

1 **Original article**

2
3
4 **Impact of endometritis on post-partum ovarian cyclicity in dairy cows**

5
6
7 Z.A. Mohammed^{a,b}, G.E. Mann^a and R.S. Robinson^{c*},

8
9
10 ^a*School of Bioscience and* ^c*School of Veterinary Medicine and Science, University of*
11 *Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK.*

12 ^b*College of Veterinary Medicine, University of Duhok, Kurdistan Regional Government*
13 *and Scientific Research, Kurdistan, Iraq.*

14
15
16
17
18 *Corresponding author. Tel.: +00 44 (0)1159516452.

19 *e-mail address:* bob.robinson@nottingham.ac.uk (R. S Robinson)

20 **Abstract**

21 Endometritis in dairy cows is a major global issue and has been associated with a decrease in
22 reproductive performance. The aim of this study was to quantify the effect of endometritis (as defined
23 by the presence of any abnormal vaginal discharge after 21 days post-partum) on post-partum ovarian
24 cyclicity in dairy cows. Milk progesterone analysis was used to monitor reproductive cyclicity in 170
25 dairy cows across 3 different commercial herds. Associations between the occurrence of endometritis
26 and the incidence risk of a variety of atypical cycle profiles during the calving to conception period
27 were investigated to establish the importance of endometritis on post-partum ovarian activity.
28 Endometritis increased the incidence odds of atypical ovarian profiles ($P<0.05$) with prolonged luteal
29 activity being the most affected ($P<0.05$) but also showed prolonged time (3 days) to onset of luteal
30 activity after parturition ($P<0.05$). In conclusion, using milk progesterone analysis, we found a
31 relatively low incidence odds for reproductive cycle problems in normal cows during the calving to
32 conception period. However, the incidence odds of cycle problems, in particular prolonged luteal
33 activity, were high in cows that had experienced endometritis, which would have significantly
34 impaired reproductive function.

35

36 *Keywords:* Dairy cows; Endometritis; Milk progesterone; Ovarian cyclicity

37

38

39

40 **Introduction**

41 To achieve a calving interval of 365 days, cows should resume their ovarian cycles within 25
42 days post calving and conceive within 85 days post-partum. Endometritis is the principle infectious
43 disease of the uterus at this time and is caused by persistent bacterial infection after calving ([Barlund
44 et al., 2008](#); [Gilbert et al., 2005](#); [Runciman et al., 2008](#); [Sheldon et al., 2009](#); [Sheldon et al., 2006](#)).
45 Often, the real impact of endometritis is only measured by subsequent analysis and, while the
46 economic impact of this disease remains to be fully quantified, it is speculated to cost many millions
47 of pounds annually for the UK dairy industry alone ([McNaughton and Murray, 2009](#)).

48 The mechanism by which uterine infection with bacterial endotoxin during the post-partum
49 period adversely affects fertility is likely to be multi-factorial influencing fertility both locally and
50 systematically. There is a disruption in the production of prostaglandin (PG) $F_{2\alpha}$ and PGE_2 by the
51 endometrium ([Herath et al., 2009](#)). At the same time, there is an alteration to immune system
52 mediators and production of cytokines, which can affect the function of the hypothalamus and
53 pituitary gland ([Sheldon et al., 2009](#)). An important observation was that uterine infection adversely
54 affected follicular growth and function ([Sheldon et al., 2002](#)) with the bacterial endotoxin
55 lipopolysaccharide (LPS) detected in follicular fluid.

56 An association between clinical signs of uterine inflammation and post-partum ovarian
57 cyclicity has been reported previously ([Mateus et al., 2003](#); [McCoy et al., 2006](#); [Opsomer et al., 2000](#);
58 [Shrestha et al., 2004](#); [Taylor et al., 2003](#); [Williams et al., 2007](#)). Milk progesterone monitoring has
59 been used widely for assessment of ovarian function, reproductive patterns and abnormalities in dairy
60 cows ([Bulman and Lamming, 1978](#); [Bulman and Lamming, 1979](#); [King et al., 1976](#)). One abnormality
61 identified by progesterone profiling is prolonged luteal activity. Abnormal ovarian profiles mainly
62 prolonged luteal activity have been associated with an abnormal uterine environment which can
63 perturb the production of prostaglandins ([Opsomer et al., 1998](#)). The risk factors for prolonged luteal
64 activity have included metritis, abnormal vaginal discharge and retained fetal membrane ([Opsomer
65 et al. 1998](#), [Opsomer et al. 2000](#)). This supports the concept that uterine inflammatory/infection

66 problems could lead to prolonged luteal activity post-partum as well as follicular dysfunctions such
67 as smaller follicles and reduced oestradiol secretions. However, the sequelae of cows having
68 endometritis on the distribution of the different atypical ovarian profiles (i.e. cessation of cyclicity,
69 delayed ovulation or prolonged luteal activity) is less clear and whether particular atypical ovarian
70 profiles are affected. Thus, the objective of this study was to quantify the incidence risk of different
71 atypical ovarian profiles in dairy cows experiencing post-partum. This was then further expanded to
72 compare the influence of different grades of endometritis on ovarian cyclicity.

