Animal (2008), **2:8**, pp 1153–1157 © The Animal Consortium 2008
doi:10.1017/S1751731108002413

animal

Effect of *Escherichia coli* infection of the bovine uterus from the whole animal to the cell*

E. J. Williams[1†], S. Herath[1], G. C. W. England[2], H. Dobson[3], C. E. Bryant[4] and I. M. Sheldon[1]

[1]*Department of Veterinary Clinical Sciences, Royal Veterinary College, Royal College Street, London, NW1 0TU, UK;* [2]*The School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK;* [3]*Department of Veterinary Clinical Science and Animal Husbandry, Faculty of Veterinary Science, University of Liverpool, Leahurst, Neston, CH64 7TE, UK;* [4]*Centre for Veterinary Science, Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, CB3 0ES, UK*

(Received 18 September 2007; Accepted 19 November 2007)

Following parturition, contamination of the uterine lumen by bacteria is ubiquitous, and uterine health is impaired in cattle because infection persists in 10% to 15% of animals as endometritis. Endometritis causes infertility for the duration of infection, and subfertility persists even after apparent successful resolution of the disease. Escherichia coli *is the pathogenic bacterium most frequently isolated from the* post partum *uterus, and is associated with increased concentrations of peripheral plasma acute phase proteins and fetid vaginal mucus. The presence of* E. coli *is also associated with slower growth of the first* post partum *dominant follicle and perturbed oestradiol secretion. Furthermore, in animals that ovulate the first dominant follicle, the corpus luteum is smaller and secretes less progesterone. The endotoxin lipopolysaccharide (LPS), which is released from* E.coli*, can pass from the uterine lumen to the peripheral circulation and LPS concentrations are increased in cows with uterine infection. Infusion of* E. coli *LPS into the uterine lumen suppresses the pre-ovulatory luteinising hormone surge and disrupts ovulation in heifers.* In vitro*, endometrial explants produce prostaglandins in response to LPS. Addition of LPS or* E. coli *to stromal or epithelial cells increases cyclooxygenase-2 mRNA expression, and stimulates the production of prostaglandin* E_2 *and prostaglandin* $F_{2\alpha}$ *. Furthermore, uterine and ovarian cells express mRNA of the molecules required for recognition of LPS, Toll-like receptor-4 and CD14. In summary,* E. coli *is a common cause of infertility involving the perturbation of the hypothalamus, pituitary and ovary in dairy cows.*

Keywords: uterus, ovary, infection, *E. coli*, immunity

Introduction

Dairy cattle are remarkable amongst domestic animals as bacterial contamination of the uterine lumen is ubiquitous after parturition (Elliott *et al.*, 1968; Griffin *et al.*, 1974; Sheldon *et al.*, 2002). As the cervix dilates to allow passage of the calf at parturition, the anatomical barrier to bacterial contamination is breached, allowing micro organisms from the cow's skin, faeces and surrounding environment to enter the uterus (Sheldon and Dobson, 1999). In one study, 93% of uteri obtained within 2 weeks of calving were contaminated with bacteria and others have shown the presence of bacteria in more than 60% of animals 3 weeks after parturition (Elliott *et al.*, 1968; Griffin *et al.*, 1974; Sheldon *et al.*, 2002). Many cows spontaneously eliminate this contamination; however, at least 20% of cows fail to clear the bacteria and in 10% to 15% of animals infection persists within the uterus, causing endometritis (Griffin *et al.*, 1974; Borsberry and Dobson, 1989; Dhaliwal *et al.*, 2001). In addition, there is emerging evidence that other microbes such as Bovine herpesvirus 4 may contribute to the disease process (Donofrio *et al.*, 2007).

Uterine infection and the associated inflammatory and immune responses compromise animal welfare, causing subfertility and infertility (Borsberry and Dobson, 1989; Sheldon and Dobson, 2004). Endometritis is associated with lower conception rates, increased intervals from calving to first service or conception, and more culls for failure to conceive (Borsberry and Dobson, 1989). Furthermore, the

* This invited paper was presented at BSAS meeting 'Fertility in Dairy Cows – bridging the gaps' 30-31 August 2007, Liverpool Hope University.

