EARLY LACTATION MILK CONSTITUENTS AND FERTILITY

| 2 | Predicting the future reproductive potential of dairy cows Cook |
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| 3 | Economic success in dairy herds relies on obtaining pregnancies at an early stage of lactation. |
| 4 | In this study we were able to categorise and predict the reproductive performance of dairy |
| 5 | cows based on a limited number of data gathered from early lactation milk records. |
| 6 | Capability to predict future reproductive outcomes has the potential to assist dairy producers |
| 7 | with the planning and targeting of reproductive management strategies such as |
| 8 | pharmacological intervention with the possibility for less reliance on such methods and better |
| 9 | justification for their use when necessary. |
| 10 | Use of early lactation milk recording data to predict the calving to conception interval |
| 11 | in dairy herds |
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| 18 | ABSTRACT |

19 Economic success in dairy herds is heavily reliant on obtaining pregnancies at an early stage

- 20 of lactation. Our objective in this study was to attempt to predict the likelihood of conception
- 21 occurring by the 100th and 150th day of lactation (**DIM**) by Markov chain Monte Carlo
- 22 (MCMC) analysis using milk recording data and reproductive records gathered

23 retrospectively from 8750 cows from 33 dairy herds located in the United Kingdom. Overall 24 65% of cows recalved with 30%, 46% and 65% of cows conceived by 100 DIM, 150 DIM and beyond 150 DIM respectively. Overall conception rate (total cows pregnant/total cows 25 26 inseminated) was 27.47%. Median and mean calving to conception intervals were 123 d and 105 d respectively. The probability of conception by both 100 DIM and 150 DIM was 27 positively associated with week 4 milk production and protein percentage measured at 0-30 28 29 DIM and 31-60 DIM. Butterfat percentage was negatively associated with the probability of conception by 100 DIM but not at 150 DIM. Increased somatic cell count (SCC) up to 60 30 31 DIM was negatively associated with the probability of conception by 100 DIM whilst increased somatic cell count at 31-60 DIM was associated with a reduced probability of 32 conception by 150 DIM. Increasing parity was associated with a reduced odds of pregnancy. 33 34 Posterior predictions of the likelihood of conception for cows categorised as having 'good' or 'poor' early lactation attributes with actual observed values indicated model fit was good. 35 The likelihood of a 'good' cow conceiving by 100 and 150 DIM were 0.39 and 0.57 36 37 respectively (observed values 0.40 and 0.59). The corresponding values for a 'poor' cow were 0.28 and 0.42 (observed values 0.26 and 0.37). Predictions of the future reproductive 38 potential of cows may be possible using a limited number of early lactation attributes. 39

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INTRODUCTION

Dairy herd profitability is highly dependent on reproductive performance (Louca and
Legates, 1968; Oltenacu et al., 1981; Giordano et al., 2011; Giordano et al., 2012) probably
because optimising the time cows spend in the most efficient part of the lactation curve has a
significant impact on revenues obtained for milk sales (Ferguson and Galligan, 1999) as well
as minimising replacement costs due to reproductive failure. The timing at which pregnancy
occurs during lactation is of utmost importance in sustaining profitability (Giordano et al., 2011); ideally this should occur between 90 and 130 days of lactation (Giordano et al., 2011).

In this context the transition period has been recognised as a time when the dairy cow experiences an abrupt and severe change in demand for energy during the transition from the dry period to the onset of lactation (Baumann and Currie, 1980). At this time the cow's dry matter intake may be limited and so the demand for energy cannot be met by dry matter intake and most cows will experience a period of negative energy balance (**NEB**). The extent of NEB increases with energy output and increasing milk yield and has been linked to poor reproduction (Buckley et al., 2003; Patton et al., 2007).

