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Measuring dairy herd heat detection: the use of inter-service intervals

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Abstract

Good oestrus detection is essential for efficient reproductive performance in dairy herds using artificial insemination. Veterinary surgeons and farmers use a variety of tools to measure and monitor oestrus detection, including the analysis of inter-service intervals (ISIs), which rely on an estimation of the normal oestrous cycle length. The normal oestrous cycle length of the cow is also important more broadly when managing and monitoring dairy herd fertility. Whilst the normal inter-ovulatory interval is typically considered to be 21 days, some studies have found alternative intervals to be more prevalent. The aim of this project was to evaluate and develop the use of inter-service interval analysis for the assessment of oestrus detection in dairy herds.

In the first study, insemination records from 167 dairy herds from across the UK were used to generate ISI profiles for each calendar year of each herd. Intervals between serves were categorised as short irregular (2-17 days), short regular (18-24 days), long irregular (25-35 days), long regular (36-48 days) or extended (>48 days). Herd calendar year performance was ranked by oestrus detection efficiency, the mean of the top quartile of herd-years had 6%, 40%, 16%, 19% and 19% of intervals in each interval category respectively. The results show a substantial difference to accepted ISI profile targets such as achieving a 6:1 ratio of short regular to long regular ISI, and will be of use when interpreting herd data and target setting for UK dairy herds.

The next study used ISI data from 159 herds across England and Wales. Univariable analysis of the subset of 114,572 intervals between 15 and 30 days (a range covering the increased frequency of ISIs occurring at the expected time of the first return to oestrus following an insemination) revealed a modal ISI of 22 days with 90% of the inseminations in this range occurring between 18 and 28 days. Multilevel regression modelling was used to evaluate the associations between cow factors and inter-service interval, whilst accounting for clustering at the herd and cow level. This revealed significant associations between predicted ISI and insemination number, days in milk, lactation 305 day milk yield, and month and year of insemination. These findings suggest the “normal” range of

ISI for modern UK dairy cows is longer than expected and that there is a large amount of unexplained variation in cycle length within individual animals over time.

The subsequent study used ISI data from 312 herds to identify associations between ISI and conception risk. Conception risk varied significantly with ISI, with a period of increased conception risk broadly corresponding to the period of increased frequency of ISIs identified in the previous study. Unsupervised machine learning was used to identify latent classes of ISIs using conception risk and frequency data. A class of ISIs was identified at 19-26 days that was associated with increased conception risk and increased numbers of inseminations. This 19-26 day period was considered a plausible candidate as a revised "normal" range for an expected ISI.

This was further developed in a study, using data from the first study to evaluate the impact of accounting for the 19-26 day period of ISIs on common ISI related metrics. The proportion of re-inseminations occurring at 18-24 and 19-26 days was compared across all herd-years as well as the relative ranking of each herd-year using both the 18-24 and 19-26 day interval. There was a significant difference in the apparent ISI profile performance of the herd-years when using the modified interval compared to the standard interval. There was a significant association between herd-year 305 day milk yield and the change in apparent performance when using the new metric. A series of almost identical random effects regression models explaining 200 day in calf rate, each with a different oestrus detection metric as an explanatory variable, were compared. The model in which return oestrus detection was represented by mean ISI explained the highest proportion of variation in 200 day in calf rate.

The final study used regression modelling on a subset of data from 28 herds identified as having good recording of clinical mastitis and lameness incidents. Logistic regression was used to identify associations between disease incidences and changes in the probability of re-insemination at either 18-24 or 19-26 days. Lameness 0-28 days after the first insemination of the interval decreased the odds of a re-insemination at an appropriate time by approximately 20%. Clinical mastitis 1-28 days

prior to the first insemination of the interval increased the odds of insemination at the expected time by approximately 20%. The associations were similar for either outcome. The effect appeared less important at a population level when the population attributable risk was calculated

This project has used several “big data” techniques applied to a large set of on farm records to improve understanding of inter-service intervals. New target levels are suggested and a new expected or ‘normal’ range is proposed. These should be useful to advisors helping to improve oestrus detection and therefore reproductive performance on farms.

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Publications and presentations

The work described in this thesis also appears in the following publications (attached as appendices).

REMNANT, J. G., HUXLEY, J. N. & HUDSON, C. D. (2015) A fresh look at inter-service intervals in UK dairy herds. **Cattle Practice** 23, 27-32

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REMNANT, J., HUXLEY, J. & HUDSON, C. (2013) What is a normal inter-service interval? Cattle Practice 21, 163

1 Introduction and literature review

1.1 Background

The United Kingdom (UK) is the ninth largest milk producer in the world, producing approximately thirteen billion ($13x10^9$) litres of milk per year (Agriculture and Horticulture Development Board 2013). Good reproductive management of dairy cows is needed to produce milk efficiently and profitably, reducing the interval between successive calvings (calving interval) and reducing the losses associated with cows that fail to conceive (Inchaisri and others 2010).

Globally, over the past few decades, milk production per cow has been increasing; at the same time there appears to have been a corresponding decline in the reproductive performance of dairy cattle (Dobson and others 2007). Various authors have suggested a direct and causative relationship between production level and fertility, arguing that by selectively breeding for increased milk production cows have been inadvertently selected for poorer fertility (Dobson and others 2007; Pryce and others 2004). Some authors suggest that the apparent relationship between production and fertility may have been overestimated as a result of changing management systems, new methods of measuring reproductive success and inherent biases in the analyses (LeBlanc 2010). It is also possible that the decline in fertility associated with increasing milk yield is caused indirectly by increased negative energy balance in combination with the increased yields and sub optimal management. More recently the decline in dairy cow fertility seems to have slowed both in North America and in the UK (Hudson and others 2010; Norman and others 2009). How much of the apparent decline in fertility of dairy cows is as a direct result of breeding for milk yield or of challenges in the management of higher yielding cows is difficult to ascertain, although a genetic association between production and fertility traits has been identified (Royal and others 2002). Whatever the role played by these, and other factors, it is clear that reproductive performance remains a key issue for many dairy farms across the globe.

The optimum calving interval has traditionally been considered to be 365 days (Esslemont 2003); although with increased milk yield and persistency in some cases the most profitable calving interval

may be longer (Arbel and others 2001). In most UK herds, average calving interval is considerably longer than 365 days and so for a very high proportion of herds, it remains likely that efficiency of production would be improved by reducing the calving interval (Hudson and others 2012b). Efficient reproduction relies on several steps: the cow must return to cyclicity as soon as possible after calving; the cow must be correctly identified in oestrus; the cow must conceive following artificial insemination or natural service; and then the cow must maintain a pregnancy to term.

Traditionally cows were mated by "running a bull", that is a bull was kept with the cows to perform natural service when cows were in oestrus. However, the use of artificial insemination is now widespread within the dairy industry, with numerous advantages over natural service, including the potential for rapid genetic progress (Vishwanath 2003). Along with improved management and nutrition, it is likely that genetic selection, made possible by the use of artificial insemination, has made a major contribution to the dramatic increases in milk production described previously as well as the apparent reversal of the trend for declining fertility. Developments in technology, such as high density genome sequencing chips which will allow genomic selection of animals (Matukumalli and others 2009), make it likely that this trend will continue. When using artificial insemination the role of identifying when, and which, cows are ready to inseminate passes from the bull to the farmer. To avoid having to identify these cows, synchronisation protocols can be implemented which allow the use of fixed-time artificial insemination (FTAI) for all inseminations carried out within a herd (Thatcher and others 2006). There is some resistance to the routine use of FTAI protocols in the UK (Higgins and others 2013) and in most EU countries it appears the routine use of FTAI is not widespread (Statham 2017; van Arendonk and Liinamo 2003). There appears to be increasing interest in this area, but concerns about public perception both in terms of food safety and animal welfare may limit the role of FTAI in the future. Without the use of reproductive management programmes using FTAI routinely on all cows in the herd (common in large units in the USA) farmers instead rely on their ability to detect and identify cows in oestrus for artificial insemination.

Efficient oestrus detection increases the number of opportunities a cow has to become pregnant. As a result it has a large impact on the reproductive performance of a herd, which in turn has a large impact on the productivity and profitability of the dairy enterprise. Inchaisri and others (2010) created a stochastic dynamic simulation model to compare good, average and poor reproductive performance in dairy herds; they showed not only that reproductive performance had an impact on economic performance but confirmed that conception rate and oestrus detection rate were the main determinants of overall reproductive performance in a herd compared to other factors such as milk yield and disease levels. Numerous other studies have also shown that oestrus detection efficiency has a large impact on reproductive efficiency and therefore profitability (Oltenacu and others 1981; Plaizier and others 1998; Vries and Conlin 2003). Good oestrus detection improves performance, not only because of the increased opportunities for cows to conceive, but also because accurate oestrus detection increases the likelihood of a cow being truly in oestrus at the time of insemination; this results in an increased conception rate, another key determinant of herd reproductive performance (Roelofs and others 2010; Roelofs and others 2005b). Oestrus detection is therefore an important area to monitor on modern dairy farms, and one in which many units have substantial scope to improve.

Over recent decades the role of the farm animal veterinary surgeon has changed. There has been a trend towards preventing rather than just treating disease, with an increasing focus on herd health management (LeBlanc and others 2006). This trend extends to reproduction with some authors suggesting the “routine fertility visit” as a starting point for herd health management (Grove-White 2004). Routine fertility visits on dairy farms are carried out by veterinary surgeons to identify those animals failing to conceive following insemination, to treat cows with reproductive tract abnormalities preventing them from becoming pregnant and to treat non-pregnant cows to induce oestrus. Increasingly, data generated from these visits, alongside farmer-recorded data on inseminations for example, is being used to analyse fertility performance in herds as well as providing an opportunity to discuss other areas of performance and management (Mee 2007).

Assessing the efficiency of oestrus detection within a herd will form a key part of this assessment. These data can also be compared between farms, which in larger datasets can allow the setting of target performance levels based on the top herds in the group (Esslemont and Kossaibati 2000).

1.2 Detecting oestrus on farm

Interest in the oestrus behaviour of cattle is not a recent phenomenon, an engraving of an aurochs bull sniffing the vulva of a cow found in a cave in the Dordogne region of France is thought to date from circa 10,000 B.C.E. (Roelofs and others 2010). Oestrus is the name given to the behavioural signs occurring in mammals around the time of ovulation. Most oestrus detection techniques rely on identifying this characteristic behaviour of a cow about to ovulate. The term oestrus is thought to originate from the ancient greek word for gadfly, '*oistros'*; the buzzing of the gadfly caused the cows to be restless and so the word was used to describe the restless behaviour of a cow in oestrus (Short 1984).

There are two broad areas that need to be addressed in a herd when attempting to maximise oestrus detection: the systems in place to detect oestrus on the farm and the expression of oestrus by the cows.

1.2.1 Approaches to oestrus detection

Traditionally, standing to be mounted was considered the most useful behavioural sign of oestrus (Roelofs and others 2005b). Whilst this is still considered the most specific behavioural sign of oestrus there are also a number of secondary signs. Van Eerdenburg and others (1996) developed a scoring system to incorporate secondary signs of oestrus alongside standing to be mounted. The system encompasses numerous signs of oestrus including, among other things, restlessness, vulva sniffing, mounting activity and standing to be mounted. Observing for oestrus behaviour remains a cornerstone of oestrus detection but it can be very labour intensive: for example, Van Eerdenburg and others (1996) were able to detect all oestrus cows in their study, but only where two-hourly observations were used. As a result of the labour requirement for extensive observation, numerous techniques have been adopted to detect oestrus using other methods.

One strategy that is frequently adopted is the use of heat mount detectors; these use various methods to identify cows which have stood to be mounted. These range from the simple application of chalk or paint to the tail head, which is rubbed off when the cow is mounted, to electronic pressure sensors (Diskin and Sreenan 2000). Other heat mount detectors include scratchcards (e.g. *Estrotect*, MAI Animal Health) and colour changing pressure sensors (e.g. *Kamar heatmount detectors*, Kamar Products Inc.).

As well as the typical behaviours expressed by a cow in oestrus, as mentioned previously, oestrus cows tend to be more restless. Cows in oestrus typically have large increases in locomotion (Arney and others 1994; Schofield and others 1991), so pedometers and other activity monitors can be used to identify cows likely to be in oestrus (Roelofs and others 2005a). It is likely these methods will continue to develop and increase as sensor technology improves. Perhaps more importantly, developments in algorithms and machine learning techniques used to interpret the sensor output are likely to lead to improvements in these technologies as well as the ability to interpret data from several sensor types simultaneously improving test accuracy, for example with the use of image analysis technology/computer vision (Tsai and Huang 2014).

Whilst all these methods of heat detection have been shown to be effective, the highest rates of oestrus detection appear to be achieved by using a combination of methods (Holman and others 2011). There are also a variety of physiological methods of oestrus detection. One of the key physiological methods relies on identifying the low level of progesterone associated with oestrus; although this is more commonly used as a method for confirming oestrus or testing accuracy of oestrus detection (McCaughey and Cooper 1980; Nebel 1988). With developments in technology, such as in line milk progesterone detection, there may be increased uptake of these methods for more routine detection of oestrus in future (Gillis and others 2002).

One relatively rare option is to delegate oestrus detection back to a bull. By using a chin marker on a sterile “teaser” bull, cows mounted by the bull when in oestrus can be detected after only brief

observation. Teaser bulls are usually prepared surgically using either vasectomy or epididymectomy to render them infertile whilst retaining their libido (Morgan and Dawson 2008); alternative strategies preventing intromission such as penile-preputial translocation are also described (Gill 1995). The popularity of teaser bulls remains limited due to the health and safety implications associated with the handling of bulls (Gordon 2006).

Other methods of oestrus detection, yet to reach widespread use include the use of vocalisation sensors (Schon and others 2007) and the use of scent detecting dogs (Fischer-Tenhagen and others 2011). Vaginal mucus electrical conductivity, cow milk yield and cow body temperature have all been identified as potential indicators of oestrus but variation between cows limits their use as automatic heat detection aids (Phillips and Schofield 1988; Schofield and others 1991). Combining data from different sensors using machine learning algorithms may enable more effective use of these systems in the future. It has also been shown that male mice increase their grooming activity in the presence of oestrus cow urine and this has been suggested as a method of oestrus detection (Kamatchi and others 2008).

Many of the recommendations made to improve oestrus detection focus on the areas described above; such as increased duration and frequency of observations and the increased use of oestrus detection aids (Diskin and Sreenan 2000; Gordon 2011). In addition to the appropriate use of oestrus detection aids, there are numerous other practical factors important for achieving effective oestrus detection on farms. Animals must be clearly identified, there must be adequate lighting to observe behaviour and to identify the cow, staff must also be trained to identify and record oestrus behaviour. Another strategy often recommended is the observation and recording of 'reference heats' prior to the desired time of breeding (Diskin and Sreenan 2000). These allow checks on animals apparently not cycling to be carried out before breeding is due, as well as enabling predictions to be made about when a cow is likely to be in oestrus again.

1.2.2 Oestrus expression

The goal of improving expression of oestrus is to minimise any potential barriers to cows performing oestrus behaviour and maximise the outward signs of oestrus which should make detection of oestrus easier. Factors affecting oestrus expression include both environmental factors and cow level factors (Diskin and Sreenan 2000).

1.2.2.1 Environmental factors

The environment a cow is kept in can have a large impact on oestrus expression. The housing system itself appears to have an effect, with one study showing cows in loose housing exhibiting more mounts per hour during oestrus than cows kept in cubicle systems and with grazing cows showing the least (De Silva and others 1981; Gwazdauskas and others 1983). However other studies have shown more oestrus expression in pasture based cows compared to housed cows, although the difference was only apparent in some oestrus behaviours (Palmer and others 2010, 2012). This discrepancy might be explained by the oestrus behaviours examined, some behaviours such as standing to be mounted appear more common in loose housed cattle, whereas reduction in lying time were more apparent in grazing cattle (Phillips and Schofield 1990). It is also possible that these changes in oestrus expression are not attributable to a type of housing system but are affected by more specific elements of the cow's environment.

A specific aspect of the housing that is associated with oestrus expression is the floor surface. Some studies have demonstrated a preference for cows to stand on soft surfaces such as rubber matting when compared to concrete (Telezhenko and others 2007; Tucker and others 2006), there is also some suggestion that this may improve oestrus expression (Platz and others 2008). However, providing rubber matting as a softer surface does not always appear to improve oestrus expression in dairy cows (Boyle and others 2007), a possible explanation for this is that the use of rubber can results in a loss of friction leading to a similar level of 'grip' to concrete. It is thought that the friction between a cow's foot and the floor surface (i.e. "grip") is more important to expressing oestrus behaviour than the floor hardness (Bell and Huxley 2009; Britt and others 1986).

As well as the floor surface, the season and temperature can also affect dairy cow reproduction, in particular high environmental temperatures have been shown to reduce oestrus expression as well as conception rates (Cavestany and others 1985). A decrease in oestrogen concentration is consistently demonstrated in heat stressed cows (Wilson and others 1998; Wolfenson and others 1997; Wolfenson and others 1995), with Wilson and others (1998) also demonstrating delays in luteolysis in heat stressed cattle. There also appear to be direct effects of heat stress on progesterone secretion and the luteinising hormone surge (Younas and others 1993). Other studies have found no difference in hormone levels between heat stressed and non-heat stressed cattle, but reduction in the duration of oestrus and in mounting behaviour have been detected in heat stressed groups (Gwazdauskas and others 1981; Pennington and others 1985). Heat stress has the potential to reduce oestrus detection as well as altering the physiology of the oestrous cycle.

In addition to the environment the cow is kept in, the other cattle in the environment may also exert an influence on the expression of oestrus. Group size and in particular the presence of other sexually active cows has been shown to exert an effect (Sveberg and others 2011). The presence of additional oestrus females increases mounting behaviour (Cutullic and others 2009; Helmer and Britt 1985; Roelofs and others 2005b). Large groups of oestrus animals also seem to increase detection of oestrus even when using heat detection aids that don't rely on mounting behaviour, such as pedometers (Roelofs and others 2005a). The presence of a bull may also influence behaviour at oestrus. Cows seem to be attracted to a bull when in oestrus and although there may not be an increase in oestrus behaviour, oestrus cows may congregate near a bull, facilitating detection (Roelofs and others 2008).

It is clear that the environment and management of the cow and the herd can impact on the cow's ability to express oestrus. Some of these factors can be improved to maximise oestrus expression on a unit, whereas others may not be practical to alter.

1.2.2.2 Cow factors

Factors specific to the cow will also affect oestrus expression, in addition to the environment and management system she is kept in. As cows get older and go through more lactations they seem to be less active around oestrus with Lopez-Gatius and others (2005) reporting a significant decline in walking activity at oestrus with increasing parity. In contrast older cows do seem to exhibit other oestrus behaviours such as mounting and standing to be mounted more intensely (De Silva and others 1981).

There can also be a genetic effect on oestrus expression. This can occur at the species level with *Bos taurus* cattle being shown to exhibit oestrus behaviour for longer periods than *Bos indicus* cattle (Galina and others 1982). Oestrus behaviour is reduced to such an extent in *Bos indicus* cattle that oestrus detection and artificial insemination is much less frequently used in these species with other reproductive technologies and fixed time artificial insemination being used instead (Bó and others 2003). Differences in oestrus expression and detection can also occur within a species and between breeds (Cutullic and others 2009; Dillon and others 2003). Although there does appear to be a genetic effect on oestrus expression, Roxström and others (2001) identified low heritability of both oestrus intensity and inter-service interval.

Variations in milk yield are a possible explanation for within and between breed differences in oestrus expression. As stated earlier over the previous few decades milk yields have increased alongside an apparent decline in dairy cow reproductive performance (Dobson and others 2008; Walsh and others 2011). Whilst some authors have suggested the decline in reproductive performance is associated with the poor management of high producing dairy cows rather than yield *per se* (LeBlanc 2010), there remain some mechanisms by which increased milk production may lead to a reduction in oestrus expression. Higher producing dairy cows have been shown to have a lower duration of oestrus compared to comparable lower yielding cows (Lopez and others 2004). The same study identifies lower circulating oestradiol and progesterone concentrations in the high yielding cattle. One widely accepted explanation for this is that as milk yield increases so does feed

consumption, liver blood flow and steroid metabolism (Parr and others 1993; Sangsritavong and others 2002). This would result in reproductive steroid hormones such as progesterone and estradiol being cleared from the circulation faster (Wiltbank and others 2006). There is also some overlap of milk yield and metabolic health: cows producing a higher milk yield are more likely to experience more pronounced and/or prolonged early lactation negative energy balance. Increased levels of mobilised non-esterified fatty acids (NEFAs, a measure of negative energy balance) have been associated with reduced walking activity (Adewuyi and others 2006).

As well as metabolic abnormalities, other diseases in dairy cows can affect oestrus expression. Mastitis (inflammation of the udder) is a common condition, usually caused by a bacterial infection of the udder. Cases of clinical and sub-clinical mastitis have been shown to delay the return to cyclicity after calving as well as leading to premature luteolysis in cycling cows, this could explain why it has also been demonstrated that cows with mastitis were more likely to exhibit abnormal oestrous cycles (Huszenicza and others 2005; Moore and others 1991; Schrick and others 2001). There are several mechanisms by which mastitis has been demonstrated to impact on ovarian function: One mechanism is that mastitis (along with other diseases) has been shown to lead to elevated levels of prostaglandin $F_{2\alpha}$ resulting in premature luteolysis (Hockett and others 2000); another potential mechanism is that cows with mastitis have been shown to have lower circulating oestrogen levels (Hockett and others 2005). As well as clinical mastitis cases, sub clinical mastitis, detected by elevated milk somatic cell counts, has been shown to reduce the intensity of oestrus expression (Morris and others 2013). As well as direct effects on oestrus expression and cyclicity, mastitis may also lead to embryonic death and/or failure to conceive which may affect the apparent oestrous cycle length, mastitis also appears to delay the oestrus to ovulation interval in some cows which may be another mechanism reducing conception rate (Hansen and others 2004; Lavon and others 2011; Lavon and others 2010).

Lameness is another common endemic condition of dairy cows, with a variety of infectious and non-infectious causes (Archer and others 2010). Cows with lameness have been shown to have poorer reproductive outcomes than those without lameness (Lucey and others 1986; Melendez and others 2003). As with mastitis, lameness has been shown to delay the resumption of ovarian activity post calving (Garbarino and others 2004). Lameness has also been shown to lead to an increased risk of the development of ovarian cysts (Melendez and others 2003). Lameness seems to reduce the proportion of cows ovulating (Morris and others 2009). As might be expected, lameness has also been shown to reduce oestrus expression (Walker and others 2008a; Walker and others 2010; Walker and others 2008b). This could be a result of physical limitation to expressing oestrus as a result of being lame, with increased steps and mounting/standing to be mounted behaviour becoming difficult to perform. However, rather than a reduction in ability to perform the behaviours Walker and others (2008b) showed that lame cows spent longer lying down, reducing the time available to perform oestrus behaviour. Lameness has also been used as a model to evaluate the effects of chronic stress on oestrus intensity and hormone profiles, showing reduced levels of progesterone prior to oestrus in lame cows, this suggested a further endocrinological mechanism for the reduction in oestrus intensity associated with lameness (Walker and others 2008a).

There are many cow factors that can affect expression of oestrus. Whilst minimising disease will always be a sensible approach and should improve oestrus expression, other factors such as parity of the cow may be harder to “improve” to maximise expression of oestrus.

1.3 Measuring oestrus detection

Given the importance of oestrus detection in herd reproductive performance and the potential for improved oestrus detection within a herd, monitoring oestrus detection is a key component of the herd health and production management approach increasingly taken by veterinary surgeons working with dairy farms (Breen and others 2009). Numerous parameters are defined in the literature to attempt to quantify oestrus detection. When evaluating oestrus detection in a herd, it is necessary to assess both the “efficiency” or sensitivity of oestrus detection (a concept commonly

defined as the proportion of oestrus events that are detected) and the “accuracy” or specificity of oestrus detection (commonly taken to refer to the proportion of oestrus events detected that were truly in oestrus) (Heersche and Nebel 1994; Morton and Wynn 2010). A frequently suggested parameter for assessing oestrus detection sensitivity is the submission rate (Breen and others 2009; Esslemont 1992; Gordon 2011). This is the proportion of eligible cows (e.g.. cows within 24 days of the end of the voluntary waiting period and those 18 to 24 days after a previous unsuccessful insemination) that are recorded as receiving an insemination in the correct or expected interval (as defined in the brackets above) or in a given time period (e.g. a three week window). It is frequently broken down into first serve submission rate (those within 24 days of the end of the voluntary waiting period) and return serve submission rate (those within 18 to 24 days of a previous unsuccessful insemination), this allows more targeted analysis of where performance can be improved (Meadows 2005). Submission rate can be monitored over time and will change relatively rapidly with changes in performance. A method of measuring oestrus detection that appears to be gaining in popularity is the 21-day insemination rate, this is often shown alongside 21-day pregnancy rate (the proportion of eligible cows becoming pregnant every three weeks) and is the proportion of cows eligible for insemination receiving an insemination in any three week period (Fetrow and others 2007). This will usually be highly correlated with submission rate.

Another method of monitoring oestrus detection efficiency is by analysing the interval to an insemination. For example the mean, median or distribution of time from calving to first service for a herd can give an indication of how rapidly early lactation cows are detected in oestrus (Breen and others 2009). When analysing return oestrus detection (the re-insemination of cows previously inseminated that did not become pregnant) intervals between successive inseminations for a given cow can also be used. The use of the mean interval between inseminations has been described but can be hard to interpret (Heersche and Nebel 1994).

The approach more commonly used to analyse the interval between inseminations is the inter-service interval (ISI) profile. This is produced by calculating the intervals between subsequent inseminations during a cow's lactation (inter-service intervals, ISIs). These are normally calculated for all cattle in the herd for fixed time period, typically 12 months. The frequency distribution of intervals between subsequent inseminations (serves) across different categories is then examined (Hudson and others 2012b; Meadows 2005). The categories are all calculated based on the expected cow oestrous cycle length of 18 to 24 days (Hartigan 2004). With some slight variation, the approach advocated is normally based on the categories described by Meadows (2005) and shown in Table 1-1. The use of ISIs in fertility assessment is widespread in the UK and is available in commonly used herd data analysis software packages (Interherd and Interherd+, PAN Livestock Services, UK; TotalVet, SUM-IT/QMMS, UK). A method for summarising ISI profiles to provide a single numeric measure to evaluate return oestrus detection using inter-service intervals is to use the Warren Equation to calculate the oestrus detection efficiency (ODE) (Gordon 2011). This is calculated using the number of inseminations in each of the categories described in Table 1-1 in the following equation:

Equation 1-1 Oestrus detection efficiency

$$ODE = \frac{(short\ regular + long\ regular)}{(short\ irregular + short\ regular + long\ irregular + 2(long\ regular + extended)}$$

ISI profiles have the benefit of allowing an assessment of both oestrus detection sensitivity (the percentage of inseminations carried out at a short regular interval) and accuracy (a lower proportion of inseminations carried out at short irregular intervals) (Heersche and Nebel 1994). An assessment of the importance of late embryonic death in a herd can also be made, usually based on the percentage of long irregular intervals although it is worth noting ISI profiles only represent cows failing to conceive to their first insemination or that do not cycle leading to a potential bias. A more accurate representation of cyclicity and oestrus detection in a herd may be achieved using serial progesterone testing of all cows to avoid this bias (Bulman and Lamming 1979).

Table 1-1 Categories of inter-service interval (ISI) commonly used in data analysis, after Meadows (2005)

Interval Range	Description	Standard interpretation
2-17 days	Short irregular	Inaccurate oestrus detection (estimates inverse of accuracy or specificity) and pathological (although these represent a minority, such as cystic ovarian disease)
18-24 days	Short regular	Accurate oestrus detection at first heat (estimates efficiency or sensitivity)
25-35 days	Long irregular	Late embryonic death and/or inaccurate oestrus detection
36-48 days	Long regular	Accurate oestrus detection following one missed heat or late embryonic death
>48 days	Extended interval	Multiple missed heats or foetal death

The approach was described by Weaver (1986) and remains in use to the present day. Despite the continued use of this method of dairy herd fertility data analysis there are limited studies investigating suggested target levels and comparing herds. In a review by Roelofs and others (2010) various manifestations of poor oestrus detection affecting the inter-service interval profile are described. They suggest that short irregular intervals exceeding 10-15%, short regular intervals less than 60-70%, long irregular intervals above 10-15%, long regular intervals above 10% and extended intervals above 5% are all signs of oestrus detection errors justifying investigation. The same authors suggest comparing the ratio of short regular intervals to long regular intervals, citing target values suggested by Heersche and Nebel (1994) and Weaver (1986) of a ratio of 6:1 with 4:1 representing acceptable performance. This target ratio was advocated when the method of analysis was proposed (Weaver 1986; Williamson 1987) and represents a theoretical target based on achieving a oestrus detection rate of 85% across all oestrus events. In the same paper Heersche and Nebel (1994) state that 85% is a gold standard for short regular intervals (an indicator of oestrus detection rate) but

that 60-70% is a much more realistic target. More recent work in the UK indicated that even the revised target of 60-70% may be over optimistic, with Hudson and others (2010) finding mean return submission rates of only 33% in a large sample of UK dairy herds.

The shortfalls of suggesting targets and intervention levels without reference to “real life” data was highlighted by Gaines and others (1992), who analysed the ISI profiles of 71 dairy herds from the United States. As would be expected from the findings described above, very few were able to achieve the suggested target values, with only seven of the 71 herds achieving a short regular to long regular ratio of over 4:1. Furthermore, Gaines and others (1992) compared the ISI profile performance of the 71 dairy herds to overall fertility indices, they were unable to find any correlation between ISI performance and overall fertility performance of the herds. This led them to question the validity of ISI profiles as a method of measuring fertility performance. Interestingly in this study of 71, some herds had as many as 25% of intervals in the long irregular category (25-35 days) and yet maintained good conception rates. This seems inconsistent with the interpretation of a high proportion of long irregular ISIs being associated with a high degree of late embryonic death; a high degree of late embryonic death would seem likely to be associated with lower herd conception risk. Some studies which did not specifically look at ISI profiles as a whole also captured insemination dates and reported some more limited results, this also revealed herds rarely perform at the desired target levels. In a study of 2,618 British Friesian cattle the ratio of short regular to long regular intervals was only 2.5 to 1 (Wood 1976). A study examining oestrus detection on 476 dairy herds in the USA revealed a ratio of 2.75 to 1, although this study only looked at the repeat insemination of cattle confirmed to be in oestrus at the first insemination using progesterone testing (Reimers and others 1985).

Whilst ISI profiles are in common use in practice, there is a lack of data on what truly constitutes normal and good performance for herds. Further work is needed to clarify both target levels as well as whether ISI analysis is a useful means of assessing herd reproductive performance.

1.4 Oestrus cycles

Effective oestrus detection relies on a cow coming in to oestrus as part of her normal oestrous cycle. The inter-service interval analysis described above is based on current understanding of the duration of the bovine oestrous cycle. Cattle are polyoestrous, coming into oestrus cyclically throughout the year. The oestrous cycle begins at puberty (around six to twelve months of age), and the duration of each cycle is approximately 21 days with a commonly accepted normal range of 18 to 24 days (Forde and others 2011; Hartigan 2004). The oestrous cycle is regulated by the interaction of hormones from the hypothalamic pituitary axis, the ovaries and the uterus. Boer and others (2011b) summarised these interactions in a deterministic mathematical model of the endocrine processes of the bovine oestrous cycle. The hormonal interactions giving rise to the bovine oestrous cycle are outlined below. The bovine oestrous cycle is characterised by a progesterone dominated 'luteal period' lasting about two weeks and an oestrogen dominated 'follicular period' period lasting about a week (Forde and others 2011). Ovulation occurs towards the end of the follicular period. The bovine oestrous cycle is well described and has been reviewed by Forde and others (2011), key steps are outlined below.

1.4.1 Follicular selection, dominance and ovulation

Gonadotrophic releasing hormone (GnRH) is produced in a pulsatile fashion by the tonic centre of the hypothalamus, this reaches the anterior pituitary gland via the hypophyseal portal system, stimulating the release of both luteinising hormone (LH) and follicle stimulating hormone (FSH) (Schally and others 1971). LH and FSH are then also released in a pulsatile fashion, acting on the ovary to stimulate growth of ovarian follicles. As these follicles develop they produce oestrogens and inhibins which act to suppress FSH release by negative feedback. As FSH decreases, the follicles regress in a process known as atresia. As the follicles undergo atresia, oestrogen and inhibin secretion declines again which in turn leads to increased production of FSH as the negative feedback is removed, this results in the development of another wave of follicles. This cyclical increase and then decrease in FSH and corresponding development of follicles on the ovary gives rise to the

follicular waves (Adams and others 1992). These follicular waves occur without ovulation (and usually without oestrus) during the progesterone dominated luteal phase. Initially, each follicular wave consists of the development of numerous follicles, however one of the emerging follicles becomes dominant (Ginther 2016). The dominant follicles shifts as it grows to become dependent on LH for development, this leads to regression of the subordinate follicles as FSH declines, suppressed by inhibin and oestradiol produced by the dominant follicle (Adams and others 2008). As the dominant follicle develops it produces increasing levels of oestrogens. When the circulating levels of oestrogen are high enough they trigger a pre-ovulatory surge in GnRH from the surge centre of the hypothalamus, in the absence of progesterone (following luteolysis) this triggers an LH surge leading to ovulation of the dominant follicle (Sunderland and others 1994). This surge in GnRH and then LH is the cause of ovulation, but a surge can only occur when there are low levels of circulating progesterone and an appropriate amplitude and frequency of the background pulsatile release of LH is needed for the follicle to reach a sufficient stage of development and oestrogen production (Mann and Lamming 1995).

1.4.2 Luteinisation and the luteal phase

After ovulation the dominant follicle collapses, becoming a corpus haemorrhagicum which forms a corpus luteum over 3-4 days. The corpus luteum develops under the influence of LH, producing increasing levels of progesterone (Henderson and Moon 1979). In the presence of high circulating levels of progesterone the pulsatile release of LH is reduced in frequency. This means that in the presence of a corpus luteum, the negative feedback effect of progesterone prevents the LH surge and therefore ovulation occurring (Milvae and others 1996). Simultaneously, the altered LH pulse frequency limits follicular development and therefore oestrogen production.