† Present address: Unit of Animal and Veterinary Biosciences, School of Agriculture, Food Science and Veterinary Medicine, Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland. E-mail: erin.williams@ucd.ie

subfertility associated with endometritis persists even after clinical resolution of the disease. In typical studies of cows treated successfully for clinical endometritis, conception rates are approximately 20% lower than in unaffected animals and an extra 3% of animals remain infertile (Borsberry and Dobson, 1989, LeBlanc *et al.*, 2002a and 2002b). The financial losses associated with uterine infection depend on the direct cost of treatment, reduced milk yields and subfertility. Despite great effort to control uterine disease in cattle, the number of animals treated annually for endometritis reported by the National Animal Disease Information Service (NADIS, the UK Veterinary Sentinel Practice Network, http://www.nadis.org.uk) has steadily increased since 2001 (Figure 1; NADIS); in 2006, almost 6% of animals required direct intervention by a veterinarian. These data, however, only reflect a proportion of animals with clinical disease and do not quantify the numbers of animals with subclinical disease. An increased incidence of uterine disease and the associated costs will have major implications for the economic sustainability of both the individual animal and the national herd. Thus, an important challenge facing reproductive biologists is to understand the mechanisms underlying uterine disease in cattle.

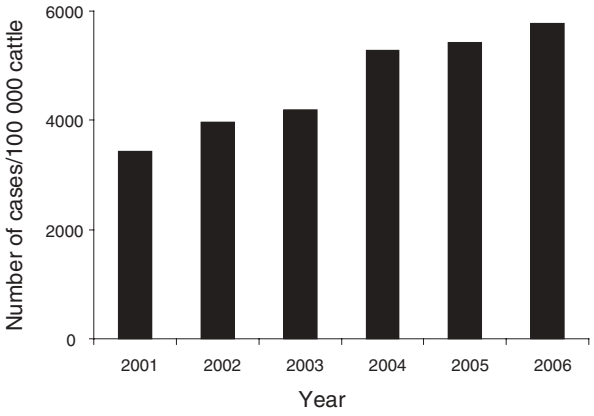

Figure 1 Cases of uterine disease requiring veterinary treatment between 2001 and 2006, as reported by 36 attending veterinarians. Data generously supplied by the National Animal Disease Information Service (NADIS).

Post partum uterine infection

The flora cultured *in vitro* from the early *post partum* uterus represents a wide spectrum of environmental contaminants and some anaerobic species, and bacteria isolated from the uterine lumen have been categorised according to their pathogenicity within the uterus (Ruder *et al.*, 1981; Olson *et al.*, 1984; Farin *et al.*, 1989; Noakes *et al.*, 1989; Bonnett *et al.*, 1991; Laven *et al.*, 2000). Bacteria are classed as 'recognised uterine pathogens' associated with uterine endometrial lesions; 'potential pathogens' frequently isolated from the bovine uterine lumen and cases of endometritis, but not commonly associated with uterine lesions; and, 'opportunist contaminants' transiently isolated from the uterine lumen but not associated with endometritis (Sheldon *et al.*, 2002; Williams *et al.*, 2005). The recognised uterine pathogens are *E. coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *Prevotella melaninogenica* and *Proteus* species, and these bacteria are associated with greater endometrial inflammation and more severe signs of clinical uterine disease (Farin *et al.*, 1989; Bonnett *et al.*, 1991; Sheldon *et al.*, 2002; Williams *et al.*, 2005). We have recently shown that potential uterine pathogens or opportunistic contaminant bacteria in the uterine lumen do not have this same relationship (Williams *et al.*, 2005).

