must also be robust to avoid breakages of antennae which could affect accuracy of position, which can be rectified by installing an internal antenna. Further potential improvements would be to increase the sampling rate; from 2Hz, twice per second, to perhaps 4Hz, relaying position 4 times per second, which has potential to smooth out any error spikes. This does however pose problems for the number of units functioning at once (20 UWB units at 2Hz, 40 UWB units at 1Hz) because when sampling rate increases, number of units able to communicate decreases. However, current improvements are being made to increase the total number of channels so that more UWB units can communicate. Therefore to strengthen the proof of concept that UWB is suitable for the purpose of oestrous detection, oestrus must be able to be monitored in a herd of cows. With further development to a specification designed for the purpose of oestrous detection UWB could be a marketable product to greatly benefit the dairy industry.

5.4.5 Implications of UWB

This study has shown proof of concept that UWB is capable of detecting oestrus in cows. Although the number of cows standing to be mounted is decreasing (Dobson *et al.*, 2008) this is still the most accurate determinant of oestrus (Orihuela, 2000) and timing of ovulation (Dransfield *et al.*, 1998). However, UWB can also detect mounting behaviour of cows in oestrus which is beneficial for detecting oestrus when cows do not display standing heat and also relates to timing of ovulation (Roelofs *et al.*, 2005). Positive implications of using UWB as a method of oestrous detection are that the detection rates can be increased, with potential to exceed the current target of 70% (DairyCo, 2009). This would lead to increased submission rate, at a more accurate time relating to onset of oestrus because UWB communicates continuously in real-time, and thus increase conception rate.

Furthermore there are other potential uses of UWB because of the 3D positioning properties. Monitoring the amount of time spent lying down can indicate health and comfort of dairy cows. Cows typically spend 11 hours per 24 hours lying down (Ito *et al.*, 2009) which is a strong identifier of cow comfort as this behaviour takes precedence over feeding and socialising (Munksgaard *et al.*, 2005). This can be used as an indicator of cow comfort; housing design, bedding and general environment. Monitoring the time spent lying down, frequency of lying bouts and duration of individual lying bouts (Haley *et al.*, 2000) can give an indication of wellbeing. Cows spent more time lying and lie down more often on mattresses than concrete surfaces (Haley *et al.*, 2001), spent more time lying down and for longer periods in wider stalls (132 vs. 112 cm) (Tucker *et al.*, 2004) and lying time increased when wet bedding was replaced with dry bedding (Fregonesi *et al.*, 2007). Lying time can however also function as an indicator of lameness. Severely lame cows are easily detected by herdsmen, but moderate lameness, which has an equal impact on production and profit, often goes undetected. Cows displaying shorter lying periods caused by high stocking density or hierarchical factors, longer times waiting for milking, and especially more time standing immobile can be predisposed to lameness (Blowey, 2005) whereas lameness can also cause more time to be spent lying down which can be used as an indicator of lameness (Walker *et al.*, 2008b). It has been reported that moderately lame cows stand up later than other cows once food has been delivered and lie down earlier after feeding, thus spend less time standing and eating, therefore can be a predictor of moderate lameness (Yunta *et al.*, 2012). UWB has the potential to monitor abnormal behaviour such as short lying time or longer lying times to indicate potential cows that may become lame, or to treat lameness. Furthermore management routines may be altered if overstocking is prevalent or if too much time is spent waiting to be milked which could be causing problems. Feeding times are often set and known, therefore real-time reports of position by UWB can indicate those cows not engaging fully in feeding behaviour and hence identifying potential ill health.

Lying time also functions as an indicator of mastitis; induced clinical mastitis resulted in reduced lying time caused by pain and discomfort in the initial 20 hours of infection (Cyples *et al.*, 2012;Siivonen *et al.*, 2011). Cows were also reported to display an altered stance in response to mastitis (Kemp *et al.*, 2008). Here UWB could automatically report any cows that have been standing longer than normal or for prolonged periods to identify mastitis.