73

74 **Materials and methods**

75 *Experimental animals*

76 The University of Nottingham Ethical Review Committee approved the study, which was
77 carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 under
78 Project Licence PPL 40/1621. A total 170 multiparous Holstein dairy cows were studied across three
79 commercial dairy units in the East Midlands, UK (Table 1). In herd 1, cows were housed all year
80 round and fed a total mixed ration based on both grass and maize silage. In herds 2 and 3, cows were
81 housed during the winter months and fed a total mixed ration based on both grass and maize silage.
82 During the summer month (May – September) cows were allowed access to grazing of grass pasture
83 with diet supplemented with a total mixed ration based on both grass and maize silage. In all systems,
84 cows were fed additional concentrates at milking according to yield.

85 In herd 1, cows were milked by robot (Lely Astronaut A3 AMS) attending the robot an
86 average of 2.5 times per day. In herds 2 and 3, cows were milked twice daily in a traditional
87 herringbone parlour. The cows recruited to the study, calved between July and April and were housed
88 and managed according to that particular commercial farm practice with no study-related treatment
89 interventions. All cows with reproductive disorders were identified by farm staff or at routine
90 veterinary visits were treated according to that farm's practice or the clinician's opinion with no
91 experimental interventions. All treatments were recorded in the herd's records by the farm staff. Cows

92 were inseminated at observed oestrus by trained AI technicians, after a 7-8 week voluntary waiting
93 period.

94

95 *Clinical investigation*

96 In all three groups, cows were checked for uterine disease through inspection of vaginal discharge
97 using a gloved hand and/or vaginal speculum. Examinations commenced at two weeks of calving
98 and were then carried out at weekly intervals until 45 days post-partum.

99 The extent and smell of vaginal discharge was scored according to [Sheldon \(2004\)](#) as:

100 VDS0: Clear or translucent mucus (normal cows)

101 VDS1: Mucus containing flecks of white or off white pus

102 VDS2: Exudates containing $\geq 50\%$ purulent material, usually white or yellow

103 Endometritis was then more precisely defined by the degree of the abnormal vaginal discharge after
104 21 days post-partum (VDS1 or VDS2) with normal cows showing no detectable discharge (VDS0).

105 VDS1 and VDS2 were categorised based on the greatest vaginal discharge that was observed in the
106 study window.

107

108 *Milk sampling*

109 Representative whole milk samples were collected for progesterone analysis two to three
110 times per week starting as soon as a particular cow was two weeks after calving and continued until
111 15 weeks post-partum or until pregnancy was confirmed by rectal palpation or ultrasonography. Milk
112 was collected in plastic tubes contain broad-spectrum preservative (Microtabs[®]II containing
113 Bronopol and Natamycin; D&F Control Systems, Inc.). The concentration of progesterone in milk
114 was determined using a competitive enzyme-linked immunosorbent assay supplied as a commercially
115 available kit (Ridgeway Science Ltd). The standards (10 μ l, 0-50ng/ml) or sample (10 μ l) were added
116 to the appropriate wells and analyzed in duplicate. Next, the progesterone-alkaline phosphatase
117 conjugate (200 μ l) was added to all wells except for the blank wells. The plate was incubated at room

118 temperature for 2 hours after which it was washed 3 times in PBS. Next, 200 μ l of alkaline
119 phosphatase substrate was added to each well and the plate left for 1 hour. The absorbance was then
120 measured at 584nm using a microplate reader (Optima). A standard curve (4-parameter logistic
121 regression) was fitted from which the unknown were interpolated and quantified. The reliable reading
122 range was from 1.0 to 10ng/ml. All samples reading below or above this range were taken to be 1.0
123 (follicular phase or anoestrus) or 10ng/ml (luteal phase), respectively. All samples with a coefficient
124 of variation >15% were repeated. The intra and inter-assay coefficients of variation were 6.3 and
125 7.7%, respectively.

126

127 *Analysis of ovarian profiles*

128 Onset of luteal activity (OLA) was defined as the first day that milk progesterone
129 concentrations were ≥ 3 ng/ml for two successive measurements that was >10 days after calving
130 (Garmo et al., 2009; Mann et al., 2005). Post-partum ovarian profiles were defined according to
131 parameters based on Mann et al. (2005) and allocated to one of five categories:

- 132 1. Typical: First rise in progesterone occurred ≤ 45 days post-partum and was followed by normal
133 cyclicity (a short luteal phase <10 days after the first ovulation was considered typical).
- 134 2. Delayed first ovulation: The first rise in progesterone (>3ng/ml) occurred more than 45 days
135 after calving.
- 136 3. Cessation of cyclicity: The first progesterone rise occurred within the normal period (≤ 45
137 days) but was followed by a period of progesterone concentrations <3ng/ml for >12 days.
- 138 4. Prolonged luteal activity. Progesterone concentration >3ng/ml for >21 days in the absence of
139 AI
- 140 5. Short luteal phase. Progesterone concentration >3ng/ml for less than 10 days (excluding the
141 first oestrous cycle).

