INTERPRETIVE SUMMARY

Variation in the inter-service intervals of UK dairy cows. Remnant

It is commonly accepted that domestic cattle have an average estrous cycle length of 21 days, with a normal range of 18 to 24 days. The cycle length is thought to be consistent within a given cow over time. This study revealed inter-service intervals to be longer than the expected 21 days in a large sample of UK dairy cows. Most of the apparent variation in cycle length was within a cow over time. The physiological mechanisms underlying this are yet to be elucidated.

INTER-SERVICE INTERVALS

Variation in the inter-service intervals of UK dairy cows

J. G. Remnant, M. J. Green, J. N. Huxley, C. D. Hudson

University of Nottingham School of Veterinary Medicine and Science, Sutton Bonington Campus, Sutton Bonington, Leicestershire LE12 5RD

Corresponding author:

John Remnant, University of Nottingham

School of Veterinary Medicine and Science, Sutton Bonington Campus, Sutton Bonington, Leicestershire LE12 5RD

Email: john.remnant@nottingham.ac.uk

http://dx.doi.org/ 10.3168/jds.2014-8366
ABSTRACT

An understanding of the normal estrous cycle length of the cow is important when managing and monitoring dairy herd fertility. Whilst the normal inter-ovulatory interval is widely considered to be 21 days, some studies have found alternative intervals to be more prevalent; previously most of the variation in interval length was expected to be between cows. The aim of this study was to assess the time between inseminations (inter-service interval, ISI), in a large number of dairy cows and to explore possible associations between cow factors and estrous cycle length. The study used ISI data from 42,252 cows in 159 herds across England and Wales. Univariate 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 estrus) following an insemination revealed a modal ISI of 22 days. Primiparous heifers had a modal ISI of 21 days. There were significant differences between the distribution of ISIs for different yield groups, parity numbers and the number of inseminations.

Multilevel regression modelling was used to evaluate the associations between cow factors and ISI, 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. Variance partition coefficients indicated that only 1% of variation in ISIs was at the herd level, 12% at the animal level and 87% at the insemination level, indicating that cycle length varies substantially more between cycles within a cow than between cows or herds. 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.

INTRODUCTION

Good reproductive performance is an essential part of any successful dairy enterprise, and heat detection is an important part of this in herds using artificial insemination. It is commonly accepted that the estrous cycle of domestic cattle (Bos taurus) is approximately 21 days long, with a normal range of between 18 and 24 days (Forde et al., 2011; Hartigan, 2004). A more accurate knowledge of normal cycle length may contribute to improved heat detection. It has been demonstrated that variation in estrous cycle length occurs primarily between cows rather than within cows (Olds and Seath, 1951). The number of follicular waves in a cow’s estrous cycle affects the inter-ovulatory interval (IOI) (Ginther et al., 1989) and the number of follicular waves in a cycle is also repeatable between cycles within a cow (Jaiswal et al., 2009). Some studies have shown improved fertility in cows following two wave cycles as opposed to three wave cycles (Townson et al., 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 heat detection (Hudson et al., 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), return to service submission rate (the proportion of cows re-inseminated 18 to 24 days after an unsuccessful insemination) and analysis of inter-service interval (ISI) profiles. 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 seasonally calving enterprises). As well as allowing useful monitoring of heat detection, awareness of the normal ISI can directly help improve heat detection by allowing more accurate prediction of the next heat. A reliable figure is also useful in research, for example for constructing simulation models of reproduction, with many authors using a fixed cycle length of 21 days in their models (Brun-Lafleur et al., 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 et al., 2007; Walsh et al., 2011). The effect of increased level of production in reducing the time and intensity of estrus expression has been well documented (Lopez et al., 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 et al., 2012). It is plausible that production may have an effect on ISI length.

The aim of this study was to assess the ISI in a large number of dairy cows, to explore the variability in estrous 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 estrous cycle length will allow identification of potential mechanisms regulating this process.
MATERIALS AND METHODS

Data Collection and Organisation

Herd management data were collected as part of a larger project (Hudson et al., 2012a; Hudson et al., 2010). The commonly used ISI based measures of estrus detection efficiency have been applied to this dataset in a separate study (Remnant et al., 2014). Anonymised herd databases were requested from twenty veterinary surgeons across England and Wales with an acknowledged interest in dairy herd health management data analysis. Data came from a variety of sources, including on-farm recording software, veterinary practice bureau-recording services and the records of national milk recording organisations. Although not a probabilistic sampling method, this convenience sample was considered appropriate as high quality data were essential for the analysis.