Three dimensional position of the dairy barn can also be mapped and thus be used to monitor feeding and drinking time and behaviour through positional coordinates which is useful for determining health and productivity. Decreased feed intake has been reported in cows responding to an experimentally induced LPS challenge simulating mastitis (Waldron *et al.*, 2006). Decreased feeding time and decreased feed intake also predispose cows to illness e.g. metritis monitored post calving. However detection of decreased feeding time and intakes was prevalent 2 weeks prior to calving, which could be used as an early indicator allowing for intervention and prevention of disease (Huzzey *et al.*, 2007). For example, at risk cows could be given access to less competitive feeding environments.

Cow social interactions can also be monitored. Competitive behaviour for food can be common in cows housed indoors (Huzzey *et al.*, 2006) and this kind of behaviour could be detected by UWB to determine illness or social stressors which can affect the productivity of cows least able to compete for food, as these are more at risk of metabolic diseases as they cannot maintain their net energy balance (Weary *et al.*, 2009). In addition dominance and aggression behaviour can be studied through activity such as feed displacements to inform of any cows predisposed to these social stressors which could result in disease. Stressors can results in illness as over activity of the hypothalamic-pituitary-adrenal axis results in increased cortisol concentrations (Koolhaas *et al.*, 1999) indicating stress which affects reproductive capacity (see section 1.4.2.3) and can result in immunosuppression in dairy cows (Hopster *et al.*, 1998).

Behaviour around calving can be a useful indicator of calving time which is necessary to assist with the calving if needed and to take management steps ensuring the smooth transition from calving to lactation in order to reduce the risk of production diseases. It has been reported that cows lay down for less time, but had increased periods of lying bouts and were more active the day before calving (Jensen, 2012). Number of lying bouts and increased activity and restlessness is a reliable indicator of calving, as these coincided with increased contractions, and occurred more commonly in the 6 hours leading up to calving therefore giving a more precise indication of time (Jensen, 2012). These behaviours are possible to monitor by UWB as height is accurately recorded and the increased frequency of lying bouts and restless behaviour could be automatically detected to alert the farmer of calving.

UWB will be a useful tool for further research into cow behaviour to increase our biological understanding of cow comfort, but also has the prospect of being developed as a diagnostic tool to identify ill health more promptly, to improve cow welfare and reduce the negative impacts of ill health and discomfort on production.

5.5 CONCLUSION

These trials have provided evidence and proof of concept that UWB can be used for the purpose of oestrous detection. The software and communications have been developed to detect oestrus in order to identify cows entering into oestrus; mounting and those in standing oestrus; standing to be mounted. This information is reported in real-time, and therefore gives provides a measure for predicting time of ovulation. Furthermore all cows identified in oestrus by UWB were confirmed in oestrus by other physiological and physical measurements. These findings are of importance to the dairy industry, where other methods of oestrous detection lack adequate efficiency and accuracy; hence UWB can fill this niche. Through continuous, automated monitoring UWB is efficient, and yet accurate at detecting the definitive sign of oestrus relating to ovulation which enhances the accuracy. The current weaknesses of UWB were identified, although these were mainly due to the prototype stage of development. With appropriate development by decreasing the size of units, increasing battery power and improving script analysis to eliminate error, UWB has the potential to revolutionise oestrous detection through novel positioning technology. Furthermore a future refined product could incorporate a sensor system to integrate UWB positioning and activity monitoring, with algorithms to analyse both data sets simultaneously to strengthen oestrous detection. The current research has provided an insight into the potential of UWB and identified areas for further investigation. Future work would be to monitor oestrous detection in larger herd sizes and investigate several herds in order to conclude whether UWB could significantly improve oestrous detection rates in more commercial scenarios. By predicting the onset of standing to be mounted in real-time and thus providing an optimal timing for insemination, further work should investigate how UWB can improve submission rates and thus conception rates.

