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1 **INTERPRETIVE SUMMARY**

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

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

9
10 **INTER-SERVICE INTERVALS**

11 **Variation in the inter-service intervals of UK dairy cows**

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

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

15 Corresponding author:

16 John Remnant, University of Nottingham

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

19 Email: john.remnant@nottingham.ac.uk

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21

22 Keywords:

23 Inter-service interval, Inter-ovulatory interval, Estrous cycle

24 **ABSTRACT**

25 An understanding of the normal estrous cycle length of the cow is important when managing
26 and monitoring dairy herd fertility. Whilst the normal inter-ovulatory interval is widely
27 considered to be 21 days, some studies have found alternative intervals to be more prevalent;
28 previously most of the variation in interval length was expected to be between cows. The
29 aim of this study was to assess the time between inseminations (inter-service interval, ISI), in
30 a large number of dairy cows and to explore possible associations between cow factors and
31 estrous cycle length. The study used ISI data from 42,252 cows in 159 herds across England
32 and Wales. Univariate analysis of the subset of 114,572 intervals between 15 and 30 days (a
33 range covering the increased frequency of ISIs occurring at the expected time of the first
34 return to estrus) following an insemination revealed a modal ISI of 22 days. Primiparous
35 heifers had a modal ISI of 21 days. There were significant differences between the
36 distribution of ISIs for different yield groups, parity numbers and the number of
37 inseminations.

38 Multilevel regression modelling was used to evaluate the associations between cow factors
39 and ISI, whilst accounting for clustering at the herd and cow level. This revealed significant
40 associations between predicted ISI and insemination number, days in milk, lactation 305 day
41 milk yield, and month and year of insemination. Variance partition coefficients indicated that
42 only 1% of variation in ISIs was at the herd level, 12% at the animal level and 87% at the
43 insemination level, indicating that cycle length varies substantially more between cycles
44 within a cow than between cows or herds. These findings suggest the “normal” range of ISI

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

47 **INTRODUCTION**

48 Good reproductive performance is an essential part of any successful dairy enterprise, and
49 heat detection is an important part of this in herds using artificial insemination. It is
50 commonly accepted that the estrous cycle of domestic cattle (*Bos taurus*) is approximately 21
51 days long, with a normal range of between 18 and 24 days (Forde et al., 2011; Hartigan,
52 2004). A more accurate knowledge of normal cycle length may contribute to improved heat
53 detection. It has been demonstrated that variation in estrous cycle length occurs primarily
54 between cows rather than within cows (Olds and Seath, 1951). The number of follicular
55 waves in a cow's estrous cycle affects the inter-ovulatory interval (IOI) (Ginther et al., 1989)
56 and the number of follicular waves in a cycle is also repeatable between cycles within a cow
57 (Jaiswal et al., 2009). Some studies have shown improved fertility in cows following two
58 wave cycles as opposed to three wave cycles (Townson et al., 2002). Explaining the
59 between-cow variation in IOI may uncover mechanisms to improve fertility.

60 The expected normal range of IOIs is used to calculate a variety of fertility parameters
61 employed by veterinarians, farmers and other professionals to monitor dairy herd heat
62 detection (Hudson et al., 2012b). These include first service submission rate (the proportion
63 of cows which are inseminated within 24 days of the end of the voluntary waiting period),
64 return to service submission rate (the proportion of cows re-inseminated 18 to 24 days after
65 an unsuccessful insemination) and analysis of inter-service interval (ISI) profiles. Expected
66 cycle length could also affect the interpretation of commonly used indices for monitoring
67 overall reproductive performance, such as the proportion of eligible cows becoming pregnant
68 every 21 days ("21 day pregnancy risk" or "fertility efficiency", common in year round

69 calving herds) or the proportion of cows pregnant within the first 21 or 42 days of the
70 breeding season (in seasonally calving enterprises). As well as allowing useful monitoring of
71 heat detection, awareness of the normal ISI can directly help improve heat detection by
72 allowing more accurate prediction of the next heat. A reliable figure is also useful in
73 research, for example for constructing simulation models of reproduction, with many authors
74 using a fixed cycle length of 21 days in their models (Brun-Lafleur et al., 2013).

75 Globally, milk yield has been increasing over time, and until recently dairy herd fertility had
76 been declining. Delayed return to normal ovarian cyclicity, reduced heat expression and poor
77 conception rates are commonly implicated in this trend (Dobson et al., 2007; Walsh et al.,
78 2011). The effect of increased level of production in reducing the time and intensity of estrus
79 expression has been well documented (Lopez et al., 2004); an association between increasing
80 milk yield and an increase in the incidence of abnormal ovarian cycles (particularly
81 prolonged luteal phases) has also been shown (Kafi et al., 2012). It is plausible that
82 production may have an effect on ISI length.