Britt (1992) suggested that the biological environment in the relatively long period of 55 follicular growth prior to ovulation is a major factor contributing to the 'syndrome of sub 56 57 fertility' in dairy cows however to date the only long term demonstrable adverse effect on fertility is the effect of heat stress (Chebel et al., 2004). Leroy (2008a, 2008b) has reviewed 58 the mechanisms by which the biochemical environment of the cow and in particular the 59 60 metabolic changes associated with negative energy balance may influence fertility but many of these effects have been investigated only in the short term because in vitro studies 61 examining long term effects of metabolism are difficult to conduct (Leroy et al., 2008a). 62

Therefore, it would seem likely then that negative energy balance in early lactation may at 63 64 least in part be responsible for the creation of the biological environment that leads to sub fertility in dairy cows. Milk recording data from individual cows has been used in several 65 studies to associate milk composition in early lactation with energy balance (Duffield et al., 66 67 1997; De Vries and Veerkamp, 2000; Heuer et al., 2001) and more recently Madouasse et al. (2010) have associated milk components in early lactation with the probability of conception 68 69 at certain intervals during lactation. In particular Madouasse et al. identified an association between lower milk production on second test day, higher percentage of protein on second 70 71 test day and higher percentage of lactose on first test day with increased probability of 72 conception before 145 DIM whilst positive associations of a smaller magnitude were

identified for percentage of protein at first test day and negative associations with first testbutterfat and SCC on both first and second test days.

More recently Shahinfar et al. (2014) have used milk yield data amongst a potential 25 other explanatory variables including genetic information and health history to predict insemination outcome success using machine learning algorithms. This research also showed that the quality of data can have a significant effect on the accuracy of predictions particularly where recording of data is subjective traits such as in the recording of health traits.

Transition management success used early lactation milk yield data was also studied by
Lukas et al. (2015) however in this study transition management success was not linked to
future reproductive outcomes.

Data from individual cow milk recordings is easily measured and readily accessible to both 83 84 producers and consultants in the dairy industry globally and offers both a non-invasive and affordable method at both individual cow and herd level of assessing and monitoring energy 85 status in early lactation as well as future reproductive potential. Readily accessible 86 information that predicts an individual cow or herd's future reproductive performance would 87 be of use to producers in managing reproductive programs as it affords the opportunity to 88 89 alter management programs to potentially optimise the use of hormonal synchronisation protocols, insemination methods (e.g., the use of sexed semen) or environmental 90 91 management.

92 The objective of this research was to evaluate the usefulness of readily available production
93 data to predict reproductive performance of dairy cows. Of particular interest was the
94 difference between production parameters to predict early pregnancy (by day 100 of
95 lactation) or later pregnancy (by day 150 of lactation).

MATERIALS AND METHODS

97 Herd Selection and Reproductive Management

Herds were identified and selected to be included in this study if they had been referred to the
technical services department of Genus ABS (Nantwich, United Kingdom) for consultation
because of perceived poor reproductive performance reported in the period 1st September
2010-31st December 2010 and as such were part of the normal daily workload of that team.

Data were available for a total of 8750 cows located in 33 herds. Mean and median herd size was 228 and 265 cows respectively. Overall 65% of cows (5693) became pregnant and re calved. The percentage of cows that had conceived by 100 days, 150 days and beyond 150 days of lactation were 30%, 46% and 65% respectively. Overall conception rate (total cows becoming pregnant/total cows served) for cows in this study was 27.47%. Mean and median calving to conception interval was 123 and 105 days respectively.

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All herds practised all year round calving and were composed of Holstein Friesian cows. 110 Herds participated in a commercial reproductive management program for heat detection, 111 insemination and fertility data recording operated by Genus ABS (RMS[®], Genus ABS). 112 Briefly this service involved a daily visit to the farm premises by a trained technician using 113 the tail chalk method to identify cows in oestrus alongside more detailed manual examination 114 115 per rectum of the reproductive tract of cows showing changes in the distribution of the chalk 116 which are indicative but not conclusive of the animal expressing oestrus since the previous visit. All cows identified in oestrus were then artificially inseminated. Cows scheduled for 117 118 fixed time insemination (TAI) in accordance with veterinary protocols were also inseminated. The technician had responsibility to collect reproductive data on all cows 119

including dates of calving, oestrus, insemination and dates and outcomes of veterinary
examinations for pregnancy. In addition to reproductive data, date of dry off and the date
when a cow is designated as 'do not breed' and removed from the reproductive management
program as well as dates of cow deaths, disposals and exits were also recorded. Data
collected was then entered into Dairy Comp 305 (Valley Agricultural Software, Tulare,
USA).