142 Examples of the various types of ovarian profiles detected during the post-partum period, based on
143 milk progesterone analysis, are shown in Fig.1. Some cows exhibited more than one type of atypical

144 ovarian profile and in this case, the cow was categorised according to the first atypical ovarian profile
145 that was exhibited only.

146

147 *Statistical analysis*

148 All statistical analyses were performed using GenStat (17th Edition, Hemel Hempstead, UK).
149 Logistic regression was used to compare the incidence risk of typical and atypical ovarian profiles
150 from calving to conception between “normal” and “cows that experienced endometritis” as well as
151 comparing the incidence risk during the same time period of typical and atypical ovarian profiles
152 between cows with different vaginal discharge scores (VDS) of 0, 1 and 2. The data are presented as
153 odds ratio (OR) with 95% confidence intervals. As this study combined data from three different
154 herds, herd was included as a fixed factor in all models. The P-values were adjusted using
155 Bonferroni’s correction for multiple-testing. In addition, binominal logistic regression was used to
156 determine odds ratio and the effect of risk factors (endometritis and/or normality of ovarian cycles)
157 on the 1st service conception rate in those animals (n=153) that were served. Survival analysis
158 (Kaplan-Meier, log-rank) was performed to compare the time of the onset of luteal activity between
159 the cows that experienced endometritis and normal cows.

160 The sample sizes were based on an increased distribution of atypical ovarian profiles from
161 10% to 40% for cows without and with endometritis, respectively, at a significance level of 5% with
162 90% power required 17 animals per group. While for conception rates, a 20% percentage point
163 decrease from the comparator normal cows (40% conception rate) with a 90% power, at 5%
164 significance level required 56 animals per group.

165

166 **Results**

167 The incidence risk of endometritis before 45 DIM did not differ between the three herds (Table
168 1). Similarly, the distribution of atypical ovarian profiles in the calving to conception interval were
169 equivalent across the three herds (Table 1). Logistic regression revealed cows that had endometritis

170 had an increased odds of atypical ovarian profiles (OR=2.38 [95% CI: 1.30-4.35]; $P<0.05$; Table 2;
171 Fig 2A). Specifically, 75% cows that experienced endometritis had atypical ovarian profiles
172 compared with 25% in normal cows. Additionally, cows that exhibited endometritis had increased
173 odds of prolonged luteal activity compared to normal cows (OR=4.60 [95% CI: 1.53-13.84]; $P<0.05$;
174 Table 2; Fig 2A). However, the odds of cessation of cyclicity, short luteal phase, and delayed 1st
175 ovulation events were not different between cows with endometritis and normal cows (Table 2; Fig
176 2A).

177 A similar profile was seen when the endometritic cows were analysed on the basis of vaginal
178 discharge score. Abnormal vaginal discharge score (VDS) of 2 but not VDS1 increased the odds of
179 atypical ovarian profiles (OR=2.44 [95% CI: 1.29-4.61]; $P<0.05$; Table 3; Fig 2A) which numerically
180 equated to 81% of cows having atypical ovarian profiles. Additionally, there was a significantly
181 increased odds of prolonged luteal activity in cows with VDS2 (OR=4.66 [95% CI: 1.48-14.6];
182 $P<0.05$) compared to cows with no vaginal discharge (Table 3, Fig 2). Incidence of prolonged luteal
183 activity in cows with VDS1 (OR=4.46 [95% CI: 1.12-17.83]) was in between that for VDS0 and
184 VDS2. However, neither VDS1 nor VDS2 had any significant effect on the odds of having delayed
185 first ovulation, cessation of cyclicity or short luteal phase events ($P>0.05$, Fig 2). There was no
186 statistical significant differences between the VDS1 or VDS2 groups with respect to risk of atypical
187 ovarian profiles or any specific problems during the calving to conception interval (Table 3, Fig 2).

188 An altered interval from calving to onset of luteal activity was associated with endometritis
189 status. Namely, normal cows resuming cyclicity post-partum earlier (median time, 25 days) compared
190 to cows that experienced endometritis (median time 28 days, $P<0.05$; Fig.3).