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

90 Data Collection and Organisation

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

100 The initial data consisted of databases from 468 dairy herds. The datasets were converted to
101 a standard format for restructuring and initial analysis. Data quality was assessed at the herd-
102 year level over eight years, with only calendar years considered acceptable included for each
103 herd. Measures of data quality included identification of herd datasets with random errors
104 (such as calving events recorded without a corresponding insemination event) and systematic
105 errors (such as under-recording of unsuccessful insemination events). Further detail is given
106 in Hudson et al. (2012a). The resulting data were from the years 2000 to 2008, originated
107 from 167 herds and included 449,471 inseminations from 67,926 cows. Mean 305 day milk
108 yield, calving index, culling rate and average herd size (estimated by multiplying the number
109 of calving events in a year by the calving index divided by 365) were calculated for each herd
110 for each calendar year.

111 The data were structured with an individual ISI (the number of days between subsequent
112 inseminations in the same cow, in the same lactation) as a line of data. For each interval, the

113 cow and herd identity were recorded, along with the 305-day adjusted milk yield, start
114 (calving) date and parity of the lactation in which the ISI occurred. The date, DIM and
115 insemination number of the insemination ending the interval were also recorded. Lactations
116 with milk yields outside the range 2,500 to 15,000 litres and ISIs ending at more than 365
117 DIM were excluded, as these were likely to represent outliers and recording errors.

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

120 **Descriptive Analysis**

121 A frequency distribution of ISIs up to 100 days was plotted. For initial univariate analysis, a
122 subset of intervals between 15 and 30 days was used. The initial distribution demonstrated a
123 clear peak at 15 to 30 days, and this is a range thought likely to contain the first return to
124 estrus following an insemination, without including subsequent cycles (occurring at extended
125 intervals as a result of failed estrus detection or resynchronisation protocols). Herds
126 contributing less than 100 ISIs within this range were excluded, leaving a sample consisting
127 of 114,573 ISIs from 42,252 cows in 159 herds. Summary herd level statistics for herd-years
128 included in the analysis are shown in Table 1. The distribution of ISIs within this sample was
129 assessed using a frequency plot. Bar charts were used to compare the distribution of ISIs
130 across different parities (grouped as 1, 2, 3 or 4+), insemination numbers (grouped as 2, 3, 4
131 or 5+ according to the number of the insemination ending the interval) and lactation 305-day
132 adjusted milk yield (grouped as <7,000 litres, 7,000 to 10,000 litres and \geq 10,000 litres, based
133 on the approximate bottom quartile (<7,021 litres), median half and top quartile (>9934 litres)
134 of all insemination-level 305 day lactation yields). First lactation heifers were excluded from
135 the univariate yield category plot. Differences between groups were tested with a Kruskal-
136 Wallis rank sum test, with P-values of \leq 0.05 considered significant.

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

139 **Statistical modelling**

140 A regression model to predict ISI was fitted using the subset of ISIs at 15 to 30 days (as
141 described earlier). A three-level random effects structure was used to account for potential
142 clustering of ISIs at the animal and herd level, with an individual interval as the lowest level
143 unit of data. The model was built by forward selection. Explanatory variables were added to
144 the model sequentially and coefficients and standard errors of coefficients estimated. For
145 continuous predictor variables, polynomial functions up to degree three were tested, as were
146 terms representing biologically plausible first-order interactions. Variables were retained in
147 the model where the estimated coefficient was greater than twice the standard error (such that
148 the 95% confidence interval for the estimate did not include zero); all rejected variables were
149 re-offered to the final model and retained if they now met these criteria. In the case of
150 categorical explanatory variables, all categories were retained in the model if one or more of
151 the categories met the criteria. The model took the conventional form:

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

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

$$v_{0k} \sim N(0, \sigma_{v_0}^2)$$

$$u_{0jk} \sim N(0, \sigma_{u_0}^2) \quad (3)$$

$$e_{0ijk} \sim N(0, \sigma_{e_0}^2)$$

152

153 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
154 of β_0 the overall intercept, v_{0k} the herd level residual for the k^{th} herd, u_{0jk} the cow level
155 residual for the j^{th} animal and e_{0ijk} the insemination level residual for the i^{th} insemination.