1.4.3 Luteolysis

As the corpus luteum develops after ovulation the high progesterone concentrations act on the endometrium of the uterus to initiate uterine receptivity to oxytocin (Kimmins and Maclaren 2001). If a conceptus is present (following successful insemination at the time of ovulation) then maternal

recognition of pregnancy should occur after 16 days, with the production of interferon tau from the conceptus (Bazer and others 1997). In the absence of a conceptus and therefore interferon tau, the uterus will produce prostaglandin-F2α (PGF2α) (Lamothe and others 1977; Mann and Lamming 2001). The PGF2α release is mediated by oxytocin, oxytocin receptors in the uterus are upregulated by oestradiol prior to luteolysis (Robinson and others 2001). Binding of oxytocin from the ovary then leads to the pulsatile release of PGF2α from the luminal epithelium (Mann and Lamming 2006). The PGF2α then passes into the uterine vein and is exchanged across a counter current mechanism to the ovarian artery. PGF2α is luteolytic and induces regression of the corpus luteum. As the corpus luteum regresses progesterone levels decline removing the negative feedback on the LH surge, this allows the development and ovulation of a follicle as oestrogen levels increase again.

1.4.4 Oestrous cycles

It is this cyclical pattern of progesterone secretion by the corpus luteum and then oestrogen secretion by the follicles and the associated negative feedback that gives rise to the oestrous cycle. The cyclical changes in hormone levels throughout the cycle are illustrated in Figure 1-1 (Forde and others 2011).

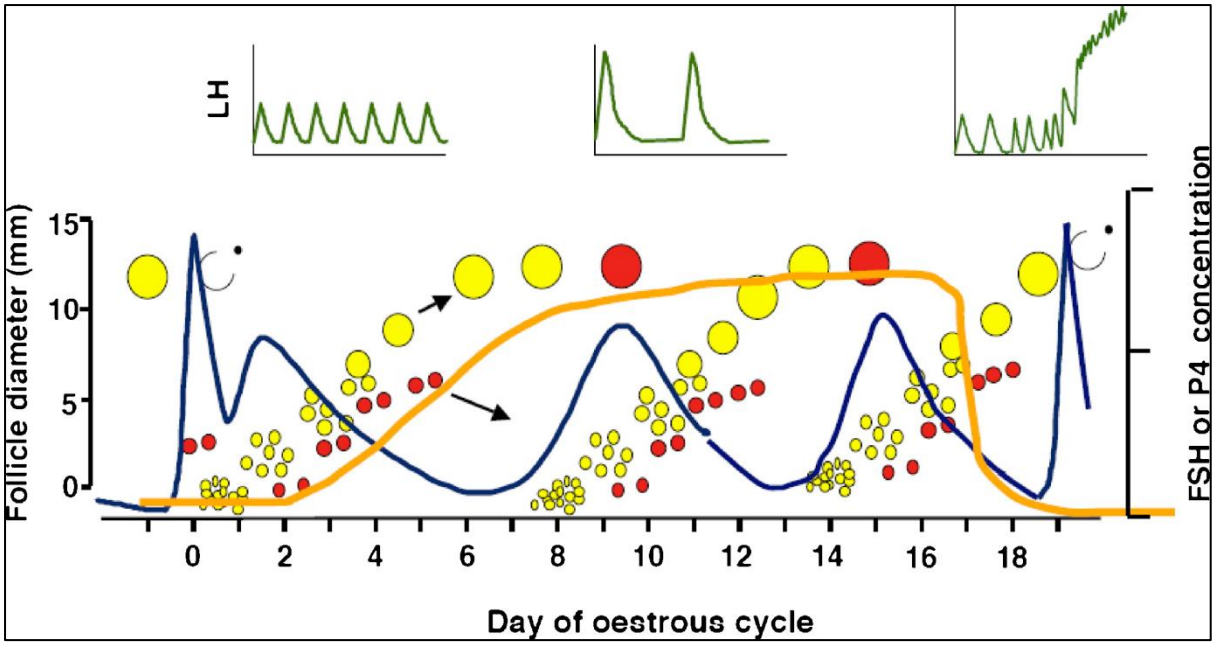


Figure 1-1 Schematic of the cyclical changes in hormones during a bovine oestrous cycle with three follicular waves: the blue line shows follicle stimulating hormone (FSH), the orange line progesterone (P4), the green line luteinising hormone (LH); yellow circles indicate developing follicles, red circles indicate atretic follicles and the open circle ovulation. Reprinted from Animal Reproduction Science 124, FORDE, N., BELTMAN, M. E., LONERGAN, P., DISKIN, M., ROCHE, J. F. & CROWE, M. A., Oestrous cycles in Bos taurus cattle, pp. 163-169 , Copyright (2011), with permission from Elsevier

The behavioural signs associated with oestrus are caused by the action of oestrogen on the brain, in the absence of progesterone but following progesterone priming (Vailes and others 1992). Hence, oestrus typically occurs just prior to ovulation as circulating oestrogen level increases with follicular growth. The effect of this lag between oestrus and ovulation on conception rate has been investigated to attempt to calculate the optimum timing of artificial insemination relative to behavioural oestrus. Most of these studies indicate delaying insemination until around twelve hours after the onset of oestrus is optimal (Gwazdauskas and others 1986; Roelofs and others 2005b). This finding has given rise to the 'am-pm' rule, whereby if a cow is seen in oestrus in the morning it is inseminated in the afternoon and if a cow is observed in oestrus in the afternoon it is inseminated the following morning. However, some studies have found no benefit to applying the am-pm rule (Foote 1979; Nebel and others 1994). It is thought that this is due to variable levels of oestrus

detection on farms, with those herds efficiently detecting the onset of oestrus benefitting most from a delay before insemination, whereas those that detect oestrus less efficiently may not detect the onset and so there is little benefit from delaying insemination (Dransfield and others 1998).

As stated previously the length of the bovine oestrus cycle is commonly thought to be 21 days with a normal range of 18 to 24 days (Forde and others 2011; Hartigan 2004). The cycle was first described by Hammond (1927, cited in Chapman and Casida 1935) after a study on three nulliparous heifers. Since this study 21 days has remained the accepted normal cycle length, however subsequent studies have often resulted in slightly longer lengths. Olds and Seath (1951) carried out a study analysing eighteen years of breeding records for a dairy farm in Kentucky, across 3,776 inter-service intervals from 278 cows, 22 days was the modal interval between inseminations. This may be explained by the findings of Chapman and Casida (1935), who found that the average cycle length of "copulatory cycles" where the cow had been inseminated at the first oestrus was 22 days, whereas for cycles where the cow had not been bred the average cycle length was 21 days. However more recent studies using endocrine testing and transrectal ultrasonography to determine the cycle length have frequently found a modal cycle length of 22 days rather than 21 in both inseminated and non-inseminated animals (Bleach and others 2004; Sartori and others 2004; Wolfenson and others 2004). However, these studies used relatively small numbers of animals on single farms (158, 60 and 39 animals per study respectively). Despite this potentially contradictory evidence, a 21 day cycle length has remained 'received wisdom'. During a review of the literature to create a reference range of the hormones of the bovine oestrous cycle Martin and others (2013) standardised their reference ranges to a 21-day cycle despite the finding that "the average cycle duration varies from 21 to approximately 23 days for Holstein cows". This was also the finding of a brief literature review included in the discussion of Sartori and others (2004), concluding that "the interovulatory interval for lactating Holstein cows in our study and from the literature was about 2 d longer than the commonly accepted 21-d average". Both these reviews of the literature refer to Holstein dairy cows, which may provide an explanation. In two older studies where different breeds were used, Holstein

dairy cows were found to have a longer oestrous cycle than the other breeds in these studies, resulting in a bimodal distribution (Britt 1995; Joubert 1954).

Another factor which appears to be related to oestrus cycle length, or which may explain the breed variation described above, is the number of follicular waves that occur in a given oestrous cycle. In a review on follicular dynamics, Adams and others (2008) state that two wave cycles are usually 19-20 days long and three wave cycles are usually 22-23 days long and that the quoted 21 day length is merely the average of the two most common follicular wave patterns. This appeared to be confirmed in a study of 91 inter-ovulatory intervals where 88% of cycles less than or equal to 21 days long consisted of two follicular waves, where as 78% of cycles greater than or equal to twenty two days were three follicular wave cycles (Jaiswal and others 2009). However, there is considerable variation in the mean cycle length associated with two and three wave cycles in the literature (Adams and others 1992; Ahmad and others 1997; Bleach and others 2004; Ginther and others 1989; Jaiswal and others 2009; Savio and others 1990; Townson and others 2002). This variation is shown in Figure 1-2. Most studies find that two follicular wave cycles are the more prevalent cycle type, with a ratio of approximately three two-wave cycles for every one three-wave cycle (Sakaguchi and others 2004; Sartori and others 2004; Wolfenson and others 2004). Although two-wave cycles appear to be more common, some authors hypothesise that three-wave cycles will lead to an increased conception risk because the oocyte is less aged. Studies investigating this have often failed to find a significant difference between two and three wave cycles (Ahmad and others 1997; Bleach and others 2004; Townson and others 2002). Although statistical significance at the 95% level was not established, no power calculations were described in the studies and in all three studies conception rates were higher for 3-wave cycles. Wolfenson and others (2004) found that despite two-wave oestrous cycles being almost as prevalent in nulliparous heifers as in lactating dairy cows, heifers still had on average a shorter cycle length even when comparing cycles with the same number of follicular waves. There remains a plausible mechanism by which different oestrous cycle lengths and therefore ISIs may be associated with varying conception rates.

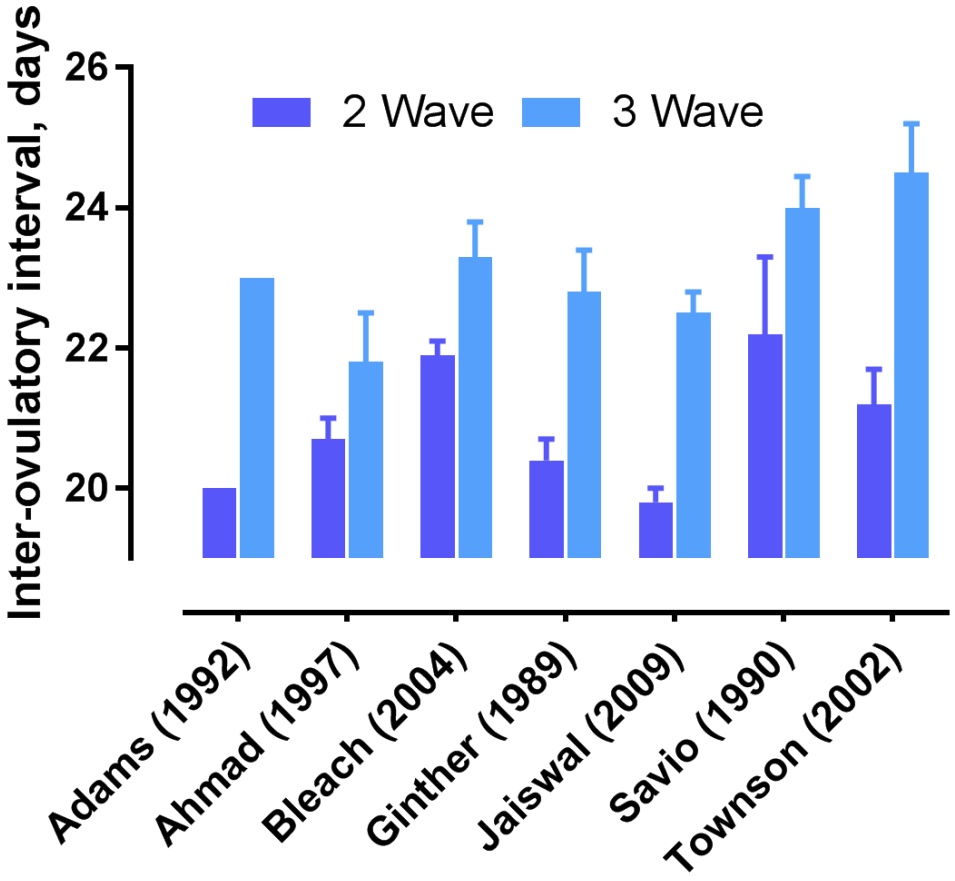


Figure 1-2 Summary of existing studies evaluating inter-ovulatory intervals in two and three follicular wave oestrous cycles, bars show the mean IOI for two and three wave cycles. Where present, error bars show stated standard error

Other factors are also known to affect cycle length. Lamming and Darwash (1998) and Kafi and others (2012) identified prolonged luteal phases occurring in cycling cows with a prevalence of 6.35% and 23.8% respectively (using slightly different definitions). The presence of prolonged luteal phases and other abnormal cycles can be further complicated when cattle are being inseminated. This can result in conception occurring and then late embryonic death delaying the return to oestrus (Britt 1995; Kafi and others 2012). As a result of the influence of abnormal oestrous cycles on the apparent cycle length, many of the older studies that pre-date the use of ultrasonography eliminated longer intervals from their datasets, excluding them as “abnormal” to give a mean of twenty one (Chapman and Casida 1935; Olds and Seath 1951; Wood 1976) perhaps explaining the

discrepancy between the accepted 21 day cycle length and the longer length often found in more recent small scale studies.

In lactating dairy cows, fertilisation rates might be slightly lower than typical for other species, but most animals that return to oestrus before being diagnosed as pregnant (or that are not detected in calf at routine pregnancy diagnosis) are still thought to have achieved fertilisation but are not detected as pregnant because the conceptus has subsequently undergone early embryonic death (Santos and others 2004). Following appropriate insemination at the time of ovulation, it is estimated that in around 90% of cases fertilisation occurs, with most embryonic loss occurring (usually before pregnancy detection) between eight and sixteen days later (Diskin and Sreenan 1980). It has been shown that from day fifteen after conception the embryo influences the lifespan of the corpus luteum, prolonging the inter oestrus interval (Northey and French 1980). It is possible that apparent cycle length appears to vary depending on whether or not the cow was inseminated at the first oestrus of the interval. Britt (1995) suggests that longer inter service intervals are used to identify embryonic death in cattle. Chapman and Casida (1935) noted that “copulatory cycles” (those following an insemination) were on average slightly longer than those where insemination was not carried out. Depending on the timing of embryo loss there is the potential for this to impact on apparent inter oestrus interval before detection of the pregnancy, it is not clear whether this is likely to be a natural service or insemination affect.

What is clear from all the studies described is that, irrespective of mean or modal oestrous cycle length and number of follicular waves per cycle, there is some degree of variation within a normal range. There is little work exploring the variability of cycle length. Olds and Seath (1951) used analysis of variance on 3,776 cycles to determine that there was significantly more between cow variation than within cow variation. Chapman and Casida (1935) also found that there was individual cow variation within the range 17 to 27 days. This is supported by the much more recent findings of Jaiswal and others (2009), who concluded that the follicular wave pattern (i.e. number of follicular

waves per oestrus cycle was repeatable within a cow, with 70% of cycles repeating the same pattern. However, when Boer and others (2011a) refined their deterministic mathematical model of the bovine oestrous cycle to account for some mechanisms that may underpin follicular wave patterns, such as follicular growth rate and time to luteal regression, one of the patterns created was an alternating pattern of two then three follicular waves.

Overall, there are numerous studies evaluating the oestrous cycle of dairy cows in a relatively small number of cows. There appears to be some discrepancy between reported inter-ovulatory interval and inter service interval in research and the commonly accepted 'normal' range. The cycle length appears to be related to the number of follicular waves in the cycle and this has been reported to be repeatable for an individual cow.

1.5 Summary

Cattle fertility is an important aspect of a global industry. Good reproductive performance can be a key driver of efficient production in dairy farms. Good oestrus detection is an essential component of this and requires regular monitoring by veterinary surgeons and other farm advisors. ISI profiles are a commonly suggested method for monitoring and better understanding oestrus detection. Despite their widespread use there are no large scale studies to assess ISI profile performance on dairy farms and the current and historic literature indicates that the recommended target levels are likely to be unachievable for most herds. Interpretation of ISI profiles is underpinned by our understanding of the bovine oestrous cycle. Whilst the cycle itself is well characterised, there appear to be some discrepancies over what constitutes a normal cycle length and how this relates to conception risk. Most studies have been carried out on small numbers of animals to gather high levels of detail on ovarian and endocrine function. There is a lack of studies on larger populations of animals attempting to quantify the normal variation of oestrous cycle length. This may impact on the most appropriate way to measure oestrus detection in dairy herds. Disease may also impact on cyclicity and affect performance, the apparent impact of disease may also vary depending on how return oestrus detection is measured.

1.6 Aims

The work described in this thesis consists of five studies:

1. From previous studies it is apparent that many farms fail to achieve recommended targets for return insemination performance as measured by inter service interval profiles. The primary aim of the first study was to address the lack of appropriate targets when analysing the ISI profiles of UK dairy herds and to explore the distribution of inter-service intervals in a large sample of UK dairy herds and establish targets for use by practitioners when interpreting ISI profiles.
2. There are varying reports of what constitutes a normal inter oestrus and therefore inter service interval in dairy cows. It is plausible that numerous cow and herd level factors may impact on the oestrus cycle of the cow. This may be affecting apparent performance as described in previous work (1). The primary aim of the second study was to assess the inter-service interval in a large number of dairy cows, to explore the variability in oestrous cycle length and to identify associations between cow factors and cycle length as a possible explanation for the earlier findings (1).
3. Inter-service intervals may be longer than expected: if these correspond to genuine oestrus events then conception risk should be similar at these intervals to that at more commonly accepted normal intervals. The length of the oestrus cycle itself may impact on conception risk. The primary aim of this study was to understand whether the common ISIs identified earlier (2) correspond to periods of increased fertility (conception risk) and whether conception risk varied with apparent cycle length.
4. Initial findings may mean that the methods of measuring oestrus detection described above may not be appropriate in UK herds. The aim of this fourth study was to identify whether accounting for the findings of the earlier work (2, 3) influenced the interpretation of the oestrus detection data from UK dairy farms as well as identifying whether the change in interpretation varied from farm to farm. This study aimed to test and evaluate a series of

new metrics to identify effective ways of measuring return oestrus detection as well as providing a model for evaluating more “diagnostic” metrics.

5. Amongst other factors, disease has been shown to influence oestrus expression and therefore detection. The aims of the final study were to quantify the impact of lameness and mastitis on return oestrus detection, to understand whether these diseases affect oestrus detection. Their impact will be measured using the traditional expected time of return as well as using an updated interval identified in earlier (2,3) to test whether both methods are as sensitive to the impact of disease.

2 Describing and benchmarking inter-service interval profiles

2.1 Introduction

Reproductive performance in dairy herds is dependent on both the rate at which cows are detected in oestrus and inseminated (submission rate) and the rate at which inseminated cows become pregnant (conception rate) (Breen and others 2009), and monitoring reproductive performance on dairy farms is a key role of veterinary surgeons. As described in Chapter One, various parameters can be used to monitor reproductive performance including multiple approaches for measuring oestrus detection (Gordon 2011). It is important to monitor both the ability to detect a cow's first oestrus after calving, and the ability to detect subsequent oestruses in cows previously inseminated but not pregnant. In both instances it is important to consider both the accuracy of detection (specificity) and the efficiency (sensitivity) of detection (Heersche and Nebel 1994; Meadows 2005).

A commonly proposed method of using farm data to monitor oestrus detection in cows returning to oestrus after a previous service is to calculate the frequency distribution of intervals between subsequent inseminations during a cow's lactation (inter-service intervals, ISIs). The use of ISI profiles to monitor oestrus detection at a herd level involves comparing the distribution of ISIs across set categories (Meadows 2005). These categories are determined based on the assumption that a normal, cyclic, non-pregnant cow will ovulate on average every 21 days, with a normal range of 18 to 24 days (Forde and others 2011; Hartigan 2004). These categories are defined in

Table 2-1.

Various target and intervention levels are suggested when examining ISI profiles. For example, Roelofs and others (2010) propose that intervals less than 18 days should account for less than 10-15% of re-serves and normal intervals of 18-24 days should account for greater than 60-70% of re-serves. Heersche and Nebel (1994) suggest a target ratio of 6:1 for 18-24 day (short regular) inseminations compared to 36-48 (long regular), with an intervention level of 4:1.

Table 2-1 Description and interpretation of inter-service interval categories (after Meadows (2005))

Interval Range	Description	Interpretation
2-17 days	Short irregular	Inaccurate oestrus detection (estimates inverse of specificity) and pathological*
18-24 days	Short regular	Accurate oestrus detection at first heat (estimates sensitivity)
25-35 days	Long irregular	Late embryonic death and inaccurate oestrus detection
36-48 days	Long regular	Accurate oestrus detection following one missed heat
49+	Extended interval	Multiple missed heats, foetal loss and abortion

**Whilst pathology (such as cystic ovarian disease) may affect the inter-oestrus interval of an individual cow, this is much rarer and is unlikely to have a dramatic influence on the herd pattern analysed using this technique*

Whilst this method is commonly described in the literature (Hanks 2008; Heersche and Nebel 1994; Meadows 2005) little work exists to assess the distribution of intervals on a large population of dairy cows and quantify the normal between-herd variation; especially in modern UK herds. One study of 71 Wisconsin dairy herds (Gaines and others 1992) found that most herds failed to achieve the commonly accepted target ISI distributions described above and that there was a poor correlation between abnormal ISIs and other reproductive parameters.

The aim of this study was to explore the distribution of inter-service intervals in a large sample of UK dairy herds and establish targets for use by practitioners when interpreting ISIs.

2.2 Materials and Methods

A set of management data from 468 English and Welsh dairy herds contributed by a group of 20 bovine practitioners as part of a larger project (Hudson and others 2012a; Hudson and others 2010) was used for this study. Although not a probabilistic sampling method, this convenience sample was considered appropriate as high quality data were essential for the analysis. Data sources were on-farm software, practice bureau recording services and milk recording organisation data. The data was amalgamated and standardised from various sources and contained health, fertility and production data for each farm. The anonymised herd datasets were individually analysed for indicators of good quality fertility data. These included identifying randomly missing events: for example by calculating the proportion of calvings for which a corresponding insemination was recorded, and calculating the lactational incidence rate of various fertility events. Systematic errors were also identified by, for example, evaluating the apparent first insemination pregnancy risk and the proportion of inseminations which were the second of a pair between milk recording test days. Data quality was evaluated at herd-year level, so that herds only contributed data for calendar years in which recording was considered acceptable. The data from one herd for one calendar year will be referred to as a "herd-year". Only data from lactations beginning in the years 2000-2008 was used.

Herds were assessed for any apparent seasonality in the number of inseminations per month. Two datasets were created, one using all herd-years with good quality data (ALL), and the subset of these herds which showed no apparent seasonal pattern of inseminations (all year round calving, AYR). This subset was created as it was considered possible that some analyses would be affected by seasonality (e.g. "falsely" extended service intervals in cows not becoming pregnant in a seasonal breeding block which were retained in the herd to be bred in the next season). After removing herds failing to meet the inclusion criteria for any year, a sample of 167 ALL herds and 103 AYR herds remained.

The data was structured so that each service event represented a line of data. The ALL group consisted of 449,471 inseminations in 181,159 lactations from 67,926 cows. The AYR consisted of 255,722 inseminations in 101,123 lactations from 40,409 cows. For each service record the date, lactation number and service number were recorded, along with calving date. Intervals between a service event and the cow's previous insemination in the same lactation were calculated, resulting in a total of 268,312 inter-service intervals (ISIs) in the ALL group and 154,599 in the AYR group. ISIs of less than two days were assumed to be related to the same oestrus event and so were excluded from the analysis (1,870 of 268,312 intervals and 1,088 of 154,599 intervals in the ALL and AYR groups respectively). As highly extended ISIs were considered likely to be related to abortion or anomalies in the records, a sensitivity analysis was carried out by repeating the analysis described below on subsets of the data excluding ISIs over 150, 200, 250 and 300 days. Results for analyses of these subsets were extremely similar, so results from the subset excluding intervals over 200 days (11,181 of 268,312 intervals and 1,525 of 154,599 intervals in the ALL and AYR groups respectively) are reported. Herd-years containing less than 100 ISIs were removed from the dataset to eliminate outliers caused by very small numbers of ISIs occurring in a herd-year (512 of 1,396 herd years were excluded in the ALL group and 210 out of 741 herd years in the AYR group).

The proportion of ISIs that fell into each of the categories described in Table 2-1 was calculated for each herd-year in both the ALL and AYR datasets. The distributions of these proportions across the herd-years were visualised using violin plots (Hintze and Nelson 1998). The proportion of ISIs within the short irregular category (intervals <18 days) was plotted against the proportion within the short regular category (18-24 days) for each herd-year. The correlation between these was evaluated using Spearman's rank correlation coefficient. This was done to identify whether herds with more 'sensitive' oestrus detection (i.e. those detecting more oestrus events at the short regular interval) tended to be less specific (i.e. inseminating more cows in the short irregular interval).

To enable achievable targets to be set for the interpretation of ISI profiles, the herd-years were ranked by an overall measure of oestrus detection. An accepted way of generating a single measure of oestrus detection based on ISI profiles is to calculate oestrus detection efficiency (ODE) using the Warren equation (Gordon 2011). ODE was calculated for each herd-year by dividing the number of ISIs falling in the short and long regular interval categories by the total number of intervals, with long regular and extended intervals weighted in the denominator by a factor of two, as shown in the following equation.

Equation 2-1 Calculating oestrus detection efficiency (ODE) from the proportion of inter service intervals falling within each category

$$ODE = \frac{short\ regular + long\ regular}{[short\ irreguar + short\ regular + long\ irregular + 2(long\ regular + extended)]}$$

Herd-years were ranked by ODE, with those having the highest ODE considered the best performing. Herd-years were then split into subsets representing the top 10%, top 25% and top 50% of the dataset by ODE. For each of these subsets, the mean percentage of ISIs falling into each of the categories was calculated, in order to represent "typical" ISI profiles for herds achieving top 10, 25 and 50% oestrus detection performance.

Average calving interval for each herd-year was calculated (using the time to next calving for cows starting the lactation in the herd-year) as a measure of overall fertility performance using the data from 655 herd-years up to 2007 (to ensure subsequent lactation start dates were available for most cattle). Spearman's rank correlation coefficient was used to evaluate the relationship between herd-year ODE and calving index to confirm whether ODE was a valid method of ranking the herd-years. Spearman's rank correlation coefficient was also used to evaluate relationships between ODE and herd-year average 305 day milk yield, average herd size and year. In all statistical tests $p<0.05$ was considered significant.

The data was restructured and amalgamated using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington) and analysis carried out in R 2.15.0 (R Core Team 2013). The vioplot package (Adler 2005) was used to produce the violin plots in R.

2.3 Results

Descriptive statistics of all the herds included in the final analysis are given in Table 2-2. Table 2-3 shows the mean, median, upper and lower quartile herd-year performance in the ALL group for each category of the ISI profile, the ODE and the short regular to long regular ratio. For example, when comparing herd-year performance in the ALL group, 25% of herd-years had more than 39% (upper quartile) of intervals in the short regular (18-24 days) category, half the herd-years had >33% (median) and 75% of herd-years had >27% (lower quartile) in this category. Both the ALL and AYR datasets showed a wider between-herd variation in the percentage of ISIs in the 18-24 day and the 49+ category compared to the remaining categories. The findings were broadly similar across the two groups and for the remaining seasonal herds (median ODE was 0.36 for all groups), and so all subsequent analysis was carried out using the ALL group.

Table 2-2 Summary production statistics for the 655 herd-years included in the calving interval and ISI category analysis

	Mean	Median	Lower Quartile	Upper Quartile
Average 305 day milk yield/litres	7599	7713	7051	8478
Average herd size*	235	201	164	256
Calving index	420	417	431	404

**Average herd size was calculated by dividing the number of cows calving by the average calving interval/365 for each herd-year.*

Table 2-3 The mean, median, upper quartile (UpperQrt) and lower quartile (LowerQrt) herd-year performance for each ISI category, oestrus detection efficiency and the ratio of short regular to long regular reserve intervals

	UpperQrt	Median	Mean	LowerQrt
2 to 17	8%	6%	6%	3%
18 to 24	39%	33%	33%	27%
25 to 35	19%	15%	16%	13%
36 to 48	21%	18%	18%	15%
49 to 200	33%	26%	27%	20%
Oestrus detection efficiency	0.41	0.36	0.36	0.31
Short regular to long regular ratio	2.52	1.86	2.01	1.32

Figure 2-1 shows the distribution of herd-year performance within each category. The vertical range of the violin plot for each category shows the range of herd-years and the width of the violin plot represents a fitted kernel density (showing the frequency distribution). The white dot represents the median herd-year and the thick black line represents the inter-quartile range. For example the herd-years range from having 11% to 68% of inseminations within the herd-year falling in to the short regular (18-24 day) category, whereas the range for the late irregular (25-35 day) category is much narrower with herd-years performance ranging from 4% to 30%. The distribution of herd-year performance also varies between categories. For example in the short irregular (2-17 day) category the median herd-year (shown by the white dot) had 6% of inseminations in this category with an inter-quartile range from 3 to 8% with half the herd-years clustered in this range, however there are a small number of herd-years with a much higher percentage of inseminations falling in this category (up to 33%), this is shown by the upward tail of the violin plot, whereas in the late irregular (25-35 day) category the herd-years are much more symmetrically distributed around the median of 15%.

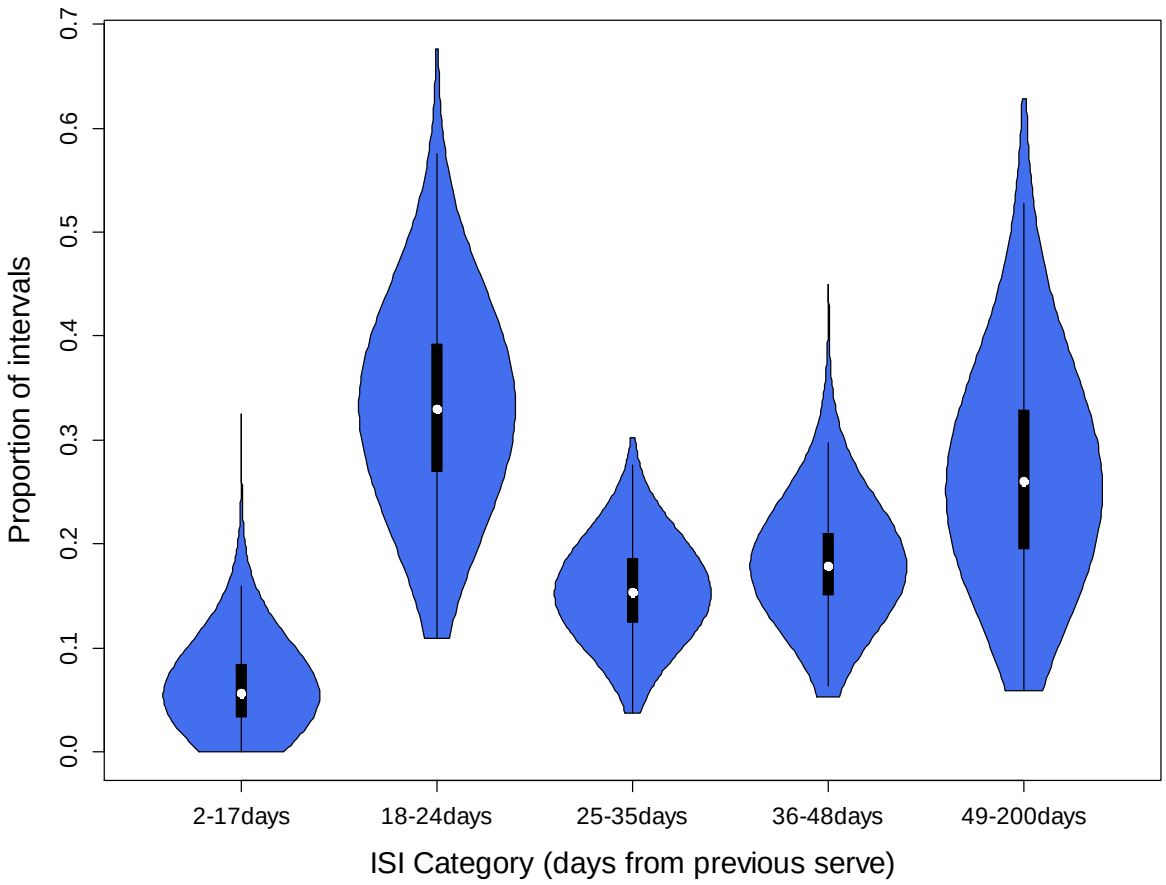


Figure 2-1 A violin plot showing the distribution inter-service interval performance calculated by herd-year from the ALL group within each category. The white dot is the median value, the thick black line the interquartile range and the vertical length of the violin is the fitted distribution. The width of the violin represents the distribution of herd-year ISI performance within this range.

There was no correlation between the proportion of ISIs in a herd-year falling in the short irregular (2-17 days) and short regular (18-24 days) category (magnitude of Spearman rho (r_s) <0.01, p=0.84) (Figure 2-2). ODE over the herd-years ranged from 0.16 to 0.63 with a median of 0.35, an upper quartile of 0.41 and a top decile of 0.47.