The initial data consisted of databases from 468 dairy herds. The datasets were converted to a standard format for restructuring and initial analysis. Data quality was assessed at the herd-year level over eight years, with only calendar years considered acceptable included for each herd. Measures of data quality included identification of herd datasets with random errors (such as calving events recorded without a corresponding insemination event) and systematic errors (such as under-recording of unsuccessful insemination events). Further detail is given in Hudson et al. (2012a). 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, 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).

**Descriptive Analysis**

A frequency distribution of ISIs up to 100 days was plotted. For initial univariate analysis, a subset of intervals between 15 and 30 days was used. 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 estrus following an insemination, without including subsequent cycles (occurring at extended intervals as a result of failed estrus detection or resynchronisation protocols). 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. Summary herd level statistics for herd-years included in the analysis are shown in Table 1. 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 univariate yield category plot. Differences between groups were tested with a Kruskal-Wallis rank sum test, with P-values of ≤0.05 considered significant.
Univariate data analysis was carried out in Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington) and R version 3.0.2 (R Core Team, 2013)

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:

\[ ISI_{ijk} = \beta_{0ijk} + \beta_1 x_{1ijk} \]

\[ \beta_{0ijk} = \beta_0 + v_{0k} + u_{0jk} + e_{0ijk} \]

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

\[ u_{0jk} \sim N(0, \sigma^2_{u0}) \]

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

where \( ISI_{ijk} \) is the \( i \)th ISI, for the \( j \)th cow in the \( k \)th herd. \( \beta_{0ijk} \) is the model intercept, comprised of \( \beta_0 \) the overall intercept, \( v_{0k} \) 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.
\( x_{ijk} \) represents the matrix of predictor variables for the \( i^{th} \) ISI in the \( j^{th} \) cow in the \( k^{th} \) herd, and \( \beta_k \) the corresponding matrix of coefficients. All potential predictor variables used in model building are shown in Table 2. 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 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.

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 identified using the diagnostic plots to assess their effect on the parameter estimates. An alternative model using a t-distribution outcome was also explored to reflect the apparently heavy-tailed distribution of the ISIs. This was compared to the initial (normal outcome) model to assess differences in 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 et al., 2013).

The main regression analysis was carried out using MLwiN version 2.10 (Rasbash et al., 2009) using iterative generalized least squares for parameter estimation, and estimation for
the alternate model with a t-distributed outcome was performed using Markov chain Monte
Carlo sampling in WinBUGS version 1.4 (Lunn et al., 2000).

RESULTS

Descriptive Analysis

Figure 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 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 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 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 2D shows the distribution of ISIs
by insemination number, the mode remained 22 days for all groups and there was a trend for
longer ISIs in later inseminations (p=0.023).

Statistical modelling

The variance partition coefficients 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 coefficients and their standard errors for the model are given in Table 3. Model fit was considered good following the assessments described above (Figure 3). There were no substantial changes in parameter estimates with outlying points removed or when modelling the outcome as a t-distribution: as a result, only the results from the conventional model are presented. 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 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 5.
DISCUSSION

In the current study most of the variation in ISI occurs at the individual ISI level within cows, which, along with the fact that the final model only explained 1% of the insemination level variance in the null model, 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 et al., 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 estrus (Diskin et al., 2011). This could also explain the trend for extended intervals in high milk-producing cows, with these cows expected to have a higher incidence of embryonic death (Sartori et al., 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 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 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 high milk-producing cows. 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 6.35% of second or subsequent estrus 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 9.92% late embryo mortality (defined as
elevated progesterone lasting for 19 days following insemination and then declining).