156 \mathbf{x}_{1ijk} represents the matrix of predictor variables for the i^{th} ISI in the j^{th} cow in the k^{th} herd, and
157 $\boldsymbol{\beta}_1$ the corresponding matrix of coefficients. All potential predictor variables used in model
158 building are shown in Table 2. To quantify the amount of variability in ISI at each level
159 (variation in ISI occurring between inseminations within the same cow; variation in ISI
160 occurring between cows within a herd; variation in average ISI between herds) variance
161 partition coefficients were calculated for each level of the model by dividing the variance of
162 the residuals at each level by the total variance. The final model was also compared to a null
163 model consisting only of herd level, animal level and insemination level random effects, to
164 calculate the percentage of the initial variance at each level which was explained by the
165 predictor variables.

166 In order to evaluate model fit, a histogram and a normal probability plot of the insemination
167 level residuals were generated to check for normality; the standardised insemination level
168 residuals were plotted against the ranked observed values to assess homoskedasticity; and the
169 predicted values were plotted against the observed values. Model parameters were re-
170 estimated following removal of outlying points identified using the diagnostic plots to assess
171 their effect on the parameter estimates. An alternative model using a t-distribution outcome
172 was also explored to reflect the apparently heavy-tailed distribution of the ISIs. This was
173 compared to the initial (normal outcome) model to assess differences in parameter estimates.

174 In order to illustrate model results, predictions were made for example scenarios, by fixing all
175 explanatory variables at their mean or reference category and then calculating the predicted
176 outcome across a range of values for a single explanatory variable at a time, with predictions
177 illustrated graphically (Archer et al., 2013).

178 The main regression analysis was carried out using MLwiN version 2.10 (Rasbash et al.,
179 2009) using iterative generalized least squares for parameter estimation, and estimation for

180 the alternate model with a t-distributed outcome was performed using Markov chain Monte
181 Carlo sampling in WinBUGS version 1.4 (Lunn et al., 2000).

182 **RESULTS**

183 **Descriptive Analysis**

184 Figure 1 shows the distribution of all the ISIs from 1 to 100 days: a clear peak in the
185 frequency of inseminations occurred at an interval of around three weeks with a smaller
186 increase around six weeks. The distribution of intervals by day within a 15 to 30 day window
187 is shown in Figure 2A; the modal ISI across the full dataset was 22 days. The accepted
188 normal range of 18 to 24 days encompassed 59% of ISIs in the 15 to 30 day window. The
189 central 90% of the ISIs fell within the range 18 to 28. Figure 2B shows the distribution of
190 intervals for different yield groups. The modal interval was 22 days for all yield groups,
191 however, there was a clear trend for longer ISIs in lactations with higher 305-day milk yield
192 ($p < 0.001$). Figure 2C shows the distribution for different parity groups. ISI appeared to
193 increase with parity ($p < 0.001$), all groups had a modal ISI of 22 days, with the exception of
194 first lactation heifers which had a mode of 21 days. Figure 2D shows the distribution of ISIs
195 by insemination number, the mode remained 22 days for all groups and there was a trend for
196 longer ISIs in later inseminations ($p = 0.023$).

197 **Statistical modelling**

198 The variance partition coefficients for the final model indicated that most of the unexplained
199 variation in ISI was at the individual insemination level. Only 1% of the variation of ISI was
200 from differences between herds, 12% was explained by differences in ISIs between animals
201 within a herd. The remaining 87% of variation was at the level of the individual insemination;
202 that is between ISIs within an animal. When comparing the final model to the null model the

203 explanatory variables included accounted for 18% of the null model herd level variance, 6%
204 of the animal level variance and 1% of the insemination level variance.

205 The coefficients and their standard errors for the model are given in Table 3. Model fit was
206 considered good following the assessments described above (Figure 3). There were no
207 substantial changes in parameter estimates with outlying points removed or when modelling
208 the outcome as a t-distribution: as a result, only the results from the conventional model are
209 presented. There was a positive association between ISI and increasing parity, with cows in
210 lactation number four or more predicted to have ISIs around half a day longer than first
211 lactation heifers. The positive association between milk yield and ISI observed in the
212 descriptive analysis was also demonstrated in the multivariable model although the
213 magnitude of the effect was very small, with a predicted increase of 0.024 days for every
214 1,000 litres of milk, when parity was accounted for. The ISI appeared to vary seasonally,
215 with a shorter ISI in the months June through to November, with predicted ISI around one
216 fifth of a day shorter in July, August and September when compared to January. There was
217 also an association with year; the ISI lengthened over the period the data were gathered, with
218 an increase in ISI of approximately 0.26 of a day in 2008 compared to the year 2000. There
219 was a quadratic relationship between DIM and ISI, with predicted ISI increasing with DIM
220 up to approximately 250 DIM and then decreasing slightly in later lactation. The predicted
221 ISI for the “average cow” increased from approximately 21 to over 23 days between 30 and
222 250 DIM; this association is illustrated in Figure 4. Another large effect size in the model
223 was the association between ISI and insemination number, with the predicted interval
224 preceding a fifth insemination or later in a lactation approximately 0.8 days shorter than the
225 interval between a first and second insemination; this effect is illustrated in Figure 5.