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All inseminations were carried out by trans-cervical artificial insemination (AI) on a once daily basis at the time of the technicians' daily visit and all herds used a local veterinary practitioner to examine cows for pregnancy from 35 days from the last insemination. All herds undertook milk sampling through National Milk records or The Cattle Information Service on a monthly basis. Milk samples were collected at 2 consecutive milkings and all herds were milked twice daily.

133 Data Collation and Analysis

134 Records of individual cow milk composition for each herd were electronically downloaded and merged with reproductive management data in Dairy Comp 305. All data downloads 135 136 were carried out in January 2012 and data were extracted for all cows with calving dates from 1st September 2009 until 1st September 2010. This ensured that every cow included had 137 sufficient time to become pregnant for this analysis (>150 days). For each cow the unique 138 identity, date of calving, lactation number, milk weights recorded at 0-30 DIM and 31-60 139 DIM milk protein percentage at 0-30 DIM and 31-60 DIM, butterfat percentage at 0-30 DIM 140 141 and somatic cell count recorded at 0-30 DIM and 31-60 DIM were electronically extracted into a spreadsheet (Excel[®], Microsoft Corporation). These data were chosen because previous 142 studies indicated that they may be useful predictors of calving to conception interval in herds 143 144 of this management type (Madousse et al 2010). In addition to these data the average daily

milk weight produced by a cow during the fourth week of lactation (Week 4 Milk) was also
extracted. Week 4 milk is a parameter which can be calculated internally in Dairy Comp 305
and has been used anecdotally to assess performance in early lactation.

148 To allow the effect of parity and season of calving to be tested as confounding variables,

149 cows were categorised by parity and calving date into groups. Group 1 contained cows only

in first parity, group 2 contained cows only in second parity and group 3 containing cows in

third parity and above. Cows were also categorised by month of calving.

152 The outcomes of interest were conception by 100 days or by 150 days of lactation.

153 Conception by these times were chosen as the outcomes of interest because previous studies

have suggested that pregnancy status at 150 DIM is a composite trait that is relatively robust

to the heterogeneity of reproductive programs that occurs between farms (Caraviello et al.,

156 2006). These points in lactation also approximate to the economic optimum time during

157 lactation for a cow to become pregnant (Giordano et al., 2011) as well as allowing the

158 differentiation of covariates that may predict pregnancy at different stages of lactation.

159 Covariates tested in each model were parity group, month of calving, and milk weights at 0-

160 30 DIM and 31-60 DIM, week 4 milk production, percentage of protein at 0-30 DIM and 31-

161 60 DIM, somatic cell count at 0-30 DIM and 31-60 DIM and percentage of fat at 0-30 DIM.

162 Farm was included as a random effect to account for confounding of cows within farm.

Initial analysis consisted of descriptive statistics and graphical assessments.
Multilevel (random effects) models (Goldstein, 1995) were specified so that correlations
within the data (cows within farms) were accounted for appropriately. Model specifications
took the form;

167 Preg_{ij} ~ Bernoulli (probability = π_{ij})

168 Logit $(\pi_{ij}) = \alpha + \beta_1 X_{ij} + \beta_2 X_j + v_j$

169
$$V_i \sim N(0, \sigma^2_v)$$

170 where the subscripts i, and j denote the ith cow and the jth farm respectively, Preg_{ij} was the 171 outcome variable (pregnant or not) for cow i in herd j either by day 100 (Model 1) or by day 172 150 (Model 2), α the regression intercept, X_{ij} the matrix of covariates at cow level, β_1 the 173 coefficients for covariates X_{ij}, X_j the matrix of farm-month level covariates, β_2 the 174 coefficients for covariates X_j and v_j the random effect to reflect residual variation between 175 farms.

