Original article

Impact of endometritis on post-partum ovarian cyclicity in dairy cows

Z.A. Mohammed[a,b], G.E. Mann[a] and R.S. Robinson[c*],

[a]School of Bioscience and [c]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK.
[b]College of Veterinary Medicine, University of Duhok, Kurdistan Regional Government and Scientific Research, Kurdistan, Iraq.

*Corresponding author. Tel.: +00 44 (0)1159516452.
e-mail address: bob.robinson@nottingham.ac.uk (R. S Robinson)

Abstract

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

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

Introduction

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

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

An association between clinical signs of uterine inflammation and post-partum ovarian cyclicity has been reported previously (Mateus et al., 2003; McCoy et al., 2006; Opsomer et al., 2000; Shrestha et al., 2004; Taylor et al., 2003; Williams et al., 2007). Milk progesterone monitoring has been used widely for assessment of ovarian function, reproductive patterns and abnormalities in dairy cows (Bulman and Lamming, 1978; Bulman and Lamming, 1979; King et al., 1976). One abnormality identified by progesterone profiling is prolonged luteal activity. Abnormal ovarian profiles mainly prolonged luteal activity have been associated with an abnormal uterine environment which can perturb the production of prostaglandins (Opsomer et al., 1998). The risk factors for prolonged luteal activity have included metritis, abnormal vaginal discharge and retained fetal membrane (Opsomer et al. 1998, Opsomer et al. 2000). This supports the concept that uterine inflammatory/infection problems could lead to prolonged luteal activity post-partum as well as follicular dysfunctions such as smaller follicles and reduced oestradiol secretions. However, the sequelae of cows having endometritis on the distribution of the different atypical ovarian profiles (i.e. cessation of cyclicity, delayed ovulation or prolonged luteal activity) is less clear and whether particular atypical ovarian profiles are affected. Thus, the objective of this study was to quantify the incidence risk of different atypical ovarian profiles in dairy cows experiencing post-partum. This was then further expanded to compare the influence of different grades of endometritis on ovarian cyclicity.

Materials and methods

Experimental animals

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

In herd 1, cows were milked by robot (Lely Astronaut A3 AMS) attending the robot an average of 2.5 times per day. In herds 2 and 3, cows were milked twice daily in a traditional herringbone parlour. The cows recruited to the study, calved between July and April and were housed and managed according to that particular commercial farm practice with no study-related treatment interventions. All cows with reproductive disorders were identified by farm staff or at routine veterinary visits were treated according to that farm's practice or the clinician's opinion with no experimental interventions. All treatments were recorded in the herd's records by the farm staff. Cows were inseminated at observed oestrus by trained AI technicians, after a 7-8 week voluntary waiting period.

Clinical investigation

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

The extent and smell of vaginal discharge was scored according to Sheldon (2004) as:

VDS0: Clear or translucent mucus (normal cows)

VDS1: Mucus containing flecks of white or off white pus

VDS2: Exudates containing ≥50% purulent material, usually white or yellow

Endometritis was then more precisely defined by the degree of the abnormal vaginal discharge after 21 days post-partum (VDS1 or VDS2) with normal cows showing no detectable discharge (VDS0). VDS1 and VDS2 were categorised based on the greatest vaginal discharge that was observed in the study window.

Milk sampling

Representative whole milk samples were collected for progesterone analysis two to three times per week starting as soon as a particular cow was two weeks after calving and continued until 15 weeks post-partum or until pregnancy was confirmed by rectal palpation or ultrasonography. Milk was collected in plastic tubes contain broad-spectrum preservative (Microtabs®II containing Bronopol and Natamycin; D&F Control Systems, Inc.). The concentration of progesterone in milk was determined using a competitive enzyme-linked immunosorbent assay supplied as a commercially available kit (Ridgeway Science Ltd). The standards (10µl, 0-50ng/ml) or sample (10µl) were added to the appropriate wells and analyzed in duplicate. Next, the progesterone-alkaline phosphatase conjugate (200µl) was added to all wells except for the blank wells. The plate was incubated at room temperature for 2 hours after which it was washed 3 times in PBS. Next, 200µl of alkaline phosphatase substrate was added to each well and the plate left for 1 hour. The absorbance was then measured at 584nm using a microplate reader (Optima). A standard curve (4-parameter logistic regression) was fitted from which the unknown were interpolated and quantified. The reliable reading range was from 1.0 to 10ng/ml. All samples reading below or above this range were taken to be 1.0 (follicular phase or anoestrus) or 10ng/ml (luteal phase), respectively. All samples with a coefficient of variation >15% were repeated. The intra and inter-assay coefficients of variation were 6.3 and 7.7%, respectively.