DISCUSSION

226
227 In the current study most of the variation in ISI occurs at the individual ISI level within cows,
228 which, along with the fact that the final model only explained 1% of the insemination level
229 variance in the null model, implies that a variable or variables not included in the model that
230 applies at the insemination level has a major impact on ISI. Previously it had been
231 documented that IOI is consistent within a cow, with most variation occurring between cows
232 (Jaiswal et al., 2009). Because the current study uses ISI as a proxy for IOI, embryonic death
233 is one possible explanation for this. Longer intervals may be a result of successful
234 conception and embryonic death delaying the second estrus (Diskin et al., 2011). This could
235 also explain the trend for extended intervals in high milk-producing cows, with these cows
236 expected to have a higher incidence of embryonic death (Sartori et al., 2002). The
237 distribution of all the ISIs between 15 and 30 days is slightly asymmetrical, with a slight
238 positive skew, which could possibly be caused by embryonic death (Figure 2A). However,
239 the shape of the distribution of ISIs from the high yield group is very similar to that of the
240 low yielding group (See Figure 2B), although centred on a higher interval. An increase in the
241 amount of embryonic death would be expected to increase the number of extended ISIs
242 (because of the extended intervals of those cows where embryonic death occurs) but would
243 not influence the number of shorter ISIs. Thus, if embryonic death accounted for the increase
244 in ISI with increased yield, the distribution of ISIs may be expected to be more right-skewed
245 in high milk-producing cows. There are possible explanations other than late embryonic
246 death for the variable and increased ISIs. Lamming and Darwash (1998) analysed
247 progesterone profiles of 1682 dairy cows and found 6.35% of second or subsequent estrus
248 events had a persistent corpus luteum (defined as a period of elevated progesterone lasting
249 more than 19 days), 12.9% had delayed ovulation (defined as periods of reduced
250 progesterone lasting more than twelve days) and 9.92% late embryo mortality (defined as

251 elevated progesterone lasting for 19 days following insemination and then declining).
252 Interestingly, the 31.72% of cows exhibiting at least one atypical cycle in this study had
253 significantly poorer fertility than those cows with normal cyclicity. Another possible
254 explanation for the variation in cycle length is changes to the follicular wave pattern of cattle:
255 three-wave cycles have been shown on average to be longer than two-wave cycles, but there
256 are contradictory reports as to which is more common (Adams et al., 2008). Previous work
257 has shown that the follicular wave pattern is repeatable for an individual cow (Jaiswal et al.,
258 2009). The unexplained variation in ISI within a cow indicates that cycle length (and
259 therefore potentially follicular wave number) may be less consistent than previously thought.
260 The findings in this study indicate that there is an unexplained and inherent variability of
261 cycle length for an individual cow which clearly warrants further studies to evaluate the
262 underlying physiological mechanisms.

263 In the current study it is likely that not all recorded insemination events will represent true
264 estrus events, and that not all true estrus events will result in a recorded insemination. In a
265 dataset this size, this effect should only introduce random background “noise” with no
266 systematic increase or decrease in ISI. This is supported by the presence of a period of
267 increased frequency of re-inseminations (ISIs) around three weeks after a previous
268 insemination (15-30 days, as shown in Figure 1). Based on physiology, these intervals would
269 be expected to represent correctly identified estrus events. Using ISI as a proxy for IOI (as
270 opposed to using insemination data as a proxy for ovulation date) also means that any
271 deliberate difference between ovulation and timing of service is likely to be applied
272 consistently to both inseminations bounding the interval. This means that the ISI should
273 correspond to the IOI even if the insemination time doesn’t coincide with ovulation.