191 The odds of conceiving at 1st service between normal cows and those that experienced
192 endometritis and whether they had typical or atypical ovarian profiles are shown in Table 4. It should
193 be recognised that the number of animals in each group is insufficient to draw definite conclusions
194 and should be interpreted with caution. There was no difference in the odds for conception at 1st
195 service between normal cows and cows with endometritis ($P>0.05$, Table 4). When the data was

196 further categorized in those with typical or atypical ovarian profiles, there was no significant effect
197 of endometritis on conception rate ($P>0.05$). However, the odds of conception at 1st AI tended to be
198 lower in cows with atypical ovarian profiles compared to those with typical ovarian profiles ($P=0.08$;
199 Table 4).

200

201 **Discussion**

202 The objective of this study was to quantify the incidence odds of atypical ovarian profiles in
203 dairy cows experiencing post-partum endometritis compared with cows that did not. In the present
204 study, some cows had more than one atypical ovarian profile but only the first presentation was
205 included in this study. This could have created a bias in the reported incidence risk of the different
206 type of atypical ovarian profiles. For example, delayed 1st ovulation is the first atypical event that can
207 occur post-partum while prolonged luteal activity only occurs once cyclicity is resumed. That said,
208 prolonged luteal activity was the profile that was associated with cows experiencing endometritis and
209 thus this potential bias does not appear to have affected these results. Equally, it is important to
210 consider that while the normal cows had no visual signs of purulent vaginal discharge and hence
211 endometritis, this does not mean that there was no subclinical signs of endometritis in these animals.
212 The diagnosis of endometritis by the presence and appearance of vaginal discharge can lead to an
213 over-diagnosis of the endometritis as these signs can also result from vaginitis or cervicitis. It has
214 been suggested that the term reproductive tract inflammatory disease is more appropriate (LeBlanc,
215 2014). The results of the present study supported the hypothesis that endometritis is associated with
216 a detrimental effect on post-partum ovarian cyclicity in Holstein dairy cows. Importantly, this was
217 observed despite some “normal” cows potentially having sub-clinical endometritis which was not
218 evaluated. Consequently, it could be expected that the results would have been drawn towards the
219 null hypothesis. However, this was not the case and strengthens the study’s conclusions but might
220 equally have masked other associations. Cows exhibiting endometritis had the highest rate of atypical
221 ovarian profiles particularly prolonged luteal activity (38.9%) compared to normal cows (9.2%). In

222 agreement with [Taylor et al. \(2003\)](#) who found that cows with abnormal vaginal discharge had a
223 47.6% incidence risk of prolonged luteal activity. The results of the current study were lower than
224 recently published by [Ghanem et al. \(2015\)](#) who observed that cows which experienced endometritis
225 had 60% prolonged luteal activity. In addition, [Ranasinghe et al. \(2011\)](#) recorded 32% of prolonged
226 luteal activity in cows with post-partum complications (retained fetal membrane, endometritis,
227 metritis and pyometra), which was lower compared to current study. Collectively, this indicates the
228 need to monitor cows that have endometritis for the presence of a subsequent persistent CL and then
229 administer the appropriate treatment.

230 It is interesting to note that, there was no relationship between endometritis and delayed first
231 ovulation, cessation of cyclicity or short luteal phase. This study showed that the incidence risk of
232 delayed first ovulation was 9.6% in cows with endometritis. This is consistent with study of [Ghanem
233 et al. \(2015\)](#) which observed there was no difference between cows with uterine bacterial infection
234 and cows without uterine infection in terms of delay to 1st ovulation. Here they reported that cows
235 positive with uterine infection are less likely to ovulate because they have a lower growth rate of the
236 dominant follicle and lower plasma oestrogen concentrations. However, the incidence risk of delay
237 to ovulation was comparatively lower compared to other studies where they observed a delay to first
238 ovulation in about 20–25% of animals ([Nakao et al., 1992](#); [Sheldon et al., 2008](#)).

239 The present study attempted to quantify the severity of inflammation symptoms by
240 examination of vaginal discharge after days 21 post-partum based on visual inspection of vaginal
241 discharge ([Gorzecka et al., 2011](#); [Williams et al., 2005](#)), which have been shown to accurately reflect
242 uterine bacterial infection and immune system. The present study observed that abnormal vaginal
243 discharge had a significant effect on subsequent ovarian profiles that was consistent with the
244 hypothesis that clinical endometritis adversely affects post-partum resumption of ovarian cyclicity.
245 As the abnormal vaginal discharge score increased, the frequency of atypical ovarian profiles was
246 increased as well. The underlying reasons behind the different levels of vaginal discharge are unclear
247 and it is feasible that cows with VDS1 had either less severe endometritis or an earlier inflammation

248 that was resolving at the start of the study. However, the differential effect of VDS1 and VDS2 is
249 consistent with a previous study using regression analysis found a significant relationship between
250 vaginal discharge index and ovarian profiles (Gorzecka et al., 2011). However, others found that there
251 was no significant relationship between vaginal discharge and abnormal ovarian profiles (McCoy et
252 al., 2006). The present study found that endometritis was an important risk factor for the development
253 of abnormal ovarian cycles, particularly prolonged luteal activity. An important point to note in the
254 present study is that there is temporal overlap in the definitions used for endometritis and atypical
255 ovarian profiles. Thus, it is feasible that abnormal cyclicity could have increased the odds of
256 endometritis as well as *vice versa*. This could be due to abnormal progesterone production resulting
257 in the bacterial contamination post-partum not fully cleared in time. Equally, it is feasible that there
258 is a common underlying cause for both atypical ovarian cyclicity and endometritis such as the
259 metabolic stress that occurs post-partum. Further mechanistic studies are required to elucidate the
260 exact relationship.