Analysis of ovarian profiles

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

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

Examples of the various types of ovarian profiles detected during the post-partum period, based on milk progesterone analysis, are shown in Fig.1. Some cows exhibited more than one type of atypical ovarian profile and in this case, the cow was categorised according to the first atypical ovarian profile that was exhibited only.

Statistical analysis

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

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

Results

The incidence risk of endometritis before 45 DIM did not differ between the three herds (Table 1). Similarly, the distribution of atypical ovarian profiles in the calving to conception interval were equivalent across the three herds (Table 1). Logistic regression revealed cows that had endometritis had an increased odds of atypical ovarian profiles (OR=2.38 [95% CI: 1.30-4.35]; $P<0.05$; Table 2; Fig 2A). Specifically, 75% cows that experienced endometritis had atypical ovarian profiles compared with 25% in normal cows. Additionally, cows that exhibited endometritis had increased odds of prolonged luteal activity compared to normal cows (OR=4.60 [95% CI: 1.53-13.84]; $P<0.05$; Table 2; Fig 2A). However, the odds of cessation of cyclicity, short luteal phase, and delayed 1st ovulation events were not different between cows with endometritis and normal cows (Table 2; Fig 2A).

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

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

The odds of conceiving at 1st service between normal cows and those that experienced endometritis and whether they had typical or atypical ovarian profiles are shown in Table 4. It should be recognised that the number of animals in each group is insufficient to draw definite conclusions and should be interpreted with caution. There was no difference in the odds for conception at 1st service between normal cows and cows with endometritis ($P>0.05$, Table 4). When the data was further categorized in those with typical or atypical ovarian profiles, there was no significant effect of endometritis on conception rate (*P>0.05*). However, the odds of conception at 1st AI tended to be lower in cows with atypical ovarian profiles compared to those with typical ovarian profiles (*P=0.08*; Table 4).

Discussion

The objective of this study was to quantify the incidence odds of atypical ovarian profiles in dairy cows experiencing post-partum endometritis compared with cows that did not. In the present study, some cows had more than one atypical ovarian profile but only the first presentation was included in this study. This could have created a bias in the reported incidence risk of the different type of atypical ovarian profiles. For example, delayed 1st ovulation is the first atypical event that can occur post-partum while prolonged luteal activity only occurs once cyclicity is resumed. That said, prolonged luteal activity was the profile that was associated with cows experiencing endometritis and thus this potential bias does not appear to have affected these results. Equally, it is important to consider that while the normal cows had no visual signs of purulent vaginal discharge and hence endometritis, this does not mean that there was no subclinical signs of endometritis in these animals. The diagnosis of endometritis by the presence and appearance of vaginal discharge can lead to an over-diagnosis of the endometritis as these signs can also result from vaginitis or cervicitis. It has been suggested that the term reproductive tract inflammatory disease is more appropriate (LeBlanc, 2014). The results of the present study supported the hypothesis that endometritis is associated with a detrimental effect on post-partum ovarian cyclicity in Holstein dairy cows. Importantly, this was observed despite some "normal" cows potentially having sub-clinical endometritis which was not evaluated. Consequently, it could be expected that the results would have been drawn towards the null hypothesis. However, this was not the case and strengthens the study's conclusions but might equally have masked other associations. Cows exhibiting endometritis had the highest rate of atypical ovarian profiles particularly prolonged luteal activity (38.9%) compared to normal cows (9.2%). In agreement with Taylor et al. (2003) who found that cows with abnormal vaginal discharge had a 47.6% incidence risk of prolonged luteal activity. The results of the current study were lower than recently published by Ghanem et al. (2015) who observed that cows which experienced endometritis had 60% prolonged luteal activity. In addition, Ranasinghe et al. (2011) recorded 32% of prolonged luteal activity in cows with post-partum complications (retained fetal membrane, endometritis, metritis and pyometra), which was lower compared to current study. Collectively, this indicates the need to monitor cows that have endometritis for the presence of a subsequent persistent CL and then administer the appropriate treatment.