274 In this sample of UK dairy cows the modal ISI was longer than the expected “normal” (IOI)
275 of 21 days (Forde et al., 2011; Hartigan, 2004). That this represents a true reflection of IOI is

276 further supported by the findings of a number of recent physiological studies revealing an IOI
277 of greater than 21 days (Bleach et al., 2004; Sartori et al., 2004; Wolfenson et al., 2004). The
278 current study indicates that the discrepancy between the average IOI observed in these
279 physiological studies on smaller numbers of animals and the commonly accepted average IOI
280 of 21 days is widespread among UK dairy cows. This brings in to question the continued use
281 of the 21 day “normal” interval particularly given that a similar finding from ISI data was
282 reported as long ago as the 1950s by Olds and Seath (1951) following analysis of records
283 from 278 cows on a research farm in Kentucky. Some of the early research on ovarian cycles
284 in cattle was conducted on nulliparous heifers (Hammond, 1927, cited in Chapman and
285 Casida, 1935; Joubert, 1954; Werner et al., 1938). In the current study, primiparous heifers
286 appear to have shorter intervals than higher parity cows and it is possible that this trend
287 would extend to nulliparous heifers. This is further supported by Sartori et al. (2004) who
288 found a shorter IOI in heifers compared to cows and suggests that findings from studies
289 carried out on the estrous cycle of heifers cannot be directly applied to later parity dairy
290 cows. Previous studies have found an effect of breed: Joubert (1954) reported a bimodal
291 distribution of IOI, attributing a second peak of IOI at 22 days to longer cycles of Friesian
292 cows; other studies have observed similarly longer cycles in Holstein-Friesian type animals
293 (Britt, 1995). With a trend away from “traditional” breeds towards Friesian and Holstein
294 genetics since the 1950s it seems possible that this may have resulted in IOIs being longer
295 than those demonstrated in older studies carried out on traditional breeds. Pragmatically, it is
296 also possible that 21 days has remained the accepted “normal” interval because a three week
297 cycle is easier to discuss than a three week and one day cycle. Whilst the difference between
298 the commonly accepted IOI of 21 days and the apparently more common interval of 22 days
299 is only one day, the normal range of 18 to 24 days appears inappropriate. A better estimation
300 of the normal range maybe the 18 to 28 day range incorporating 90% of the ISIs between 15

301 and 30 in this study. In some instances it may be appropriate to use a different “normal”
302 range for heifers than for multiparous cows.

303 As well as the strong association of ISI with parity, many other associations became apparent
304 in the current study. There was a relatively large negative association of insemination
305 number with ISI. DIM is already accounted for in the model and so this effect is separate to
306 any effect of increasing insemination numbers corresponding to increasing DIM. This is a
307 relatively large and consistent effect (illustrated in Figure 5) and yet is hard to explain
308 physiologically. This is an area that warrants further investigation, to establish the
309 mechanism that appears to be shortening the ISI of cows that have received multiple
310 inseminations. The association between milk yield and ISI appears relatively small in
311 magnitude once confounding factors are accounted for. Results from the multivariate
312 regression analysis suggest that the apparent relationship illustrated in Figure 2B was mostly
313 explained by the association with parity, despite primiparous animals being excluded from
314 this figure. Although lactation 305 day milk yield exhibited a small effect size there was a
315 much larger effect of DIM. The predicted difference in ISI between a high (15,000kg) and
316 low (2,500kg) yielding cow was 0.3 days, whereas the predicted ISI varied by around two
317 days over the observed range of DIM. As shown in Figure 4, ISI appears to vary throughout
318 lactation, gradually increasing up to approximately 250 days in milk. In the current study
319 only lactation yield data was available for the sample analysed and it has been demonstrated
320 that the impact of production on fertility is often related to the extent of negative energy
321 balance in early lactation and not the total milk produced (Wathes et al., 2007). DIM may
322 better represent any effect of milk yield at the time of the insemination than lactation 305 day
323 milk yield in this model; however the largest effect of DIM at 250 days does not coincide
324 with the expected peak in production. In the future, similar analyses using the nearest test
325 day milk yield to the insemination may represent this effect better. There was a small but

326 significant seasonal pattern of ISIs, with a trend for shorter intervals in the summer months.
327 There is also a trend for ISIs extending through time, with longer intervals found in lactations
328 starting in 2008 than those starting in 2000. This trend is harder to explain, but may represent
329 a longer term change to ISIs in dairy cows, perhaps related to selective breeding and changes
330 in genetics.

331 **CONCLUSIONS**

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

341

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

344 Figure 2. Bar charts showing the distribution of 114,573 inter-service intervals (ISIs) between
345 15 and 30 days within different sub-groups of 42,252 cows from 159 UK dairy herds: A,
346 shows all ISIs between 15 and 30 days; B, shows 305 day lactation milk yield groups, low
347 (<7,000 litres), medium (7,000 to 10,000 litres) and high yielding (>10,000 litres) in
348 multiparous cows; C, parity groups; D, grouped by insemination number (within a cow,
349 within a lactation).