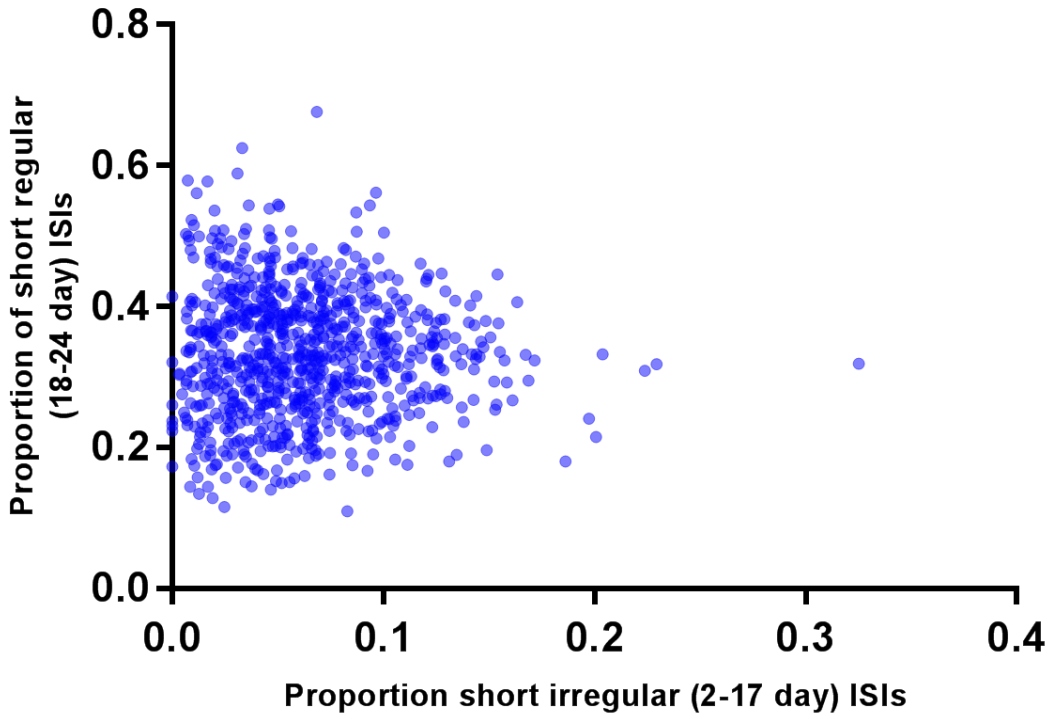


Figure 2-2 The relationship between herd-year short regular and short irregular interval categories (Spearman's Rho= -.0066)

Herd-year ODE negatively correlated with herd-year mean calving interval (r_s=-0.41, p<0.01). The ratio between short regular and long regular inseminations correlated less strongly with mean calving interval (r_s=-0.24, p<0.01), with both the percentage of inseminations in the short regular and extended interval categories having a stronger correlation with mean calving interval (r_s=-0.36, p<0.01 and r_s=0.39, p<0.01 respectively). The relationships between each individual measure and calving interval are shown in Figure 2-3. There was no significant correlation between herd-year ODE and average herd size, 305day milk yield or year.

The mean percentage of ISIs in each category for the top 10%, 25% and 50% of herd-years by ODE are shown in Figure 2-4. The top 10% of herd-years averaged 48% short regular intervals and 5% short irregular intervals.

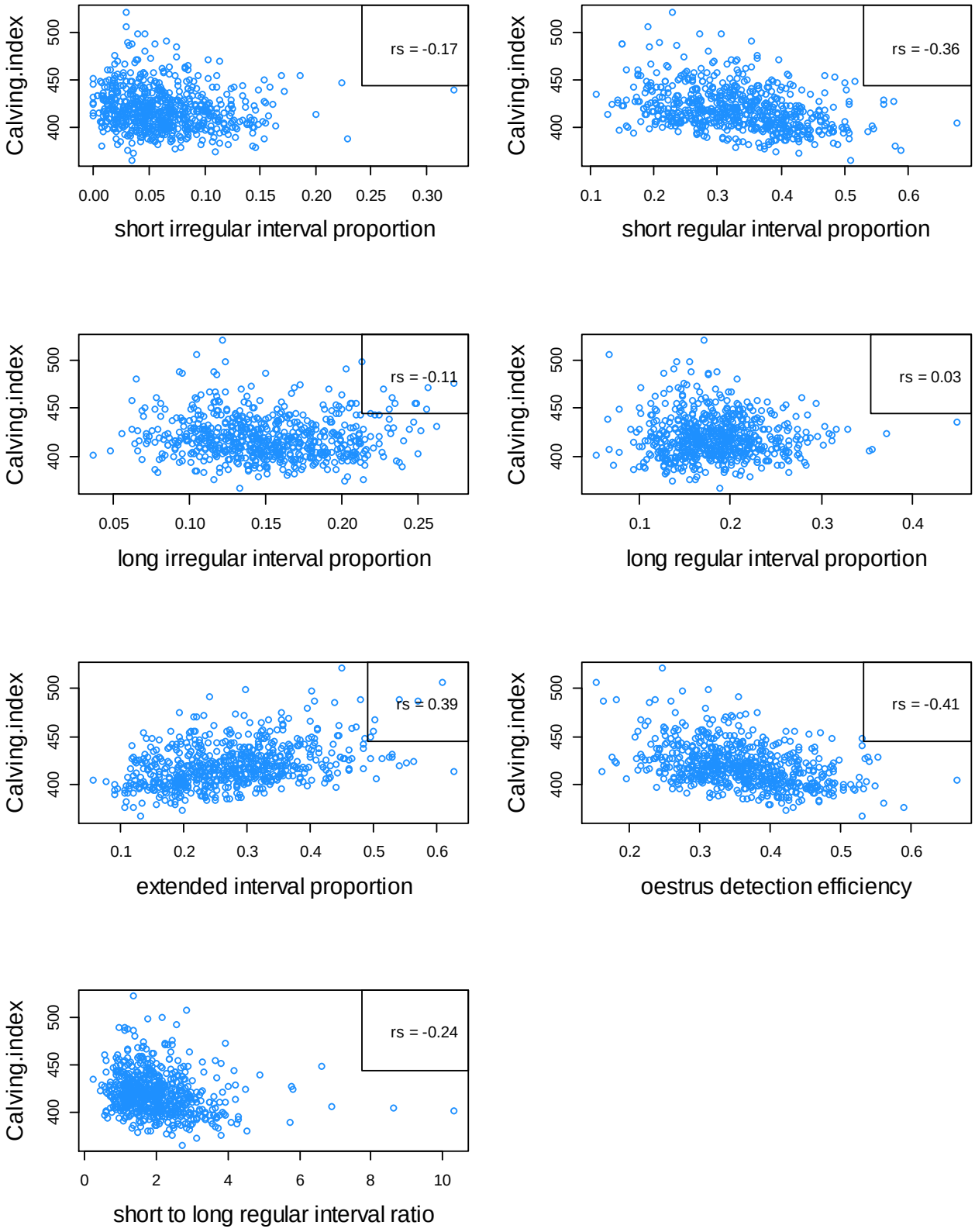


Figure 2-3 Scatterplots showing the relationship between proportion of ISIs in each category, Oestrus detection efficiency and short to long regular ratio with calving index (rs = Spearmans rho)

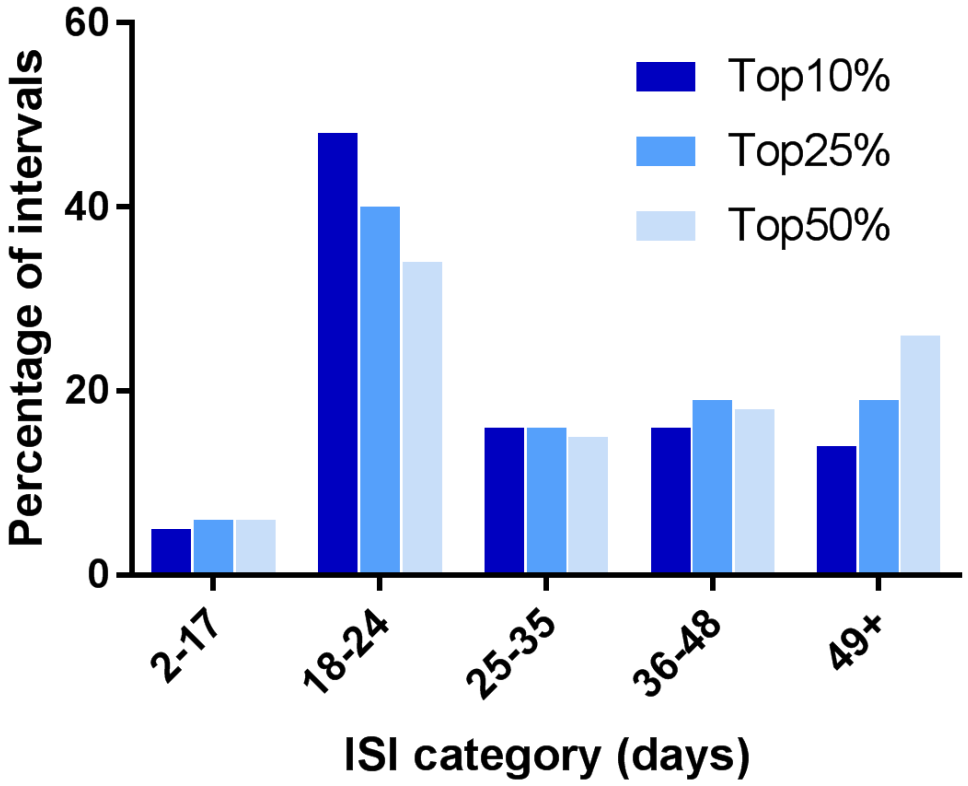


Figure 2-4 The mean percentage of intervals falling in each category for the top 10, 25 and 50 % of herd-years when ranked by performance as measured by oestrus detection efficiency

2.4 Discussion

The analysis of ISI category distribution between herd-years in this study shows that most UK herds in this sample achieve the proposed target of less than 10-15% of intervals occurring at less than 18 days (Roelofs and others 2010), but that very few achieve a target of 60-70% of re-serves occurring at 18-24 days and only 25 out of 879 herd-years exceed the accepted intervention level of a 4:1 ratio for inseminations in the 18-24 category compared to the 36-48 category. The average herd-year in the top quartile (by ODE) in this study achieves short irregular intervals of 6%, short regular intervals of 40%, long irregular intervals of 16%, long regular intervals of 19% and extended intervals of 19%. These could be considered more appropriate targets when interpreting inter-service intervals than the suggested targets previously described. It should be remembered that any performance targets should be adapted to the herd being assessed: clearly there are already some herds exceeding this

level of performance. However, having an appreciation of wider performance is a good context for such target setting.

There is no perfect measure of overall fertility performance. Herd-year mean calving interval was used in this study to give an idea of herd performance because it was available in the data (from lactation start dates), however it will not account for fertility culling rates. This also meant restricting the sample size by excluding herd-years where subsequent lactation start dates were not known. The correlation between ODE and calving interval supports the idea that this was an appropriate method of ranking herd-years in this study. As would be expected due to the multifactorial nature of herd fertility, ODE does not fully predict calving interval. ISI profiles can only ever provide information on re-serves and will not incorporate return to cyclicity, oestrus detection for first insemination or conception risk. These other factors will also influence the overall outcome as well as potentially being related to ISI, for example conception risk is inherently linked to oestrus detection.

The apparently good (i.e. low) proportion of short irregular intervals in herds in this study and the apparently disappointing proportion of short regular intervals suggest that specificity of detection is often acceptable in UK herds, but that sensitivity of detection is very often sub-optimal compared to existing targets. This indicates that sensitivity is very often more limiting than specificity and this should be kept in mind when trying to improve oestrus detection on a unit. This may be because the signs of oestrus have been well documented (Roelofs and others 2010) and that farmers are confident in identifying those that are definitely in oestrus. It could also be because this method looks at the sensitivity of detecting oestrus in those cows that have already been inseminated, farmers may be less willing to inseminate cows that may potentially be pregnant compared to those that have not yet been inseminated. It may just be that achieving good sensitivity with oestrus detection is more challenging than achieving good specificity.

With many tests, sensitivity and specificity are often negatively correlated: this would lead to the assumption that herds which detect a high proportion of returns to oestrus may also have high numbers of heats incorrectly diagnosed and vice versa. This would imply a correlation between the number of incorrectly identified heats (short irregular) and the number of correctly identified heats (short regular). The lack of correlation between short irregular and short regular inseminations (Figure 2-2) suggests that these two are relatively independent, and that herds are capable of increasing sensitivity without compromising specificity. It is important to remember that farmer psychology (i.e. not wanting to serve cows at the "wrong" time) may also influence this. In a clinical setting it would be important to monitor conception rate in these scenarios to ensure inseminations are carried out appropriately.

Care should be taken when extrapolating the results of any study to other UK herds. The herds used in this study may not be representative of all UK herds, the fertility data quality controls could plausibly select better managed herds. This possibility is supported by the summary statistics described in Table 2-2, with median herd size being considerably above the mean herd size of 125 in 2012 (Agriculture and Horticulture Development Board 2013), and above the median herd size in a sample of 500 milk recording herds (Hanks and Kossaibati 2012), although median, upper and lower quartile 305 day yield and calving index figures are very similar to those in the latter, larger sample. Increased herd size may also have been selected for by excluding herd-years with less than 100 ISIs. However despite this potential bias the median percentage of re-serves occurring at 18-24 days in our study (33%) is similar to the 31% median found by Hanks and Kossaibati (2012) in their larger sample of herds: this analysis is clearly only possible in herds with accurate records. It is also possible that these patterns will have changed since the data was gathered in 2009. However this data still provides a more current reference than the commonly accepted targets described previously, and the similarity with the findings of Hanks and Kossaibati (2012) suggest that there has been no dramatic change in performance between 2008 and 2012. However, there does appear to have been some improvement since this analysis was undertaken, with Hanks and Kossaibati (2017)

finding a median percentage of re-serves occurring at 18-24 days of 42% in the twelve months ending August 2017.

2.5 Conclusions

ISI profiles appear to be a valid method to consider as part of a package of methods to monitor oestrus detection as a component of dairy herd fertility. Currently accepted targets seem optimistic and the results of this study will be of use for practitioners when interpreting ISI profiles.

3 Evaluating the variation in inter service intervals

3.1 Introduction

It is commonly accepted that the oestrus cycle of domestic cattle (*Bos taurus*) is approximately 21 days long, with a normal range of between 18 and 24 days (Forde and others 2011; Hartigan 2004). It has been demonstrated that variation in oestrous cycle length occurs primarily between cows rather than within cows (Olds and Seath 1951). The number of follicular waves in a cow's oestrous cycle affects the inter-ovulatory interval (IOI) (Ginther and others 1989) and the number of follicular waves in a cycle is also repeatable between cycles within a cow (Jaiswal and others 2009). Some studies have shown improved fertility in cows following two wave cycles as opposed to three wave cycles (Townson and others 2002). Explaining the between-cow variation in IOI may uncover mechanisms to improve fertility.

The expected normal range of IOIs is used to calculate a variety of fertility parameters employed by veterinarians, farmers and other professionals to monitor dairy herd oestrus detection (Hudson and others 2012b). These include first service submission rate (the proportion of cows which are inseminated within 24 days of the end of the voluntary waiting period), in particular return service submission rate (the proportion of cows reserved 18 to 24 days after an unsuccessful insemination) and analysis of inter-service interval (ISI) profiles which will both be highly and directly affected by the choice of normal range. Expected cycle length could also affect the interpretation of commonly used indices for monitoring overall reproductive performance, such as the proportion of eligible cows becoming pregnant every 21 days ("21 day pregnancy risk" or "fertility efficiency", common in year round calving herds) or the proportion of cows pregnant within the first 21 or 42 days of the breeding season (in block calving enterprises), although in these cases 21 days only really functions as an arbitrary unit of time. As well as allowing useful monitoring of oestrus detection, awareness of the normal ISI can directly help improve oestrus detection by allowing more accurate prediction of the timing of the next heat. A reliable figure is also useful in research, for example for constructing

simulation models of reproduction, with some authors using a fixed cycle length of 21 days in their models (Boer and others 2011b; Brun-Lafleur and others 2013).

Globally, milk yield has been increasing over time, and until recently dairy herd fertility had been declining. Delayed return to normal ovarian cyclicity, reduced heat expression and poor conception rates are commonly implicated in this trend (Dobson and others 2007; Walsh and others 2011). The effect of increased level of production in reducing the time and intensity of oestrus expression has been well documented (Lopez and others 2004); an association between increasing milk yield and an increase in the incidence of abnormal ovarian cycles (particularly prolonged luteal phases) has also been shown (Kafi and others 2012). It is plausible that production may have an effect on ISI length.

The aim of this study was to assess the inter-service interval in a large number of dairy cows, to explore the variability in oestrous cycle length and to identify associations between cow factors and cycle length. A more accurate understanding of the normal ISI of a cow would enable this knowledge to be used when interpreting herd production parameters. Understanding the variability of oestrous cycle length will allow identification of potential mechanisms regulating this process.

3.2 Materials and Methods

3.2.1 Data Collection and Organisation

Herd management data were collected as part of a larger project as described in Chapter Two. The insemination data from the same herd-years used in Chapter Two were used for this analysis. The resulting data were from the years 2000 to 2008, originated from 167 herds and included 449,471 inseminations from 67,926 cows. Mean 305 day milk yield, calving index, culling rate and average herd size (estimated by multiplying the number of calving events in a year by the calving index divided by 365) were calculated for each herd for each calendar year.

The data were structured with an individual ISI (the number of days between subsequent inseminations in the same cow, in the same lactation) as a line of data. For each interval, the cow and herd identity were recorded, along with the 305-day adjusted milk yield, start (calving) date and

parity of the lactation in which the ISI occurred. The date, days in milk (DIM) and insemination number of the insemination ending the interval were also recorded. Lactations with milk yields outside the range 2,500 to 15,000 litres and ISIs ending at more than 365 DIM were excluded, as these were likely to represent outliers and recording errors.

Data restructuring was carried out in Microsoft Access 2010 (Microsoft Corporation, Redmond, Washington).

3.2.2 Descriptive Analysis

A frequency distribution of ISIs up to 100 days was plotted. The initial distribution demonstrated a clear peak at 15 to 30 days, and this is a range thought likely to contain the first return to oestrus following an insemination, without including subsequent cycles. Therefore a subset of intervals between 15 and 30 days was used for further univariable analysis. Herds contributing less than 100 ISIs within this range were excluded, leaving a sample consisting of 114,573 ISIs from 42,252 cows in 159 herds. The distribution of ISIs within this sample was assessed using a frequency plot. Bar charts were used to compare the distribution of ISIs across different parities (grouped as 1, 2, 3 or 4+), insemination numbers (grouped as 2, 3, 4 or 5+ according to the number of the insemination ending the interval) and lactation 305-day adjusted milk yield (grouped as <7,000 litres, 7,000 to 10,000 litres and ≥10,000 litres, based on the approximate bottom quartile (<7,021 litres), median half and top quartile (>9934 litres) of all insemination-level 305 day lactation yields). First lactation heifers were excluded from the univariable yield category plot. Differences between groups were tested with a Kruskal-Wallis rank sum test, with P-values of ≤0.05 considered significant.

Univariable data analysis was carried out in Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington) and R version 3.0.2 (R Core Team 2013)

3.2.3 Statistical modelling

A regression model to predict ISI was fitted using the subset of ISIs at 15 to 30 days (as described earlier). A three-level random effects structure was used to account for potential clustering of ISIs at the animal and herd level, with an individual interval as the lowest level unit of data. The model was

built by forward selection. Explanatory variables were added to the model sequentially and coefficients and standard errors of coefficients estimated. For continuous predictor variables, polynomial functions up to degree three were tested, as were terms representing biologically plausible first-order interactions. Variables were retained in the model where the estimated coefficient was greater than twice the standard error (such that the 95% confidence interval for the estimate did not include zero); all rejected variables were re-offered to the final model and retained if they now met these criteria. In the case of categorical explanatory variables, all categories were retained in the model if one or more of the categories met the criteria. The model took the conventional form:

Equation 3-1 Structure of random effect regression model of ISI (1. model structure, 2. model intercept and random effects, 3. model herd and cow random effects and residual variation)

$$ISI_{ijk} = \beta_{0ijk} + \boldsymbol{\beta_1 x_{1}}_{ijk} \quad (1)$$

$$\beta_{0ijk} = \beta_0 + v_{0k} + u_{0jk} + e_{0ijk} \quad (2)$$

$$v_{0k} \sim N(0, \sigma^2_{v0})$$

$$u_{0jk} \sim N(0, \sigma^2_{u0}) \quad (3)$$

$$e_{0ijk} \sim N(0, \sigma^2_{e0})$$

where ISI_{ijk} is the i^{th} ISI, for the j^{th} cow in the k^{th} herd. β_{0ijk} is the model intercept, comprised of β_0 the overall intercept, v_{ok} the herd level residual for the k^{th} herd, u_{0jk} the cow level residual for the j^{th} animal and e_{0ijk} the insemination level residual for the i^{th} insemination. $\mathbf{x_1}_{ijk}$ represents the vector of predictor variables for the i^{th} ISI in the j^{th} cow in the k^{th} herd, and $\boldsymbol{\beta_1}$ the corresponding vector of coefficients. All potential predictor variables used in model building are shown in Table 3-1. To quantify the amount of variability in ISI at each level (variation in ISI occurring between inseminations within the same cow; variation in ISI occurring between cows within a herd; variation in average ISI between herds), variance partition coefficients (VPCs) were calculated for each level of

the model by dividing the variance of the residuals at each level by the total variance. The final model was also compared to a null model consisting only of herd level, animal level and insemination level random effects, to calculate the percentage of the initial variance at each level which was explained by the predictor variables.

Table 3-1 Potential predictor variables used for building a multi-level regression model of inter-service interval (ISI)

Variable	Variable type
305 day milk yield (x1000 litres)	Continuous (centred around population mean)
Year in which the lactation began	Categorical (2000, 2001…2008)
Calendar month in which the ISI ends	Categorical (January, February…December)
Lactation number	Categorical (parity 1,2,3,4+)
Days in milk at the end of the ISI	Continuous
Number of inseminations in the lactation including the insemination ending the ISI	Categorical (insemination number 2,3,4,5+)

In order to evaluate model fit, a histogram and a normal probability plot of the insemination level residuals were generated to check for normality; the standardised insemination level residuals were plotted against the ranked observed values to assess homoskedasticity; and the predicted values were plotted against the observed values. Model parameters were re-estimated following removal of outlying points on the diagnostic plots to assess their effect on the parameter estimates.

In order to illustrate model results, predictions were made for example scenarios, by fixing all explanatory variables at their mean or reference category and then calculating the predicted

outcome across a range of values for a single explanatory variable at a time, with predictions illustrated graphically (Archer and others 2013).

The main regression analysis was carried out using MLwiN version 2.10 (Rasbash and others 2009) using iterative generalized least squares for parameter estimation.

To identify underlying distributions in the frequency distribution of ISIs a finite Gaussian mixture model was fit to the data using an Expectation-Maximisation algorithm in the Mixtools R package (Benaglia and others 2009); models with two, three and four underlying distributions were tested. Identified underlying distributions were illustrated in a density plot.

3.3 Results

3.3.1 Descriptive Analysis

Summary herd level statistics for herd-years included in this analysis are shown in Table 3-2.

Table 3-2 Summary statistics for 1,275 herd-years from 159 herds included in the analysis of inter-service intervals (following data quality screening)

	305day milk Yield (Kg)	**Calving index (days)**	**Cull rate**	**Herd size**
Mean	7437	415	25%	190
Median	7534	412	23%	167
Upper quartile	8344	427	30%	222
Lower Quartile	6735	399	17%	116

Figure 3-1 shows the distribution of all the ISIs from 1 to 100 days: a clear peak in the frequency of inseminations occurred at an interval of around three weeks with a smaller increase around six weeks. The distribution of intervals by day within a 15 to 30 day window is shown in Figure 3-2A; the modal ISI across the full dataset was 22 days. The accepted normal range of 18 to 24 days encompassed 59% of ISIs in the 15 to 30 day window. The central 90% of the ISIs fell within the

range 18 to 28. Figure 3-2B shows the distribution of intervals for different yield groups. The modal interval was 22 days for all yield groups, however, there was a clear trend for longer ISIs in lactations with higher 305-day milk yield ($p<0.001$). Figure 3-2C shows the distribution for different parity groups. ISI appeared to increase with parity ($p<0.001$), all groups had a modal ISI of 22 days, with the exception of first lactation heifers which had a mode of 21 days. Figure 3-2D shows the distribution of ISIs by insemination number, the mode remained 22 days for all groups and there was a significant association for longer ISIs in later inseminations ($p=0.023$).

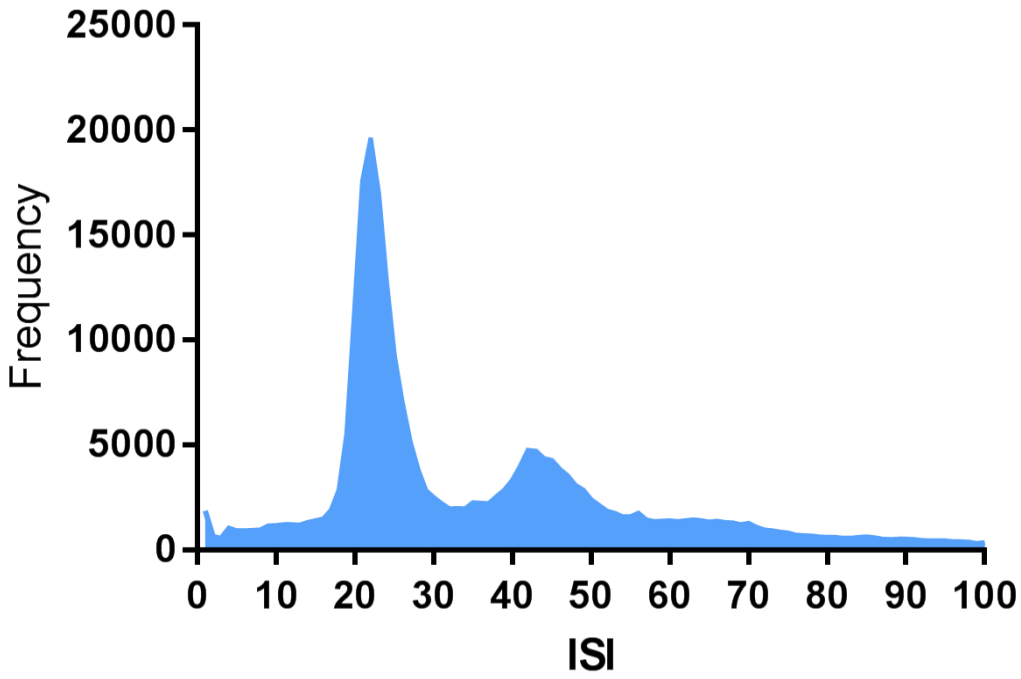


Figure 3-1 Frequency distribution of inter-service intervals (ISIs) between 1 and 100 days, recorded between the years 2000 and 2008 in 167 UK dairy herds

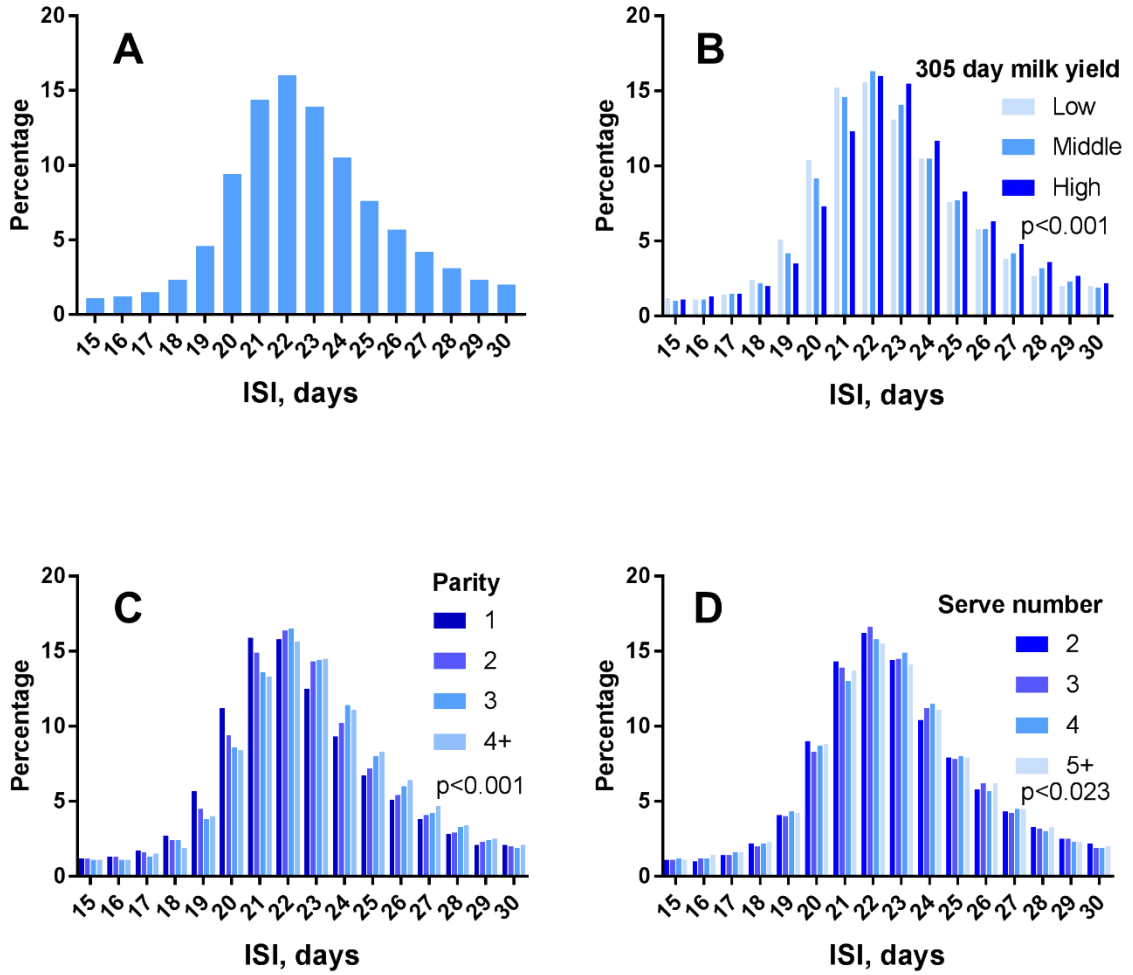


Figure 3-2 Bar charts showing the distribution of 114,573 inter-service intervals (ISIs) between 15 and 30 days within different sub-groups of 42,252 cows from 159 UK dairy herds: A, shows all ISIs between 15 and 30 days; B, shows 305 day lactation milk yield groups, low (<7,000 litres), medium (7,000 to 10,000 litres) and high yielding (>10,000 litres) in multiparous cows; C, parity groups; D, grouped by insemination number (within a cow, within a lactation).

3.3.2 Statistical modelling

The coefficients and their standard errors for the model are given in Table 3-3. Model fit was considered good following the assessments described above (Figure 3-3). There was a positive association between ISI and increasing parity, with cows in lactation number four or more predicted to have ISIs around half a day longer than first lactation heifers. The positive association between milk yield and ISI observed in the descriptive analysis was also demonstrated in the multivariable model although the magnitude of the effect was very small, with a predicted increase of 0.024 days

for every 1,000 litres of milk, when parity was accounted for. The ISI appeared to vary seasonally, with a shorter ISI in the months June through to November, with predicted ISI around one fifth of a day shorter in July, August and September when compared to January. There was also an association with year; the ISI lengthened over the period the data were gathered, with an increase in ISI of approximately 0.26 of a day in 2008 compared to the year 2000. There was a quadratic relationship between DIM and ISI, with predicted ISI increasing with DIM up to approximately 250 DIM and then decreasing slightly in later lactation. The predicted ISI for the "average cow" increased from approximately 21 to over 23 days between 30 and 250 DIM; this association is illustrated in Figure 3-4. Another large effect size in the model was the association between ISI and insemination number, with the predicted interval preceding a fifth insemination or later in a lactation approximately 0.8 days shorter than the interval between a first and second insemination; this effect is illustrated in Figure 3-5.

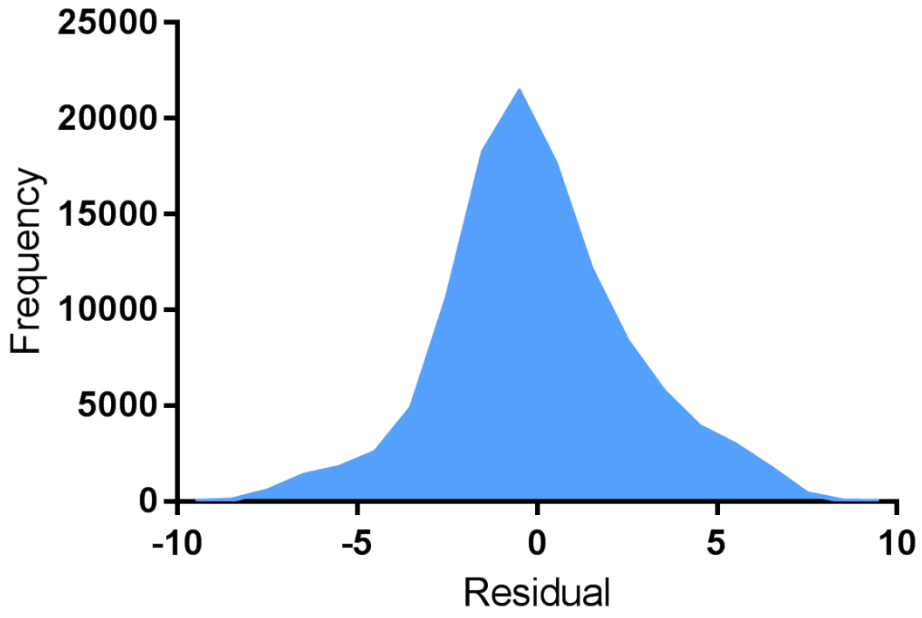


Figure 3-3 Distribution of 114,573 insemination level residuals for a multi-level regression model with the outcome inter-service interval (ISI) based on data from 42,252 cows in 159 UK dairy herds between the years 2000 and 2008.