Initial covariate assessment was carried out using MLwiN (Centre for Multilevel 176 modelling, University of Bristol) (Browne, 2009) with penalised quasi-likelihood for 177 parameter estimation (Rasbash et al., 2005). Final models, with the same specification as that 178 above, were constructed in a Bayesian framework using MCMC for parameter estimation in 179 180 WinBUGS (Spiegelhalter et al., 2004), to avoid the potential biased estimates that can arise from quasi-likelihood methods with binary data (Browne and Draper, 2006). Final model 181 182 parameters were estimated using MCMC with a burn-in of at least 2,000 iterations during 183 which time model convergence had occurred. Parameter estimates were based on a minimum further 8,000 iterations. Vague prior distributions were used for β (Normal (0, 10⁶)) and σ^2_v 184 (Gamma (0.001, 0.001)) (Green et al., 2004). Investigation of model fit was made from plots 185 of cumulated fitted probabilities and residuals (Langford and Lewis, 1998; Green et al., 186 187 2004). To further assess model fit and predictive value, posterior predictive assessments were used (Gelman et al., 1996; Green et al., 2008) to evaluate the probabilities of pregnancy for 188 cows with different production characteristics. This allowed ready comparisons to be made 189 190 between cows with 'good' and 'poor' production attributes, as identified in the statistical models, and to place these into a clinical context. 191

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RESULTS

193 The final models are presented in Tables 1 and 2. Increased milk yield in the fourth week of lactation was associated with an increased odds of a cow becoming pregnant by day 100 and 194 150 of lactation. Protein percentage up to 60 days of lactation remained in both models with 195 196 increased protein percentage being associated with an increased odds of pregnancy. Fat percentage was associated with reduced odds of a cow becoming pregnant by day 100 but not 197 by day 150. Increased somatic cell count up to 60 days of lactation was associated with a 198 199 decreased odds of a cow becoming pregnant by day 100 of lactation and increased somatic cell count 31- 60 days of lactation was associated with a decreased odds of a cow becoming 200 201 pregnant by day 150 of lactation. In both models, increasing parity was associated with a reduced odds of pregnancy. 202

Posterior predictive assessments indicated model fit for both models was good. Posterior 203 predictions for the likelihood of pregnancy were made from both models using cows with 204 205 relatively good and poor production attributes. 'Good' cows were defined as those with a milk yield in week four >30Kg, protein percentage 0-30 days in milk >3.2% and protein 206 207 percentage 31-60 days in milk >3.2%. 1337 cows were classified as 'good'. 'Poor' cows were defined as those with a milk yield in week four <21Kg, protein percentage 0-30 days in 208 milk >3.0% and protein percentage 31-60 days in milk >3.0%. The posterior predicted 209 probability of a 'good' cow becoming pregnant by day 100 of lactation was 0.39 (95% 210 credible interval 0.35-0.43) and this compared to the observed value of 0.40. The posterior 211 predicted probability of a 'poor' cow becoming pregnant by day 100 of lactation was 0.28 212 (95% credible interval 0.23-0.33) and this compared to the observed value of 0.26. The 213 posterior predicted relative risk of pregnancy in a 'good' cow in relation to a 'poor' cow was 214 1.40 (95% credible interval 1.14-1.76). 215

The posterior predicted probability of a 'good' cow becoming pregnant by day 150 of

217 lactation was 0.57 (95% credible interval 0.54-0.61) and this compared to the observed value

of 0.59. The posterior predicted probability of a 'poor' cow becoming pregnant by day 150 of
lactation was 0.42 (95% credible interval 0.37-0.48) and this compared to the observed value
of 0.37. The posterior predicted relative risk of pregnancy in a 'good' cow in relation to a
'poor' cow was 1.36 (95% credible interval 1.18-1.59).

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DISCUSSION

223 Many studies have reported associations between milk yields, constituents and energy

balance, consistent with the idea that milk composition reflects energy status (Grieve et al.,

1986; Duffield et al., 1997; De Vries and Veerkamp, 2000; Heuer et al., 2001) and NEB has

been linked to poor reproduction (Buckley et al., 2003; Patton et al., 2007).