261 This study found that cows with endometritis and vaginal discharge score >1 were 2.8 times
262 more likely to show a prolonged luteal activity than other atypical ovarian profile. In agreement to
263 the findings of the previous study which observed that cows with post-partum complications
264 (endometritis, metritis and pyometra) were 5 times more likely to show prolonged luteal activity
265 (Ranasinghe et al., 2011). Four major risk factors for prolonged luteal activity have been previously
266 identified; metritis, abnormal vaginal discharge, retained placenta and parity (Opsomer et al., 2000).
267 The same study observed that metritis is the most important factor and cows with metritis were 11
268 times more at risk of developing prolonged luteal activity. In agreement with these data support the
269 concept that prolonged luteal activity were associated with endometritis as well.

270 The precise mechanism through which post-partum endometritis increases the incidence risk
271 of prolonged luteal in dairy cows is unknown. It is feasible though that it is through the disturbance
272 of the luteolytic process that results in the extended CL lifespan (Lamming and Darwash, 1998;
273 Sheldon et al., 2008; Williams et al., 2008). In addition, endometritis caused an increase in PGE₂

274 concentration in uterine fluid of cows (Mateus et al., 2003), with epithelial cell of endometrium
275 exposed to bacterial endotoxin undergo an endocrine switch from luteolytic PGF_{2α} to luteotrophic
276 PGE₂ production (Herath et al., 2009). Increased PGE₂ concentrations in uterine fluid caused luteal
277 persistence in cows (Thibodeaux et al., 1992). This suggests that in cows with endometritis, aberrant
278 prostaglandin production plays a local role in the pathogenesis of prolonged luteal function.

279 In addition to metritis, abnormal vaginal discharge and difficult calving have a negative
280 impact on the post-partum ovarian cyclicity by day 50 post-partum (Opsomer et al., 2000) and on
281 time to resume ovarian cyclicity (El-Din Zain et al., 1995). The impact of metritis on cyclicity may
282 be partly explained by the relation between metritis and negative energy balance (Galvão et al., 2010;
283 Hammon et al., 2006; Huzzey et al., 2007) and by the release of bacterial endotoxin (LPS) into the
284 uterine lumen, and then enters into the blood stream (Herath et al., 2009; Mateus et al., 2003). As
285 reported previously, LPS can affect GnRH and LH release, follicle development, oestradiol
286 production and ovulation. In this study, once reproductive cycles have re-commenced, after a time of
287 post-partum anoestrus, many cows continue to cycle at regular intervals until they are successfully
288 inseminated. However, cows with endometritis had statistically significant 3 day longer interval of
289 onset of luteal activity compared to normal cows. This is in agreement with previous reports where
290 cows with endometritis had delayed onset of ovarian activity compared to healthy cows (Borsberry
291 and Dobson, 1989; Holt et al., 1989). Equally, how biologically significant an extra 3 day to the onset
292 of luteal activity is unclear especially given that most cows will have a voluntary waiting period.

293

294

295 **Conclusions**

296 This study demonstrated that endometritis had a clear negative impact on the post-partum
297 ovarian cyclicity in Holstein dairy cows. Cows, which experienced endometritis, had a quantifiable
298 3 day longer onset of luteal activity and a 3-fold increased odds of an atypical ovarian profile in the
299 calving to conception interval. In particular, cows experiencing endometritis were associated with

300 increased odds of prolonged luteal activity which if undiagnosed or treated could extend the calving-
301 to-conception interval and potentially increased costs to the farmer. Moreover, those cows with
302 visually more severe vaginal discharge were particularly affected. This emphasizes the importance of
303 monitoring cyclicity in cows that have had endometritis.

304

305 **Conflict of interest statement**

306 There are no conflicts of interest to report.

307

308 **Acknowledgments**

309 We are grateful to Oli Maxwell, John Remnant and the staff of Nottingham University Dairy Centre
310 for their assistance with sample collection. This work has funded by the Ministry of Higher Education
311 and Scientific Research, Kurdistan Regional Government.