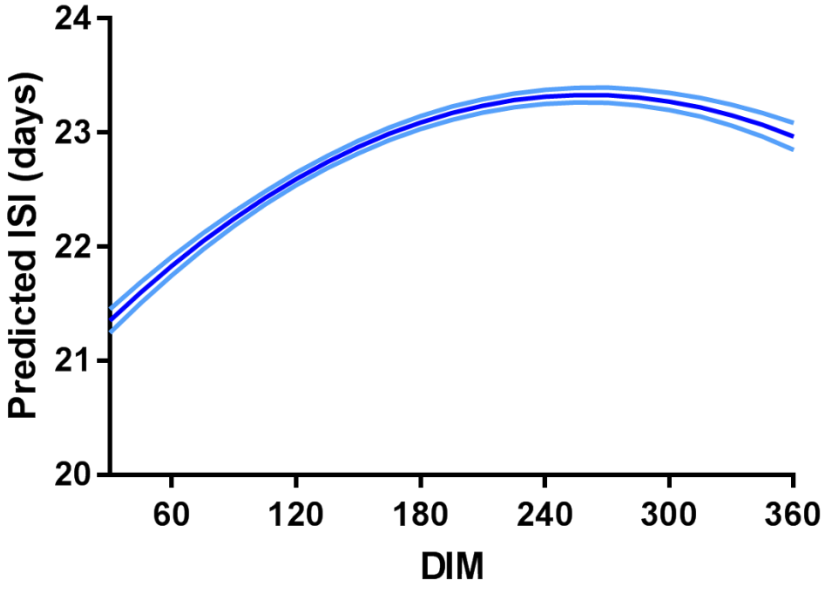


Figure 3-4 Predicted inter-service interval (ISI) from a multi-level regression model based on data from 42,252 cows in 159 UK dairy herds across a range of days in milk; the light blue lines show the 95% confidence interval for the prediction

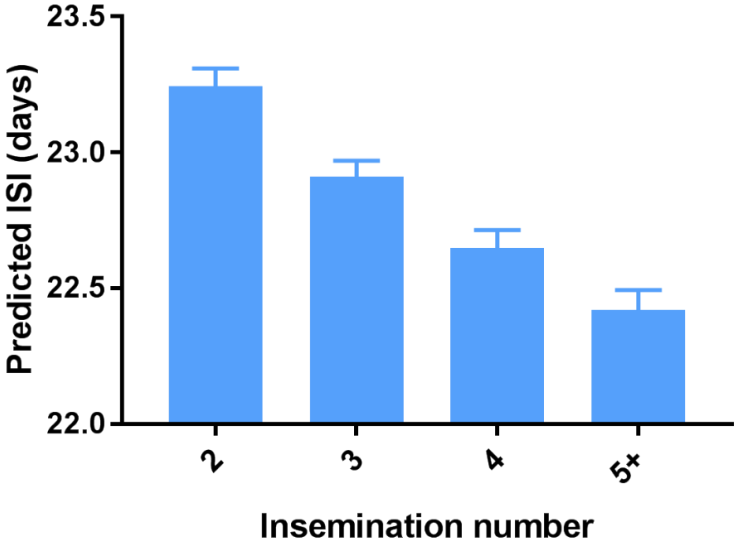


Figure 3-5 Predicted inter-service interval (ISI) by insemination number category (number of inseminations occurring within the same cow within a lactation, insemination number) from a multi-level regression model based on data from 42,252 cows in 159 UK dairy herds, error bars represent 95% confidence intervals for each prediction

Table 3-3 Parameter estimates for a multilevel regression model predicting inter-service interval (ISI) based on data from 42,252 cows in 159 UK dairy herds

Model term	Coefficient	Standard error
ISI	Outcome	
intercept	20.764	0.087
Fixed effects:		
305 day milk yield ('000s litres)	0.024	0.006
Year 2000	Reference	
Year 2001	0.088	0.065
Year 2002	0.126	0.061
Year 2003	0.158	0.059
Year 2004	0.154	0.058
Year 2005	0.142	0.057
Year 2006	0.157	0.057
Year 2007	0.235	0.056
Year 2008	0.263	0.056
Month 1	Reference	
Month 2	0.027	0.038
Month 3	0.02	0.039
Month 4	0.038	0.041
Month 5	-0.071	0.041
Month 6	-0.195	0.042
Month 7	-0.203	0.042
Month 8	-0.184	0.043
Month 9	-0.219	0.043
Month 10	-0.162	0.041
Month 11	-0.111	0.04
Month 12	0	0.038
Parity 1	Reference	
Parity 2	0.227	0.026
Parity 3	0.408	0.029
Parity 4	0.519	0.026
Days in Milk	0.019	0.001
Days in Milk^2	-3.7×10^{-05}	1.7×10^{-06}
Insemination number 2	Reference	
Insemination number 3	-0.334	0.025
Insemination number 4	-0.596	0.032
Insemination number 5	-0.823	0.037
Random effects:		
Herd level variance	0.089	0.012
Animal level variance	1.057	0.029
Insemination level variance	7.562	0.038

The VPCs for the final model indicated that most of the unexplained variation in ISI was at the individual insemination level. Only 1% of the variation of ISI was from differences between herds, 12% was explained by differences in ISIs between animals within a herd. The remaining 87% of variation was at the level of the individual insemination; that is between ISIs within an animal. When comparing the final model to the null model the explanatory variables included accounted for 18% of the null model herd level variance, 6% of the animal level variance and 1% of the insemination level variance.

The finite Gaussian mixture model with three underlying distributions had the best fit to the data (models with two and four distributions were also tested). Three latent distributions of ISIs were identified, with means (and standard deviations) of 22.4 (2.0), 24.2 (10.9) and 44.0 (3.5). The distributions are illustrated in Figure 3-6.

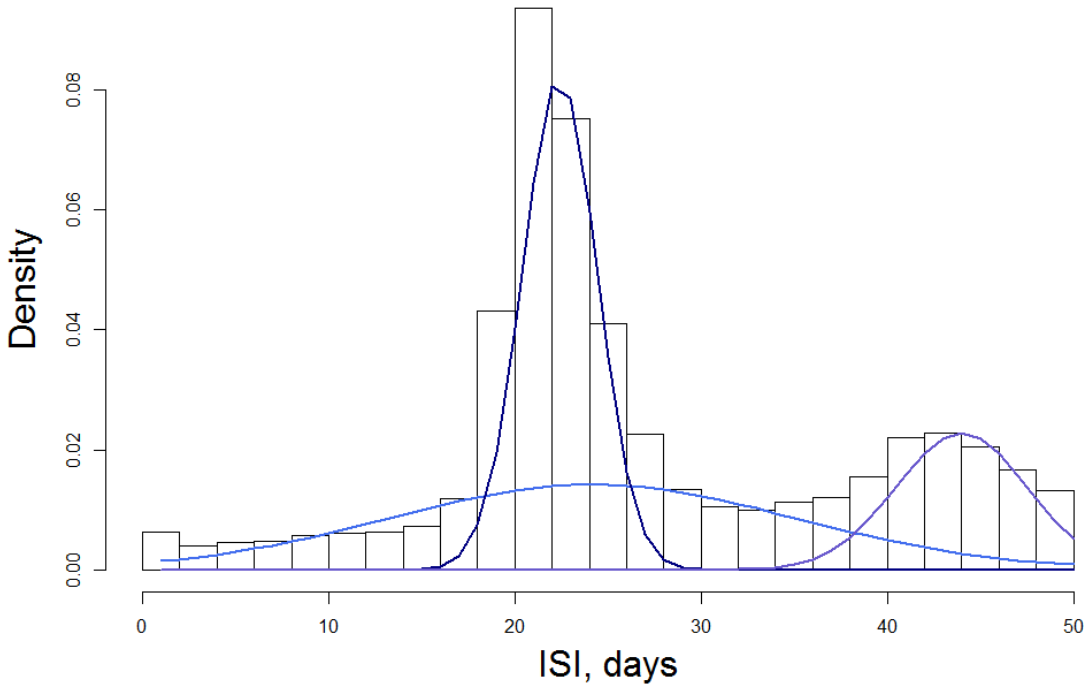


Figure 3-6 Histogram of ISIs showing three latent distributions identified by finite Gaussian mixture model

3.4 Discussion

In the current study most of the variation in ISI occurs at the individual ISI level within cows. Along with the fact that the final specified model only explained 1% of the insemination level variance in the null model (i.e. after adding the explanatory variables to the model only an extra 1% of the variation was explained), this implies that a variable or variables not included in the model that applies at the insemination level has a major impact on ISI. Previously it had been documented that IOI is consistent within a cow, with most variation occurring between cows (Jaiswal and others 2009). Because the current study uses ISI as a proxy for IOI, embryonic death is one possible explanation for this. Longer intervals may be a result of successful conception and embryonic death delaying the second oestrus (Diskin and others 2011). This would also explain the trend for extended intervals in higher yielding cows, with these cows expected to have a higher incidence of embryonic death (Sartori and others 2002). The distribution of all the ISIs between 15 and 30 days is slightly asymmetrical, with a slight positive skew, which could possibly be caused by embryonic death (Figure 3-2A). However, the shape of the distribution of ISIs from the high yield group is very similar to that of the low yielding group (see Figure 3-2B), although centred on a higher interval. An increase in the amount of embryonic death would be expected to increase the number of extended ISIs (because of the extended intervals of those cows where embryonic death occurs) but would not influence the number of shorter ISIs. Thus, if embryonic death accounted for the increase in ISI with increased yield, the distribution of ISIs may be expected to be more right-skewed in higher yielders. This is also supported by the latent distributions identified in the mixture model; the normal distribution most likely to represent the expected return to oestrus has a mean of 22.4 days. There are possible explanations other than late embryonic death for the variable and increased ISIs. Lamming and Darwash (1998) analysed progesterone profiles of 1682 dairy cows and found 6.4% of second or subsequent oestrus events had a persistent corpus luteum (defined as a period of elevated progesterone lasting more than 19 days), 12.9% had delayed ovulation (defined as periods of reduced progesterone lasting more than twelve days) and 9.9% late embryo mortality (defined as

elevated progesterone lasting for 19 days following insemination and then declining. Interestingly, the 31.7% of cows exhibiting at least one atypical cycle in this study had significantly poorer fertility than those cows with normal cyclicity (Lamming and Darwash 1998). Another possible explanation for the variation in cycle length is changes to the follicular wave pattern of cattle: three-wave cycles have been shown on average to be longer than two-wave cycles, but there are contradictory reports as to which is more common (Adams and others 2008), see Figure 1-2. Previous work has shown that the follicular wave pattern is repeatable for an individual cow (Jaiswal and others 2009). The unexplained variation in ISI within a cow indicates that cycle length (and therefore potentially follicular wave number) may be less consistent than previously thought. The findings in this study indicate that there is an unexplained and inherent variability of cycle length for an individual cow which clearly warrants further studies to evaluate the underlying physiological mechanisms.

In the current study it is likely that not all recorded service events will represent true oestrus events, and that not all true oestrus events will result in a recorded insemination. In a dataset this size, this effect should only introduce random background "noise", and it is hard to see how this could introduce a systematic increase or decrease in ISI. This is supported by the presence of a period of increased frequency of re-serves (ISIs) around three weeks after a previous insemination (15-30 days, as shown in Figure 3-1). These intervals would be expected to represent correctly identified oestrus events. Using ISI as a proxy for IOI (as opposed to using service data as a proxy for ovulation date) also means that any deliberate difference between ovulation and timing of service is likely to be applied consistently (or at least randomly) to both inseminations bounding the interval. This means that the ISI should correspond to the IOI even if the insemination time doesn't coincide with ovulation (for example due to application of the "am-pm" rule).

In this sample of UK dairy cows the modal ISI was longer than the expected "normal" (IOI) of 21 days (Forde and others 2011; Hartigan 2004). That this represents a true reflection of IOI is further supported by the findings of a number of recent physiological studies revealing an IOI of greater

than 21 days (Bleach and others 2004; Sartori and others 2004; Wolfenson and others 2004) as well as the mixture model illustrated in Figure 3-6. The current study indicates that the discrepancy between the average IOI observed in these physiological studies on smaller numbers of animals and the commonly accepted average IOI of 21 days is widespread among UK dairy cows, this is also supported by studies published since this analysis was undertaken (Blavy and others 2016). This brings in to question the continued use of the 21 day "normal" interval particularly given that a similar finding from ISI data was reported as long ago as the 1950s by Olds and Seath (1951) following analysis of records from 278 cows on a research farm in Kentucky. Some of the early research on ovarian cycles in cattle was conducted on nulliparous heifers (Hammond, 1927, cited in Chapman and Casida 1935; Joubert 1954; Werner and others 1938). In the current study, primiparous heifers appear to have shorter intervals than higher parity cows and it is possible that this trend would extend to nulliparous heifers. This is further supported by Sartori and others (2004) who found a shorter IOI in nulliparous heifers compared to cows and suggests that findings from studies carried out on the oestrous cycle of heifers cannot be directly applied to later parity dairy cows. Previous studies have found an effect of breed: Joubert (1954) reported a bimodal distribution of IOI, attributing a second peak of IOI at 22 days to longer cycles of Friesian cows; other studies have observed similarly longer cycles in Holstein-Friesian type animals (Britt 1995). With a trend away from "traditional" breeds towards Friesian and Holstein genetics since the 1950s it seems possible that this may have resulted in IOIs being longer than those demonstrated in older studies carried out on traditional breeds. Pragmatically, it is also possible that 21 days has remained the accepted "normal" interval because a three-week cycle is easier to discuss than a three-week-and-one-day cycle. Whilst the difference between the commonly accepted IOI of 21 days and the apparently more common interval of 22 days is only one day, the normal range of 18 to 24 days appears inappropriate. A better estimation of the normal range maybe the 18 to 28 day range incorporating 90% of the ISIs between 15 and 30 in this study, or the middle 95% of the latent

distribution identified at the expected return to oestrus (22.4 ± 3.9). It is possible it may be appropriate to use a different "normal" range for heifers than for multiparous cows.

As well as the strong association of ISI with parity, many other associations became apparent in the current study. There was a relatively large negative association of insemination number with ISI. DIM is already accounted for in the model and so this effect is separate to any effect of increasing insemination numbers corresponding to increasing DIM. This is a relatively large and consistent effect (illustrated in Figure 3-5) and yet is hard to explain physiologically. This is an area that warrants further investigation, to establish the mechanism that appears to be shortening the ISI of cows that have received multiple inseminations. By definition cows receiving more inseminations are likely to be less fertile, this may be associated with progesterone levels and other endocrine factors that may be associated with cycle length (Mann and Lamming 1995). The association between milk yield and ISI appears relatively small in magnitude once confounding factors are accounted for. Results from the multivariable regression analysis suggest that the apparent relationship illustrated in Figure 3-2B was mostly explained by the association with parity, despite primiparous animals being excluded from this figure. Although lactation 305 day milk yield exhibited a small effect size there was a much larger effect of DIM. The predicted difference in ISI between a high (15,000Kg) and low (2,500Kg) yielding cow was 0.3 days, whereas the predicted ISI varied by around two days over the observed range of DIM. As shown in Figure 3-4, ISI appears to vary throughout lactation, gradually increasing up to approximately 250 days in milk. In the current study only lactation yield data was available for the sample analysed and it has been demonstrated that the impact of production on fertility is often related to the extent of negative energy balance in early lactation and not the total milk produced (Wathes and others 2007). DIM may better represent any effect of milk yield at the time of the insemination than lactation 305 day milk yield in this model; however the largest effect of DIM at 250 days does not coincide with the expected peak in production. In the future, similar analyses using the nearest test day milk yield to the insemination may represent this effect better. There was a small but significant seasonal pattern of ISIs, with a trend for shorter

intervals in the summer months. This could be a physiological effect, for example of photoperiod, or more management related, for example shorter window available for artificial insemination on the farm due to fewer hours of daylight. There is also a trend for ISIs extending through time, with longer intervals found in lactations starting in 2008 than those starting in 2000. This trend is harder to explain, but may represent a longer term change to ISIs in dairy cows, perhaps related to selective breeding and changes in genetics.

3.5 Conclusions

In a large sample of UK dairy herds, most variation in ISI length occurred between cycles within cows (87%) compared to the variation between cows within a herd (12%). The most common interval between inseminations was 22 days rather than the accepted 21 day "normal" interval with a range of 18-28 days incorporating 90% of ISIs, or the 19-26 days identified by the mixture model potentially being more appropriate. Various factors have an association with ISI and would be expected to have a similar association with IOI, including parity, DIM and insemination number. There was a small association with production as measured by lactation 305 day yield. Further work is needed to elucidate the physiological mechanisms behind these associations and behind the unexplained within cow variation in cycle length.

4 Associations between inter-service interval and probability of conception

4.1 Introduction

Better insight into expected interval between oestrus events can help with accurate oestrus detection monitoring, and has potential to inform improved oestrus detection strategies on farm. Exploring associations between inter-service interval (ISI) and subsequent fertility may provide insights which help to improve conception risk (the probability of an insemination resulting in a pregnancy).

The average length of the oestrous cycle of the cow is commonly quoted as 21 days, with a normal range of 18 to 24 days (Forde and others 2011; Hartigan 2004). A number of small-scale studies have identified mean oestrous cycle lengths in excess of 21 days (Bleach and others 2004; Sartori and others 2004; Wolfenson and others 2004). The work in Chapter Three, evaluating the time between successive inseminations in the same cow in the same lactation, suggests that ISIs in excess of the normal range are frequent, with 22 days being the modal interval and that the traditional normal range of 18 to 24 days may poorly reflect the observed distribution of intervals in the population; a similar pattern has been identified in progesterone profiles (Blavy and others 2016). Some of these longer ISIs could be the result of late embryonic death (Northey and French 1980; Ricci and others 2017; Wijma and others 2016), this may impact on the cow's chance of conception at the next insemination. It is not clear whether cows inseminated at these apparently abnormal intervals are as likely to conceive as those inseminated within the traditional expected range.

Oestrous cycles in *Bos taurus* cattle typically consist of two or three follicular waves, with three wave cycles tending to result in an inter-ovulatory interval (IOI) longer than that of two wave cycles (Adams and others 2008). Some authors have hypothesised that due to the relatively increased time taken for development of the pre-ovulatory follicle in two wave cycles that these may be less fertile than three wave cycles. However there is no clear consensus with some studies finding reduced conception risk in two wave cycles (Townson and others 2002) and others finding no difference

(Ahmad and others 1997; Bleach and others 2004). There are several potential mechanisms by which ISI could plausibly impact on conception risk as well as late embryonic death. Whilst follicular wave number, IOI and ISI may not be well correlated, there are potential mechanisms for conception risk to vary with ISI. To the authors' knowledge, no studies exist evaluating the relationship between ISI and conception risk on a large number of cows.

The aim of this study was to investigate the variation in conception risk by ISI in a large number of dairy cows in order to further our understanding of the possible effects of ISI on fertility, and to explore the expected oestrous cycle length of a previously inseminated modern dairy cow.

4.2 Materials and methods

4.2.1 Data collection, organisation and descriptive analysis

Management data was collected from farms that were clients of one of twenty veterinary surgeons in England and Wales as part of larger project (Hudson and others 2012a; Hudson and others 2010). Records for 468 dairy farms considered to have good quality data were collated and converted to a standard format. An assessment of data quality was carried out to identify herds with accurate recording of calving and insemination. Measures used to screen for data quality included the proportion of calving events with corresponding inseminations and the proportion of inseminations leading to a pregnancy (as described previously, 2.2). This left data for 257,396 inseminations in 75,745 cows from 312 herds. The data were structured with a single insemination as a line of data, with the insemination, cow, parity and herd identity recorded. For each insemination, the date of insemination as well as the variables shown in Table 4-1 were recorded or calculated. The interval between subsequent inseminations in the same lactation from the same cow was calculated (inter-service interval, ISI); first inseminations were excluded. Inseminations were categorised as successfully resulting in a pregnancy when the cow was recorded as having calved 266 to 296 days after insemination, based on the expected range of gestation length for the common dairy breeds (McGuirk and others 1998, 1999). Where two inseminations occurred within this range, the closest

to 283 days gestation was categorised as successful. Plots were produced to examine the distribution of ISIs between 3 and 50 days to visualise the increased frequency of insemination occurring at the expected time of around 21-22 days. The distribution of the proportion of inseminations resulting in a pregnancy (conception risk) at each ISI between 3 and 50 days was also plotted using GraphPad Prism (Version 7.02, California, USA); with confidence intervals for risk at each ISI calculated using the modified Wilson interval (Brown and others 2001). From this distribution, a range of 16 to 28 days was selected for further exploration as it appeared to encompass the range of increased conception risk and insemination frequency at which most first returns would be expected, predominantly reflecting accurately detected oestrus events. This final dataset, used to fit the regression model, contained 60,094 ISIs from 33,122 cows in 312 herds. Initial data restructuring and analysis was carried out in Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington).

4.2.2 Statistical modelling

A logistic multivariable regression model with the binary event of insemination success as the outcome variable was fitted to the data, with ISI as an explanatory variable to test the hypothesis that ISI was associated with conception risk. A multivariable method was used to account for the potential effect of other measurable confounding variables on conception risk. A multilevel model was used with a three level structure, with insemination as the bottom level, and cow- and herd-level random effects used to account for clustering at each level. The model was created by stepwise forward selection, with each variable being offered to the model and retained if the magnitude of its estimated coefficient was at least double the standard error of the estimate (equivalent to $p<0.05$). ISI was forced in to the model as a categorical variable, with each discrete one-day interval represented as a category. An ISI of 25 days was selected as the reference ISI as it represented a conception risk in the middle of the range. Where one or more categories of a variable were significant, all the categories for that variable were retained in the model. A list of all variables offered to the model is given in Table 4-1. Polynomial functions were tested for all continuous

variables up to power three. Biologically plausible first order interaction effects were also tested, including ISI with milk yield, days in milk, parity and service number. All rejected variables were re-offered to the final model, and retained if they met the criteria described above.

The model took the conventional form shown in Equation 4-1.

Equation 4-1 Structure of random effects logistic regression model of pregnancy (1. distribution of pregnancy, 2. logit transformation and model, 3. herd level random effect, 4. cow level random effect)

$$Pregnancy_{ijk} \sim Bernoulli\left(mean = \pi_{ijk}\right) \quad (1)$$

$$\ln\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \beta_0 + \boldsymbol{\beta x}_{ijk} + v_{0k} + u_{0jk} \quad (2)$$

$$v_{0k} \sim N(0, \sigma_{v0}^2) \quad (3)$$

$$u_{0jk} \sim N(0, \sigma_{u0}^2) \quad (4)$$

where $Pregnancy_{ijk}$ is whether the i^{th} insemination in the j^{th} cow in the k^{th} herd resulted in a pregnancy; π_{ijk} is the fitted probability of $Pregancy_{ijk}$; β_0 is the regression intercept, **β** is the vector of coefficients for the vector of predictor variables **x**; v_{0k} and u_{0jk} are the random effects to represent herd and cow level variation respectively.

Table 4-1 Variables tested for inclusion in a logistic regression model with the outcome of conception risk

Variable	Type
Pregnancy	Outcome variable, binary
ISI, days	Categorical (16 days, 17 days…28 days)
Month of insemination ending ISI	Categorical
Year of insemination ending ISI	Categorical
Parity	Categorical (1,…,5+)
305 day lactation milk yield	Continuous
Days in milk at insemination ending ISI	Continuous
Number of inseminations (in this lactations)	Categorical (2,3+)
Cow ID	Random effect
Herd ID	Random effect

The model was fitted using MLwiN version 2.35 (Rasbash and others 2009). Initial parameter estimates were calculated using iterative generalised least squares (IGLS) and final parameter estimates generated using a Bayesian approach, Markov Chain Monte Carlo (MCMC) with Gibbs sampling (Browne 2012; Browne and Draper 2006). A burn-in length of 5,000 iterations was used followed by a monitoring chain of 50,000 iterations. MCMC chains for the parameter estimates were visually checked to ensure adequate convergence. Model fit was checked by comparing observed and predicted number of pregnancies for each decile of risk (Hosmer and Lemeshow 1980).

To aid in interpretation, the model was used to predict the probability of achieving pregnancy for an insemination following an ISI of each length, whilst all other explanatory variables were fixed at their

population mean values. These predictions were illustrated graphically by plotting predicted probability for each ISI as a bar chart, along with the corresponding 95% credible intervals.

An unsupervised latent class analysis (a machine learning technique) was conducted to identify and define clusters of similar ISIs based on the frequency of inseminations occurring at that interval and the probability of conception. This analysis was carried out in R version 3.3.1 (R Core Team 2013) using the *mclust* package (Fraley and others 2002, 2012). A finite Gaussian mixture model was fitted to the insemination frequency and conception risk data using an expectation-maximisation algorithm, the optimum number of classifications was selected based on maximising the Bayesian Information Criterion (BIC) (Fraley and others 2002, 2012). The probability of each ISI falling within each classification was calculated and graphical plots used to illustrate the results. ISIs were assigned to the cluster that they had the highest probability of belonging to, the uncertainty of this classification was also illustrated by plotting the probability that a given interval fits within each cluster.

4.3 Results

Figure 4-1 shows the distribution of ISIs between 3 and 50 days: a clear increase in the frequency of insemination occurring around 22 days is evident. Figure 4-1 also shows the observed conception risk across the same range. A peak in conception risk is apparent around multiples of the expected oestrous cycle (around three and six weeks). The conception risk is low at 16 days (27%) and increases from 18 days up to a peak of 44% at 21 days, there is then a more gradual decline to a plateau of around 35% from 27 days ISI.

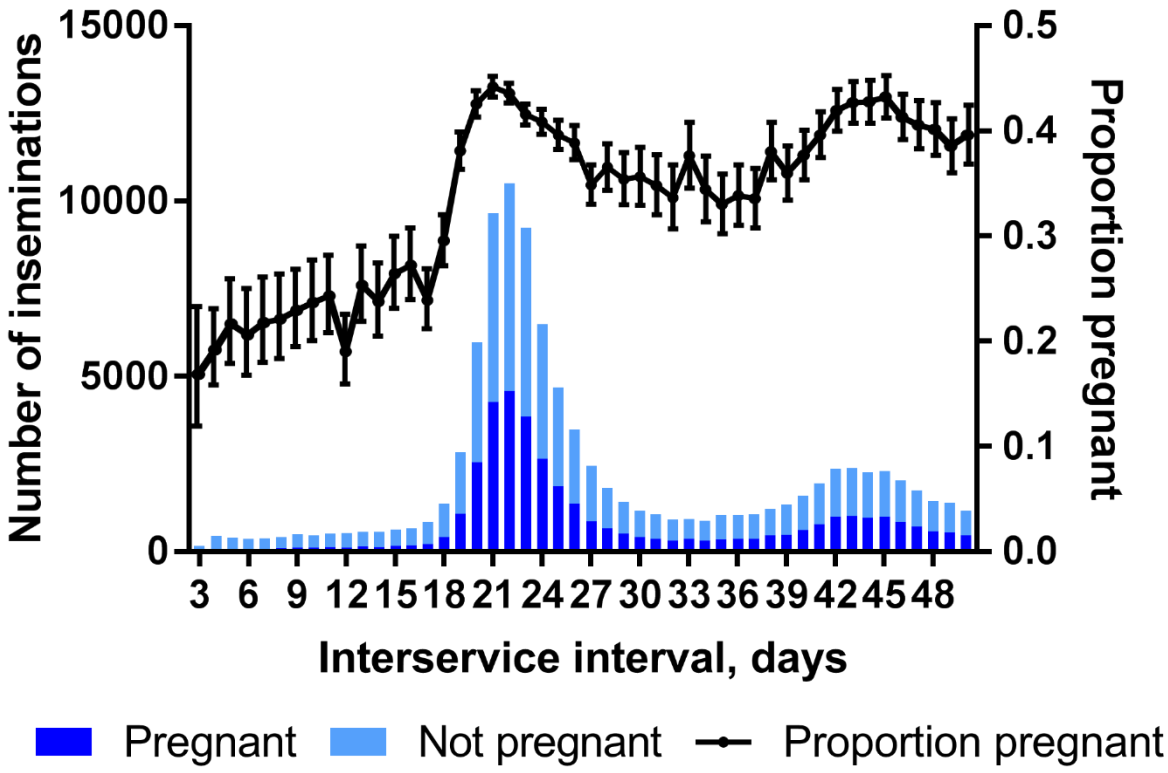


Figure 4-1 The distribution of interservice intervals (ISIs) from a large dataset of UK dairy cows, showing the number of inseminations (left axis) both resulting in a pregnancy (dark blue bars), the number not resulting in a pregnancy (light blue bars) and the change in mean conception risk across the range of inter-service intervals (line) with 95% confidence intervals (error bars)(right axis)

The final parameter estimates for the logistic regression model (with the outcome of a pregnancy (yes or no) resulting from an insemination) are shown in Table 4-2. The distribution of predicted mean conception risk by ISI is shown in Figure 4-2. Predicted conception risk was significantly lower for ISIs of 16, 17 and 18 days (mean predicted conception risk of 30, 27 and 32% respectively), whereas predicted mean conception risk for ISI of days 20, 21 and 22 were significantly higher than the reference interval of 25 days. There was no significant difference in predicted mean conception risk between ISIs of 21 or 22 days (47 and 46% respectively).

Table 4-2 Parameter estimates for a Bayesian multilevel logistic regression model with conception risk as an outcome variable and the inter-service interval (ISI) preceding the insemination forced in to the model as a categorical variable.

Variable	Coefficient estimate	Standard error	Odds ratio (95% credible interval)	Absolute predicted risk* (95% credible interval)
Pregnancy	Outcome			
Fixed Part				
Intercept	0.155	0.058		
ISI_16	-0.531	0.095	0.58(0.48-0.70)	0.3 (0.27-0.34)
ISI_17	-0.722	0.087	0.48(0.40-0.57)	0.27 (0.24-0.3)
ISI_18	-0.446	0.068	0.64(0.56-0.73)	0.32 (0.3-0.35)
ISI_19	-0.082	0.05	0.92(0.83-1.01)	0.4 (0.38-0.43)
ISI_20	0.098	0.04	1.10(1.01-1.19)	0.45 (0.43-0.46)
ISI_21	0.167	0.037	1.18(1.09-1.27)	0.46 (0.45-0.48)
ISI_22	0.152	0.037	1.16(1.08-1.25)	0.46 (0.45-0.47)
ISI_23	0.075	0.037	1.07(1.00-1.15)	0.44 (0.43-0.45)
ISI_24	0.054	0.04	1.05(0.97-1.14)	0.44 (0.42-0.45)
ISI_25	Reference			0.42 (0.41-0.44)
ISI_26	-0.034	0.047	0.96(0.88-1.05)	0.41 (0.4-0.43)
ISI_27	-0.205	0.053	0.81(0.73-0.90)	0.37 (0.36-0.4)
ISI_28	-0.144	0.058	0.86(0.77-0.97)	0.39 (0.37-0.41)
305 day milk yield ('000s of litres)	-0.022	0.005	0.97(0.96-0.98)	Continuous
Parity 1	Reference			0.45 (0.44-0.46)
Parity 2	-0.028	0.025	0.97(0.92-1.02)	0.45 (0.43-0.46)
Parity 3	-0.052	0.027	0.94(0.90-1.00)	0.44 (0.43-0.45)
Parity 4	-0.077	0.031	0.92(0.87-0.98)	0.43 (0.42-0.45)
Parity 5+	-0.276	0.026	0.75(0.72-0.79)	0.39 (0.38-0.4)
January	Reference			0.45 (0.44-0.47)
February	-0.038	0.035	0.96(0.89-1.03)	0.44 (0.43-0.46)
March	-0.068	0.036	0.93(0.87-1.00)	0.43 (0.42-0.45)
April	-0.12	0.039	0.88(0.82-0.95)	0.42 (0.4-0.44)
May	-0.053	0.041	0.94(0.87-1.02)	0.44 (0.42-0.46)
June	-0.126	0.042	0.88(0.81-0.95)	0.42 (0.4-0.44)
July	-0.202	0.045	0.81(0.74-0.89)	0.4 (0.38-0.42)
August	-0.188	0.047	0.82(0.75-0.90)	0.41 (0.39-0.43)
September	-0.27	0.046	0.76(0.69-0.83)	0.39 (0.37-0.41)
October	-0.079	0.041	0.92(0.85-1.00)	0.43 (0.41-0.45)
November	-0.094	0.037	0.91(0.84-0.97)	0.43 (0.41-0.44)
December	-0.037	0.033	0.96(0.90-1.02)	0.44 (0.43-0.46)
2002	Reference			0.49 (0.47-0.51)

2003	-0.063	0.04	0.93(0.86-1.01)	0.47 (0.46-0.49)
2004	-0.201	0.039	0.81(0.75-0.88)	0.44 (0.43-0.45)
2005	-0.265	0.039	0.76(0.71-0.82)	0.42 (0.41-0.44)
2006	-0.319	0.039	0.72(0.67-0.78)	0.41 (0.4-0.42)
2007	-0.327	0.038	0.72(0.66-0.77)	0.41 (0.4-0.42)
2008	-0.372	0.047	0.68(0.62-0.75)	0.4 (0.38-0.41)
Insemination number 2	Reference			0.44 (0.43-0.46)
Insemination number >2	-0.136	0.022	0.87(0.83-0.91)	0.41 (0.4-0.42)
Log_eDiM	0.17	0.035	1.18(1.10-1.26)	Continuous
$(Log_eDiM)^2$	-0.345	0.071	0.70(0.61-0.81)	Continuous
Random Part				
Herd level variance	0.06	0.008		
Cow level variance	0.001	0		

*Absolute predicted risk has been calculated for all categorical variables by fixing all other model variables at their population means, and predicting absolute risk (with a 95% credible interval for the prediction) for each of the categories within the variable. Absolute predicted risks are not presented for continuous variables.

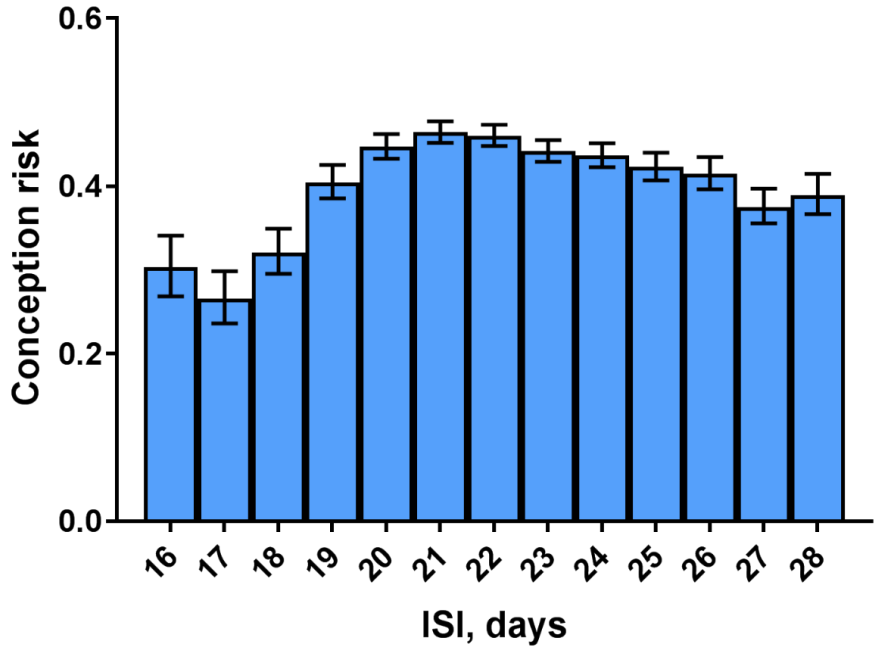


Figure 4-2 Predicted mean conception risk from multilevel model for different inter-service intervals, error bars show the 95% Bayesian credible interval. Bar height shows the average predicted conception risk at inseminations of different inter-service intervals (ISI) with all other explanatory variables fixed at the mean value

There were significant associations of milk yield, parity, days in milk, insemination number, month and year of insemination with conception risk. As milk yield increased conception risk tended to decrease, there was a trend for decreasing conception risk with increasing parity, with a significant decline in conception risk for parity five or greater. There was a quadratic association between the natural logarithm of days in milk and conception risk: conception risk tended to increase up to around 160 days in milk and then decline in later lactation. Third or later inseminations were less likely to result in pregnancy than second inseminations.