CHAPTER 6 – Overall Discussion & Conclusions

6.1 OVERALL DISCUSSION

Declining fertility in dairy cows has become an international problem strongly associated with genetic selection for high milk yield with little selection for other traits (Royal *et al.*, 2000a;Butler, 2003;Pryce *et al.*, 2004). A major part of the decline in fertility can be attributed to poor detection of oestrus (Van Eerdenburg *et al.*, 2002). It is crucially important for cows to express oestrus and for oestrus to be detected in order for AI to occur at an appropriate time relative to ovulation (Evans and Walsh, 2011). Therefore detection of oestrus is a key determinant of profitability in dairy herds (Pecsok *et al.*, 1994). Poor detection of oestrus can be attributed to the decline in oestrous expression. The number of cows standing to be mounted has declined from 80 to 50% (Dobson *et al.*, 2008) and oestrus is less intense and of shorter duration, averaging only 7 hours in Holsteins (Dransfield *et al.*, 1998). The decline in oestrous detection has been associated with larger herd sizes with the average herd size in the UK increasing from 75 cows in 1996 to 123 cows by 2011 (DairyCo, 2012c). This does vary worldwide with some herds in China and America having 15000 cows. Less labour per cow and a general lack of time dedicated to oestrous expression may also contribute to poor oestrous detection rates. Currently the national average for oestrous detection rate is only 50% where there is potential to be much higher and achieve a realistic target of over 70% (DairyCo, 2009).

Traditionally oestrous detection was performed by visual observation and although this method is accurate at detecting oestrus events which occur during observation periods (Van Vliet and Van Eerdenburg, 1996), due to time constraints and impracticality it is largely inefficient (Lehrer *et al.*, 1992). Continuous visual observation of synchronised cows (Chapter 5) detected all cows in oestrus, but continuous observation is not a viable method for commercial use. Many oestrous detection aids are available, with different detection efficiencies and accuracies (Roelofs *et al.*, 2010). Automated methods of detection are becoming increasingly popular, but there is still scope for improvement of detection rates.

Activity increases at oestrus (Farris, 1954;Kiddy, 1977) and activity monitoring is a convenient way of detecting oestrus. Detection rates for activity monitoring are often 80 to 90% (Firk *et al.*, 2002), some even achieving 100% (At-Taras and Spahr, 2001). Error rate remains high however, ranging between 17 and 55% (Firk *et al.*, 2002). Cow factors which can affect expression of oestrus by activity monitoring are reported in Chapter 2.

Activity monitoring was used to confirm oestrus in Chapter 5 and correctly identified all cows showing oestrus according to visual observation. Milk progesterone measurements (Chapter 5) suggested that some oestruses were missed however, both with activity monitoring and visual observation. Changes in milk progesterone concentration suggested 2 potential incidences of silent oestrus where overt oestrus was not displayed. Progesterone monitoring gives an accurate assessment of the cows physiological status and eligibility for oestrus, but is weak at predicting the precise time of oestrus and timing of insemination relative to ovulation (Lovendahl and Friggens, 2008). Current methods of detection are not proven to significantly increase detection rates and so do not improve dairy cow fertility, when compared with the traditional technique of visual observation.

In order to improve upon current methods of detection this thesis investigated possible ways to improve oestrous expression by genetic selection and alternative methods of oestrous detection to improve detection rates.

Fertility traits are of low heritability, $h^2<0.05$ (Berglund, 2008) and genetic gain is slow which hinders improvements through selection for fertility (Flint *et al.*, 2008). Incorporating measures of fertility into selection programs is limited because environmental and management factors influence many fertility traits, which results in low heritability. However using hormone measurements and traits less affected by environmental factors may be a possible solution. The heritability of several endocrine parameters has been investigated, demonstrating the high heritability of endocrine fertility traits over traditional fertility traits. Commencement of luteal activity, as measured by progesterone, was reported to be heritable, $h^2=0.16$, alongside other aspects of the progesterone curve which are also heritable, including length of first luteal phase and probability of a persistent CL (Royal *et al*., 2002). In a similar study the heritability of calving to commencement of luteal activity as measured by progesterone was also reported as $h^2=0.30$ (Petersson *et al.*, 2007). Using progesterone measurements has also revealed a similar heritability for days to first oestrus, h^2=0.27 (Lovendahl *et al.*, 2008). In addition, the heritability of oestrous behaviour verified by pedometer data has been reported: days to first activity increase, h^2=0.18, duration of oestrus, h^2=0.02 and strength of oestrus, h^2=0.04 (Lovendahl and Chagunda, 2009). Heritability is low, however, compared to production traits and to enhance selection for dairy cow fertility genomic selection would be a suitable alternative to traditional selection methods.