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

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

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

360 Table 1. Summary statistics for 1,275 herd-years from 159 herds included in the analysis of
 361 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

362

363

364 Table 2. Potential predictor variables used for building a multi-level regression model of
 365 inter-service interval (ISI)

Variable	Variable type
305 day milk yield (x1000kg)	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+)

366

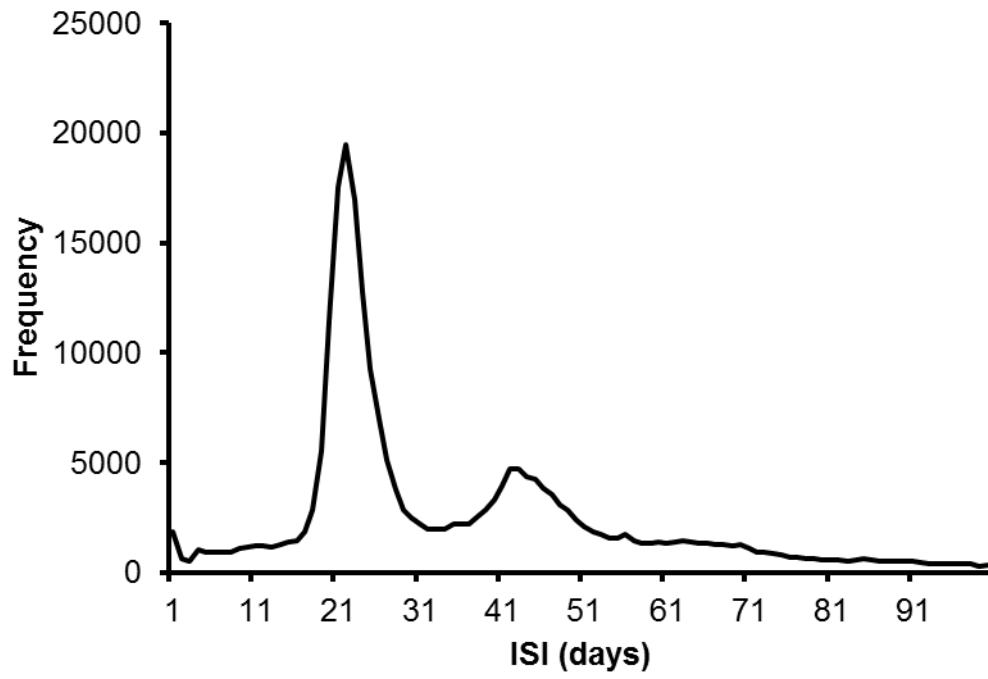
367

368 Table 3. Parameter estimates for a multilevel regression model predicting inter-service
 369 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 kg)	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