Three latent classes (clusters) were identified in the data, broadly corresponding to intervals with a lower chance of conception with lower number of inseminations (LL), a higher chance of conception and lower number of inseminations (HL), a higher chance of conception and a higher number of inseminations (HH). Figure 4-3 illustrates the estimated probability for each ISI (day) being a member of each latent class. Inseminations carried out between 19 and 26 days were most likely to fall in the HH classification. Figure 4-4 illustrates the clustering of each ISI by conception rates and number of inseminations on each day.

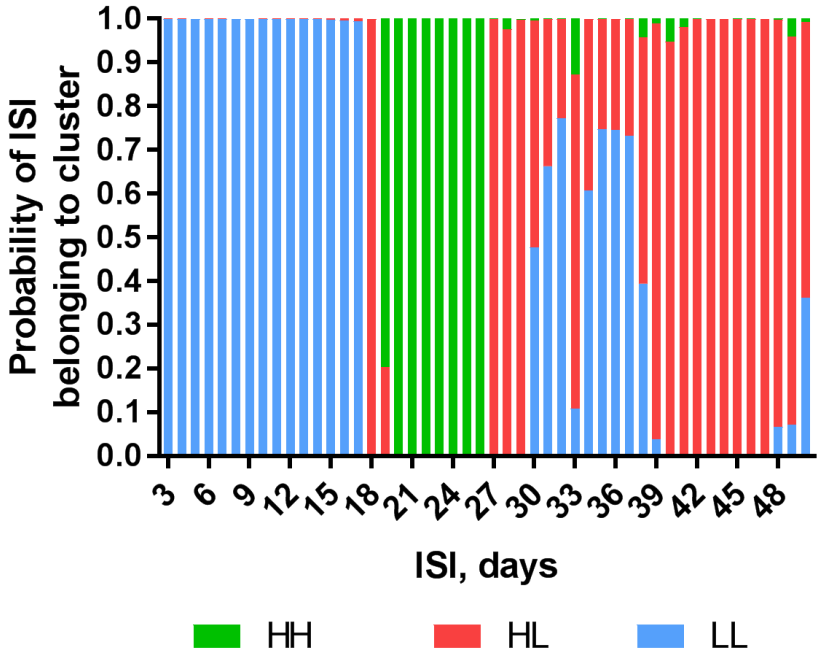


Figure 4-3 Probability of each ISI falling within the finite Gaussian model classifications: low conception rate, low number of inseminations (LL); High conception rate, low number of inseminations (HL); high number of inseminations and higher conception rate (HH)

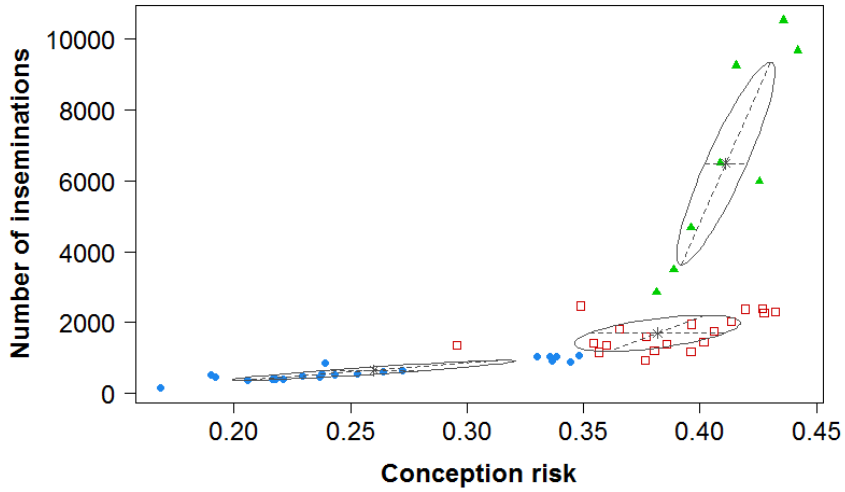


Figure 4-4 Finite Gaussian mixture model classification of ISIs by average conception risk and number of inseminations at each ISI, the identified clusters are shown by the different symbols (blue dots, LL; red squares, HL with; green triangles, HH) the mean of each cluster identified with the star

4.4 Discussion

These findings are useful in reconsidering estimates of normal or expected ISI. The lowest conception risk identified in the analyses were for inseminations given at ISIs of less than 18 days (Figure 4-1). This supports the common interpretation that these inseminations frequently represent inaccurately detected oestrus events, with either the first or the second insemination of the interval having occurred when the cow was not truly in oestrus (Meadows 2005). However, it is interesting to note that ISIs of 18 days (traditionally considered to be within the normal cycle range) had outcomes similar to the "short" intervals of 16-17 days and significantly lower than ISIs of 19 to 26 days. This is supported by the outcome of the machine learning element, where a cluster of ISI with high insemination frequency and high conception rate was confidently identified using unsupervised cluster analysis, covering intervals of between 19 and 26 days. This would seem to support the idea that 18 day ISIs more often represent inaccurate oestrus detection than a normal cycle of this duration. It also remains plausible that 18 day cycles predominantly represent true pairs of oestrus events, but are associated with reduced fertility (shown in Figure 4-2), although the fact that 18 day ISIs are similar to shorter intervals, in terms of frequency, would tend to support the theory that these are much more commonly the result a "false positive" detection of oestrus. In this case, 18 to 24 days would not be an appropriate expected range by which to define a normal ISI in the modern dairy cow and 19-26 days may be more appropriate. This may have implications for the interpretation of some standard methods for assessing distribution of ISIs as a measure of oestrus detection performance, as well as for the generation of expected heat dates in on-farm management systems.

The conception risk appears to peak at multiples of the expected cycle length (i.e around 21 and 42 days, Figure 4-1); it is likely these intervals predominantly represent pairs of true oestrus events. However, the conception risk remains markedly higher for intervals of 28-37 days (i.e. those between the approximate expected lengths of one and two cycles) than for intervals shorter than 18 days. This is likely to be due to different possible explanations for ISIs within this range. This is also

reflected in the uncertainty of classifying these ISIs in the mixture model. In some cases, these intervals are likely to be the result of cows being inseminated outside of true oestrus events. In this case, a missed oestrus and a wrongly identified oestrus would need to occur in succession. As these could occur in either order, the second insemination of the interval may or may not be given when the cow is not in oestrus, and a lower conception risk would therefore be expected. These intervals may also occur following late embryonic death (Northey and French 1980; Ricci and others 2017; Wijma and others 2016), or as a result of pharmacological cycle manipulation following early negative pregnancy diagnosis (Pursley and others 1995). Unfortunately pharmacological intervention was not captured in the dataset, it seems unlikely that few animals would receive prostaglandin treatments less than 35 days after insemination. In either case, the second insemination is likely to relate to a true ovulation event, but would in some cases perhaps be expected to have a somewhat reduced risk of conception (depending on the cause of LED, or the type of cycle manipulation employed). Whilst the use of fixed time AI was not captured in the data, the authors would expect the use of these techniques (particularly resulting in re-insemination within 30 days of a previous insemination) to be minimal in UK dairy herds at the time of data capture: this is supported by the clear increase in frequency of ISIs at the expected unmanipulated cycle lengths of three and six weeks. Any impact of cycle manipulation would also fall outside of the 16-28 day window of interest. Finally, it is also possible that these may represent normal physiological cycles of longer length than traditionally expected, sometimes classified as abnormal e.g. DOVII and PCLII described by Lamming and Darwash (1998). The current results suggest that 26 day intervals are associated with similar outcomes to the 25 day reference category (Table 4-2), as are those at 23 and 24 days. Intervals of 27 or 28 days were associated with a significantly decreased odds of pregnancy, which is likely to reflect the combined impact of the possible explanations described above.

This study used ISI as a proxy for IOI; this was considered useful as it enabled a much larger number of herds to be studied than has previously been the case in this field. However, since this study

excludes the first insemination of each lactation (where there is no ISI), it is likely that late embryonic death (as described above) will mean that the distribution of ISIs shown in Figure 4-1 is not likely to reflect accurately the distribution of IOIs in the same population. Without a marker for the presence of the embryo, or a group of "control" cattle that are not inseminated, it is impossible to know to what extent the results are affected by embryonic death and how much is due to oestrous cycle length. However, recent work evaluating progesterone profiles of 1,418 oestrous cycles in 1009 lactations has shown a markedly similar pattern in oestrous cycle length (Blavy and others 2016) independent of whether cows were inseminated. This is also consistent with studies on smaller numbers of cattle where oestrous cycles were found to be longer than expected (Sartori and others 2004). This suggests that the increased frequency of cows being inseminated at intervals slightly greater than 24 days and the decreased frequency of inseminations carried out at intervals of 18 days from a previous insemination is consistent with the cow return oestrous cycle being longer than the traditionally accepted 18-24 days. This is important clinically, but also in research, where some studies may categorise oestrous cycles greater than 24 days as abnormal (Blavy and others 2016) when they may represent normal variation.

Individual studies commonly find significant differences between the durations of two- and three-wave cycles, the mean IOI in each group varies substantially between studies, with some reporting the mean duration of a three-wave cycle at around 22 days (Ahmad and others 1997) whilst others found this to be greater than 24 days (Townson and others 2002), this variation in reported inter-ovulatory interval is shown in Figure 1-2. Some authors have hypothesised that fertility will be reduced in cows undergoing two-wave (and therefore shorter) cycles, as a result of longer follicular maturation time (Adams and others 2008). The potential for substantial overlap between the distributions of two- and three-wave cycles makes it impossible to accurately separate cycles into follicular wave categories by ISI. This is especially difficult where data comes from a large number of herds and is confounded by factors such as embryonic death discussed previously. Cycle length is also likely to be driven by timing of luteolysis and corpus luteum duration (Mann and Lamming

2006). In this study, slightly shorter cycles (20-21 days) tended to be slightly more fertile rather than less (compared to 23 days); although speculative this would seem not to provide support for the two- versus three-wave hypothesis as an important factor in determining conception risk across a large, multi-herd dataset.

Other findings in this study are consistent with existing work, showing a negative association of conception risk with increasing parity and increasing number of inseminations as well as a non-linear association between days in milk and conception risk (Chebel and others 2004). There was a statistically significant but small negative association between increasing milk production and conception risk. For categorical variables effect size can be seen in Table 4-2 using the predicted absolute risk, for example although month effects were significant they were very small effect sizes as the predicted risk is very similar across months. This appeared to show a seasonal effect, with conception risks declining in the summer. This is consistent with the likely effects of heat stress or nutritional changes during the summer (Rensis and Scaramuzzi 2003). Care needs to be taken when interpreting these associations and there are many confounding factors which cannot be measured or were not available to include in the model (LeBlanc 2010).

The data collected in this study represent a convenience sample of those farms where data quality was sufficient for the analysis. It is possible that this would select for farms with better management which may influence the results. Whilst care needs to be taken before generalising these findings to all farms it seems unlikely that farm management would influence the results to a great degree, and the hierarchical multilevel structure of the regression model would account for any between farm variations in “baseline” ISI.

4.5 Conclusions

Inseminations carried out at intervals of 19-26 days following previous insemination were significantly more likely to result in a pregnancy than those carried out at shorter or longer intervals, within the 16 to 28 day range. This work also provides support for the hypothesis that the expected

range for inter-service interval in the modern dairy cow should be considered longer than the traditional 18-24 days, with an alternative range of 19-26 being supported in analysis where machine learning is used to identify clusters of intervals with similar properties (in terms of both frequency and outcome).

5 Evaluating methods of interpreting inter-service interval data

5.1 Introduction

Numerous methods are available and described for monitoring the two keys steps of dairy herd reproduction, insemination and conception, as well as methods of measuring overall reproductive performance (Breen and others 2009). As described in Chapter One, measuring the effectiveness of oestrus detection can be done by looking at the farm's ability to detect and inseminate cows immediately after the voluntary waiting period (first inseminations) or their ability to detect and inseminate cows following a return to oestrus after a previous unsuccessful insemination (return inseminations) (Hudson and others 2012b). The use of inter service interval analysis is focussed on return oestrus detection. The inter service interval profile analysis is based on the idea that traditionally a cow can be expected to return to oestrus (if it does not become pregnant) between 18 and 24 days after an insemination (Meadows 2005) as described in Chapter One and Chapter Two (1.3 and 2.1). The results described in this thesis so far, as well as other literature, suggest that this expected range may not be appropriate and that normal ISIs may in fact be longer than 18-24 days (Blavy and others 2016; Sartori and others 2004).

As well as visualising the distribution of inter-service intervals, metrics have also been developed which are based on inter service intervals across a cohort of inseminations. Examples of single metrics include Oestrus Detection Efficiency (calculated using the Warren equation, as described in Chapters One and Two – 1.3 and 2.1) and the short regular to long regular interval described in Chapter Two (2.1). These cohort metrics can be valuable to simplify the data as well as facilitate benchmarking.

The aims of this study were threefold: To investigate the potential impact of the use of a longer "normal" ISI on the use of ISI based metrics to examine dairy herd reproductive performance, to identify which (if any) herds would be affected, and to explore novel metrics to represent these data more appropriately. Investigating the potential impact of accounting for the longer expected interval

identified earlier is important to explore whether current methods of evaluating return oestrus detection are inaccurate and could result in invalid conclusions or the over or under estimation of performance on some units. Identifying whether some herds are more likely to be affected will help to place these findings in context as well as potentially enabling the prediction of herds where an alternative approach is needed or where over or under estimation of performance should be considered. Exploring some novel metrics serves two purposes. Firstly, by evaluating a number of existing and novel methods of analysis a preferred option can be identified and recommended. Secondly, the types of metric that are more or less effective may help inform conclusions on the relevance of the extended intervals at a herd level i.e. if metrics that are independent of a preferred interval perform better than those with a preferred interval.

5.2 Materials and methods

5.2.1 Data collection and analysis

Data from the same herd-years used in the benchmarking study of ISI profiles of UK dairy herds described in Chapter Two was used. These data were collected as part of a larger project from 468 UK dairy farms (Hudson and others 2010). The subset of data used for this study consisted of 449,471 insemination records from 67,926 cows in 167 herds between the year 2000 and 2008 where insemination data was considered reliable. Data quality checks and the source data are described in Chapter Two. For every second or subsequent insemination to the same cow in the same lactation, an ISI was calculated. Each ISI was categorised according to the categories described in Table 1-1. The ISI profile (the proportion of ISIs falling in each category) was then calculated for each herd for each calendar year, and this unit of data referred to as a herd-year. ISIs were allocated to a year based on year of insemination, lactation level data were allocated to a calendar year by lactation start date, this was chosen as it was thought most likely to represent the approach used in common software packages as well as ensuring the overall measurements of reproduction reflected those at the time of insemination. Data from 779 herd-years was included in the final analysis. An

alternative “normal” category based on the findings of the work described in Chapter Three and Chapter Four was defined and the ISI profile was recalculated, based on the proportion of ISIs in a herd-year falling between 19 and 26 days. The distribution of the proportion ISIs for each herd-year falling in the 18-24 period and the 19-26 days period were compared, a paired t-test was used to test for a significant difference between the two distributions, with $p<0.05$ considered significant.

For each herd-year an estimated average herd size was calculated by dividing the number of cows calving in the year by a denominator calculated by dividing 365 by the calving index. Culling rate (proportion of cows calving exiting the herd), calving index, voluntary wait period (estimated based on earliest insemination DIM), and proportion of cows in calf by 200 DIM were also calculated. The average 200 day in calf rate was calculated for each herd-year as a marker of overall reproductive performance in that herd-year that was also likely to be affected by return oestrus detection performance (Anon. 2017). Average 305 day milk yield for each herd year was recorded, as well as the proportion of cows calving that were in their first parity.

5.2.2 Identifying herd factors affecting interpretation of metrics

Herd-years were ranked by the proportion of ISIs in the traditional 18-24 day and the proportion of ISIs in the new 19-26 day category and allocated a numerical rank position for each in descending order (i.e the herd-year ranked as number one was the one with the highest proportion of re-inseminations occurring within the target window). Where two or more herd-years had the same proportion of ISIs in the category they were given the average numeric rank position. The change in numeric rank position between the new 19-26 day proportion and the traditional 18-24 day proportion was calculated as an indicator of the size of the difference between the two methods. A negative number indicated an increase in apparent performance relative to other herd-years when using the 19-26 day interval. In order to identify herds in which using the 19-26 day as opposed to 18-24 day ISI had a major impact on their ranked position, herd-years with an absolute change in rank position of greater than 77.9 (i.e. a change in herd-year rank position of more than one decile in

either direction, representing one tenth of 779 herd-years) were identified. Scatter plots were used to visualise both the change in proportion and the change in rank position for each herd-year.

To identify factors that influenced the change in the herd-year proportion of ISIs falling in the 18-24 day or 19-26 day expected ranges, two regression models were specified. The first model used change in rank position as a continuous outcome and the second model used absolute change in proportion as a continuous outcome. The variables 305-day milk yield, the proportion of ISIs from first lactation heifers and the average DiM for each ISI were tested as explanatory variables as the findings of Chapter Three suggested they may impact on ISI. A random effect for herd was included to account for clustering at the herd level. The models were built by stepwise forward selection, with variables offered to the model and retained if the estimated coefficient was more than twice its standard error (i.e. the 95% confidence interval did not cross zero, equivalent to $p<0.05$). Model parameters were estimated using iterative generalised least squares (IGLS) in MLwiN version 2.35 (Rasbash and others 2009). Model fit was checked by comparing the distribution of bottom level residuals to an expected normal distribution. Predictions were used to visualise the model outcomes by fixing all variables at their mean and predicting the outcome across the range of the variable in question.

5.2.3 Evaluating accepted and novel metrics

A series of novel metrics (shown in Table 5-2), were developed to use ISI data to measure oestrus detection performance, some of which were independent of any choice of “normal” interval. These metrics were calculated for each herd-year in the data set along with the oestrus detection efficiency (Gordon 2011).

Table 5-1 Definitions of accepted and novel return oestrus detection metrics

Metric	Description
19-26 day proportion	The proportion of ISIs occurring between 19 and 26 days after a previous insemination
18-24 day proportion	The proportion of ISIs occurring between 18 and 24 days after a previous insemination
MaxRate	The maximum proportion of reserves on a single day between 16-28 days after a previous insemination
3d Max Rate	The maximum rolling three day average proportion of reserves between 16-28 days after a previous insemination
Max sum proportion	The maximum proportion of reserves in a three day window between 16 and 28 days
Max Rate 200	The maximum proportion of reserves on a single day occurring between 2 and 200 days after a previous insemination
Max Rate 200 3d	The maximum rolling three day average proportion of re-serves between 2 and 200 days after a previous insemination
ODE traditional	Oestrus detection efficiency*, calculated using an 18-24 day normal interval
ODE modified	Oestrus detection efficiency*, calculated using an 19-26 day normal interval
Average ISI	The mean inter-service interval for all inseminations between 2-200 days
Ratio traditional	Short regular (18-24) to Long regular (36-48) ratio
Ratio modified	Short regular (19-26) to Long regular (38-52) ratio

*** where proportion of ISIs in each category (see Table 2-1) are denoted as a, b, c, d and e respectively, ODE = (b + d)/(a + b + c + 2(d + e))**

To identify the metric which most closely reflected overall herd reproductive performance, the metrics were evaluated alongside a measure of overall performance. An interval based measurement is prone to bias because only cows that calve again are included in the calculation and so interval-based outcomes were not preferred. The 100 day in calf rate is a popular measurement of overall reproductive performance but was considered less likely to be influenced by return oestrus detection (and therefore ISIs) than 200 day in calf rate. This was because return inseminations will tend to occur later in lactation; it was considered that the 100 day in calf rate would be much more dependent on first insemination efficiency. The 200 day in calf rate was selected as the outcome as it avoids the bias inherent in interval based metrics (such as calving index) and is an effective measure of overall reproductive performance and has been shown to be correlated to the 100 day in calf rate (Löf and others 2012) but has been suggested as a more reflective measure of reproductive performance throughout lactation (Anon. 2017).

Evaluation of the ISI-based metrics was carried out by constructing a series of multiple regression models with 200 day in calf rate as the outcome variable for each. All models included the explanatory variables average herd-year conception risk, 305-day milk yield and estimated voluntary wait period as well as a measurement of oestrus detection, in order to account for key measurable features likely also to contribute to overall reproductive performance. Fitted models were identical except for the oestrus detection metric, with each metric used in one model. An additional 'control' model was also constructed which contained no oestrus detection metric. Model fit was checked by examining the distribution of residuals on a quantile-quantile plot to ensure the assumption of normality was met. Fitted models, each containing a different oestrus detection metric, were compared using the deviance and adjusted R^2 to assess which best fit the data. Deviance is calculated from the model likelihood (-2 x log likelihood); the likelihood is the maximum probability of getting the observed data given the model parameters (Dohoo and others 2003). Adjusted R^2 is also a measure of model fit with a more intuitive interpretation (the proportion variation in observed data explained by the model) but is corrected for the number of terms in the model (to

account for the fact that the 'un-adjusted' R^2 will always increase with additional variables included in the model). Adjusted R^2 was calculated using the following formula:

Equation 5-1 Calculating adjusted R^2

$$Adjusted\ R^2 = 1 - \left[\frac{(1 - R^2)(n - 1)}{n - k - 1}\right]$$

where n is the number of observations in the dataset and k is the number of variables in the model (Dohoo and others 2003).

The first model (with no oestrus detection metric) was built by stepwise forward selection, with variables offered to the model and retained if the coefficient was more than twice the standard error (equivalent to $p<0.05$). All significant variables were then retained in the subsequent models to test each oestrus detection metric, which was forced in to the model (i.e. retained irrespective of the relative sizes of coefficient and standard error). Model parameters were estimated using iterative generalised least squares (IGLS) in MLwiN version 2.35 (Rasbash and others 2009).

For all tested metrics the mean, median and the boundary of the 'best' (numerically highest or lowest, depending on the metric) quartile and decile of the herd-years were calculated to allow benchmarking and target setting.

5.3 Results

5.3.1 Descriptive data

Descriptive data for the 779 herd-years included in the study are shown in Table 5-2.

Table 5-2 Descriptive data for the 779 herd-years included in the analysis

	Herd year average 305 day milk yield (litres)	Herd year average herd size	Herd year average calving interval (days)	Herd year 200 day in calf rate
Mean	7738	233	414	0.64
Maximum	11421	870	521	0.85
Minimum	3804	60	355	0.29

Figure 5-1 shows the distributions of proportions of inter-service intervals across the herd-years using both the accepted and modified 'normal' range, there is a significant difference between the two metrics ($p<0.001$). Figure 5-2 compares the proportion of intervals falling within each of the accepted (18-24) and modified (19-26) intervals for all herd-years. Figure 5-3 compares the rank positions of all herd-years by 18-24 day proportion and 19-26 day proportion. For 151 herd-years of the 779 herd-years included in the study, rank by the modified 19-26 interval was more than a decile different to the rank by the accepted 18-24 interval.

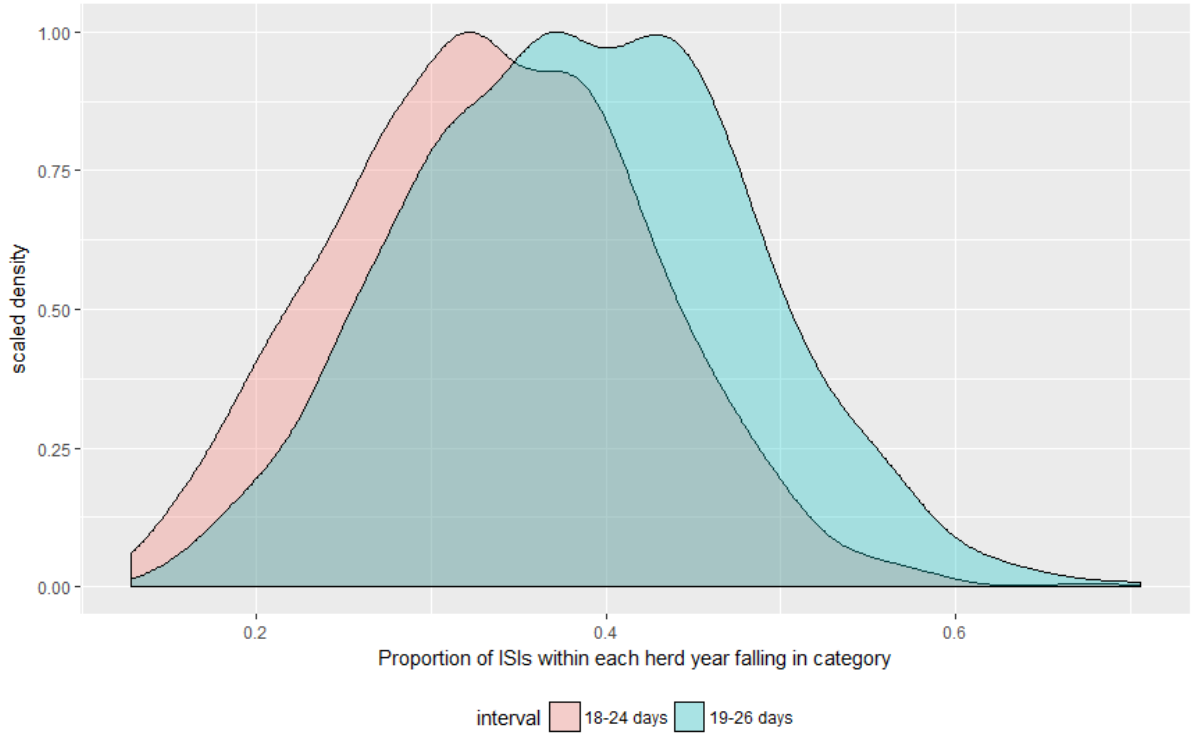


Figure 5-1 Density plots showing the distribution of proportion of ISIs falling within each category for all herd-years using both the accepted (18-24 day) interval and the modified (19-26 day) interval

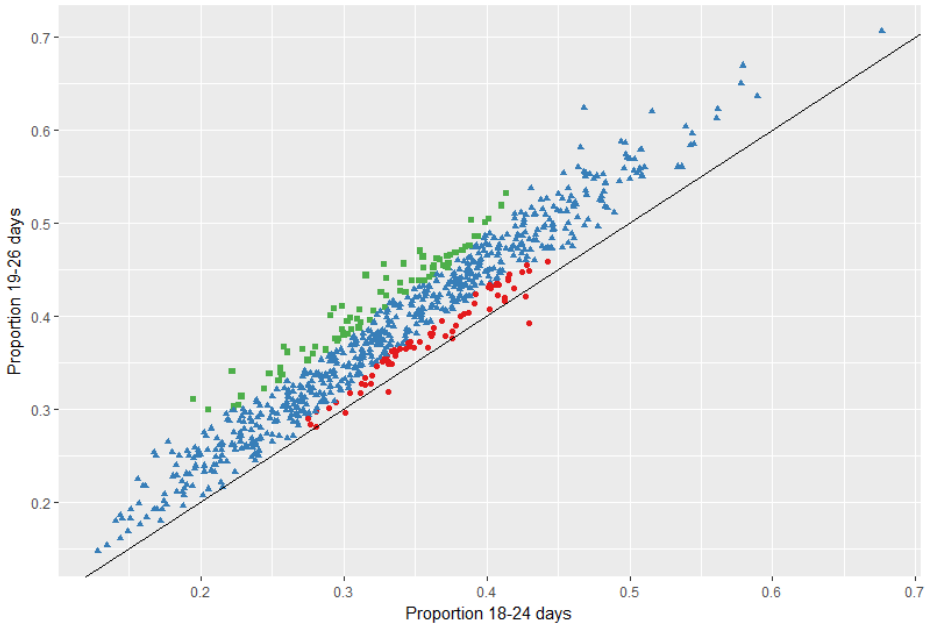


Figure 5-2 The distribution of herd years by the proportion of all ISIs falling with 18-24 days and the proportion of all ISIs falling within 19-26 days for 779 herd-years, herd-years are shown in blue those moving more than a decile in rank between each metric are shown as green squares (relative performance increased), and red circles (relative performance decreased), the black line (x=y) shows the line where the two metrics are equal



Figure 5-3 The distribution of herd years by the relative rank position by proportion of ISIs falling with 18-24 days and relative rank position by the proportion of ISIs falling within 19-26 days for 779 herd-years, herd-years are shown in blue, those moving more than a decile in rank between each metric are shown as green squares (relative performance increased), and red circles (relative performance decreased), the black line (x=y) shows the line where the herd-year ranks are equal

5.3.2 Identifying herd factors affecting interpretation of metrics

Results from regression models predicting the change in the absolute proportion of ISIs and the change in rank position with the longer "normal" interval are shown in Table 5-3 and Table 5-4 respectively. These results show the impact of herd level variables on the change in performance when changing from 18-24 to 19-26 day intervals. Model fit was considered acceptable for both models. There was a significant association between the average herd-year 305 day milk yield and both the change in the absolute proportion and the rank position of the herd year. Relative oestrus detection performance appeared to be underestimated in higher yielding herd-years by using 18-24 days as the reference interval, with a 1000 litre change in average yield associated with an average change in rank of 12 places. The proportion of first lactation heifers in the herd also had a significant effect on the change in proportion but not the relative ranking.

Table 5-3 Coefficients from a regression model with the difference in proportion of intervals in the 18-24 day and modified 19-26 day range for a herd year as the outcome

Variable	Coefficient (Standard error)	p-value
Change in proportion	Outcome variable	
Intercept	0.0512 (0.00121)	
Average 305 day milk yield ('000s litres)	3.86×10^{-3} (0.961×10^{-3})	<0.01
Proportion of heifers	-0.0235 (0.00864)	<0.01
Average days in milk at insemination	No significant effect	
Herd level random effect, sd	0.116×10^{-3} (0.248×10^{-4})	
Model R^2	0.350	

Table 5-4 Coefficients from a regression model with the difference in rank position by proportion of intervals in the 18-24 day and modified 19-26 day range for a herd year as the outcome

Variable	Coefficient (Standard error)	p-value
Change in rank	Outcome variable	
Intercept	0.373 (2.643)	
Average 305 day milk yield ('000s litres)	12 (2)	<0.01
percentage of heifers	No significant effect	
Average days in milk	No significant effect	
Herd level random effect, sd	402.692 (117.871)	
Model R^2	0.267	

5.3.3 Evaluating accepted and novel metrics

The first regression model predicting 200 day in calf rate was constructed without using any oestrus detection metric as an explanatory variable; this outcome was significantly associated with herd year 305 day milk yield, herd year average conception rate, herd year estimated voluntary wait period and herd year culling rate. These variables were forced into all subsequent models as each oestrus detection metric in turn was tested. The coefficients for all variables in all the models along with the model deviance (-2 x log-likelihood) and adjusted R^2 values as a measure of model fit are shown in Table 5-5. All tested oestrus detection metrics were significantly associated with the 200 day in calf rate except for the metrics MaxRate, 3dMaxRate and MaxSum proportion. The highest proportion of herd-year variation in 200 day in calf rate was explained using mean ISI as a measure of oestrus detection, followed by modified ODE and then traditional ODE. In absolute terms, the proportion of outcome variation explained was similar between models, with the 'control' (no oestrus detection metric) model explaining 83% of the variation and the best (Mean ISI) model explaining 84% of the variance (see Table 5-5). This suggests that there is limited difference between metrics and that (as expected) many other factors are associated with overall reproduction. Benchmarking values for these metrics are shown in Table 5-6, with the best quartile providing a useful target level.

Table 5-5 Coefficient estimates from a series of similar regression models predicting 200 day in calf rate and substituting in different oestrus detection metrics as well as likely herd level reproduction confounding variables, p-values for the oestrus detection metrics and model deviance and adjusted r^2 are also shown

Oestrus detection metric tested*	Oestrus detection metric	P-value (HD metric)	305 day milk yield (litres)	Average Conception Rate	Estimated VWP	Average culling rate	Deviance	Adjusted r^2
No HD			7.57×10^{-6} (3.26×10^{-6})	0.419 (0.0431)	-6.29×10^{-4} (1.81×10^{-4})	-0.192 (0.0169)	-2060	0.828
18-24 day proportion	0.271 (0.0311)	<0.01	8.14×10^{-6} (3.01×10^{-6})	0.469 (0.0417)	-7.4×10^{-4} (1.74×10^{-4})	-0.195 (0.0163)	-2130	0.836
19-26 day proportion	0.265 (0.0286)	<0.01	7.19×10^{-6} (3.00×10^{-6})	0.473 (0.0415)	-7.39×10^{-4} (1.73×10^{-4})	-0.199 (0.0163)	-2140	0.839
ODE traditional	0.395 (0.0355)	<0.01	6.86×10^{-6} (2.86×10^{-6})	0.49 (0.0407)	-7.72×10^{-4} (1.7×10^{-4})	-0.197 (0.0161)	-2170	0.839
ODE modified	0.384 (0.0333)	<0.01	5.79×10^{-6} (2.85×10^{-6})	0.492 (0.0405)	-8.05×10^{-4} (1.7×10^{-4})	-0.201 (0.016)	-2180	0.841
Average of ISI	-0.00579	<0.01	4.20×10^{-6} (2.64×10^{-6})	0.583 (0.0401)	-6.79×10^{-4} (1.64×10^{-4})	-0.200 (0.0156)	-2230	0.844
MaxRate	0.0285 (0.0566)	0.615	7.56×10^{-6} (3.26×10^{-6})	0.415 (0.0437)	-6.3×10^{-4} (1.81×10^{-4})	-0.193 (0.0171)	-2060	0.828
3dMaxRate	0.0358 (0.107)	0.737	7.60×10^{-6} (3.26×10^{-6})	0.418 (0.0433)	-6.31×10^{-4} (1.82×10^{-4}	-0.192 (0.0169)	-2060	0.828
Max sum proportion	0.0344 (0.109)	0.753	7.59×10^{-6} (3.26×10^{-6})	0.418 (0.0433)	-6.31×10^{-4} (1.82×10^{-4})	-0.192 (0.0169)	-2060	0.828
MaxRate200	0.56 (0.0963)	<0.01	7.29×10^{-6} ($3.15 \times 10^{-6)}$	0.425 (0.0423)	-6.93×10^{-4} (1.78×10^{-4})	-0.192 (0.0166)	-2100	0.833
MaxRate200 3d	0.804 (0.13)	<0.01	7.65×10^{-6} (3.12×10^{-6})	0.448 (0.0424)	-7.14×10^{-4} (1.78×10^{-4})	-0.184 (0.0167)	-2100	0.832
Ratio traditional	0.013 (0.003)	<0.01	8.55×10^{-6} (3.2×10^{-6})	0.437 (0.0427)	-6.63×10^{-4} (1.79×10^{-4})	-0.195 (0.0167)	-2058	0.832
Ratio modified	0.015 (0.003)	<0.01	8.08×10^{-6} (3.17×10^{-6})	0.448 (0.0426)	-6.60×10^{-4} (1.79×10^{-4})	-0.195 (0.0167)	-2094	0.833

* Metric definitions are provided in Table 2

Table 5-6 Mean, median, boundary of the best quartile and decile values for various ISI metrics used across 779 herd-years

	Mean	Median	Best Quartile	Best Decile
ODE traditional	0.36	0.36	0.41	0.46
ODE modified	0.42	0.41	0.47	0.52
18-24 day proportion	0.33	0.33	0.39	0.45
19-26 day proportion	0.39	0.38	0.45	0.51
Average of ISI*	41.4	40.69	36.32	33.12
MaxRate	0.21	0.2	0.22	0.26
3dMaxRate	0.16	0.16	0.18	0.19
Max sum proportion	0.17	0.17	0.19	0.2
MaxRate200	0.09	0.09	0.1	0.12
MaxRate200 3d	0.07	0.07	0.09	0.1
Ratio traditional	2.03	1.88	2.52	3.14
Ratio modified	2.09	1.92	2.6	3.27

* For Average ISI numerically lower values are 'best'; Metric definitions are provided in Table 2

5.4 Discussion

The first aim of this study was to investigate the potential impact of accounting for longer ISIs on apparent herd reproductive performance. There was a significant difference between using the 18-24 day and modified 19-26 day range, with the 19-26 day range generally giving a higher apparent performance. Using a modified "normal" interval of 19-26 days instead of the traditional 18-24 days changes both the apparent absolute performance of herds, as well as their performance relative to each other as measured by their rank. This suggests that the finding that longer intervals occur more than would be expected also impacts on interpretation of herd reproductive data (Blavy and others 2016). Whilst the changes in absolute performance are to be expected as the new range of intervals

is one day longer, the changes in relative performance are independent of the width of the interval and may result in different interpretation of herd data.