Genomic selection provides a rapid, permanent solution to declining oestrous expression which reaps cumulative gains (Hayes *et al.*, 2009). Genomic selection has advantages over current genetic selection methods, especially for traits of low heritability because accuracy of SNP selection is high (Muir, 2007), so there is potential to improve fertility whilst milk yield is maintained (Veerkamp *et al.*, 2000). Molecular markers for fertility have already been reported in the bovine genome (Hastings *et al.*, 2006;Khatib *et al.*, 2008a), yet in this study no true significant associations were found between SNPs and the increase in activity. There is potential to investigate further the association between SNPs and oestrous expression to provide an opportunity, with effective interpretation and integration, for incorporation of genomic breeding values for oestrous expression into breeding programs to allow selection of animals that show strong oestrous expression.

Alternative methods of oestrous detection are required to improve detection rates. An accurate and effective method of detection is required to fulfil the criteria described by Senger (1994), where detection rate has the potential to be 100% using an effective system of continuous monitoring of all oestrous symptoms (Van Vliet and Van Eerdenburg, 1996).

A commercial technique developed to improve oestrous detection rates is online monitoring of milk progesterone concentration. The HerdNavigator system has additional elements to detect health, fertility and metabolic status (Lovendahl and Friggens, 2008). Monitoring of hormones gives an accurate representation of the cow's physiological status (Friggens and Chagunda, 2005) and oestrous detection rates using the progesterone model described by Friggens and Chagunda (2005) are comparable with other automated techniques; 99.2% and 93.7% detection rates (Friggens *et al.*, 2008). However precise timing of oestrus is unknown, so monitoring of oestradiol would be advantageous (Lopez *et al.*, 2002). Studies investigating conception rate based on insemination at the time of low progesterone concentration report low pregnancy rates (Eddy and Clark, 1987), but online monitoring of progesterone is an effective method for detecting silent oestrus (Ranasinghe *et al.*, 2010). It can also confirm errors in detection when combined with other methods which can positively affect detection rates (Nebel, 1988).

To overcome the need for detection of oestrus the use of oestrous synchronisation and timed AI are becoming widespread among production systems which avoids the practical difficulties associated with detection (Macmillan, 2010;Pursley and Martins, 2012). The original and most basic programme is the Ovsynch protocol which uses timed injections of GnRH and $PGF_{2\alpha}$ to control follicle development, luteolysis and ovulation, and finally timed AI to eliminate oestrous detection altogether (Thatcher *et al.*, 1989;Pursley *et al.*, 1995). However, cows detected in oestrus without hormone treatment are reported to have higher conception rates than those undergoing synchronisation and timed AI (Stevenson *et al.*, 1999a;Macmillan, 2010). The calving to conception interval is decreased by using synchronisation, although the incidence of anoestrus and early embryonic death is increased (Macmillan, 2010). Progesterone treatments (PRIDs, CIDRs etc.) are also used for synchronisation, but still require oestrous detection, yet at a more precise time, and can result in reduced fertility depending on length and precise timing of treatment (Yavas and Walton, 2000). Synchronisation can reduce the incidence of problem cows not showing oestrus such as anoestrus and cystic cows (Lucy *et al.*, 2004). However there are many negatives associated with use of hormone treatments; availability of hormones due to cost, legislation and regulations and also public perception of hormone use. Using hormone treatments may improve submission rates, but conception rates are not necessarily higher, although pregnancy rates may be increased due to increased submission for AI (Lucy *et al.*, 2004). It is clear that although online progesterone monitoring can detect oestrus, and manipulation of the oestrous cycle removes the need for oestrous detection, neither is a simple solution to improving dairy cow fertility within current management systems for the modern dairy cow. In order to improve reproductive performance the novel approaches discussed in this thesis are advantageous over current methods.