227 In this study we were able to use a limited number of early lactation production

characteristics to accurately assess and predict the risk of pregnancy occurring by 100 or 150

229 DIM. Clear and sizeable differences were also demonstrated in the risk of pregnancy

230 occurring at either 100 or 150 DIM between cows categorised as showing either 'good' or

231 'poor' early lactation production characteristics suggesting that these parameters may be of

use for incorporation into decision support models to allow early identification of a cow's
likely reproductive potential soon after calving and importantly, before the breeding period
begins. Management interventions such as feeding strategies, synchronisation protocols and
the use of sexed semen could then be targeted with better efficiency.

In contrast to some other studies (Shahinfar et al., 2014) this study sought to predict future reproductive outcomes from a limited number of early lactation production characteristics that are readily available on most dairies. Week 4 Milk in particular can be calculated from milk weights automatically collected at every milking from every cow via milk meters located in the milking parlour and as such is less subject to data quality issues. 241 More notably the findings of this study and in particular the large difference between 'good' and 'poor' cow's demonstrates that a cows reproductive potential may be determined some 242 weeks in advance of the breeding period before the active efforts by managers to obtain a 243 pregnancy begins and that the overall reproductive success of these herds is at least in part 244 determined by the proportion of cows falling into each category. Although outside the scope 245 of this study it is also interesting to speculate upon which factors may interact to determine 246 247 which category a cow falls into and over what time period that impact occurs. The 33 herds participating in this study all reported recent disappointing reproductive outcomes but it 248 249 seems highly unlikely that the same combinations of management factors were present on each dairy at a similar time. 250

In particular the degree and extent to which TAI was used in different herds and at different 251 times may have produced a bias in our findings as cows subjected to TAI may be more likely 252 253 to become pregnant earlier in lactation. However it is also likely that any bias would have been in favour of the 'poor' cows as they are perhaps less likely to have resumed normal 254 255 oestrus cyclicity post-partum and been inseminated to a natural oestrus (Santos et al., 2010). Pregnancy status at 150 DIM is also a composite trait that is relatively robust to the 256 heterogeneity to the extent of hormonal synchronisation and duration of the voluntary waiting 257 258 period (Caraviello et al., 2006). Giordano et al (2012) have also shown that the influence of the extent of hormonal synchronisation on overall herd reproductive performance as 259 measured by 21d pregnancy rate (Ferguson and Galligan, 1999) is minimal at the conception 260 rates achieved by the herds in this study unless 100% of inseminations are carried out to TAI. 261 262 Reductions in milk proteins are a consistent feature of cases of NEB. Milk protein is synthesised in the mammary gland, a process requiring energy and amino acids supplied via 263 the blood stream. Attempts to predict milk protein content from amino acid supply however 264 265 have been disappointing and it is now becoming clearer that the signalling pathways

266 responsible for milk protein synthesis are capable of responding to a variety of nutritional and hormonal signals such that activation of the pathways responsible for protein translation in 267 bovine mammary epithelial cells are capable of being modulated by signals other than simple 268 269 amino acid supply (Burgos et al., 2010). During the physiological change from the dry period 270 to the lactating state there are substantial changes to both the nutrient supply and hormonal environment and it is not unreasonable to suggest that at this time these factors become 271 272 limiting for the production of milk protein synthesis resulting in cows with poor transition demonstrating lower milk protein production in early lactation which may then allow for 273 274 early lactation milk proteins to be used as an indicator of both the nutrient and hormonal state of the cow as she entered lactation and her future reproductive potential. 275

The link between high milk production and poorer reproduction is equivocal with some 276 studies indicating a negative effect whilst others show no effect (Lucy, 2001). Uniquely in 277 278 this study week 4 milk production was used as an early indicator of production and a positive association was identified between week 4 milk and the probability of pregnancy at both 100 279 280 and 150 DIM. Lactose is the major sugar in milk composed of a single molecule of glucose and one of galactose. Synthesis takes place in the mammary gland from glucose supplied via 281 the blood stream and secretion of lactose by the mammary epithelial cell into milk has a 282 283 major impact on milk volume via its osmotic effect. Cows in good energy balance will be able to sustain better supplies of glucose to the mammary gland to drive lactose synthesis and 284 milk volume. 285