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

The present study attempted to quantify the severity of inflammation symptoms by examination of vaginal discharge after days 21 post-partum based on visual inspection of vaginal discharge (Gorzecka et al., 2011; Williams et al., 2005), which have been shown to accurately reflect uterine bacterial infection and immune system. The present study observed that abnormal vaginal discharge had a significant effect on subsequent ovarian profiles that was consistent with the hypothesis that clinical endometritis adversely affects post-partum resumption of ovarian cyclicity. As the abnormal vaginal discharge score increased, the frequency of atypical ovarian profiles was increased as well. The underlying reasons behind the different levels of vaginal discharge are unclear and it is feasible that cows with VDS1 had either less severe endometritis or an earlier inflammation that was resolving at the start of the study. However, the differential effect of VDS1 and VDS2 is consistent with a previous study using regression analysis found a significant relationship between vaginal discharge index and ovarian profiles (Gorzecka et al., 2011). However, others found that there was no significant relationship between vaginal discharge and abnormal ovarian profiles (McCoy et al., 2006). The present study found that endometritis was an important risk factor for the development of abnormal ovarian cycles, particularly prolonged luteal activity. An important point to note in the present study is that there is temporal overlap in the definitions used for endometritis and atypical ovarian profiles. Thus, it is feasible that abnormal cyclicity could have increased the odds of endometritis as well as *vice versa*. This could be due to abnormal progesterone production resulting in the bacterial contamination post-partum not fully cleared in time. Equally, it is feasible that there is a common underlying cause for both atypical ovarian cyclicity and endometritis such as the metabolic stress that occurs post-partum. Further mechanistic studies are required to elucidate the exact relationship.

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

The precise mechanism through which post-partum endometritis increases the incidence risk of prolonged luteal in dairy cows is unknown. It is feasible though that it is through the disturbance of the luteolytic process that results in the extended CL lifespan (Lamming and Darwash, 1998; Sheldon et al., 2008; Williams et al., 2008). In addition, endometritis caused an increase in PGE_2

concentration in uterine fluid of cows (Mateus et al., 2003), with epithelial cell of endometrium exposed to bacterial endotoxin undergo an endocrine switch from luteolytic $PGF_{2\alpha}$ to luteotrophic PGE_2 production (Herath et al., 2009). Increased PGE_2 concentrations in uterine fluid caused luteal persistence in cows (Thibodeaux et al., 1992). This suggests that in cows with endometritis, aberrant prostaglandin production plays a local role in the pathogenesis of prolonged luteal function.

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

Conclusions

This study demonstrated that endometritis had a clear negative impact on the post-partum ovarian cyclicity in Holstein dairy cows. Cows, which experienced endometritis, had a quantifiable 3 day longer onset of luteal activity and a 3-fold increased odds of an atypical ovarian profile in the calving to conception interval. In particular, cows experiencing endometritis were associated with increased odds of prolonged luteal activity which if undiagnosed or treated could extend the calving-to-conception interval and potentially increased costs to the farmer. Moreover, those cows with visually more severe vaginal discharge were particularly affected. This emphasizes the importance of monitoring cyclicity in cows that have had endometritis.

Conflict of interest statement

There are no conflicts of interest to report.

Acknowledgments

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

References

Barlund, C.S., Carruthers, T.D., Waldner, C.L., Palmer, C.W., 2008. A comparison of diagnostic techniques for post-partum endometritis in dairy cattle. Theriogenology 69, 714-723.

Borsberry, S., Dobson, H., 1989. Periparturient diseases and their effect on reproductive performance in five dairy herds. Veterinary Record 124, 217-219.

Bulman, D.C., Lamming, G.E., 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. Journal of Reproduction and Fertility 54, 447-458.