The final section of this thesis describes the development (Chapter 4) and proof of concept (Chapter 5) studies addressing a novel technique for detection of oestrus. UWB technology has been developed to detect oestrus in dairy cows by using precise positioning to monitor cow interactions in 3 dimensions. Height changes and the horizontal relationship between 2 cows can be determined which has been translated into data about oestrus to detect mounting and standing to be mounted. Automated analysis of UWB positioning data can then detect whether cows are in oestrus or not, and report this information in real-time. The work in this thesis provides evidence that UWB can detect cows in oestrus and shows proof of concept that UWB can be used for the purpose of oestrous detection.

In summary enhancing oestrous expression could achieve improvements in oestrous detection rate as animals display stronger oestrus and are therefore more likely to be detected in oestrus. However, no true significant associations were found in this study and a larger cohort of cows is required to improve the investigation in Chapter 3 into SNPs related to oestrous expression. Genomic selection is a rapid and permanent method of enhancing oestrous expression and incorporating measures of oestrus in selection programs such as activity monitoring and progesterone measurements (Lovendahl *et al.*, 2008) and it could be used to permanently improve oestrous expression in herds of cows. Endocrine parameters have been investigated for their heritability in relation to oestrus. The GnRH response, measured as production of LH which is directly linked to production of oestradiol, was measured to predict fertility and oestrus activity in heifers. The GnRH response had a high heritability, h^2=0.51, and data are available at an early age for both bulls and heifers (Royal *et al.*, 2000b). However improvements in oestrous expression would be of little benefit without an effective method for detecting cows in oestrus.

UWB can greatly benefit detection of oestrus as demonstrated in Chapter 5 where 100% detection rate was achieved. However we have to be careful with interpretation of these results due to the limited number of cows used in the study. Here we have demonstrated proof of concept of UWB for oestrous detection using precise positioning technology to detect oestrous behaviour, standing oestrus and mounting, in dairy cows. UWB is promising because standing to be mounted can be detected and is the most accurate sign of oestrus, with only 2% error in wrongly identifying cows in oestrus (Kiddy, 1977). Hence it should be able to greatly improve the accuracy of detection. This method of detection therefore improves upon existing methods by monitoring 2 different behaviours (standing to be mounted and mounting), with the potential to include activity monitoring in future developments to UWB. This follows from research suggesting that detection rates are improved when methods of detection are combined (Peralta *et al.*, 2005;Lovendahl *et al.*, 2008) for use as one complete method.

Another previously reported method of oestrous detection, which is similar to UWB in the way it monitors the cows in all dimensions for both primary and secondary oestrous behaviour is 4sight. 4sight monitors cows through an image database containing images of 4 sides of the cow for identification, and is an optical digital surveillance system identifying cows in heat when they break a photosensitive beam. This method has reported 90% heat detection rate in a commercial situation, although conception rate is reportedly poor (Esslemont, 2006). However this method has not succeeded widely in the commercial sector compared to the various methods of activity monitoring available. UWB has the potential to succeed because of its ability to monitor individual cows in 3D with precision. Detection rates are strongly linked to conception rate (Roelofs *et al.*, 2010), thereby improving detection rate enhances the potential to improve conception rate, and provides an opportunity to adhere to strict calving intervals. Furthermore, although the detection rate reported for UWB is comparable with existing methods such as activity monitoring (At-Taras and Spahr, 2001) and current commercial systems of automated online progesterone monitoring (Friggens *et al.*, 2008) it has the advantage of reporting oestrus in real-time. Therefore unlike retrospective reports of 2 hourly activity units and weak correlations with time of ovulation, the onset of oestrus is known and insemination can occur within 4 to 12 hours (Dransfield *et al.*, 1998). In addition UWB monitors mounting and standing to be mounted which are both strongly linked to the timing of ovulation (Roelofs *et al.*, 2005) and the likelihood of UWB improving conception rates via accurate detection is increased.