The second aim of this study was to identify which herds were most affected when evaluating apparent oestrus detection performance using the modified interval compared to the accepted interval. The difference in absolute performance (i.e. difference between the proportion of ISIs within the 18-24d range and the proportion within the 19-26d range) for herd-years with a lower proportion of inseminations to first lactation heifers was significantly higher than herd-years with a higher proportion of inseminations to heifers, although the effect size was very small and unlikely to be clinically relevant. For example, a reduction in the percentage of inseminations to first lactation heifers of 10 percentage points for a herd-year, would be associated with an increase in the difference in proportion between the 18-24 and 19-26 day interval of 0.2 percentage points. Although the change in absolute performance was associated with the proportion of inseminations to first lactation heifers, this did not significantly affect rank position relative to other herd-years, which is plausible given the small effect size described in the previous model. Herd-year milk yield had a small but significant association with changes in both relative and absolute proportion, with higher yielding herd-years tending to improve in apparent performance when accounting for the longer intervals. For example, a difference in herd-year 305 day milk yield of 5,000 litres would be associated with a positive change of difference in absolute performance of 1.9 percentage points and a positive change in relative rank position of 60 (equivalent to over seven percentiles). This corresponds with the findings in Chapter Three that heifers typically have shorter ISIs than multiparous cows and that milk yield appears to have a small effect on typical ISI at an individual cow level. Although these findings were statistically significant the model with an outcome of the change in absolute proportion only explains about one third of the variation and the model with an outcome of change in rank position only explains about a quarter of the variation. There is a large amount of unexplained variation both at the herd and herd-year level. Although a lot of the variation is unexplained, level of milk production seems to be the best, readily-available predictor of whether

a herd is more likely to improve in apparent performance when accounting for the longer intervals. This may help identify herds where use of the 18-24 day interval may have resulted in inaccurate conclusions about performance level.

The third aim of this study was to propose and evaluate new metrics for evaluating return oestrus detection. Overall metrics using the modified interval (19-26 day proportion, modified ratio and modified ODE) appear to predict herd-year reproductive performance better than the corresponding traditional metric. This is particularly interesting given shorter ISIs will generally lead to more inseminations in a given time period, and so even when penalising against these shorter ISIs the 19-26 day metrics still predict 200 day in calf rate better. The best performing metric was the mean ISI, followed by modified ODE and then traditional ODE. Of the novel rate metrics developed that were independent of a "normal" interval (MaxRate etc.) those calculated over a wider window of time (e.g. 200 days) were more effective than those looking for peak performance within a narrower range (e.g. 16-28). When extending the window, the denominator for the metric increases to include the extended and other abnormal inseminations. These findings highlight the importance of accounting for not just the efficiency of detecting the first return oestrus, but of accounting for the relative frequency of extended returns when evaluating the success of return oestrus detection on dairy herds. Metrics that include a component not just of efficiency at detecting return oestrus, but also a measurement of how delayed detection is, appear to predict overall herd performance better. Although oestrus detection methods seem to explain only a small part of the variation in herd reproductive performance (as the adjusted R^2 varied very little between different metrics and compared to a model with no oestrus detection metric), this could be because in the null model (without including a variable accounting for oestrus detection), some of the herd level clustering accounted for by the herd level random effect may be accounting for between herd differences in oestrus detection performance.

This work highlights some of the challenges of selecting and validating metrics when measuring dairy herd performance. This process is much easier for those metrics used for monitoring performance than those used "diagnostically" to identify potential explanations for poor performance. Metrics that are used or developed for monitoring are usually dependent on a clear desirable or negative outcome, such as insemination, pregnancy or disease. Diagnostic metrics are usually used to explore changes in an overall reproductive performance metric, for example if less cows are becoming pregnant per unit time (i.e. performance overall is decreasing), then inter-service interval profiles may be analysed to identify whether return oestrus detection or late embryonic death might be a factor. The challenge with diagnostic metrics relates to the lack of a single, clear "gold standard" outcome to compare too. When comparing measurements designed for monitoring overall reproductive performance (for example 21-day pregnancy risk or 100 day in calf rate), the measurement can be compared to clear, desirable reproductive outcomes such as proportion of cows becoming pregnant or calving index and culling rate. With metrics such as the use of inter-service intervals, it is much harder to define a gold standard outcome. These metrics are generally not used to monitor ongoing performance towards a known outcome. This chapter proposes one method of testing and comparing these diagnostic metrics; by using a multivariable approach the metrics can be compared to an overall outcome of value whilst accounting for confounding by other factors. It is impossible to eliminate all confounders by this method, for example it is likely that in this study, this approach will suggest shorter ISIs are better whether accurate or not because this will tend to result in more inseminations – this is especially true given the model accounts for any decline in conception rate which might offset any drop in performance due to inaccurate oestrus detection. This approach still does not reveal the value of these metrics as a purely diagnostic tool, for example, whether inter-service intervals useful for identifying an increased rate of late embryonic death (Meadows 2005). This highlights the importance of advisors both being critical of the metrics that are used to make decisions about dairy herds, but also suggests that the metrics should be viewed holistically and related to other data and the clinical presentation on the farm –

Chapter Two showed that where metrics and associated targets are developed without reference to real data they may end up inappropriate and unachievable. Setting targets from benchmarking datasets is a recognised approach (Hanks 2008; Hanks and Kossaibati 2017). The top quartile of performance from real herds would be an appropriate level for most herds to aspire to, with very high performing herds perhaps aiming for top decile performance. Appropriate targets are shown in Table 5-6.

Whilst target setting is facilitated by having a numeric metric to consider (for example oestrus detection rate or average inter-service interval in this instance), the author would suggest when considering return oestrus detection in a herd there is merit to viewing the distribution of ISIs on a continuous axis rather than by the categories described in Table 1-1. This is how the use of ISIs appears to have been originally proposed (Williamson 1987). This approach is independent of a preferred "expected" interval. If single numeric values are preferred, the values in Table 5-6 provide guidance as to appropriate levels. Where a numeric value is required, mean ISI would appear to be the most valuable in giving information about the herd's likely overall reproductive performance and should help identify whether return oestrus detection is likely to be an issue (although it will not identify why). Metrics relying on a preferred normal interval may not be as valuable. The chosen preferred interval seems to make little difference to the interpretation, although it is worth noting that where metrics had been modified to use a 19-26 day interval rather than a traditional 18-24 day interval they tended to explain more variation than the traditional equivalent. This is probably particularly important in higher yielding herds (which was accounted for separately in the model).

This work focussed on reviewing ISI based metrics to summarise return oestrus detection in a cohort of lactations. Interestingly the best performing summary metric, mean ISI, is in this context mathematically related to one of the preferred monitoring metrics, 21-day insemination risk. The 21-day insemination risk measures the proportion of cows eligible for insemination that are inseminated in a 21 day period and (similar to 21-day pregnancy risk) it is often recommended as an

ongoing monitoring metric as it can be calculated with little lag or bias (Fetrow and others 2007). It is not possible to calculate insemination risk directly from the lactation cohort interval data used in this study. However, a retrospective approximation of average return insemination risk can be calculated and is correlated to mean ISI. An example cow is shown in Figure 5-4.

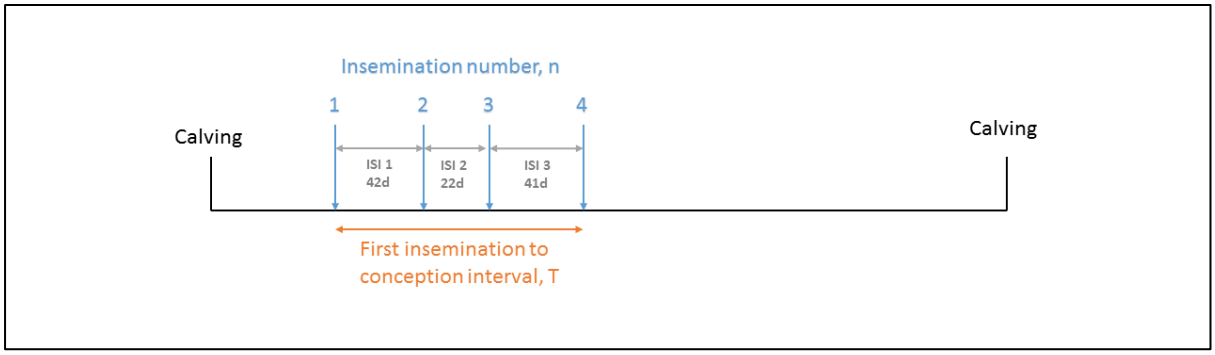


Figure 5-4 A diagram illustrating ISIs in an example cow

In the example cow the mean ISI can be calculated by dividing the sum of all the ISIs by the number of ISIs using the following formula:

Equation 5-2 Calculating mean ISI

$$Mean\ ISI = \frac{42 + 22 + 41}{3} = \frac{T}{n_{max} - 1}$$

where *T* is the time between first and last insemination and n_{max} is the total number of inseminations in the lactation. The retrospective average return insemination risk across the 21 day periods can be estimated by dividing the number of return inseminations by the number of days of eligibility for a return insemination divided by the 21 day period of interest using the following formula:

Equation 5-3 Calculating average return insemination risk

$$Average\ return\ insemination\ risk = \frac{3}{(42 + 22 + 41)\ \div\ 21} = (\frac{n_{max} - 1}{T}) \times 21$$

It can be seen that the return insemination risk is the reciprocal of the average ISI multiplied by 21. Although it remains more rational to measure mean ISI in retrospective cohort data this inherent

relationship with insemination risk further supports the value of both metrics. Particular given that mean ISI can be confusing and is perhaps not very tangible, these findings also support the use of an increasingly popular metric, 21-day insemination risk, which is more intuitive and exhibits less lag and bias in measurement and has a clear association with 21 day pregnancy risk.

5.5 Conclusions

Accounting for an increase in length of a "normal" ISI (19-26 compared to 18-24) appears to improve the apparent performance of most herds. Herds with higher yields were most likely to change in relative performance as measured by herd-year ranking, although it was not possible to completely predict the herd-years that would be most affected. Viewing the distribution of ISIs on a continuous scale may facilitate interpretation. Where a numeric metric is preferred, Average ISI appears to explain more of the variation in herd performance than other existing and proposed metrics when taking in to account other confounding factors, and is mathematically related to 21-day insemination risk.

6 The association of lameness and mastitis with return oestrus detection in the dairy cow

6.1 Introduction

The effectiveness of oestrus detection on a dairy farm can be measured in numerous ways. Some approaches focus on trends over time, for example the proportion of cows eligible for insemination that are inseminated in a 21 day period (21-day insemination risk). Other approaches look at the timing of inseminations, for example by days in milk or in relation to previous inseminations (Hudson and others 2012b; Meadows 2005). Oestrus detection is also frequently divided into first insemination oestrus detection and return insemination oestrus detection (for subsequent inseminations). First insemination oestrus detection focuses on the efficiency with which cows are inseminated at the start of lactation following the end of the voluntary waiting period. Return oestrus detection focuses on the detection of oestrus after a previous (unsuccessful) insemination. Return oestrus detection is usually measured by evaluating the proportion of cows that are re-inseminated at an appropriate interval (usually 18-24 days) from a previous, unsuccessful insemination. Alongside the findings of Chapter Three, there is some evidence that longer intervals than the expected 18-24 are more common (Blavy and others 2016), with Chapter Four suggesting that an interval of 19-26 days may be more appropriate.

It is well accepted that disease in cattle will impact on their reproductive performance (Fourichon and others 2000; Walsh and others 2011). Both mastitis and lameness are common problems in dairy cows. Clear negative associations with overall reproductive performance have been demonstrated for both clinical mastitis and elevated milk somatic cell counts (SCC) (Ahmadzadeh and others 2009; Pinedo and others 2009) and for lameness (Hernandez and others 2001; Hultgren and others 2004; Sprecher and others 1997).

The associations of these diseases with overall reproductive performance could be related to effects on conception, oestrus detection or both. Clinical mastitis has been shown to reduce pregnancies per AI (Fuenzalida and others 2015), a similar reduction in conception rate has been demonstrated

in cows with elevated somatic cell counts (Albaaj and others 2017; Lavon and others 2011). Mastitis also has the potential to impact on the apparent (measured) oestrus detection efficiency by leading to embryonic death and irregular returns (Hansen and others 2004) or by direct effects on ovarian function (Huszenicza and others 2005; Lavon and others 2010). This includes potential impact on the apparent inter-ovulatory interval of the cow (Moore and others 1991). Similar findings have been demonstrated for cases of lameness, with evidence to support a decrease in conception rate in lame animals (Hernandez and others 2001) and other studies showing a decrease in oestrus behaviour (Walker and others 2008b).

Whilst it is clear that both lameness and mastitis have a negative association with reproductive performance their impact on return oestrus detection specifically has not been evaluated on a large scale. The aim of this study was to explore and quantify the impact of lameness and mastitis on return oestrus detection at an individual cow level as well as exploring the impact of a different "expected" interval on any apparent associations.

6.2 Materials and methods

6.2.1 Data collection and organisation

As in previous chapters, farm management data were collected as part of a wider project (Hudson and others 2012a; Hudson and others 2010). Data were contributed by 20 farm animal veterinary surgeons from across England and Wales from a total of 468 dairy herds considered to have good quality records. These data were converted into a common format and screened for fertility data quality as described in Chapter Two. A subset of these herds was then selected that contained regular lameness treatment records, clinical mastitis records with a plausible incidence rate and consistent recording and milk recording data collected at a regular monthly interval. These data were structured so that each insemination was a single line of data along with the animal and herd identity, cow parity, days in milk, 305 day milk yield for that lactation, the number of inseminations so far that lactation, the year the cow calved and the month of the insemination. Lameness and

clinical mastitis records kept according to normal farm detection and recording procedures were converted to give a number of days to the disease event relative to the insemination. These disease records were then converted to binary categories by timeframe relative to the insemination (whether there was a case of clinical mastitis or lameness 29-100 days before the insemination, 1-28 days before the insemination, 0-28 days after the insemination and 29-100 days after an insemination). Neither clinical mastitis aetiology or lameness lesion identification were collected. Where a milk recording was carried out within 31 days prior to the insemination and 31 days after the insemination, the individual cow SCC both before and after the insemination were recorded and the natural logarithm of SCC treated as a continuous variable. SCC status was also categorised based on whether the SCC before and after insemination stayed below 200,000 cells/ml (uninfected), passed from below 200,000 cells/ml (new infection), decreased from above 200,000 cells/ml to below (cure) or stayed above 200,000 cells/ml (chronic). The interval to the next insemination in that cow in that lactation was calculated in days (Inter-service interval, ISI). Any inseminations not followed by a subsequent insemination, likely to be due to pregnancy or culling, were excluded from the data, as were ISIs of 1 or 2 days as these were considered likely be related to the same oestrus event. ISIs of over 200 days were also excluded as they were considered likely to represent recording errors or abortion events. As a result, only cows where there was an apparent intention to re-inseminate were included in the study. Two binary outcome variables were calculated from this ISI corresponding to whether the ISI was within the expected range of 18-24 days or not and whether it was within the alternative range 19-26 days or not as a way of measuring return oestrus detection. The final data set contained 19,011 inseminations for 6,749 cows calving between 2000 and 2008 from 28 dairy herds.

6.2.2 Regression modelling

Logistic multivariable regression models were built with the outcome representing whether or not a cow received a re-insemination at the expected interval. Two similar models were fitted, one with an expected interval of 18-24 days and one with an expected interval of 19-26 days. Herd was included

as a random effect to account for variation in herd level oestrus detection efficiency. Cow was also tested as a random effect, but model fit was poor, and so a two level structure was used (inseminations within herds), this was thought to be caused by the cow level random effect being strongly associated with disease. Both models were developed by stepwise forward selection, with each variable being offered to the models and retained if the magnitude of its estimated coefficient was at least double the standard error of the estimate (equivalent to $p<0.05$). All rejected variables were re-offered to the final models, and retained if they met the criteria described above. Variables offered to the models are shown in Table 6-1. Biologically plausible interactions with the variables of interest (significant lameness and clinical mastitis variables with milk yield and month) were also tested. The model took the conventional form

Equation 6-1 Structure of a random effects logistic regression model with an outcome of re-insemination at the appropriate time (1. Outcome distribution, 2. Logit transformation with model structure, 3. Herd level random effect)

$$re - insemination_{ij} \sim Bernoulli\left(mean = \pi_{ij}\right) \quad (1)$$

$$\ln\left(\frac{\pi_{ij}}{1-\pi_{ij}}\right) = \beta_0 + \boldsymbol{\beta}\boldsymbol{x}_{ij} + u_{0j} \quad (2)$$

$$u_{0j} \sim N(0, \sigma^2_{u0}) \quad (3)$$

where re-insemination$_{ij}$ is whether the ith insemination in the jth herd was followed by a re-insemination at either 18-24 days (model 1) or 19-26 days (model 2); π_{ij} is the fitted probability of re-insemination$_{ij}$; β_0 is the regression intercept, **β** is the vector of coefficients for the vector of predictor variables **x**; u_{0j} is the random effect to represent herd level variation.

The model was fitted using MLwiN version 2.35 (Rasbash and others 2009). Initial parameter estimates were calculated using iterative generalised least squares (IGLS) and final parameter estimates generated using a Bayesian approach, Markov Chain Monte Carlo (MCMC) with Gibbs sampling (Browne 2012; Browne and Draper 2006). A burn-in length of 1,000 iterations was used

followed by a monitoring chain of 10,000 iterations. MCMC chains for the parameter estimates were visually checked to ensure adequate convergence. Model fit was checked by comparing observed and predicted number of re-inseminations for each decile of risk using a modified Hosmer-Lemeshow approach (Hosmer and Lemeshow 1980).

To aid in interpretation, each model was used to predict the probability of cow receiving a re-insemination at the appropriate interval for the different disease scenarios, with all other variables fixed at their population means. Predictions were illustrated using bar charts, showing the mean predicted effect as well as the 95% credible interval around the mean prediction.

Table 6-1 Variables tested for inclusion in a logistic model with the outcome of whether a re-insemination occurs

Variable	Type
Parity	Categorical (1,…,4+)
Days in milk at insemination	Continuous
305 day lactation milk yield	Continuous
Year of calving	Categorical (2000,…,2007)
Month of insemination	Categorical (Jan,…,Dec)
Insemination number	Categorical (1,…,5)
Lameness 29-100 before insemination	Binary
Lameness 1-28 before insemination	Binary
Lameness 0-28 after insemination	Binary
Lameness 29-100 after insemination	Binary
Clinical mastitis 29-100 days before insemination	Binary
Clinical mastitis 28-1 days before insemination	Binary
Clinical mastitis 0-28 days after insemination	Binary
Log_e SCC before	Continuous
Log_e SCC after	Continuous
SCC Status	Categorical (Uninfected, new infection, chronic infection, cure)
Inseminated again at 18-24 days?	Binary outcome (traditional)
Inseminated again at 19-26 days?	Binary outcome (modified)

6.2.3 Population attributable risk

To aid understanding of the potential effect of disease at a population level accounting for effect size and prevalence of lameness and mastitis, the population attributable risk was calculated (Hanley 2001). A prediction was produced for each insemination in the dataset at each iteration of the MCMC chains. The process was then repeated to give a population level predicted outcome in a hypothetical situation where there was no mastitis (i.e. a posterior prediction where every line of data was changed to have no case of clinical mastitis in the 28 days prior to the first insemination). The median values and 95% confidence intervals of these posterior predictions were calculated and compared. The same process was repeated for lameness variables, giving three sets of posterior predictions – the study population as it was, the study population in a hypothetical situation with no mastitis and the study population in a hypothetical situation with no lameness. This in effect created three identical datasets (i.e. with the same number of ISIs), the original and then one where the outcome predicted is as if there were no cases of mastitis and another with no cases of lameness.

6.3 Results

6.3.1 Descriptive data

Of the 19,011 inseminations included in the analysis, 7,693 (40.5%) were followed by an insemination at 18-24 days and 8,741 (46.0%) were followed by an insemination at 19-26 days. The mean 305 day yield for lactations included in the final analysis was 8,854 litres. There were 1,188 inseminations with a case of lameness recorded 29-100 days before, 565 inseminations with a case 1-28 days before, 656 inseminations with a case 0-28 days after and 1,615 inseminations with a case of lameness recorded 29-100 days after. There were 2,157 inseminations with a case of clinical mastitis recorded 29-100 days before, 902 inseminations with a case 28-1 days before, 982 inseminations with a case 0-28 days after and 1,843 inseminations with a case of clinical mastitis recorded 28-100 days after.

6.3.2 Regression modelling

The parameter estimates for each of the regression models are shown in Table 6-2. There was no significant association of 305 day milk yield, days in milk or year of calving with either outcome. Parity was not significantly associated with the probability of re-insemination when using 19-26 day ISIs as an outcome but was when using 18-24 days. In both the 18-24 day model and the 19-26 day model there was a significant negative association of lameness treatments carried out 0-28 days after the first insemination. Odds of re-insemination at 18-24 days were reduced by 18% and those of re-insemination at 19-26 days by 17%. There was no significant association with lameness treatments occurring in other time periods. There was a significant positive association of clinical mastitis recorded 1-28 days before the first insemination with the odds of re-insemination at 18-24 days increased by 21% and those of re-insemination at 19-26 days by 19%. There was no significant association with clinical mastitis at other time periods or of SCC.

Predicted probabilities from the models are illustrated in Figure 6-1. Effect sizes were similar in both models. Effect sizes for month of service and insemination number were similar between the two models, with increasing numbers of inseminations having a positive association and August having a negative association on the probability of re-insemination.

6.3.3 Population attributable risk

The effect of both lameness and mastitis were considered similar in both models and so population attributable risk was calculated for model 2 (19-26 day interval outcome). The median predicted probability of being re-served at 19-26 days was 45.3% (95% credible interval 29.3-66.6%). In a hypothetical scenario, the same population with no cases of lameness in the 0-28 day window after an insemination would lead to a probability of 45.4% (95% credible interval 29.5-66.7%), the same population with no cases of clinical mastitis in the 1-28 days window before an insemination would lead to a probability of 45.1% (95% credible interval 29.2%-66.3%).

Table 6-2 Model parameters and odds ratios from two logistic regression models predicting whether an insemination is followed by another insemination

Variable	Model 1		Model 2	
	Coefficient (Standard error)	Odds ratio (95% credible interval)	Coefficient (Standard error)	Odds ratio (95% credible interval)
Outcome	re-inseminated at 18-24 days		re-inseminated at 19-26 days	
Intercept	-0.5 (0.09)		-0.41 (0.08)	
Parity 1	reference category		reference category	
Parity 2	-0.01 (0.04)	0.99 (0.91-1.08)	not significant	
Parity 3	-0.1 (0.05)	0.91 (0.83-0.99)*	not significant	
Parity 4+	-0.18 (0.04)	0.84 (0.77-0.91)*	not significant	
January	reference category		reference category	
February	-0.07 (0.07)	0.93 (0.81-1.07)	-0.05 (0.07)	0.95 (0.83-1.09)
March	-0.1 (0.07)	0.91 (0.79-1.04)	-0.14 (0.07)	0.87 (0.76-0.99)*
April	-0.09 (0.07)	0.92 (0.79-1.06)	-0.06 (0.07)	0.94 (0.82-1.08)
May	-0.02 (0.08)	0.98 (0.84-1.13)	0.01 (0.07)	1.01 (0.87-1.16)
June	-0.11 (0.08)	0.89 (0.77-1.04)	-0.15 (0.07)	0.87 (0.75-0.99)*
July	-0.07 (0.07)	0.94 (0.81-1.08)	-0.07 (0.07)	0.94 (0.81-1.08)
August	-0.21 (0.08)	0.81 (0.69-0.94)*	-0.24 (0.08)	0.79 (0.68-0.91)*
September	0.05 (0.08)	1.05 (0.9-1.21)	0.04 (0.07)	1.04 (0.9-1.19)
October	0.03 (0.07)	1.03 (0.89-1.18)	0.05 (0.07)	1.05 (0.91-1.2)
November	-0.02 (0.07)	0.98 (0.85-1.12)	-0.01 (0.07)	0.99 (0.87-1.13)
December	-0.03 (0.07)	0.97 (0.84-1.11)	0 (0.07)	1 (0.88-1.15)
First insemination	reference category		reference category	
Second insemination	0.2 (0.04)	1.22 (1.14-1.31)*	0.26 (0.04)	1.29 (1.2-1.39)*
Third insemination	0.35 (0.04)	1.42 (1.31-1.55)*	0.43 (0.04)	1.53 (1.4-1.67)*
Fourth insemination	0.56 (0.06)	1.74 (1.55-1.96)*	0.66 (0.06)	1.93 (1.72-2.17)*
Fifth+ insemination	0.88 (0.07)	2.4 (2.09-2.76)*	0.91 (0.07)	2.48 (2.16-2.84)*
Not lame 0-28 days post insemination	reference category		reference category	
lame 0-28 days post insemination	-0.2 (0.09)	0.82 (0.69-0.97)*	-0.19 (0.08)	0.83 (0.71-0.97)*
no CM 28-1 day previous	reference category		reference category	
CM 28-1day previous	0.19 (0.07)	1.21 (1.05-1.39)*	0.17 (0.07)	1.19 (1.03-1.36)*
Herd level random effect	0.08 (0.03)		0.08 0.03)	

* $p<0.05$

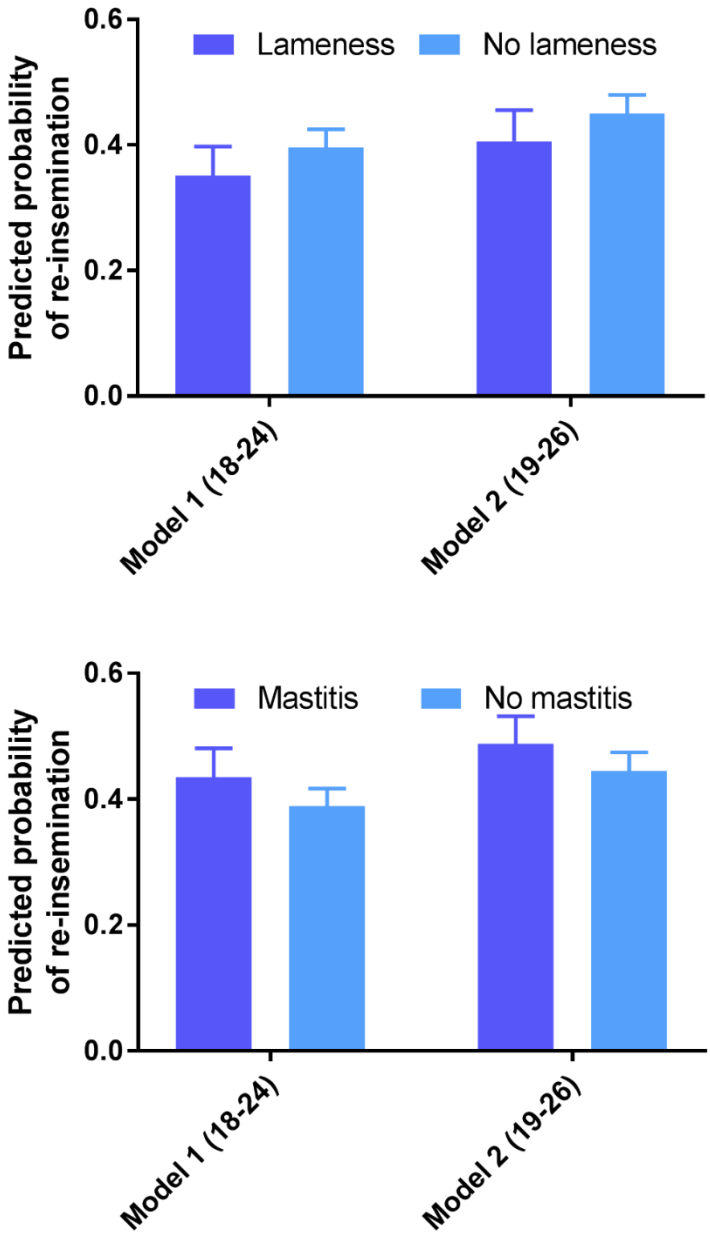


Figure 6-1 Bar chart showing the predicted probabilities and 95% credible interval from two logistic regression models predicting the probability a cow is re-inseminated at 18-24 (model 1) or 19-26 (model 2) days following an insemination for cows that have a case of lameness within 28 days of the first insemination (top) or a case of clinical mastitis in the 28 days preceding the first insemination (bottom)

6.4 Discussion

Cases of both lameness and clinical mastitis appear to be associated with the probability of a cow being re-inseminated at the expected time. Cases of lameness after the first insemination of the interval are associated with a reduced risk of re-insemination. Cases of clinical mastitis before the first insemination of the interval are associated with an increased risk of subsequent insemination at the expected time. These associations were extremely similar whether the traditional (18-24 day) or modified (19-26 day) expected interval was used. The effect of disease on the probability of re-insemination was statistically significant at an individual cow level, with a decrease in odds of nearly 20% and an increase in odds of re-insemination of 20% for lameness and mastitis cases respectively, however the impact was much smaller at a population level. This is supportive of other work in this area suggesting that at a herd level reducing lameness or mastitis is unlikely to have a clinically relevant increase in herd level reproductive performance (Hudson and others 2015; Hudson and others 2014). It is worth noting that herds with this level of data recording may have higher health performance than average and the impact of disease is likely be higher in herds with very high lameness prevalence or mastitis incidence (in this study average lactation level incidence of mastitis was 26% and lameness was 19%). This is because the population attributable risk calculated in this work uses the prevalence of disease in the study population to estimate the impact of eliminating that disease.

The apparent negative association of lameness at cow level with oestrus detection is supported by the existing literature. Lame cows have a longer calving to first service interval (Collick and others 1989); have been shown to spend more time lying and less time standing, walking and expressing oestrus behaviour (Walker and others 2008b) and appear to express oestrus less intensely (Walker and others 2008a). Lameness has been shown to reduce the time oestrus cows are mounted by their herd mates and to reduce the intensity of oestrus behaviour, Walker and others (2010) showed that lame cows were approximately a third less likely to be observed in oestrus. These studies suggest an explanation for the temporal relationship between lameness cases and oestrus detection identified

in the current study. If the presence of lameness reduces the expression of oestrous behaviour then the greatest impact on return oestrus detection will occur when the lameness occurs before the second oestrus is due. The current study has shown a relatively small effect size at an individual cow level, with the probability re-insemination at the expected time decreasing by approximately 10% (Figure 6-1).

It is likely that this represents a conservative estimate as the current study relied on farmer recorded lameness treatments. It has been shown that farmer recorded lameness treatments often represent an underestimate due to delays in detection and treatment (Alawneh and others 2012; Fabian and others 2014; Leach and others 2012). This may have resulted in the misclassification of lame animals in this dataset, with some lame animals being recorded as non-lame because they were not treated or recorded as lame. Conversely, it is also possible that this recording bias would select for more severe cases, potentially leading to the overestimation of the association of return insemination submission rate and lameness. This delayed treatment and recording may also mean that the temporal association of the onset of lameness may be different to the temporal association with lameness treatments. Further studies examining the relationship between oestrus detection and lameness using mobility score data are warranted.

These findings also highlight the value of calculating the predicted relative risk. Classically, findings from logistic regression models are presented as odds ratios (as in Table 6-2), as these are easier to calculate directly from the model coefficients. However, odds ratios can be harder to interpret as humans tend to find probability and risk more intuitive than odds (Bland and Altman 2000). In this study the odds ratios show a change of about 20% whereas the predicted risk only changes by about 10%. The population attributable risk is then useful to put these findings in the context of the whole population (Rockhill and others 1998).