Bulman, D.C., Lamming, G.E., 1979. The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryonic mortality in dairy cows. British Veterinary Journal 135, 559-567.

El-Din Zain, A., Nakao, T., Abdel Raouf, M., Moriyoshi, M., Kawata, K., Moritsu, Y., 1995. Factors in the resumption of ovarian activity and uterine involution in post-partum dairy cows. Animal Reproduction Science 38, 203-214.

Galvão, K.N., Frajblat, M., Butler, W.R., Brittin, S.B., Guard, C.L., Gilbert, R.O., 2010. Effect of early post-partum ovulation on fertility in dairy cows. Reproduction in Domestic Animals 45, e207-e211.

Garmo, R.T., Martin, A.D., Thuen, E., Havrevoll, Ø., Steinshamn, H., Prestløkken, E., Randby, Å., Eknaes, M., Waldmann, A., Reksen, O., 2009. Characterization of ovarian profiles in fall-calving Norwegian Red cows. Journal of Dairy Science 92, 4919-4928.

Ghanem, M.E., Tezuka, E., Devkota, B., Izaike, Y., Osawa, T., 2015. Persistence of uterine bacterial infection, and its associations with endometritis and ovarian function in post-partum dairy cows. Journal of Reproduction and Development 61, 54.

Gilbert, R.O., Shin, S.T., Guard, C., Erb, H.N., Frajblat, M., 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64, 1879-1888.

Gorzecka, J., Friggens, N.C., Ridder, C., Callesen, H., 2011. A universal index of uterine discharge symptoms from calving to 6 weeks post-partum. Reproduction in Domestic Animals 46, 100-107.

Hammon, D.S., Evjen, I.M., Dhiman, T.R., Goff, J.P., Walters, J.L., 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Veterinary Immunology and Immunopathology 113, 21-29.

Herath, S., Lilly, S.T., Fischer, D.P., Williams, E.J., Dobson, H., Bryant, C.E., Sheldon, I.M., 2009. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. Endocrinology 150, 1912-1920.

Holt, L., Whittier, W.D., Gwazdauskas, F.C., Vinson, W.E., 1989. Early post-partum reproductive profiles in Holstein cows with retained placenta and uterine discharges. Journal of Dairy Science 72, 533-539.

Huzzey, J.M., Veira, D.M., Weary, D.M., Von Keyserlingk, M.A.G., 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. Journal of Dairy Science 90, 3220-3233.

King, G.J., Hurnik, J.F., Robertson, H.A., 1976. Ovarian function and estrus in dairy cows during early lactation. Journal of Animal Science 42, 688-692.

Lamming, G.E., Darwash, A.O., 1998. The use of milk ovarian profiles to characterise components of subfertility in milked dairy cows. Animal Reproduction Science 52, 175-190.

LeBlanc, S.J., 2014. Reproductive tract inflammatory disease in postpartum dairy cows. Animal 8, 54-63.

Mann, G.E., Keatinge, R., Hunter, M., Hedley, B.A., Lamming, G.E., 2005. The use of milk progesterone to monitor reproductive function in beef suckler cows. Animal Reproduction Science 88, 169-177.

Mateus, L., Lopes da Costa, L.L., Diniz, P., Ziecik, A.J., 2003. Relationship between endotoxin and prostaglandin (PGE2 and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. Animal Reproduction Science 76, 143-154.

McCoy, M.A., Lennox, S.D., Mayne, C.S., McCaughey, W.J., Edgar, H.W.J., Catney, D.C., Verner, M., Mackey, D.R., Gordon, A.W., 2006. Milk ovarian profiles and their relationship with fertility, production and disease in dairy cows in Northern Ireland. Animal Science 82, 213-222.

McNaughton, A.P., Murray, R.D., 2009. Structure and function of the bovine fetomaternal unit in relation to the causes of retained fetal membranes. Veterinary Record 165, 615-622.

Nakao, T., Moriyoshi, M., Kawata, K., 1992. The effect of post-partum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. Theriogenology 37, 341-349.

Opsomer, G., Coryn, M., Deluyker, H., Kruif, A.D., 1998. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on ovarian profiles. Reproduction in Domestic Animals 33, 193-204.

Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H., de Kruif, A., 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 53, 841-857.

Ranasinghe, R., Nakao, T., Yamada, K., Koike, K., Hayashi, A., Dematawewa, C.M.B., 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. Journal of Dairy Science 94, 116-127.

Runciman, D.J., Anderson, G.A., Malmo, J., Davis, G.M., 2008. Use of post-partum vaginoscopic (visual vaginal) examination of dairy cows for the diagnosis of endometritis and the association of endometritis with reduced reproductive performance. Australian Veterinary Journal 86, 205-213.

Sheldon, I.M., 2004. The post-partum uterus. Veterinary Clinics of North America 20, 569.

Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., Schuberth, H., 2009. Defining post-partum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biology of Reproduction 81, 1025-1032.

Sheldon, I.M., Lewis, G.S., LeBlanc, S., Gilbert, R.O., 2006. Defining post-partum uterine disease in cattle. Theriogenology 65, 1516-1530.

Sheldon, I.M., Noakes, D.E., Rycroft, A.N., Pfeiffer, D.U., Dobson, H., 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123, 837-845.

Sheldon, I.M., Williams, E.J., Miller, A.N.A., Nash, D.M., Herath, S., 2008. Uterine diseases in cattle after parturition. Veterinary Journal 176, 115-121.

Shrestha, H.K., Nakao, T., Higaki, T., Suzuki, T., Akita, M., 2004. Resumption of post-partum ovarian cyclicity in high-producing Holstein cows. Theriogenology 61, 637-649.

Taylor, V.J., Beever, D.E., Bryant, M.J., Wathes, D.C., 2003. Metabolic profiles and progesterone cycles in first lactation dairy cows. Theriogenology 59, 1661-1677.

Thibodeaux, J.K., Roussel, J.D., Myers, M.W., Godke, R.A., 1992. Intrauterine infusion of prostaglandin E2 and subsequent luteal function in cattle. Prostaglandins 44, 531-541.

Williams, E.J., Fischer, D.P., Noakes, D.E., England, G.C.W., Rycroft, A.N., Dobson, H., Sheldon, I.M., 2007. The relationship between uterine pathogen growth density and ovarian function in the post-partum dairy cow. Theriogenology 68, 549-559.

Williams, E.J., Fischer, D.P., Pfeiffer, D.U., England, G.C.W., Noakes, D.E., Dobson, H., Sheldon, I.M., 2005. Clinical evaluation of post-partum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63, 102-117.

Williams, E.J., Herath, S., England, G.C.W., Dobson, H., Bryant, C.E., Sheldon, I.M., 2008. Effect of Escherichia coli infection of the bovine uterus from the whole animal to the cell. Animal 2, 1153-1157.

Table 1

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

Herd ID	**Cows**	**Normal Cows**		**Endometritic Cows**		**Typical ovarian profiles**		**Atypical ovarian profiles**	
	n	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***

Table 2

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

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

Table 3

Incidence odds of typical and atypical ovarian profiles during the calving to conception interval in 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 1st 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*

[a]$P<0.05$; vs VDS0

No significant differences were detected between VDS1 and VDS2 groups

Table 4

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

Class	Normal cows	Endometritic cows	P-value
All cows	38.6% (n=34/88)	30.8% (n=20/65)	*P=0.78*
Typical ovarian profile	43.8% (n=28/64)	47.1% (n=8/17)	*P=0.29*
Atypical ovarian profiles	25.0% (n=6/24)	25.0% (n=12/48)	*P=0.42*

	Typical ovarian profile	Atypical ovarian profile	
All cows	44.4% (n=36/81)	25.0% (n=18/72)	*P=0.08*

Figure legends

Fig.1: Schematic illustration of the types of reproductive cycles identified in Holstein dairy cows by analysis of progesterone in milk: These represent: A) typical ovarian profile, B) prolonged luteal activity, C) cessation of cyclicity, D) short luteal phase and E) delayed 1st ovulation. Grey boxes represent the period during which specified problem occurred. The dashed line represents the limit of progesterone for luteal activity (3ng/ml). AI: time of artificial insemination; $PGF_2\alpha$: prostaglandin $F_2\alpha$.

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

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