312

313

314 **References**

- 315 Barlund, C.S., Carruthers, T.D., Waldner, C.L., Palmer, C.W., 2008. A comparison of diagnostic
316 techniques for post-partum endometritis in dairy cattle. *Theriogenology* 69, 714-723.
317
- 318 Borsberry, S., Dobson, H., 1989. Periparturient diseases and their effect on reproductive performance
319 in five dairy herds. *Veterinary Record* 124, 217-219.
320
- 321 Bulman, D.C., Lamming, G.E., 1978. Milk progesterone levels in relation to conception, repeat
322 breeding and factors influencing acyclicity in dairy cows. *Journal of Reproduction and*
323 *Fertility* 54, 447-458.
324
- 325 Bulman, D.C., Lamming, G.E., 1979. The use of milk progesterone analysis in the study of oestrus
326 detection, herd fertility and embryonic mortality in dairy cows. *British Veterinary Journal* 135,
327 559-567.
328
- 329 El-Din Zain, A., Nakao, T., Abdel Raouf, M., Moriyoshi, M., Kawata, K., Moritsu, Y., 1995. Factors
330 in the resumption of ovarian activity and uterine involution in post-partum dairy cows. *Animal*
331 *Reproduction Science* 38, 203-214.
332
- 333 Galvão, K.N., Frajblat, M., Butler, W.R., Brittin, S.B., Guard, C.L., Gilbert, R.O., 2010. Effect of
334 early post-partum ovulation on fertility in dairy cows. *Reproduction in Domestic Animals* 45,
335 e207-e211.
336

- 337 Garmo, R.T., Martin, A.D., Thuen, E., Havrevoll, Ø., Steinshamn, H., Prestløkken, E., Randby, Å.,
338 Eknaes, M., Waldmann, A., Reksen, O., 2009. Characterization of ovarian profiles in fall-
339 calving Norwegian Red cows. *Journal of Dairy Science* 92, 4919-4928.
340
- 341 Ghanem, M.E., Tezuka, E., Devkota, B., Izaike, Y., Osawa, T., 2015. Persistence of uterine bacterial
342 infection, and its associations with endometritis and ovarian function in post-partum dairy
343 cows. *Journal of Reproduction and Development* 61, 54.
344
- 345 Gilbert, R.O., Shin, S.T., Guard, C., Erb, H.N., Frajblat, M., 2005. Prevalence of endometritis and its
346 effects on reproductive performance of dairy cows. *Theriogenology* 64, 1879-1888.
347
- 348 Gorzecka, J., Friggens, N.C., Ridder, C., Callesen, H., 2011. A universal index of uterine discharge
349 symptoms from calving to 6 weeks post-partum. *Reproduction in Domestic Animals* 46, 100-
350 107.
351
- 352 Hammon, D.S., Evjen, I.M., Dhiman, T.R., Goff, J.P., Walters, J.L., 2006. Neutrophil function and
353 energy status in Holstein cows with uterine health disorders. *Veterinary Immunology and*
354 *Immunopathology* 113, 21-29.
355
- 356 Herath, S., Lilly, S.T., Fischer, D.P., Williams, E.J., Dobson, H., Bryant, C.E., Sheldon, I.M., 2009.
357 Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2 α to
358 prostaglandin E2 in bovine endometrium. *Endocrinology* 150, 1912-1920.
359
- 360 Holt, L., Whittier, W.D., Gwazdauskas, F.C., Vinson, W.E., 1989. Early post-partum reproductive
361 profiles in Holstein cows with retained placenta and uterine discharges. *Journal of Dairy*
362 *Science* 72, 533-539.
363
- 364 Huzzey, J.M., Veira, D.M., Weary, D.M., Von Keyserlingk, M.A.G., 2007. Prepartum behavior and
365 dry matter intake identify dairy cows at risk for metritis. *Journal of Dairy Science* 90, 3220-
366 3233.
367
- 368 King, G.J., Hurnik, J.F., Robertson, H.A., 1976. Ovarian function and estrus in dairy cows during
369 early lactation. *Journal of Animal Science* 42, 688-692.
370
- 371 Lamming, G.E., Darwash, A.O., 1998. The use of milk ovarian profiles to characterise components
372 of subfertility in milked dairy cows. *Animal Reproduction Science* 52, 175-190.
373
- 374 LeBlanc, S.J., 2014. Reproductive tract inflammatory disease in postpartum dairy cows. *Animal* 8,
375 54-63.
376
- 377 Mann, G.E., Keatinge, R., Hunter, M., Hedley, B.A., Lamming, G.E., 2005. The use of milk
378 progesterone to monitor reproductive function in beef suckler cows. *Animal Reproduction*
379 *Science* 88, 169-177.
380
- 381 Mateus, L., Lopes da Costa, L.L., Diniz, P., Ziecik, A.J., 2003. Relationship between endotoxin and
382 prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with
383 puerperal endometritis. *Animal Reproduction Science* 76, 143-154.
384
- 385 McCoy, M.A., Lennox, S.D., Mayne, C.S., McCaughey, W.J., Edgar, H.W.J., Catney, D.C., Verner,
386 M., Mackey, D.R., Gordon, A.W., 2006. Milk ovarian profiles and their relationship with
387 fertility, production and disease in dairy cows in Northern Ireland. *Animal Science* 82, 213-
388 222.