The changes in milk yield and constituents and their association with energy balance has also become a basis for on farm monitoring using indicators such as fat to protein ratio. In this study fat percentage at 0-30 DIM was negatively associated with the probability of pregnancy at 100 DIM but not at 150 DIM, a finding which suggests that the use of fat percentage in predicting energy balance may be limited and that early lactation changes in fat percentage 291 may be indicative of energy balance problems but that the impairment may be relatively mild and appears to be recoverable deeper into lactation whereas protein percentage in early 292 lactation shows a stronger and longer lasting association with the predicted probability of 293 294 pregnancy possibly indicative of a more serious and damaging impact on energy balance during transition. The findings of this study also indicate that fat to protein ratio may be 295 confounded as variations may be due to changes in fat percentage, protein percentage or both 296 297 and will be further affected as the normal variations in fat percentage observed throughout lactation and season are of greater magnitude than changes in protein percentage and will 298 299 have a greater influence on changes in the ratio observed.

300 Somatic cell count around the time of insemination has been shown to influence the probability of conception (Lavon et al., 2011) however in this study the somatic cell count in 301 early lactation was shown to have an influence on the probability of pregnancy much deeper 302 303 into lactation and there may be a number of explanations for this finding. Changes in cell count were not tracked into lactation in this study and it is possible that cows with high 304 305 somatic cell counts early in lactation are infected and retain their infected status into lactation 306 so that the cell count remains high around the time of insemination. Alternatively it may be that the elevated cell count observed in early lactation in these cows may be indicative of 307 308 excess body condition loss, more severe NEB and more severe metabolic issues in early lactation (Van Straten et al 2009) which predisposes them to udder inflammation. It is 309 interesting to note that in this study high somatic cell count at both 0-30 DIM and 31-60 DIM 310 had a negative effect on pregnancy outcome by 100 d and 150 d of lactation but only cell 311 count at 31-60 DIM had an influence on pregnancy outcome by 150 d, perhaps suggesting 312 that in cows with elevated cell counts only at 0-31 days the cell count indicates mild early 313 lactation body condition loss which recovers as lactation progresses whilst cows with 314

elevated cell counts at 31-60 days may be either cows with persistent mammary infections orcows that suffered a more severe body condition loss requiring a more prolonged recovery.

The final models also indicated that month of calving had no influence on the probability of 317 318 pregnancy occurring by 100 DIM but did have an influence at 150 DIM. This finding is difficult to explain but may be related to several factors. Firstly it has been shown that both 319 milk yield and constituents vary with season in dairy herds located in England and Wales 320 where the herds in this study were located (Madouasse et al 2010) and that feeding regimen is 321 known to influence butterfat (Baumann and Griinari, 2003). The grazing practices of the 322 herds in this study may have varied considerably and it is common for seasonal grazing and 323 324 pasture to form a part of the feeding management of dairy herds in England and Wales. This was not accounted for in the study design and may have contributed to this finding. All the 325 herds in this study utilized a program of tail chalking (RMS[®], Genus ABS) to identify cows 326 327 expressing heat and it is known that cows which remain metabolically stable around calving are likely to return to cycling behaviour and be detected in heat and inseminated well before 328 329 100 days of lactation (Santos et al., 2010). The reproductive behaviour of these cows is 330 predictable. In the UK it is not common practice for cows that fail to express heat and be inseminated by a certain days in milk to be enrolled to synchronisation programs to ensure a 331 332 timely first insemination (Higgins et al., 2013). Instead they are more commonly presented for veterinary examination by ultrasound when individual treatments would be applied to 333 each cow based on the ultrasonic appearance of the ovaries and uterus. It is likely that the 334 cows presented for such examination are cows that passed through transition in a relatively 335 poor metabolic state and so fail to return to normal cycling behaviour. The reproductive 336 behaviour of these cows will be less predictable, they will be less likely to be detected in heat 337 by the chalking program and perhaps more exposed to seasonal influences. In the UK it is 338 also common practice for lists of cows scheduled for veterinary examination to be heavily 339

edited by herdsman and also for routine veterinary visits to be rescheduled when there is a
conflict with staff holidays or other farm tasks considered of greater importance such as
silage making are taking place. Any or all of these circumstances could have been present at
differing times on the farms represented in this study.