Interestingly, the 31.72% of cows exhibiting at least one atypical cycle in this study had significantly poorer fertility than those cows with normal cyclicity. 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 et al., 2008). Previous work has shown that the follicular wave pattern is repeatable for an individual cow (Jaiswal et al., 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 insemination events will represent true estrus events, and that not all true estrus events will result in a recorded insemination. In a dataset this size, this effect should only introduce random background “noise” with no systematic increase or decrease in ISI. This is supported by the presence of a period of increased frequency of re-inseminations (ISIs) around three weeks after a previous insemination (15-30 days, as shown in Figure 1). Based on physiology, these intervals would be expected to represent correctly identified estrus events. Using ISI as a proxy for IOI (as opposed to using insemination 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 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.

In this sample of UK dairy cows the modal ISI was longer than the expected “normal” (IOI) of 21 days (Forde et al., 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 et al., 2004; Sartori et al., 2004; Wolfenson et al., 2004). 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 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 et al., 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 et al. (2004) who found a shorter IOI in heifers compared to cows and suggests that findings from studies carried out on the estrous 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. In some instances 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 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 inseinations. The association between milk yield and ISI appears relatively small in magnitude once confounding factors are accounted for. Results from the multivariate regression analysis suggest that the apparent relationship illustrated in Figure 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 (15,000 kg) and low (2,500 kg) 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 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 et al., 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. 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.

**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. 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.
Figure 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 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).

Figure 3. Histogram 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 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 dashed lines show the 95% confidence interval for the prediction.

Figure 5. Predicted inter-service interval (ISI) by insemination number category (number of inseminations occurring within the same cow within a lactation, serve 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 1. Summary statistics for 1,275 herd-years from 159 herds included in the analysis of inter-service intervals (following data quality screening)

<table>
<thead>
<tr>
<th></th>
<th>305day milk Yield (kg)</th>
<th>Calving index (days)</th>
<th>Cull rate</th>
<th>Herd size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7437</td>
<td>415</td>
<td>25%</td>
<td>190</td>
</tr>
<tr>
<td>Median</td>
<td>7534</td>
<td>412</td>
<td>23%</td>
<td>167</td>
</tr>
<tr>
<td>Upper quartile</td>
<td>8344</td>
<td>427</td>
<td>30%</td>
<td>222</td>
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<tr>
<td>Lower Quartile</td>
<td>6735</td>
<td>399</td>
<td>17%</td>
<td>116</td>
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Table 2. Potential predictor variables used for building a multi-level regression model of inter-service interval (ISI)

<table>
<thead>
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<th>Variable</th>
<th>Variable type</th>
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<tr>
<td>305 day milk yield (x1000kg)</td>
<td>Continuous (centred around population mean)</td>
</tr>
<tr>
<td>Year in which the lactation began</td>
<td>Categorical (2000, 2001…2008)</td>
</tr>
<tr>
<td>Calendar month in which the ISI ends</td>
<td>Categorical (January, February…December)</td>
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<tr>
<td>Lactation number</td>
<td>Categorical (parity 1,2,3,4+)</td>
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<tr>
<td>Days in milk at the end of the ISI</td>
<td>Continuous</td>
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<tr>
<td>Number of inseminations in the lactation</td>
<td>Categorical (insemination number 2,3,4,5+)</td>
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<td>including the insemination ending the ISI</td>
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Table 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

<table>
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<tr>
<th>Model term</th>
<th>Coefficient</th>
<th>Standard error</th>
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<td>ISI</td>
<td>Outcome</td>
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<td>intercept</td>
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<td>0.087</td>
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<td></td>
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<tr>
<td>305 day milk yield ('000s kg)</td>
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<td>0.006</td>
</tr>
<tr>
<td>Year 2000</td>
<td>Reference</td>
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</tr>
<tr>
<td>Year 2001</td>
<td>0.088</td>
<td>0.065</td>
</tr>
<tr>
<td>Year 2002</td>
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<td>Month 7</td>
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Random effects:

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Remnant, Figure 1

![Graph showing frequency against ISI (days)]
Remnant, Figure 3
Remnant, Figure 4

![Graph showing predicted SI (days) over DIM (days). The graph displays two curves representing different models or conditions, with the predicted SI increasing up to a peak and then decreasing.]
Remnant, Figure 5

![Graph showing predicted ISI (days) for different service numbers. The x-axis represents service number (2 to 5), and the y-axis represents predicted ISI in days. The graph illustrates a decrease in predicted ISI as the service number increases.](image-url)
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