Further development to the UWB prototype is required and future work would be to monitor oestrous detection in several larger herds. This would determine the possible improvements to oestrous detection rate and by using the real-time properties of UWB investigate the effect on conception rate. By using UWB in a commercial situation a true estimation of the efficacy of UWB as a method of oestrous detection will be gained and comparisons can be made between UWB and current detection methods.

Further research possibilities using UWB are discussed in Chapter 5 and could be used to measure behaviour as an indicator of cow welfare and health. The novelty of UWB is 3D position recording and relaying of information in real-time. Cows typically spend 11 hours per 24 hours lying down (Ito *et al.*, 2009) which is a strong indicator of cow comfort (Munksgaard *et al.*, 2005). Using 3D positioning we can determine whether cows have an increased lying time which can indicate lameness (Walker *et al.*, 2008b), and particular times when cows spend more time lying down such as feeding time can be identified which can be used as an indicator of moderate lameness (Yunta *et al.*, 2012). Furthermore UWB may be able to alert the farmer to cases of clinical mastitis due to decreased lying times caused by pain and discomfort (Siivonen *et al.*, 2011;Cyples *et al.*, 2012). Therefore UWB can not only be used for research opportunities but also can be used to advise management and veterinary practices resulting in increased productivity and profit.

UWB fulfils the criteria described by Senger (1994) for the optimal method of oestrous detection and is both efficient and accurate at detecting oestrus. UWB has the potential to increase the current oestrous detection rate from 50% (DairyCo, 2009). Increasing detection rate benefits the dairy industry by maximising productivity and increasing profit by adhering to desired calving intervals. If oestrus goes undetected then the strict 365 day calving interval, in which the aim is to produce 1 calf per cow per year, is extended, in turn decreasing overall productivity. Moreover extended calving intervals lead to an increase in forced culling; a significant contributor to greenhouse gas emissions as more animals must be reared to deliver the same level of production. It is reported that an increase of just 10% in oestrous detection rate can reduce the calving to conception interval by 4 days, and the incidence of cows culled for failure to conceive by 6% (DairyCo, 2009). Furthermore poor oestrous detection has other associated costs; extra labour for oestrous detection, more inseminations to get cows in calf, extra semen straws and technicians to artificially inseminate, and veterinary costs all reducing the net profit per cow, decreasing herd profitability (Roche, 2006). Importantly the financial implications of improving oestrous detection rates are large; a mere 10%

increase in oestrous detection rate can result in gains of 0.81 pence per litre in the average (6000 litre) cow (DairyCo, 2009). Therefore improvements in oestrous detection rate, which could be realised using UWB, will yield greater profit especially in higher yielding herds.

Therefore oestrous detection is of major importance to maximising yields and productivity and contributing to the economic sustainability and reduced environmental impact of the dairy industry. Conception rate is only 40% (Royal *et al.*, 2000a), combined with 50% oestrous detection (DairyCo, 2009), meaning only 20% of all ovulations result in pregnancy. Therefore strategies to improve the oestrous detection rate can help to arrest the decline in dairy cow fertility.

6.2 OVERALL CONCLUSIONS

This work in this thesis has highlighted that several factors can affect the activity increase at oestrus:

i. Parity ($P<0.001$) is inversely related to activity at oestrus; as parity increases, activity at oestrus decreases. Time period when oestrus occurred ($P=0.007$) is also associated with activity increase; activity at oestrus is greater with increased day length.
ii. As milk yield at oestrus ($P=0.002$) and average daily milk yield to oestrus ($P=0.003$) increase, the smaller the activity increase at oestrus.

This work has also focussed on the development of a novel technology to improve oestrous detection. These studies have demonstrated that:

iii. UWB can, in real-time, accurately identify cows approaching oestrus (mounting) and cows in oestrus (standing to be mounted) and distinguish those from cows not in oestrus.

This work has highlighted novel solutions for improvement of oestrous expression and detection. A larger population of cows is needed to determine the effect of SNPs on oestrous expression and use of UWB with larger herd sizes would provide a more definite measure of oestrous detection rate and improvements to conception rate. However, this study provides an insight the potential of UWB for increasing oestrous detection rate. This could arrest the worldwide decline in dairy cow fertility contributing to sustainability of the dairy industry.

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