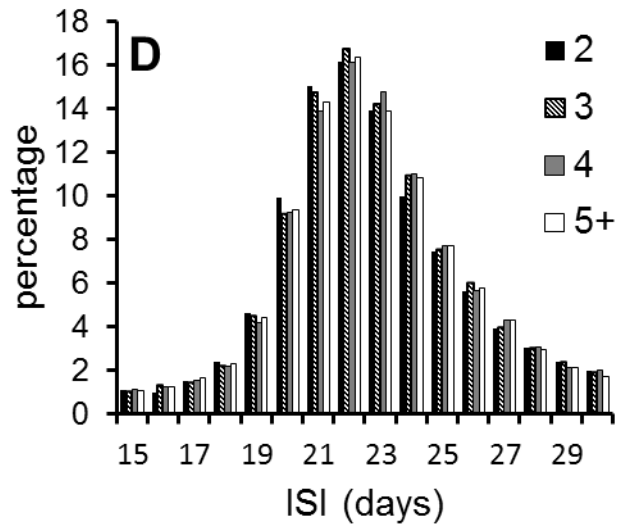
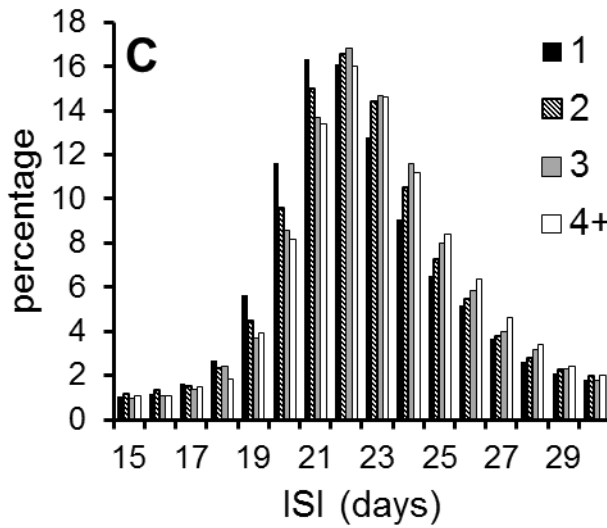
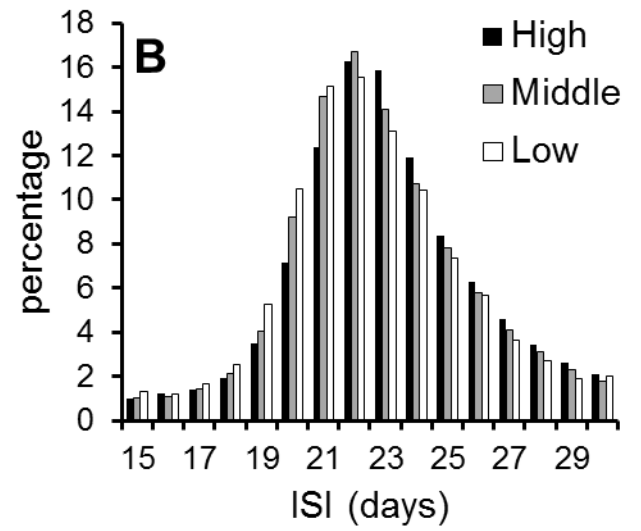
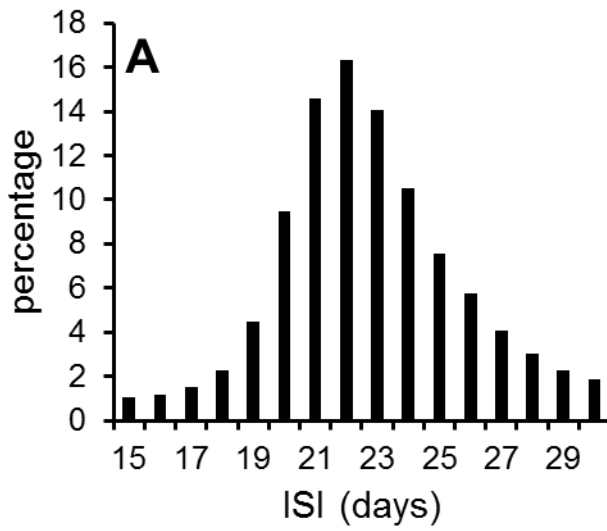
Month 9	-0.219	0.043
Month 10	-0.162	0.041
Month 11	-0.111	0.04
Month 12	0	0.038
Parity 1	Reference	
Parity 2	0.227	0.026
Parity 3	0.408	0.029
Parity 4	0.519	0.026
Days in Milk	0.019	0.001
Days in Milk ²	-3.7x10 ⁻⁰⁵	1.7x10 ⁻⁰⁶
Insemination number 2	Reference	
Insemination number 3	-0.334	0.025
Insemination 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

371 Remnant, Figure 1



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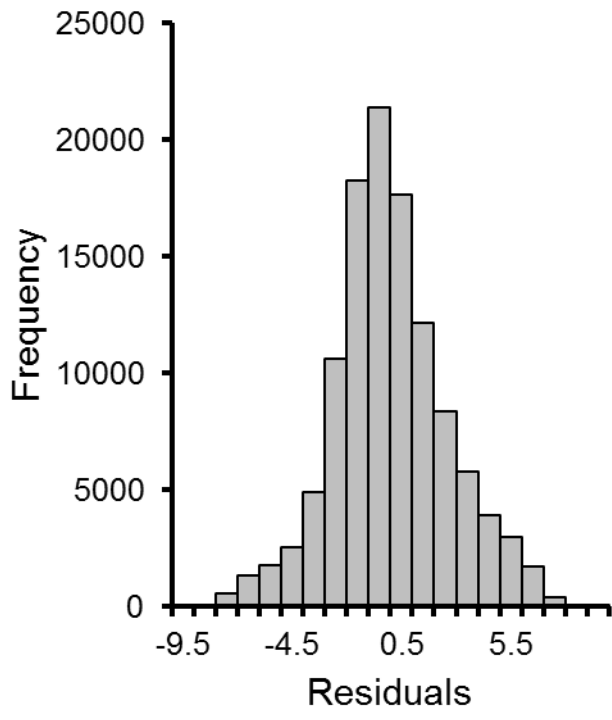