389
390 McNaughton, A.P., Murray, R.D., 2009. Structure and function of the bovine fetomaternal unit in
391 relation to the causes of retained fetal membranes. *Veterinary Record* 165, 615-622.
392
393 Nakao, T., Moriyoshi, M., Kawata, K., 1992. The effect of post-partum ovarian dysfunction and
394 endometritis on subsequent reproductive performance in high and medium producing dairy
395 cows. *Theriogenology* 37, 341-349.
396
397 Opsomer, G., Coryn, M., Deluyker, H., Kruif, A.D., 1998. An analysis of ovarian dysfunction in high
398 yielding dairy cows after calving based on ovarian profiles. *Reproduction in Domestic*
399 *Animals* 33, 193-204.
400
401 Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H., de Kruif, A., 2000. Risk factors for
402 post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study.
403 *Theriogenology* 53, 841-857.
404
405 Ranasinghe, R., Nakao, T., Yamada, K., Koike, K., Hayashi, A., Dematawewa, C.M.B., 2011.
406 Characteristics of prolonged luteal phase identified by milk progesterone concentrations and
407 its effects on reproductive performance in Holstein cows. *Journal of Dairy Science* 94, 116-
408 127.
409
410 Runciman, D.J., Anderson, G.A., Malmo, J., Davis, G.M., 2008. Use of post-partum vaginoscopic
411 (visual vaginal) examination of dairy cows for the diagnosis of endometritis and the
412 association of endometritis with reduced reproductive performance. *Australian Veterinary*
413 *Journal* 86, 205-213.
414
415 Sheldon, I.M., 2004. The post-partum uterus. *Veterinary Clinics of North America* 20, 569.
416
417 Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., Schuberth, H., 2009. Defining post-partum uterine
418 disease and the mechanisms of infection and immunity in the female reproductive tract in
419 cattle. *Biology of Reproduction* 81, 1025-1032.
420
421 Sheldon, I.M., Lewis, G.S., LeBlanc, S., Gilbert, R.O., 2006. Defining post-partum uterine disease in
422 cattle. *Theriogenology* 65, 1516-1530.
423
424 Sheldon, I.M., Noakes, D.E., Rycroft, A.N., Pfeiffer, D.U., Dobson, H., 2002. Influence of uterine
425 bacterial contamination after parturition on ovarian dominant follicle selection and follicle
426 growth and function in cattle. *Reproduction* 123, 837-845.
427
428 Sheldon, I.M., Williams, E.J., Miller, A.N.A., Nash, D.M., Herath, S., 2008. Uterine diseases in cattle
429 after parturition. *Veterinary Journal* 176, 115-121.
430
431 Shrestha, H.K., Nakao, T., Higaki, T., Suzuki, T., Akita, M., 2004. Resumption of post-partum
432 ovarian cyclicity in high-producing Holstein cows. *Theriogenology* 61, 637-649.
433
434 Taylor, V.J., Beever, D.E., Bryant, M.J., Wathes, D.C., 2003. Metabolic profiles and progesterone
435 cycles in first lactation dairy cows. *Theriogenology* 59, 1661-1677.
436
437 Thibodeaux, J.K., Roussel, J.D., Myers, M.W., Godke, R.A., 1992. Intrauterine infusion of
438 prostaglandin E2 and subsequent luteal function in cattle. *Prostaglandins* 44, 531-541.
439

- 440 Williams, E.J., Fischer, D.P., Noakes, D.E., England, G.C.W., Rycroft, A.N., Dobson, H., Sheldon,
441 I.M., 2007. The relationship between uterine pathogen growth density and ovarian function in
442 the post-partum dairy cow. *Theriogenology* 68, 549-559.
443
- 444 Williams, E.J., Fischer, D.P., Pfeiffer, D.U., England, G.C.W., Noakes, D.E., Dobson, H., Sheldon,
445 I.M., 2005. Clinical evaluation of post-partum vaginal mucus reflects uterine bacterial
446 infection and the immune response in cattle. *Theriogenology* 63, 102-117.
447
- 448 Williams, E.J., Herath, S., England, G.C.W., Dobson, H., Bryant, C.E., Sheldon, I.M., 2008. Effect
449 of *Escherichia coli* infection of the bovine uterus from the whole animal to the cell. *Animal* 2,
450 1153-1157.

451 **Table 1**

452 The number of normal and cows that experienced endometritis as well as the percentage of typical
 453 and atypical ovarian profiles across the three commercial dairy herds included in the study.