The positive association of clinical mastitis cases with the probability of a return insemination at the expected interval is harder to explain. Clinical mastitis has been shown to reduce reproductive

performance in dairy cows (Ahmadzadeh and others 2009; Barker and others 1998) although other studies have found no effect (Fourichon and others 2000). Moore and others (1991) found that clinical mastitis resulted in a greater number of abnormal interoestrus intervals, defined as those falling outside of the expected 18-24 day range. However, this was not consistent and of the two herds studied, the effect was much stronger in the herd with predominantly gram negative mastitis cases. Another possible explanation for the difference in findings is the study methodology. In contrast to the current study Moore and others (1991) looked for the interval around a case of clinical mastitis (i.e. with the case occurring after the first insemination and before the second). In the current study the only significant association of return oestrus detection with clinical mastitis was when the case of mastitis occurred prior to the first insemination of the interval. Clinical mastitis has consistently been shown to reduce the chance of conception and maintenance of pregnancy after artificial insemination (Ahmadzadeh and others 2009; Fuenzalida and others 2015). A possible explanation for the positive association detected in the current study is that the case of clinical mastitis reduced the chance of conception at the first insemination. This could potentially reduce the likelihood of an abnormal return to oestrus due to embryonic death; the mastitis may have prevented fertilisation leading to a “normal” cycle length. Alternatively, Hockett and others (2005) demonstrated that some cows with experimentally induced mastitis failed to express oestrus behaviour and that in these cows cyclicity was abnormal. It is possible that in the current study, using return oestrus detection as an outcome selected for cows that cycled normally in the presence of clinical mastitis (i.e. cows with clinical mastitis prior to the first insemination would not have had a first insemination if it affected their oestrous cycle). It is important to note by eliminating inseminations not followed by another insemination in this study, the impact of disease on oestrus detection and not on established pregnancy is being measured.

There was a very similar association of both clinical mastitis and lameness with the traditional (18-24 day) and modified (19-26 day) outcome interval. This suggests that these approaches are comparable and that neither is more or less sensitive to the effect of disease on return oestrus

detection as well as suggesting that these associations are not a result of the interval that is selected. It is also suggestive that embryonic death is not the cause of the longer 'expected' interval identified in this project, as it would seem likely that if it were the impact of disease would vary more with the chosen expected interval. Of note is the confounding of parity when using the traditional interval which did not occur when using the modified interval, given the similar associations with disease these supports the suggestion that 19-26 days may be a more appropriate interval to use for most cows when analysing dairy herd reproduction data at least in the context of the impact of disease, as parity does not appear to influence the use of the 19-26 day interval.

6.5 Conclusion

Cases of lameness and clinical mastitis are respectively, negatively and positively associated with re-insemination at the expected time at an individual cow level. At a population level the impact of these conditions on return oestrus detection appears very small. These associations are very similar whether a traditional expected interval of 18-24 days or a modified expected interval of 19-26 days is used as the outcome.

7 General discussion

The aim of this project was to evaluate and develop the use of inter-service interval analysis for the assessment of oestrus detection in dairy herds. Another aim of this project was to see whether "big data" techniques on large scale epidemiological datasets could be used to improve our understanding of the impact of physiological processes at a population level, in this case the length of the bovine oestrous cycle and its associations with disease and likelihood of conception, typically evaluated on smaller more detailed studies. The literature reviewed in Chapter One indicated that traditionally accepted target levels of return oestrus detection performance as measured by inter-service intervals were not likely to be achieved in most herds. There was also suggestion from some small scale studies that the assumptions about normal oestrous cycle length that underpin the current common approach to inter-service interval analysis may not be correct. A number of factors were identified that may influence both oestrous cycle length in inseminated cattle and the inter-service interval profile of a herd.

This project used farm management data collected from a large number of UK dairy farms to identify trends and associations that are likely to be representative of the UK dairy cow population. The data primarily consisted of farm recorded insemination records. This is the same source of data used by farmers and farm advisors when examining inter-service intervals, and so the data used in this study is exactly the same type of data used by farm advisors. As a result, results should be directly applicable to this sort of analysis.

Whilst this thesis focuses on the evaluation of inter-service intervals, the approach taken could be used in the evaluation of other dairy herd metrics and indices. The results of this project highlight the importance of being critical of metrics used in the evaluation of dairy herd performance, ensuring that target levels are set appropriately and that underlying assumptions are appropriate.

The work of Gaines and others (1992) indicated that the inter-service interval profile targets that were proposed historically by Weaver (1986) and Williamson (1987) may be over optimistic.

Chapter Two confirms that this is the case for a large sample of UK dairy herds as well as suggesting appropriate target levels and benchmarking data for use by dairy herd managers and their advisors. By setting targets based on population distributions, instead of using theoretical or physiological assumption, advisors now that proposed targets are achievable. In contrast to the similar work by Gaines and others (1992) the work carried out in Chapter Two did identify a correlation between inter-service interval profile and overall herd reproductive performance (as measured by calving interval). This increased confidence that the method of ranking herds using oestrus detection efficiency (ODE) was valid and the resulting target values illustrated in Figure 2-4 should guide practitioners when assessing oestrus detection on their clients' farms. Whilst care needs to be taken extrapolating findings to other farms, the scale of the dataset should give practitioners confidence in the proposed target values. The targets established allow practitioners to identify areas of oestrus detection that are sub-optimal, building on the evidence base for interventions to improve performance would also be of benefit. The data used in this project is also now relatively old (2000-2008), it appears that in the intervening time performance has improved (Hanks and Kossaibati 2017). As technology and recording software improve it may be possible to set up data aggregation systems that allow the automatic updating of benchmarking analyses such as the one described in Chapter Two as opposed to the periodic manual updating currently implemented in some systems (Hanks and Kossaibati 2017). The literature described in Chapter One describes many management processes that can be used to improve oestrus detection on farms overall, it is likely that these will all also act to improve return oestrus detection and therefore oestrus detection as measured using ISI profiles. At a more general level, this study also highlights the importance of ensuring target and reference values for herd health metrics are generated from real herd performance data, this ensures target levels are achievable. From a research perspective maintaining a target based on what might be theoretically or biologically possible may still be useful to innovate and develop the industry. This should avoid creating unrealistic target levels which may result in inappropriate advice being given. Proposed new target levels are shown below (Table 7-1).

Table 7-1 Proposed targets for measuring inter-service interval profiles of dairy herds (based on top quartile performance)

	Metric	Target
Traditional	2 to 17	0.08
	18 to 24	0.39
	25 to 35	0.19
	36 to 48	0.21
	49 to 200	0.33
	Oestrus detection efficiency	0.41
	Short regular to long regular ratio	2.52
Modified	ODE modified (using 19-26)	0.47
	19-26 day proportion	0.45
	Average of ISI	36.32
	Short regular to long regular ratio (using 19-26)	2.6

The more detailed analysis of the variation in ISIs at the cow level carried out in Chapter Three also benefits from the large size of the dataset. Reviewing the literature revealed a discrepancy between observed oestrous cycle length in several studies (Bleach and others 2004; Sartori and others 2004; Wolfenson and others 2004) and the expected normal length of 21 days (Forde and others 2011). However, these studies focussed on exploring physiological mechanisms in detail and so tended to include a limited number of animals. The work described here has identified that this trend for longer intervals occurs across a large number of UK dairy cows and herds. Potential mechanisms influencing the apparent length of the bovine oestrous cycle have also been identified by this work and are described in Chapter Three. They include seasonal changes, age related changes and an apparent link with production. Whilst this type of study does not allow the underlying mechanisms to be established, it has identified potential lines of enquiry that warrant further exploration.

Perhaps of more interest than the factors that were shown to be associated with cycle length was the remaining unexplained variation. Previously it had been though that oestrus cycle length was relatively consistent within a cow (Jaiswal and others 2009), whereas most of the variation in this much larger dataset was within a cow. The use of random effects multiple regression models has provided a robust way of measuring this variation, taking in to account confounding factors and partitioning the variance across the different 'levels' of the whole dataset. The study by Jaiswal and others (2009) used a sample of 15 heifers and compared proportions of alternating and repeated follicular wave patterns over 75 inter-ovulatory intervals measured using ultrasonography, it is possible this approach underestimated variability across a more diverse population of cattle. Understanding the cause of this variation may become useful when trying to understand and improve fertility on dairy farms, for example it may allow targeted modifications of oestrus synchronisation protocols to different oestrus cycle lengths, or selection of cows that may have better fertility on account of their ovarian follicular dynamics.

Oestrous cycle length appears to be affected by the number of follicular waves in the cycle. The potential links between follicular wave number and subsequent conception risk are currently equivocal (Townson and others 2002), being able to understand this process should prove useful. Although there is no way to confirm it using the data from this project, the effect of follicular wave number on conception risk is a potential explanation for the decreasing cycle length associated with increasing insemination number found in the study. With each additional insemination a population of potentially less fertile cows is being selected (by virtue of the fact they fail to become pregnant), the findings of the study indicate that this population also tend to have shorter oestrous cycles which would fit with the hypothesis that two wave cycle cows are less fertile.

This hypothesis was investigated further in Chapter Four. Identifying the association of ISI with the probability of conception was considered useful to investigate whether this increase in frequency of inseminations coincided with a period of increased fertility (true oestrus) as well as investigating the

potential for different apparent cycle lengths to result in varying probabilities of conception. The findings suggest that although some minor differences may be present there was no clear indication of a clinically important difference in fertility between cycles likely to be two or three wave. From a performance point of view, inseminating a cow that is in oestrus is more important.

In Chapter Four the period of increased fertility and increased frequency of insemination associated with the expected return to oestrus three weeks after the first insemination was further explored. Machine learning was used to identify latent classes of ISIs within the distribution, this identified a cluster of ISIs that occurred with both a high frequency and a high probability of conception. The technique used unsupervised machine learning and so the classifications were not informed by the operator. The number of latent classes identified was also optimised used machine learning algorithms again without 'supervision' from the operator. It is highly plausible that this cluster represents a more appropriate range of 'normal' ISIs. This new expected return to oestrus was identified as 19-26 days (Figure 4-4). This is a different range to the traditionally accepted 18-24 days. If we accept the hypothesis that 19 to 26 represents a more plausible 'normal' range of ISIs than 18 to 24 days this will clearly affect interpretation of ISI data from farms. However, if the difference in proportions changes similarly for every herd when accounting for the longer intervals it will not change the interpretation and so accounting for the different range may not be clinically important. It was necessary to identify the relevance of this finding to those analysing reproductive data from dairy farms.

The data evaluated for benchmarking in Chapter Two using the traditional normal range for ISI of 18-24 days was re-evaluated using the alternative normal range (19-26 days) identified in Chapter Five. This revealed that accounting for the new, longer range had the potential to make a meaningful difference to the interpretation of the herd's reproductive data (with 19% of herd-years changing in relative ranking by more than a decile). Accounting for this range also increased the apparent performance of the herd-years analysed. This was highly likely to occur given the range is wider and

so will inevitably include more inseminations. It is also possible that not accounting for these 'normal' ISIs falling outside the traditional range could explain some of the underperformance identified in Chapter Two. Herd yield appeared to be associated with a larger change in apparent performance, with higher yielding herds appearing to improve in performance more when accounting for the new longer interval. This is consistent with the finding in Chapter Three that there was a small but significant increase in average ISI associated with increased cow level milk yield. To allow easier benchmarking and target setting, several new metrics were tested in an attempt to identify whether overall inter service interval metrics that were independent of a preferred interval were more strongly associated with overall herd reproductive performance. Average ISI was identified as the metric most associated with overall reproductive performance. This finding also supports the use of 21 day insemination risk, an increasingly popular measurement of overall oestrus detection, although it is not directly calculated from ISIs. Average ISI and several of the other metrics tested accounted not only for the proportion of return inseminations occurring at the "right" or expected time, but also accounted for how late those falling outside the target range are. Whilst this might be expected to be important (for example an ISI of 42 days is better for herd performance than an ISI of 100 days even though neither fall in the preferred or expected range) there appears to only a be a limited benefit of accounting for this. This could be because it is likely that the proportion of ISIs falling at the correct time period, i.e. the first return to oestrus, will correlate with the proportion that are detected at subsequent oestrus events meaning that herd with good return oestrus events will have more inseminations at the right time and less very extended intervals. Accounting for inseminations falling at incorrect times, i.e. not multiples of the expected range, also seems to have little impact on the amount of variation in herd level reproduction explained. This could be because these ISIs would be expected to result in a lower conception rate, and this would be accounted for in the model. This suggests that examining ISI profiles and then encouraging farmers to modify oestrus detection practices as a result of an apparent large proportion of inaccurate serves is unlikely to be as beneficial as evaluating ISI profiles for inaccurate oestrus

detection once poor conception rates have been identified (i.e. when it is plausible that inaccurate oestrus detection is affecting overall reproduction).

Chapter Five presented a challenge with evaluating metrics such as inter-service interval. ISI profiles are often used "diagnostically" rather than for routine monitoring. That is to say, they are used to make conclusions about the causes of poor performance, for example identifying that there is likely to be elevated late embryonic death in a herd. This makes evaluating their value and performance challenging as there is no "gold standard" to compare to. Metrics such as 21-day pregnancy rate, calving index or 100 day in calf rate or even submission rate are much more clearly related to the reproductive goals of a dairy farm, i.e. inseminating cows and generating pregnancies efficiently, than the ISI profile. The methodology used in Chapter Five provides an approach for testing these diagnostic metrics. By using a multiple regression model with an outcome that represents overall reproductive performance and accounting for key confounding variables in the model, the association between a given metric and an outcome can be measured. This gives a guide as to how much the metric explains the variation in overall performance (whilst accounting for other components of overall performance). Unfortunately this approach still does not validate any subsequent diagnoses of underlying problems made after reviewing the metric, for example it does not confirm whether analysis of ISI profiles is a sensitive and specific way of identifying herds with a high degree of embryonic death.

Chapter Six also tests the application of the newly identified expected range of ISIs alongside the primary aim of quantifying the association between lameness and mastitis events and return oestrus detection (as measured by ISI). Lameness and clinical mastitis have both been shown to affect reproductive performance, and oestrus detection specifically (Walker and others 2010). Chapter Six demonstrated that this association was apparent in this dataset as well as demonstrating a consistent association between lameness and a reduction in return oestrus events occurring within the expected interval, whichever range was used to define a normal interval. The association of

clinical mastitis cases with return oestrus detection measured using ISI was in an unexpected direction, clinical mastitis was associated with an increase in inseminations occurring at the appropriate time. This could potentially be explained by confounding with embryonic death. Like the association between lameness and the proportion of inseminations occurring in the expected interval, the association was present and similar in magnitude which ever target range of ISIs was used. Although there was a statistically significant association of both lameness and clinical mastitis with the proportion of ISI at the expected range, they did not appear important factors at a population level. This is consistent with other large scale epidemiological studies in this area published during this study (Hudson and others 2012a; Hudson and others 2014). These studies highlight the importance of reviewing data not just at an individual level but also reviewing the data at a population level.

This study uses several "big data" techniques to evaluate routinely collected farm data (for example random effects multiple regression modelling and machine learning). In the time since this project was started the data routinely collected on dairy farms has continued to increase in both availability and quality. "Smart farming" and "big data" approaches are increasing in use and capability (Wolfert and others 2017). Techniques used in this study, such as random effects regression modelling and latent class analysis, which allow the evaluation of associations between outcomes and multiple variables from multiple farms simultaneously will become increasingly useful in research to understand patterns on farm. The associations found by this type of study can provide valuable insights in to factors influencing performance on farms and can be used as the basis for evidence based veterinary medicine. As data from individual farms improves these techniques can also be applied at a herd level to understand factors influencing performance, helping to guide advice to dairy producers. In this instance, these epidemiological techniques have also given some insights in to dairy cow physiology, the findings described can be used to inform smaller experimental studies in this area. Techniques like calculating population attributable risk, as described in Chapter Six can also help to put these findings in a wider population level context. With large datasets, statistically

significant associations are often found but need to be put in context to understand their clinical relevance.

7.1 Study limitations

The data used in this work was collected from a convenience sample of UK dairy herds. This was necessary to ensure data were likely to be of high enough quality. Data from a large number of herds were used in this project, however, the convenience sampling strategy has the potential to influence the results. This may limit the potential to generalise the findings described to other UK dairy farms. By selecting herds thought likely to have good record keeping and to be working closely with their veterinary surgeon some bias may have been introduced. It seems plausible that herds working with their veterinary surgeon and with good data recording would be expected to have better performance than those with poor records. This could be a causal relationship where insights gained from good data lead to improved performance, alternatively good data recording may be reflective of farmers with greater attention to detail and better management skills. This potential bias is most likely to impact on the results of Chapter Two when data is used for benchmarking and setting targets. This could mean that the data used already represents a subset of higher performing herds. It is not possible to identify whether this is true across all areas of performance, as without the data it is impossible to measure and compare the performance of those farms without good enough recording. It is possible to compare some measurements, for example herd size and yield which are measured across larger populations. In Chapter Two the subset of data used gave a mean 305 day milk yield of 7599 litres and a mean herd size of 235 cows. The milk production level is comparable to other datasets at the time, with Agriculture and Horticulture Development Board (2013) stating national average milk yields of 7445 litres however at the same time average herd size was 125 in the UK compared to 235 in Chapter Two. This suggest there is a bias towards larger herds.

Whilst this selection bias may exist, the impact on the conclusions drawn from work described in Chapter Three and Chapter Four is likely to be minimal. It seems unlikely that herds recording more thoroughly would have different oestrous cycles and any bias in yield or herd age profile would be

accounted for by the model. The targets suggested in Chapter Two may represent over estimations of average performance when accounting for all herds, however given the standards described are demonstrably achievable and that benchmarking is only possible for herds with appropriate data they still represent a useful guide for UK farm advisors. The impact on the conclusions of Chapter Five are harder to predict, the development of novel metrics may have been influenced by those metrics most appropriate in better recording herds, although again this sort of analysis is only possible in those herds. Chapter Six used an even smaller subset of very well recorded herds that had both lameness and clinical mastitis records, although it seems unlikely that the level of recording in a herd changes the relationship between disease and oestrus detection.

Farms expected to have good records were selected, but no on-farm records will be perfect. Although systematic errors in the data were screened for (for example the removal of herd data recording only one insemination per pregnancy described in Chapter Two) and herds with unacceptable data excluded, some random error is impossible to identify (for example one missing insemination record, or the misclassification of a lame cow). To some extent this random error will be compensated for by having a larger sample size, reducing the potential impact on the conclusions. Chapter Six has the most potential to be affected by poor recording. Fewer herds were used in this chapter because of the additional recording and data quality required. Under recording of lameness is well accepted and appears prevalent in this dataset too with many herds not having adequate lameness records for inclusion in the analysis (Alawneh and others 2012; Fabian and others 2014). It is plausible that this may have affected both temporal association with return oestrus detection as well as the size of the association. The under recording of lameness would result in some lame animals being misclassified as non-lame, this is likely to impact on the size of the association as not all lame cows would be included in the lame category and some would be included in the non-lame category. It seems most likely that this will result in an underestimate of the size of the association, although it remains plausible that this under recording may result in only selecting more severe cases so it may have results in overestimation of the association with all

lameness events. The temporal association may have also been influenced by the under recording, studies have shown that cows identified as lame by independent researchers actively mobility scoring a herd are often not detected and treated by the farmer for several weeks (Leach and others 2012).

A potential confounding factor in this study is the potential for embryonic death to be extending the apparent oestrus cycle length or ISI. This is because by definition the ISI starts and end with an insemination, and so every interval has the potential to be influenced by insemination and therefore conception. It has previously been suggested that longer ISIs can be used to as a measure of embryonic death (Britt 1995; Meadows 2005). It has also been demonstrated that apparent oestrus cycles tended to be longer following natural mating (Chapman and Casida 1935). It is not possible to determine how frequently this has occurred in the dataset as fertilisation/conception was not routinely measured before 21 days of gestation. The potential impact on the conclusions is perhaps greater for more physiological statements about oestrus cycle length and variability. An attempt was made to understand this in Chapter Four, where a latent class model was used to try and identify underlying distributions. Figure 4-3 highlights the uncertainty in classifying some of the longer intervals, although there was very little uncertainty in the 19-26 day range. When looking at the distribution of ISIs, Figure 4-1 and Figure 3-1 both show a slight skew in the distribution of ISIs towards longer intervals. This could be a result of embryonic death leading to some additional longer intervals. Although a slight skew is present, the distributions are surprisingly symmetrical, it is likely that any late embryonic death would occur randomly and result in some additional ISIs beyond 26 days rather than concentrating in the 25-26 day range. This is also supported by the mixture model illustrated in Figure 3-6, where the whole observed distribution is well represented by three (symmetrical) normal distributions. Despite this slight skew towards longer intervals, the left hand (shorter interval) side of the distributions also does not support a normal range beginning with 18 days, there is limited increase in either frequency of insemination or probability of conception at this stage. This is supported by the findings described in Chapter One from several studies with a more

detailed physiological focus (Blavy and others 2016; Bleach and others 2004; Sartori and others 2004; Wolfenson and others 2004).Whilst this remains a potential confounder, the evidence generated by this project and the existing literature suggest that embryonic death is not a major factor influencing the conclusions made. In terms of the clinical applications of the findings of this thesis there should be little impact of embryonic death confounding the findings, this is because it is not currently possible to establish the role of embryonic death definitively in a dairy herd either and so this confounder is inherent in clinical data as well, meaning 19-26 does represent a normal range for an ISI even if not an oestrous cycle.

One of the most common ways of assessing the potential impact of embryonic death at a herd level is the use of ISI profiles as described in Chapter Two, specifically comparing the proportion of ISIs falling within the 25-35 day range to the other categories (Meadows 2005). The findings of Chapter Three and Chapter Four may indicate that some of the long irregular intervals attributed to embryonic death are in fact a normal return to oestrus and purely a reflection of 18 to 24 days underestimating the length of a normal oestrus cycle, possibly including a 'normal' amount of embryonic death. This needs to be taken in to account when examining ISI profiles from herds. Consideration should be given to viewing the whole distribution to assess whether an increased long irregular category is primarily caused by 25-26 days intervals or whether there is a more random distribution of late returns across this range which would seem more likely to reflect embryonic death.

A universal challenge with studies of this type is that causation cannot be established. The large dataset enables many associations to be explored and identified but it is not possible to explain the mechanisms that underlie these associations or even to attribute causality. This is true of all studies of this sort and highlights the need for complementary physiological studies to unravel the mechanisms underpinning these findings. In this case, some of the key findings of this project (the longer expected range of ISIs) are supported by the findings of smaller scaler studies identifying

oestrus cycles that are longer than traditionally expected (Blavy and others 2016; Bleach and others 2004; Ricci and others 2017; Sartori and others 2004). This combination increases confidence in the findings of both types of work. The larger scale epidemiological work described here suggests the findings of the smaller physiological studies were not anomalies due to small sample sizes but are likely to represent a true reflection of the modern dairy cow. Similarly, the findings of the smaller, more detailed physiological studies that used ultrasonography or hormone testing to identify true oestrus give confidence that the findings of this project are not an artefact of the way insemination data is recorded or a result of using insemination as a proxy for oestrus. This highlights the value of both types of study, the large scale epidemiological scale studies are useful for generating hypotheses and confirming findings are consistent across large numbers of cattle. The more detailed physiological studies enable more understanding of underpinning mechanisms and can be carried out in a more controlled way.

7.2 Future work

The work described in this thesis could underpin several avenues of potential future work. Two broad approaches could be taken to answering questions raised by these results. Firstly, further epidemiological style studies using large data sets collected from farms, and secondly, smaller scale studies focussing on unravelling the causative pathways and physiological mechanisms underpinning these findings.

The findings of Chapter Two describe the performance of a large number of herds, although the factors driving this performance have not been established. Supplementing a large dataset such as the one used in this thesis with further detailed farm management practices, such as method(s) of oestrus detection, housing design and system would allow similar methods to be used to identify associations between management practices and performance. An understanding of the methods used by higher performing herds would facilitate decision making for herds with poorer performance. Both Chapter Two and Chapter Five have highlighted the importance of reviewing

metrics and commonly accepted targets. The approach taken in this project could be applied to other measures of dairy herd (or other farmed species) performance to provide better benchmarks and set more appropriate targets. The approach described in Chapter Five in particular provides a model for the assessment of more 'diagnostic' metrics by using a multivariable approach to evaluate the degree of variation in a key outcome that they explain.

Whilst challenging to acquire appropriate and robust data, further work to test these 'diagnostic' metrics on herds with a known disease status would be of value. This would provide a gold standard with which to judge the metrics or method of analysis – for example herds experiencing endemic infectious disease such as Bovine Viral Diarrhoea and experiencing high levels of embryonic death as a result could be compared to healthier herds to test the sensitivity and specificity of ISI profiles for identifying embryonic death allowing an 'abnormal' level of irregular returns to be established. The challenge for this type of study would be accurately measuring levels of embryonic death in a large number of herds. ISI profiles could be compared to other indicators of embryonic death for example the number of cows diagnosed as pregnant but not calving or being inseminated again using these later indicators of embryonic death as a sentinel to compare to variations in ISI profile likely to be caused by high embryonic death prevalence. Collecting data from herds with better disease recording would also enable more accurate estimations of the impact of mastitis and particularly lameness on oestrus detection. With the increased use of mobility scoring on UK dairy herds the approach in Chapter Six could be repeated using mobility score data in addition to treatment data to more accurately define a lame cow, with increased electronic records on farms this could be included in a large multivariable model alongside other disease states. This would enable a wider variety of diseases to be evaluated as well as accounting for any confounding or interactions between diseases, for example ketosis predisposing to displaced abomasum or hypocalcaemia predisposing to mastitis.

In terms of more physiological studies, the findings of Chapter Three and Chapter Four have identified several associations of cow factors with ISI. These warrant further investigation. Since commencement of this work, Blavy and others (2016) have used serial progesterone testing to accurately measure oestrus cycle length. Ricci and others (2017) have used synchronisation, hormone measurements and transrectal ultrasonography to study follicular waves and timing of ovulation in more detail. Both these approaches could be used to compare oestrous cycles with and without insemination at the start and also for cows of differing production levels and breeds. This could help explain the influence of prior insemination (and possible embryonic death) on the apparent oestrous cycle length, as well as identifying possible mechanisms for the factors associated with ISI identified in Chapter Three such as milk yield.

7.3 Conclusions

This work has added to knowledge in this area by identifying potential for misinterpretation in the analysis of dairy herds' ISI profiles, an existing approach for the assessment of return oestrus detection in dairy herds. Current targets appear unrealistic and new benchmarks are suggested. A modification to the commonly accepted target interval is proposed after identifying that ISIs tend to be longer than expected and that this does impact on the interpretation of herd level data. Using a target interval of 19-26 days instead of 18-24 is more appropriate. A series of new metrics have been tested and evaluated and target levels for these identified with Average ISI giving the best overview of ISI performance. The impact of disease on return oestrus detection has also been evaluated. This work should enable better interpretation of ISI data from farms, in turn leading to better oestrus detection performance. Oestrus detection is a key component of efficient reproductive performance on dairy farms, and therefore the efficient production of milk which is likely to become even more important as the world's population grows and increased demand for food places pressure on resources and the environment. ISI profiles are an effective way of evaluating return oestrus detection in dairy herds and should be used alongside other measures of herd reproductive performance.

References

ADAMS, G. P., JAISWAL, R., SINGH, J. & MALHI, P. (2008) Progress in understanding ovarian follicular dynamics in cattle. Theriogenology 69, 72-80

ADAMS, G. P., MATTERI, R. L., KASTELIC, J. P., KO, J. C. H. & GINTHER, O. J. (1992) Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. Journal of Reproduction and Fertility 94, 177-188

ADEWUYI, A. A., ROELOFS, J. B., GRUYS, E., TOUSSAINT, M. J. M. & VAN EERDENBURG, F. J. C. M. (2006) Relationship of Plasma Nonesterified Fatty Acids and Walking Activity in Postpartum Dairy Cows. Journal of Dairy Science 89, 2977-2979

ADLER, D. (2005) vioplot: Violin Plot. R package version 0.2

AGRICULTURE AND HORTICULTURE DEVELOPMENT BOARD (2013) Dairy Statistics: An insider's guide 2013. Kenilworth, DairyCo

AHMAD, N., TOWNSEND, E. C., DAILEY, R. A. & INSKEEP, E. K. (1997) Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. Animal Reproduction Science 49, 13-28

AHMADZADEH, A., FRAGO, F., SHAFII, B., DALTON, J. C., PRICE, W. J. & MCGUIRE, M. A. (2009) Effect of clinical mastitis and other diseases on reproductive performance of Holstein cows. Animal Reproduction Science 112, 273-282

ALAWNEH, J. I., LAVEN, R. A. & STEVENSON, M. A. (2012) Interval between detection of lameness by locomotion scoring and treatment for lameness: A survival analysis. The Veterinary Journal 193, 622-625

ALBAAJ, A., FOUCRAS, G. & RABOISSON, D. (2017) High somatic cell counts and changes in milk fat and protein contents around insemination are negatively associated with conception in dairy cows. Theriogenology 88, 18-27

ANON. (2017) InCalf book (2nd Ed.). Dairy Australia Limited

ARBEL, R., BIGUN, Y., EZRA, E., STURMAN, H. & HOJMAN, D. (2001) The Effect of Extended Calving Intervals in High Lactating Cows on Milk Production and Profitability. Journal of Dairy Science 84, 600-608

ARCHER, S., BELL, N. & HUXLEY, J. (2010) Lameness in UK dairy cows: a review of the current status. In Practice 32, 492-504

ARCHER, S. C., MC COY, F., WAPENAAR, W. & GREEN, M. J. (2013) Association between somatic cell count early in the first lactation and the longevity of Irish dairy cows. Journal of Dairy Science 96, 2939-2950

ARNEY, D. R., KITWOOD, S. E. & PHILLIPS, C. J. C. (1994) The increase in activity during oestrus in dairy cows. Applied Animal Behaviour Science 40, 211-218

BARKER, A. R., SCHRICK, F. N., LEWIS, M. J., DOWLEN, H. H. & OLIVER, S. P. (1998) Influence of Clinical Mastitis During Early Lactation on Reproductive Performance of Jersey Cows. Journal of Dairy Science 81, 1285-1290

BAZER, F. W., SPENCER, T. E. & OTT, T. L. (1997) Interferon Tau: A Novel Pregnancy Recognition Signal. American Journal of Reproductive Immunology 37, 412-420

BELL, N. J. & HUXLEY, J. N. (2009) The use of rubber floor matting on dairy units: A critical review. Cattle Practice 17, 142-147

BENAGLIA, T., CHAUVEAU, D., HUNTER, D. R. & YOUNG, D. S. (2009) mixtools: An R Package for Analyzing Mixture Models. 2009 32, 29

BLAND, J. M. & ALTMAN, D. G. (2000) The odds ratio. BMJ : British Medical Journal 320, 1468-1468

BLAVY, P., DERKS, M., MARTIN, O., HÖGLUND, J. K. & FRIGGENS, N. C. (2016) Overview of progesterone profiles in dairy cows. Theriogenology 86, 1061-1071

BLEACH, E. C. L., GLENCROSS, R. G. & KNIGHT, P. G. (2004) Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. Reproduction 127, 621-629

BÓ, G. A., BARUSELLI, P. S. & MARTíNEZ, M. F. (2003) Pattern and manipulation of follicular development in Bos indicus cattle. Animal Reproduction Science 78, 307-326
BOER, H. M. T., RÖBLITZ, S., STÖTZEL, C., VEERKAMP, R. F., KEMP, B. & WOELDERS, H. (2011a) Mechanisms regulating follicle wave patterns in the bovine estrous cycle investigated with a mathematical model. Journal of Dairy Science 94, 5987-6000
BOER, H. M. T., STÖTZEL, C., RÖBLITZ, S., DEUFLHARD, P., VEERKAMP, R. F. & WOELDERS, H. (2011b) A simple mathematical model of the bovine estrous cycle: Follicle development and endocrine interactions. Journal of Theoretical Biology 278, 20-31
BOYLE, L. A., MEE, J. F. & KIERNAN, P. J. (2007) The effect of rubber versus concrete passageways in cubicle housing on claw health and reproduction of pluriparous dairy cows. Applied Animal Behaviour Science 106, 1-12
BREEN, J. E., HUDSON, C. D., BRADLEY, A. J. & GREEN, M. J. (2009) Monitoring dairy herd fertility performance in the modern production animal practice. Cattle Practice 17, 196-201
BRITT, J. H. (1995) The relationship between postpartum estrous cycles, estrus cycle length, and early embryonic death. Cattle Practice 3, 85-88
BRITT, J. H., SCOTT, R. G., ARMSTRONG, J. D. & WHITACRE, M. D. (1986) Determinants of Estrous Behavior in Lactating Holstein Cows. Journal of Dairy Science 69, 2195-2202
BROWN, L. D., CAI, T. T. & DASGUPTA, A. (2001) Interval Estimation for a Binomial Proportion, 101-133
BROWNE, W. J. (2012) MCMC estimation in MLwiN, v2.25. Centre for Multilevel Modelling, University of Bristol
BROWNE, W. J. & DRAPER, D. (2006) A comparison of Bayesian and likelihood-based methods for fitting multilevel models. Bayesian Analysis 1, 473-514
BRUN-LAFLEUR, L., CUTULLIC, E., FAVERDIN, P., DELABY, L. & DISENHAUS, C. (2013) An individual reproduction model sensitive to milk yield and body condition in Holstein dairy cows. Animal 7, 1332-1343
BULMAN, D. C. & LAMMING, G. E. (1979) The Use of Milk Progesterone Analysis in the Study of Oestrus Detection, Herd Fertility and Embryonic Mortality in Dairy Cows. British Veterinary Journal 135, 559-567
CAVESTANY, D., EL-WISHY, A. B. & FOOTE, R. H. (1985) Effect of Season and High Environmental Temperature on Fertility of Holstein Cattle. Journal of Dairy Science 68, 1471-1478
CHAPMAN, A. & CASIDA, L. (1935) Factors associated with breeding efficiency in dairy cattle. Journal of Animal Science 1935, 57-62
CHEBEL, R. C., SANTOS, J. E. P., REYNOLDS, J. P., CERRI, R. L. A., JUCHEM, S. O. & OVERTON, M. (2004) Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Animal Reproduction Science 84, 239-255
COLLICK, D. W., WARD, W. R. & DOBSON, H. (1989) Associations between types of lameness and fertility. Veterinary Record 125, 103-106
CUTULLIC, E., DELABY, L., CAUSEUR, D., MICHEL, G. & DISENHAUS, C. (2009) Hierarchy of factors affecting behavioural signs used for oestrus detection of Holstein and Normande dairy cows in a seasonal calving system. Animal Reproduction Science 113, 22-37
DE SILVA, A. W. M. V., ANDERSON, G. W., GWAZDAUSKAS, F. C., MCGILLIARD, M. L. & LINEWEAVER, J. A. (1981) Interrelationships With Estrous Behavior and Conception in Dairy Cattle. Journal of Dairy Science 64, 2409-2418
DILLON, P., SNIJDERS, S., BUCKLEY, F., HARRIS, B., O'CONNOR, P. & MEE, J. F. (2003) A comparison of different dairy cow breeds on a seasonal grass-based system of milk production: 2. Reproduction and survival. Livestock Production Science 83, 35-42
DISKIN, M. G., PARR, M. H. & MORRIS, D. G. (2011) Embryo death in cattle: an update. Reproduction, Fertility, and Development 24, 244-251
DISKIN, M. G. & SREENAN, J. M. (1980) Fertilization and embryonic mortality rates in beef heifers after artificial insemination. Journal of Reproduction and Fertility 59, 463-468