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377 Remnant, Figure 3

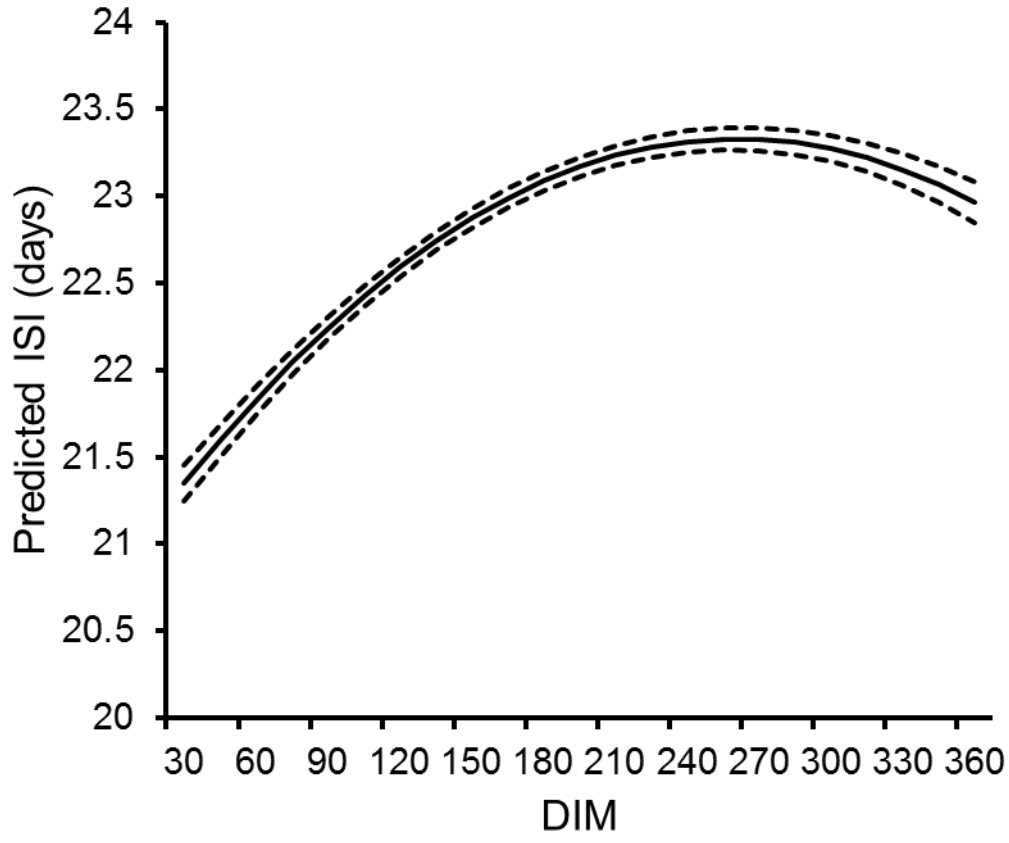
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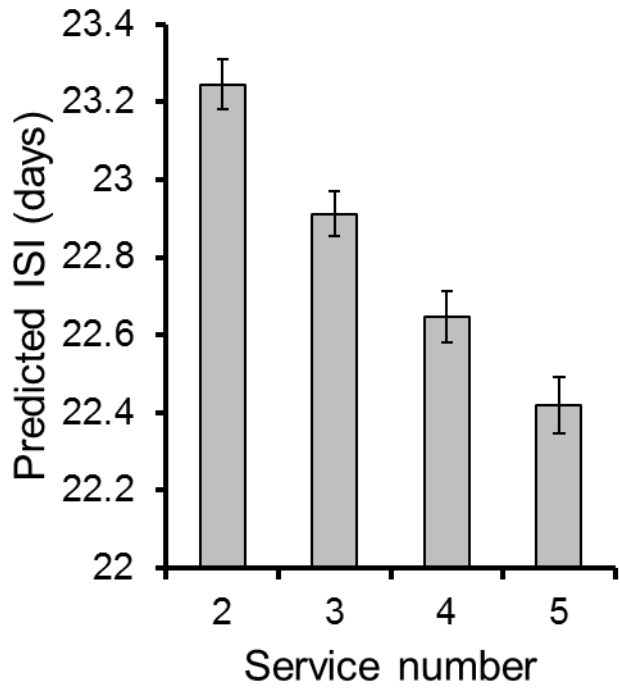
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380 Remnant, Figure 4

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