The findings of this study should be interpreted with caution as the relatively small number of farms in this study were recruited because of perceived poor reproductive performance and as such may not be representative of all herds. Further work would be needed to clarify if these findings are truly representative of all herds.

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CONCLUSIONS

Milk protein percentage between 0-30 and 30-60 days in milk, week 4 milk yield, milk fat 350 percentage in the first 30 days of lactation, parity and somatic cell count at 0-30 days and 30-351 352 60 days of lactation were found to be significant predictors of the probability of pregnancy by 100 days of lactation. Of these only 0-30 day and 30-60 day milk protein percentage, week 4 353 milk, parity and somatic cell count along with month of calving were shown to be significant 354 predictors of pregnancy by 150 days of lactation. Selecting a limited number of these 355 parameters with the strongest associations with the outcomes of interest we were then able to 356 categorise cows and use posterior predictions to accurately predict the probabilities of the 357 occurrence of pregnancy. Sizeable and significant differences in reproductive potential were 358 shown between cows categorised as having 'good' or 'poor' production characteristics in 359 360 early lactation.

Table 1. Final multilevel logistic regression model with response variable pregnant by

100 days in milk

| Model | Coefficient | Odds Batio | Credible Interval | |
|----------------------|-------------|---------------|----------------------|-------|
| | | 1 | 2.5% | 97.5% |
| | | | | |
| Intercept | -2.87 | | | |
| Week 4 milk yield (k | Kg) | 1.02 | 1.01 | 1.02 |
| Protein % 0-30 DIM | | 1.35 | 1.30 | 1.39 |
| Protein % 31-60 DIN | 1 | 1.91 | 1.86 | 1.96 |
| Fat % 0-30 DIM | | 0.92 | 0.90 | 0.95 |
| Parity 1 Reference | | | | |
| category | | 0.57 | 0 - 51 | 0 = 1 |
| Parity 2 | | 0.67 | 0.61 | 0.74 |
| Parity 3+ | | 0.53 | 0.47 | 0.60 |
| Log SCC 0-30 DIM | _ | 0.92 | 0.91 | 0.93 |
| Log SCC 31-60 DIM | | 0.95 | 0.93 | 0.96 |
| Random effect; | 0.38 | | | |
| between farm variand | ce 0.50 | | | |
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Table 2. Final multilevel logistic regression model with response variable pregnant by

150 days in milk

| Model terms | Coefficient | Odds Ratio | Credible Interval | |
|----------------------------|-------------|---------------|----------------------|-------|
| | | | 2.5% | 97.5% |
| | | | | |
| Intercept | -2.95 | | | |
| Week 4 milk yield (Kg) | | 1.02 | 1.02 | 1.0 |
| Protein % 0-30 | DIM | 1.27 | 1.17 | 1.3 |
| Protein % 31-60 |) DIM | 2.17 | 2.05 | 2.3 |
| Parity 1 Referen | nce | | | |
| category | | | | |
| Parity 2 | | 0.68 | 0.60 | 0.7 |
| Parity 3+ | | 0.47 | 0.43 | 0.5 |
| Log SCC 31-60 | DIM | 0.92 | 0.89 | 0.9 |
| MONTH 1 | | 0.94 | 0.75 | 1.1 |
| MONTH 2 | | 0.87 | 0.70 | 1.0 |
| MONTH 3 | | 0.99 | 0.80 | 1.2 |
| MONTH 4 | | 0.84 | 0.68 | 1.0 |
| MONTH 5 | | 1.01 | 0.80 | 1.2 |
| MONTH 6 | | 1.05 | 0.82 | 1.3 |
| MONTH 7 | | 0.84 | 0.65 | 1.0 |
| MONTH 8 | | 0.76 | 0.61 | 0.9 |
| MONTH 9 | | 0.89 | 0.70 | 1.1 |
| MONTH 10 | | 0.99 | 0.80 | 1.2 |
| MONTH 11 | | 0.99 | 0.78 | 1.2 |
| MONTH 12 R | eference | | | |
| category Random effect; | 0.57 | | | |
| between farm V | analice | | | |

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