DISKIN, M. G. & SREENAN, J. M. (2000) Expression and detection of oestrus in cattle. Reproduction, Nutrition, Development 40, 481-491
DOBSON, H., SMITH, R. F., ROYAL, M. D., KNIGHT, C. H. & SHELDON, I. M. (2007) The high-producing dairy cow and its reproductive performance. Reproduction in Domestic Animals 42, 17-23
DOBSON, H., WALKER, S. L., MORRIS, M. J., ROUTLY, J. E. & SMITH, R. F. (2008) Why is it getting more difficult to successfully artificially inseminate dairy cows? Animal 2, 1104-1111
DOHOO, I. R., MARTIN, W. & STRYHN, H. (2003) Veterinary epidemiologic research, AVC Incorporated Charlottetown, Canada
DRANSFIELD, M. B. G., NEBEL, R. L., PEARSON, R. E. & WARNICK, L. D. (1998) Timing of Insemination for Dairy Cows Identified in Estrus by a Radiotelemetric Estrus Detection System. Journal of Dairy Science 81, 1874-1882
ESSLEMONT, R. (1992) Measuring dairy herd fertility. Veterinary Record 131, 209-212
ESSLEMONT, R. J. (2003) The Costs of Poor Fertility and What to Do about Reducing Them. Cattle Practice 11, 237-250
ESSLEMONT, R. J. & KOSSAIBATI, M. A. (2000) The use of databases to manage fertility. Animal Reproduction Science 60–61, 725-741
FABIAN, J., LAVEN, R. A. & WHAY, H. R. (2014) The prevalence of lameness on New Zealand dairy farms: A comparison of farmer estimate and locomotion scoring. The Veterinary Journal 201, 31-38
FETROW, J., STEWART, S., EICKER, S. & RAPNICKI, P. (2007) CHAPTER 61 - Reproductive Health Programs for Dairy Herds: Analysis of Records for Assessment of Reproductive Performance A2 - YOUNGQUIST, ROBERT S. In Current Therapy in Large Animal Theriogenology (Second Edition). Ed W. R. THRELFALL. Saint Louis, W.B. Saunders. pp 473-489
FISCHER-TENHAGEN, C., WETTERHOLM, L., TENHAGEN, B. A. & HEUWIESER, W. (2011) Training dogs on a scent platform for oestrus detection in cows. Applied Animal Behaviour Science 131, 63-70
FOOTE, R. H. (1979) Time of Artificial Insemination and Fertility in Dairy Cattle. Journal of Dairy Science 62, 355-358
FORDE, N., BELTMAN, M. E., LONERGAN, P., DISKIN, M., ROCHE, J. F. & CROWE, M. A. (2011) Oestrous cycles in Bos taurus cattle. Animal Reproduction Science 124, 163-169
FOURICHON, C., SEEGERS, H. & MALHER, X. (2000) Effect of disease on reproduction in the dairy cow: a meta-analysis. Theriogenology 53, 1729-1759
FRALEY, C., RAFTERY, A. E., MURPHY, T. B. & SCRUCCA, L. (2002) Model-based Clustering, Discriminant Analysis and Density Estimation. Journal of the American Statistical Association 97, 611-631
FRALEY, C., RAFTERY, A. E., MURPHY, T. B. & SCRUCCA, L. (2012) mclust Version 4 for R: Normal Mixture Modeling for Model-Based Clustering, Classification, and Density Estimation. In Technical Report No. 597. Department of Statistics, University of Washington
FUENZALIDA, M. J., FRICKE, P. M. & RUEGG, P. L. (2015) The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows. Journal of Dairy Science 98, 3791-3805
GAINES, J. D., THOMAS, C. B., EICKER, S., RHODA, D., GOEDTEL, M., LARSON, P. L., LINDNER, E. R. & LINDBORG, M. (1992) Interestrous interval profiles of 71 Wisconsin dairy herds and relationship to days to pregnancy. Preventive Veterinary Medicine 12, 47-58
GALINA, C. S., CALDERÓN, A. & MCCLOSKEY, M. (1982) Detection of signs of estrus in the charolais cow and its Brahman cross under continuous observation. Theriogenology 17, 485-498
GARBARINO, E. J., HERNANDEZ, J. A., SHEARER, J. K., RISCO, C. A. & THATCHER, W. W. (2004) Effect of Lameness on Ovarian Activity in Postpartum Holstein Cows*. Journal of Dairy Science 87, 4123-4131
GILL, M. S. (1995) Surgical Techniques for Preparation of Teaser Bulls. Veterinary Clinics of North America: Food Animal Practice 11, 123-136

GILLIS, E. H., GOSLING, J. P., SREENAN, J. M. & KANE, M. (2002) Development and validation of a biosensor-based immunoassay for progesterone in bovine milk. Journal of Immunological Methods 267, 131-138
GINTHER, O. J. (2016) The theory of follicle selection in cattle. Domestic Animal Endocrinology 57, 85-99
GINTHER, O. J., KNOPF, L. & KASTELIC, J. P. (1989) Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. Journal of Reproduction and Fertility 87, 223-230
GORDON, P. (2011) Oestrus detection in dairy cattle. In Practice 33, 542-546
GORDON, P. J. (2006) A study on the use of vasectomised bulls as an aid to heat detection in 5 dairy herds. Cattle Practice 14, 37-39
GROVE-WHITE, D. (2004) Healthcare in the modern dairy herd. In Practice 26, 368-376
GWAZDAUSKAS, F. C., LINEWEAVER, J. A. & MCGILLIARD, M. L. (1983) Environmental and Management Factors Affecting Estrous Activity in Dairy Cattle. Journal of Dairy Science 66, 1510-1514
GWAZDAUSKAS, F. C., THATCHER, W. W., KIDDY, C. A., PAAPE, M. J. & WILCOX, C. J. (1981) Hormonal patterns during heat stress following PGF2α-tham salt induced luteal regression in heifers. Theriogenology 16, 271-285
GWAZDAUSKAS, F. C., WHITTIER, W. D., VINSON, W. E. & PEARSON, R. E. (1986) Evaluation of Reproductive Efficiency of Dairy Cattle with Emphasis on Timing of Breeding. Journal of Dairy Science 69, 290-297
HANKS, J. (2008) Using milk recording data to investigate herd fertility. UK Vet: Livestock 13, 37-40
HANKS, J. & KOSSAIBATI, M. (2012) Key Performance Indicators for the UK national dairy herd. Ed VETERINARY EPIDEMIOLOGY & ECONOMICS RESEARCH UNIT (VEERU). University of Reading
HANKS, J. & KOSSAIBATI, M. (2017) Key Performance Indicators for the UK national dairy herd. Ed VETERINARY EPIDEMIOLOGY & ECONOMICS RESEARCH UNIT (VEERU). University of Reading
HANLEY, J. A. (2001) A heuristic approach to the formulas for population attributable fraction. Journal of Epidemiology and Community Health 55, 508-514
HANSEN, P. J., SOTO, P. & NATZKE, R. P. (2004) Mastitis and Fertility in Cattle – Possible Involvement of Inflammation or Immune Activation in Embryonic Mortality*. American Journal of Reproductive Immunology 51, 294-301
HARTIGAN, P. J. (2004) Reproductive Physiology in Cattle. In Bovine Medicine: Diseases and Husbandry of Cattle. 2nd edn. Eds A. H. ANDREWS, R. W. BLOWEY, H. BOYD, R. G. EDDY. Oxford, England, Blackwell Science Ltd. pp 471-505
HEERSCHE, G., JR. & NEBEL, R. L. (1994) Measuring efficiency and accuracy of detection of estrus. Journal of Dairy Science 77, 2754-2761
HELMER, S. D. & BRITT, J. H. (1985) Mounting Behavior as Affected by Stage of Estrous Cycle in Holstein Heifers1. Journal of Dairy Science 68, 1290-1296
HENDERSON, K. M. & MOON, Y. S. (1979) Luteinization of bovine granulosa cells and corpus luteum formation associated with loss of androgen-aromatizing ability. Journal of Reproduction and Fertility 56, 89-97
HERNANDEZ, J., SHEARER, J. K. & WEBB, D. W. (2001) Effect of lameness on the calving-to-conception interval in dairy cows. Journal of the American Veterinary Medical Association 218, 1611-1614
HIGGINS, H. M., FERGUSON, E., SMITH, R. F. & GREEN, M. J. (2013) Using Hormones to Manage Dairy Cow Fertility: The Clinical and Ethical Beliefs of Veterinary Practitioners. PloS One 8, e62993
HINTZE, J. L. & NELSON, R. D. (1998) Violin Plots: A Box Plot-Density Trace Synergism. The American Statistician 52, 181-184
HOCKETT, M. E., ALMEIDA, R. A., ROHRBACH, N. R., OLIVER, S. P., DOWLEN, H. H. & SCHRICK, F. N. (2005) Effects of induced clinical mastitis during preovulation on endocrine and follicular function. Journal of Dairy Science 88, 2422-2431

HOCKETT, M. E., HOPKINS, F. M., LEWIS, M. J., SAXTON, A. M., DOWLEN, H. H., OLIVER, S. P. & SCHRICK, F. N. (2000) Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. Animal Reproduction Science 58, 241-251
HOLMAN, A., THOMPSON, J., ROUTLY, J. E., CAMERON, J., JONES, D. N., GROVE-WHITE, D., SMITH, R. F. & DOBSON, H. (2011) Comparison of oestrus detection methods in dairy cattle. Veterinary Record 169, 47
HOSMER, D. W. & LEMESHOW, S. (1980) Goodness of fit tests for the multiple logistic regression model. Communications in Statistics - Theory and Methods 9, 1043-1069
HUDSON, C. D., BRADLEY, A. J., BREEN, J. E. & GREEN, M. J. (2012a) Associations between udder health and reproductive performance in United Kingdom dairy cows. Journal of Dairy Science 95, 3683-3697
HUDSON, C. D., BRADLEY, A. J., BREEN, J. E. & GREEN, M. J. (2015) Dairy herd mastitis and reproduction: Using simulation to aid interpretation of results from discrete time survival analysis. The Veterinary Journal 204, 47-53
HUDSON, C. D., BREEN, J. E., BRADLEY, A. J. & GREEN, M. J. (2010) Fertility In UK Dairy Herds: Preliminary Findings Of A Large-Scale Study. Cattle Practice 18, 89-94
HUDSON, C. D., HUXLEY, J. N. & GREEN, M. J. (2014) Using Simulation to Interpret a Discrete Time Survival Model in a Complex Biological System: Fertility and Lameness in Dairy Cows. PloS One 9, e103426
HUDSON, C. D., KERBY, M., STATHAM, J. & WAPENAAR, W. (2012b) Managing Herd Reproduction. In Dairy Herd Health. Ed M. GREEN. Oxfordshire, UK, CABI
HULTGREN, J., MANSKE, T. & BERGSTEN, C. (2004) Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. Preventive Veterinary Medicine 62, 233-251
HUSZENICZA, G., JANOSI, S., KULCSAR, M., KORODI, P., REICZIGEL, J., KATAI, L., PETERS, A. R. & RENSIS, F. D. (2005) Effects of clinical mastitis on ovarian function in post-partum dairy cows. Reproduction in Domestic Animals 40, 199-204
INCHAISRI, C., JORRITSMA, R., VOS, P. L. A. M., WEIJDEN, G. C. V. D. & HOGEVEEN, H. (2010) Economic consequences of reproductive performance in dairy cattle. Theriogenology 74, 835-846
JAISWAL, R. S., SINGH, J., MARSHALL, L. & ADAMS, G. P. (2009) Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. Theriogenology 72, 81-90
JOUBERT, D. M. (1954) The influence of high and low nutritional planes on the oestrous cycle and conception rate of heifers. Journal of Agricultural Science 45, 164-172
KAFI, M., MIRZAEI, A., TAMADON, A. & SAEB, M. (2012) Factors affecting the occurrence of postpartum prolonged luteal activity in clinically healthy high-producing dairy cows. Theriogenology 77, 421-429
KAMATCHI, R., SHANMUGAM, A., KANDASAMY, K. & GOVINDARAJU, A. (2008) Ability of mice to detect estrous odor in bovine urine: roles of hormones and behavior in odor discrimination. Zoological Science 25, 349-354
KIMMINS, S. & MACLAREN, L. A. (2001) Oestrous Cycle and Pregnancy Effects on the Distribution of Oestrogen and Progesterone Receptors in Bovine Endometrium. Placenta 22, 742-748
LAMMING, G. E. & DARWASH, A. O. (1998) The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. Animal Reproduction Science 52, 175-190
LAMOTHE, P., BOUSQUET, D. & GUAY, P. (1977) Cyclic variation of F prostaglandins in the uterine fluids of the cow. Journal of Reproduction and Fertility 50, 381-382
LAVON, Y., EZRA, E., LEITNER, G. & WOLFENSON, D. (2011) Association of conception rate with pattern and level of somatic cell count elevation relative to time of insemination in dairy cows. Journal of Dairy Science 94, 4538-4545
LAVON, Y., LEITNER, G., VOET, H. & WOLFENSON, D. (2010) Naturally occurring mastitis effects on timing of ovulation, steroid and gonadotrophic hormone concentrations, and follicular and luteal growth in cows. Journal of Dairy Science 93, 911-921

LEACH, K. A., TISDALL, D. A., BELL, N. J., MAIN, D. C. J. & GREEN, L. E. (2012) The effects of early treatment for hindlimb lameness in dairy cows on four commercial UK farms. The Veterinary Journal 193, 626-632
LEBLANC, S. (2010) Assessing the association of the level of milk production with reproductive performance in dairy cattle. J Reprod Dev 56 Suppl, S1-7
LEBLANC, S. J., LISSEMORE, K. D., KELTON, D. F., DUFFIELD, T. F. & LESLIE, K. E. (2006) Major Advances in Disease Prevention in Dairy Cattle. Journal of Dairy Science 89, 1267-1279
LÖF, E., GUSTAFSSON, H. & EMANUELSON, U. (2012) Evaluation of two dairy herd reproductive performance indicators that are adjusted for voluntary waiting period. Acta Veterinaria Scandinavica 54, 5-5
LOPEZ-GATIUS, F., SANTOLARIA, P., MUNDET, I. & YANIZ, J. L. (2005) Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology 63, 1419-1429
LOPEZ, H., SATTER, L. D. & WILTBANK, M. C. (2004) Relationship between level of milk production and estrous behavior of lactating dairy cows. Animal Reproduction Science 81, 209-223
LUCEY, S., ROWLANDS, G. J. & RUSSELL, A. M. (1986) The association between lameness and fertility in dairy cows. The Veterinary record 118, 628-631
MANN, G. & LAMMING, G. (1995) Progesterone inhibition of the development of the luteolytic signal in cows. Journal of Reproduction and Fertility 104, 1-5
MANN, G. & LAMMING, G. (2001) Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 121, 175-180
MANN, G. E. & LAMMING, G. E. (2006) Timing of prostaglandin F2α release episodes and oxytocin receptor development during luteolysis in the cow. Animal Reproduction Science 93, 328-336
MARTIN, O., FRIGGENS, N. C., DUPONT, J., SALVETTI, P., FRERET, S., RAME, C., ELIS, S., GATIEN, J., DISENHAUS, C. & BLANC, F. (2013) Data-derived reference profiles with corepresentation of progesterone, estradiol, LH, and FSH dynamics during the bovine estrous cycle. Theriogenology 79, 331-343.e334
MATUKUMALLI, L. K., LAWLEY, C. T., SCHNABEL, R. D., TAYLOR, J. F., ALLAN, M. F., HEATON, M. P., O'CONNELL, J., MOORE, S. S., SMITH, T. P. L., SONSTEGARD, T. S. & VAN TASSELL, C. P. (2009) Development and Characterization of a High Density SNP Genotyping Assay for Cattle. PloS One 4, e5350
MCCAUGHEY, W. & COOPER, R. (1980) An assessment by progesterone assay of the accuracy of oestrus detection in dairy cows. Veterinary Record 107, 508-510
MCGUIRK, B. J., GOING, I. & GILMOUR, A. R. (1998) The genetic evaluation of beef sires used for crossing with dairy cows in the UK: 1. Sire breed and non-genetic effects on calving survey traits. Animal Science 66, 35-45
MCGUIRK, B. J., GOING, I. & GILMOUR, A. R. (1999) The genetic evaluation of UK Holstein Friesian sires for calving ease and related traits. Animal Science 68, 413-422
MEADOWS, C. (2005) Reproductive Record Analysis. Veterinary Clinics of North America: Food Animal Practice 21, 305-323
MEE, J. F. (2007) The role of the veterinarian in bovine fertility management on modern dairy farms. Theriogenology 68, Supplement 1, S257-S265
MELENDEZ, P., BARTOLOME, J., ARCHBALD, L. F. & DONOVAN, A. (2003) The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 59, 927-937
MILVAE, R. A., HINCKLEY, S. T. & CARLSON, J. C. (1996) Luteotropic and luteolytic mechanisms in the bovine corpus luteum. Theriogenology 45, 1327-1349
MOORE, D. A., CULLOR, J. S., BONDURANT, R. H. & SISCHO, W. M. (1991) Preliminary field evidence for the association of clinical mastitis with altered interestrus intervals in dairy cattle. Theriogenology 36, 257-265
MORGAN, G. L. & DAWSON, L. J. (2008) Development of Teaser Bulls Under Field Conditions. Veterinary Clinics of North America: Food Animal Practice 24, 443-453

MORRIS, M. J., KANEKO, K., UPPAL, S. K., S.L.WALKER, JONES, D. N., ROUTLY, J. E., SMITH, R. F. & DOBSON, H. (2013) Effects of high somatic cell counts in milk on reproductive hormones and oestrus behaviour in dairy cows with special reference to those with concurrent lameness. Animal Reproduction Science 141, 20-25
MORRIS, M. J., WALKER, S. L., JONES, D. N., ROUTLY, J. E., SMITH, R. F. & DOBSON, H. (2009) Influence of somatic cell count, body condition and lameness on follicular growth and ovulation in dairy cows. Theriogenology 71, 801-806
MORTON, J. M. & WYNN, P. C. (2010) Assessing ovulation detection performance in commercial dairy herds using progesterone concentrations from limited numbers of strategically collected milk samples. Journal of Dairy Science 93, 3019-3030
NEBEL, R. L. (1988) On-Farm Milk Progesterone Tests. Journal of Dairy Science 71, 1682-1690
NEBEL, R. L., WALKER, W. L., MCGILLIARD, M. L., ALLEN, C. H. & HECKMAN, G. S. (1994) Timing of Artificial Insemination of Dairy Cows: Fixed Time Once Daily Versus Morning and Afternoon. Journal of Dairy Science 77, 3185-3191
NORMAN, H. D., WRIGHT, J. R., HUBBARD, S. M., MILLER, R. H. & HUTCHISON, J. L. (2009) Reproductive status of Holstein and Jersey cows in the United States. Journal of Dairy Science 92, 3517-3528
NORTHEY, D. L. & FRENCH, L. R. (1980) Effect of Embryo Removal and Intrauterine Infusion of Embryonic Homogenates on the Lifespan of the Bovine Corpus Luteum. Journal of Animal Science 50, 298-302
OLDS, D. & SEATH, D. M. (1951) Repeatability of the Estrous Cycle Length in Dairy Cattle. Journal of Dairy Science 34, 626-632
OLTENACU, P. A., ROUNSAVILLE, T. R., MILLIGAN, R. A. & FOOTE, R. H. (1981) Systems Analysis for Designing Reproductive Management Programs to Increase Production and Profit in Dairy Herds. Journal of Dairy Science 64, 2096-2104
PALMER, M. A., OLMOS, G., BOYLE, L. A. & MEE, J. F. (2010) Estrus detection and estrus characteristics in housed and pastured Holstein-Friesian cows. Theriogenology 74, 255-264
PALMER, M. A., OLMOS, G., BOYLE, L. A. & MEE, J. F. (2012) A comparison of the estrous behavior of Holstein-Friesian cows when cubicle-housed and at pasture. Theriogenology 77, 382-388
PARR, R. A., DAVIS, I. F., MILES, M. A. & SQUIRES, T. J. (1993) Feed intake affects metabolic clearance rate of progesterone in sheep. Research in Veterinary Science 55, 306-310
PENNINGTON, J. A., ALBRIGHT, J. L., DIEKMAN, M. A. & CALLAHAN, C. J. (1985) Sexual Activity of Holstein Cows: Seasonal Effects1. Journal of Dairy Science 68, 3023-3030
PHILLIPS, C. J. C. & SCHOFIELD, S. A. (1988) A comparison of oestrus detection by pedometer, milk progesterone content, electrical conductivity of vaginal mucus and milk yield changes. Helsinki, Finnish Animal Breeding Associations
PHILLIPS, C. J. C. & SCHOFIELD, S. A. (1990) The effect of environment and stage of the oestrous cycle on the behaviour of dairy cows. Applied Animal Behaviour Science 27, 21-31
PINEDO, P. J., MELENDEZ, P., VILLAGOMEZ-CORTES, J. A. & RISCO, C. A. (2009) Effect of high somatic cell counts on reproductive performance of Chilean dairy cattle. Journal of Dairy Science 92, 1575-1580
PLAIZIER, J. C. B., KING, G. J., DEKKERS, J. C. M. & LISSEMORE, K. (1998) Modeling the relationship between reproductive performance and net-revenue in dairy herds. Agricultural Systems 56, 305-322
PLATZ, S., AHRENS, F., BENDEL, J., MEYER, H. H. D. & ERHARD, M. H. (2008) What Happens with Cow Behavior When Replacing Concrete Slatted Floor by Rubber Coating: A Case Study. Journal of Dairy Science 91, 999-1004
PRYCE, J. E., ROYAL, M. D., GARNSWORTHY, P. C. & MAO, I. L. (2004) Fertility in the high-producing dairy cow. Livestock Production Science 86, 125-135
PURSLEY, J. R., MEE, M. O. & WILTBANK, M. C. (1995) Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44, 915-923

R CORE TEAM (2013) R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing
RASBASH, J., CHARLTON, C., BROWNE, W. J., HEALY, M. & CAMERON, B. (2009) MLwiN Version 2.1. Centre for Multilevel Modelling, University of Bristol, Bristol, UK
REIMERS, T. J., SMITH, R. D. & NEWMAN, S. K. (1985) Management Factors Affecting Reproductive Performance of Dairy Cows in the Northeastern United States. Journal of Dairy Science 68, 963-972
RENSIS, F. D. & SCARAMUZZI, R. J. (2003) Heat stress and seasonal effects on reproduction in the dairy cow - a review. Theriogenology 60, 1139-1151
RICCI, A., CARVALHO, P. D., AMUNDSON, M. C. & FRICKE, P. M. (2017) Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination. Journal of Dairy Science 100, 9851-9860
ROBINSON, R., MANN, G., LAMMING, G. & WATHES, D. (2001) Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. Reproduction 122, 965-979
ROCKHILL, B., NEWMAN, B. & WEINBERG, C. (1998) Use and misuse of population attributable fractions. American Journal of Public Health 88, 15-19
ROELOFS, J., LOPEZ-GATIUS, F., HUNTER, R. H. F., EERDENBURG, F. J. C. M. V. & HANZEN, C. (2010) When is a cow in estrus? Clinical and practical aspects. Theriogenology 74, 327-344
ROELOFS, J. B., EERDENBURG, F. J. C. M. V., SOEDE, N. M. & KEMP, B. (2005a) Pedometer readings for estrous detection and as predictor for time of ovulation in dairy cattle. Theriogenology 64, 1690-1703
ROELOFS, J. B., SOEDE, N. M., VOSKAMP-HARKEMA, W. & KEMP, B. (2008) The effect of fenceline bull exposure on expression of oestrus in dairy cows. Animal Reproduction Science 108, 226-235
ROELOFS, J. B., VAN EERDENBURG, F. J. C. M., SOEDE, N. M. & KEMP, B. (2005b) Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. Theriogenology 63, 1366-1377
ROXSTRÖM, A., STRANDBERG, E., BERGLUND, B., EMANUELSON, U. & PHILIPSSON, J. (2001) Genetic and environmental correlations among female fertility traits, and between the ability to show oestrus and milk production in dairy cattle. Acta Agriculturae Scandinavica, Section A-Animal Science 51, 192-199
ROYAL, M. D., FLINT, A. P. F. & WOOLLIAMS, J. A. (2002) Genetic and Phenotypic Relationships Among Endocrine and Traditional Fertility Traits and Production Traits in Holstein-Friesian Dairy Cows. Journal of Dairy Science 85, 958-967
SAKAGUCHI, M., SASAMOTO, Y., SUZUKI, T., TAKAHASHI, Y. & YAMADA, Y. (2004) Postpartum Ovarian Follicular Dynamics and Estrous Activity in Lactating Dairy Cows. Journal of Dairy Science 87, 2114-2121
SANGSRITAVONG, S., COMBS, D. K., SARTORI, R., ARMENTANO, L. E. & WILTBANK, M. C. (2002) High Feed Intake Increases Liver Blood Flow and Metabolism of Progesterone and Estradiol-17β in Dairy Cattle. Journal of Dairy Science 85, 2831-2842
SANTOS, J. E. P., THATCHER, W. W., CHEBEL, R. C., CERRI, R. L. A. & GALVÃO, K. N. (2004) The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science 82-83, 513-535
SARTORI, R., HAUGHIAN, J. M., SHAVER, R. D., ROSA, G. J. M. & WILTBANK, M. C. (2004) Comparison of Ovarian Function and Circulating Steroids in Estrous Cycles of Holstein Heifers and Lactating Cows. Journal of Dairy Science 87, 905-920
SARTORI, R., SARTOR-BERGFELT, R., MERTENS, S. A., GUENTHER, J. N., PARRISH, J. J. & WILTBANK, M. C. (2002) Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. Journal of Dairy Science 85, 2803-2812
SAVIO, J. D., BOLAND, M. P. & ROCHE, J. F. (1990) Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. Journal of Reproduction and Fertility 88, 581-591

SCHALLY, A. V., ARIMURA, A., KASTIN, A. J., MATSUO, H., BABA, Y., REDDING, T., NAIR, R., DEBELJUK, L. & WHITE, W. (1971) Gonadotropin-releasing hormone: one polypeptide regulates secretion of luteinizing and follicle-stimulating hormones. Science 173, 1036-1038
SCHOFIELD, S. A., PHILLIPS, C. J. C. & OWENS, A. R. (1991) Variation in the milk production, activity rate and electrical impedance of cervical mucus over the oestrous period of dairy cows. Animal Reproduction Science 24, 231-248
SCHON, P. C., HAMEL, K., PUPPE, B., TUCHSCHERER, A., KANITZ, W. & MANTEUFFEL, G. (2007) Altered vocalization rate during the estrous cycle in dairy cattle. Journal of Dairy Science 90, 202-206
SCHRICK, F. N., HOCKETT, M. E., SAXTON, A. M., LEWIS, M. J., DOWLEN, H. H. & OLIVER, S. P. (2001) Influence of Subclinical Mastitis During Early Lactation on Reproductive Parameters. Journal of Dairy Science 84, 1407-1412
SHORT, R. V. (1984) Oestrous and menstrual cycles. In Reproduction in mammals 3: Hormonal control of reproduction (2nd Ed). Eds C. R. AUSTIN, R. V. SHORT. Cambridge, Cambridge University Press
SPRECHER, D., HOSTETLER, D. & KANEENE, J. (1997) A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47, 1179-1187
STATHAM, J. M. E. (2017) Sustainable use of hormones and reproductve management in practce. Cattle Practice 25, 113-114
SUNDERLAND, S., CROWE, M., BOLAND, M., ROCHE, J. & IRELAND, J. (1994) Selection, dominance and atresia of follicles during the oestrous cycle of heifers. Journal of Reproduction and Fertility 101, 547-555
SVEBERG, G., REFSDAL, A. O., ERHARD, H. W., KOMMISRUD, E., ALDRIN, M., TVETE, I. F., BUCKLEY, F., WALDMANN, A. & ROPSTAD, E. (2011) Behavior of lactating Holstein-Friesian cows during spontaneous cycles of estrus. Journal of Dairy Science 94, 1289-1301
TELEZHENKO, E., LIDFORS, L. & BERGSTEN, C. (2007) Dairy Cow Preferences for Soft or Hard Flooring when Standing or Walking. Journal of Dairy Science 90, 3716-3724
THATCHER, W. W., BILBY, T. R., BARTOLOME, J. A., SILVESTRE, F., STAPLES, C. R. & SANTOS, J. E. P. (2006) Strategies for improving fertility in the modern dairy cow. Theriogenology 65, 30-44
TOWNSON, D. H., TSANG, P. C., BUTLER, W. R., FRAJBLAT, M., GRIEL, L. C., JR., JOHNSON, C. J., MILVAE, R. A., NIKSIC, G. M. & PATE, J. L. (2002) Relationship of fertility to ovarian follicular waves before breeding in dairy cows. Journal of Animal Science 80, 1053-1058
TSAI, D.-M. & HUANG, C.-Y. (2014) A motion and image analysis method for automatic detection of estrus and mating behavior in cattle. Computers and Electronics in Agriculture 104, 25-31
TUCKER, C. B., WEARY, D. M., DE PASSILLÉ, A. M., CAMPBELL, B. & RUSHEN, J. (2006) Flooring in Front of the Feed Bunk Affects Feeding Behavior and Use of Freestalls by Dairy Cows. Journal of Dairy Science 89, 2065-2071
VAILES, L. D., WASHBURN, S. P. & BRITT, J. H. (1992) Effects of various steroid milieus or physiological states on sexual behavior of Holstein cows. Journal of Animal Science 70, 2094-2103
VAN ARENDONK, J. A. M. & LIINAMO, A.-E. (2003) Dairy cattle production in Europe. Theriogenology 59, 563-569
VAN EERDENBURG, F. J. C. M., LOEFFLER, H. S. H. & VAN VLIET, J. H. (1996) Detection of oestrus in dairy cows: A new approach to an old problem. Veterinary Quarterly 18, 52-54
VISHWANATH, R. (2003) Artificial insemination: the state of the art. Theriogenology 59, 571-584
VRIES, A. D. & CONLIN, B. J. (2003) Economic value of timely determination of unexpected decreases in detection of estrus using control charts. Journal of Dairy Science 86, 3516-3526
WALKER, S. L., SMITH, R. F., JONES, D. N., ROUTLY, J. E. & DOBSON, H. (2008a) Chronic stress, hormone profiles and estrus intensity in dairy cattle. Hormones and Behavior 53, 493-501
WALKER, S. L., SMITH, R. F., JONES, D. N., ROUTLY, J. E., MORRIS, M. J. & DOBSON, H. (2010) The effect of a chronic stressor, lameness, on detailed sexual behaviour and hormonal profiles in milk and plasma of dairy cattle. Reproduction in Domestic Animals 45, 109-117

WALKER, S. L., SMITH, R. F., ROUTLY, J. E., JONES, D. N., MORRIS, M. J. & DOBSON, H. (2008b) Lameness, Activity Time-Budgets, and Estrus Expression in Dairy Cattle. Journal of Dairy Science 91, 4552-4559
WALSH, S. W., WILLIAMS, E. J. & EVANS, A. C. O. (2011) A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science 123, 127-138
WATHES, D. C., FENWICK, M., CHENG, Z., BOURNE, N., LLEWELLYN, S., MORRIS, D. G., KENNY, D., MURPHY, J. & FITZPATRICK, R. (2007) Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68, S232-S241
WEAVER, L. D. (1986) Evaluation of reproductive performance in dairy herds. Comp. Count. Educa. Prac. Vet. 8, S247-S254
WERNER, G., CASIDA, L. & RUPEL, I. (1938) Estrus, ovulation, and artificial insemination in cattle. Journal of Animal Science 1938, 54-57
WIJMA, R., STANGAFERRO, M. L., KAMAT, M. M., VASUDEVAN, S., OTT, T. L. & GIORDANO, J. O. (2016) Embryo Mortality Around the Period of Maintenance of the Corpus Luteum Causes Alterations to the Ovarian Function of Lactating Dairy Cows. Biology of Reproduction 95, 112
WILLIAMSON, N. B. (1987) The interpretation of herd records and clinical findings for identifying and solving problems of infertility. The Compendium on continuing education for the practicing veterinarian 9, F14-F24
WILSON, S. J., MARION, R. S., SPAIN, J. N., SPIERS, D. E., KEISLER, D. H. & LUCY, M. C. (1998) Effects of Controlled Heat Stress on Ovarian Function of Dairy Cattle. 1. Lactating Cows1. Journal of Dairy Science 81, 2124-2131
WILTBANK, M., LOPEZ, H., SARTORI, R., SANGSRITAVONG, S. & GÜMEN, A. (2006) Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65, 17-29
WOLFENSON, D., INBAR, G., ROTH, Z., KAIM, M., BLOCH, A. & BRAW-TAL, R. (2004) Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. Theriogenology 62, 1042-1055
WOLFENSON, D., LEW, B. J., THATCHER, W. W., GRABER, Y. & MEIDAN, R. (1997) Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Animal Reproduction Science 47, 9-19
WOLFENSON, D., THATCHER, W. W., BADINGA, L., SAVIO, J. D., MEIDAN, R., LEW, B. J., BRAW- TAL, R. & BERMAN, A. (1995) Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biology of Reproduction 52, 1106-1113
WOLFERT, S., GE, L., VERDOUW, C. & BOGAARDT, M.-J. (2017) Big Data in Smart Farming – A review. Agricultural Systems 153, 69-80
WOOD, P. D. P. (1976) A note on detection of oestrus in cattle bred by artificial insemination, and the measurement of embryonic mortality. Animal Science 22, 275-278
YOUNAS, M., FUQUAY, J. W., SMITH, A. E. & MOORE, A. B. (1993) Estrous and Endocrine Responses of Lactating Holsteins to Forced Ventilation During Summer1. Journal of Dairy Science 76, 430-436