454

Herd ID	Cows	Normal Cows		Endometritic Cows		Typical ovarian profiles		Atypical ovarian profiles	
		n	%	n	%	n	%	n	%
1	89	49	55.1	40	44.9	42	47.2	47	52.8
2	44	29	65.9	15	34.1	29	65.9	15	34.1
3	37	20	54.1	17	45.9	15	40.5	22	59.5
Total	170	98	57.6	72	42.4	86	50.6	84	49.4

455

456

457

458

459 **Table 2**

460 The percentages of typical and atypical ovarian profiles between normal cows and those cows that
 461 experienced endometritis.

Class	Normal		Endometritis		<i>P-value</i>
	(n)	%	(n)	%	
Typical ovarian profile	68	69.4	18	25.0	<i>P</i> <0.05
Atypical ovarian profile	30	30.6	54	75.0	
Incidence risk of specific problems					
Delayed 1 st ovulation	5 (n=98)	5.1	7 (n=72)	9.7	<i>P</i> =0.54
Prolonged luteal activity	9 (n=98)	9.2	28 (n=72)	38.9	<i>P</i> <0.05
Cessation of cyclicity	9 (n=98)	9.2	11 (n=72)	15.3	<i>P</i> =1.00
Short luteal phase	7 (n=98)	7.1	8 (n=72)	11.1	<i>P</i> =1.00

462

463 **Table 3**

464 Incidence odds of typical and atypical ovarian profiles during the calving to conception interval in
 465 normal cows and cows with vaginal discharge scores (VDS) of 0, 1 or 2.

Class	VDS0		VDS1		VDS2	
	(n)	%	(n)	%	(n)	%
Typical ovarian profile	68	69.4	10	32.3	8	19.5
Atypical ovarian profile	30	30.6	21	67.7	33	80.5 ^a
Incidence of specific problems						
Delayed 1 st ovulation	5	5.1	1	3.2	6	14.6
Prolonged luteal activity	9	9.2	11	35.5	17	41.5 ^a
Cessation of cyclicity	9	9.2	5	16.1	6	14.6
Short luteal phase	7	7.1	4	12.9	4	9.8

466 ^a $P < 0.05$; vs VDS0

467 No significant differences were detected between VDS1 and VDS2 groups

468

469

470

471

472

473 **Table 4**

474 Conception rate in those cows that received a 1st insemination during the monitoring period that
 475 were either normal or endometritic and exhibited typical or atypical ovarian profiles.

476

Class	Normal cows	Endometritic cows	P-value
All cows	38.6% (n=34/88)	30.8% (n=20/65)	<i>P=0.78</i>
Typical ovarian profile	43.8% (n=28/64)	47.1% (n=8/17)	<i>P=0.29</i>
Atypical ovarian profiles	25.0% (n=6/24)	25.0% (n=12/48)	<i>P=0.42</i>
	Typical ovarian profile	Atypical ovarian profile	
All cows	44.4% (n=36/81)	25.0% (n=18/72)	<i>P=0.08</i>

477

478

479

480

481 **Figure legends**

482

483 **Fig.1:** Schematic illustration of the types of reproductive cycles identified in Holstein dairy cows by
484 analysis of progesterone in milk: These represent: A) typical ovarian profile, B) prolonged luteal
485 activity, C) cessation of cyclicity, D) short luteal phase and E) delayed 1st ovulation. Grey boxes
486 represent the period during which specified problem occurred. The dashed line represents the limit of
487 progesterone for luteal activity (3ng/ml). AI: time of artificial insemination; PGF₂α: prostaglandin
488 F₂α.

489

490 **Fig.2:** Binominal logistic regression analysis for the association between endometritis/vaginal
491 discharge score (VDS) on the chance of having typical or atypical ovarian profiles. The data was
492 nested by each herd. A) Cows with endometritis had significantly increased odds of atypical ovarian
493 profiles (*; $P<0.05$) and an increased odds of prolonged luteal activity (*; $P<0.05$), however,
494 endometritis had no effect on the odds having the other defined atypical ovarian profiles ($P>0.05$).
495 B) Odds ratios of vaginal discharge score 1 vs normal; cows with VDS1 had similar odds of having
496 the different ovarian profiles. C) Cows with VDS2 had increased odds of atypical ovarian profiles (*;
497 $P<0.05$) and increased risk of prolonged luteal activity (*; $P<0.05$). There were no differences
498 between VDS1 and VDS2. Note: Types of ovarian profiles are not shown when the numbers of
499 animals was less than 5.

500

501 **Fig.3:** Survival analysis to compare the time of the onset of luteal activity between normal cows
502 (n=98) and those cows that experienced endometritis (n=72). Cows that experienced endometritis had
503 a longer onset of luteal activity (median, 28 days) compared to normal cows (median, 25 days) by 3
504 days ($P<0.05$).

505