The innate immune system in the uterus is responsible for host recognition of pathogens. Pattern recognition receptors (PRRs) recognise a wide host of pathogens via their pathogen-associated molecular patterns (PAMPs). A key group of receptors that recognise PAMPs are the Toll-like receptors (TLRs), which were first identified on immune cells such as macrophages, but have since been identified on other cell types (Akira and Takeda, 2004; Akira *et al.*, 2006; Beutler, 2003 and 2004). Engagement of these receptors initiates a signalling cascade stimulating cytokine release. Production of immune mediators such as tumour necrosis factor α (TNFα) and nitric oxide (NO), orchestrate the resultant immune response to clear bacterial infection. Cytokines including TNFα are potent stimulants of acute phase proteins production by liver hepatocyte cells (Baumann and Gauldie, 1994). Acute phase proteins, such as α_1-acid glycoprotein (AGP), act at the site of infection to limit tissue damage and promote tissue repair (Baumann and Gauldie, 1994). The severity of uterine bacterial contamination, as determined by the bacterial growth density, is correlated with the peripheral circulating concentrations of the acute phase protein AGP (Sheldon *et al.*, 2001). Furthermore, concentrations of AGP are particularly increased in those animals from which *E. coli* are isolated from the uterine lumen, but not other bacteria.

The bacterium *E. coli* is the most commonly isolated pathogen from the *post partum* uterus and, in the first few days after calving, dominates the uterine flora (Hussain *et al.*, 1990; Huszenicza *et al.*, 1999). Of greater importance is the infection with *E. coli*, which appears to increase the susceptibility of the endometrium to subsequent infection with *A. pyogenes* (Williams *et al.*, 2007); and *A. pyogenes* is associated with inflammation of the endometrium both *in vivo* and *in vitro* (Bonnett *et al.*, 1991; Miller *et al.*, 2007). These observations highlight the substantial impact *E. coli* has on *post partum* uterine health.

Effects of *E. coli* in the whole animal

Uterine bacterial infection or bacterial products suppress pituitary luteinising hormone (LH) secretion, and are associated with inhibition of folliculogenesis, decreased ovarian steroidogenesis and abnormal luteal phases (Peter *et al.*, 1989; Huszenicza *et al.*, 1999; Opsomer *et al.*, 2000; Mateus *et al.*, 2002 and 2003; Sheldon *et al.*, 2002; Williams *et al.*, 2007). In the field, infections are associated with changes in

Table 1 *Mean ± s.e. (a) follicle diameter and plasma oestradiol concentrations on days 11, 13 and 15* post partum *and (b) corpus luteum (CL) diameters and plasma progesterone concentrations on days 22, 24 and 26* post partum *in animals with high* v. *low uterine pathogen load*

(a)						
Day *post partum*	11		13		15	
Bacterial load	High	Low	High	Low	High	Low
Follicle diameter (mm)	9.1 ± 0.3	9.9 ± 0.7	10.9 ± 0.4	12.4 ± 0.7*	12.5 ± 0.5	13.8 ± 0.5*
Plasma E_2 (pg/ml)	1.1 ± 0.2	1.1 ± 0.3	1.0 ± 0.2	2.7 ± 1.1*	1.6 ± 0.3	2.0 ± 0.3**
(b)						
Day *post partum*	22		24		26	
Bacterial load	High	Low	High	Low	High	Low
CL diameter (mm)	19.4 ± 1.6	20.7 ± 0.7	18.8 ± 1.1	25.1 ± 0.6*	21.5 ± 1.1	26.7 ± 1.7*
Plasma P_4 (ng/ml)	1.5 ± 0.5	4.0 ± 1.6	1.6 ± 0.4	5.6 ± 1.6*	1.1 ± 0.6	5.0 ± 1.8*

Values differ between groups within day $*P < 0.05$, $**P < 0.01$.

luteal phase length and more cystic ovarian disease (Peter *et al.*, 1989; Opsomer *et al.*, 2000); and the first *post partum* ovarian follicle is smaller and produces less oestradiol in animals with higher numbers of bacteria in the *post partum* uterus (Sheldon *et al.*, 2002). More recently, we have shown that animals with high numbers of the recognised uterine pathogens including *E. coli* have retarded ovarian follicle growth, lower peripheral plasma concentrations of oestradiol and an inability to form a competent corpus luteum (CL) (Table 1; Williams *et al.*, 2007).

Lipopolysaccharide (LPS), the main pathogenic moiety of *E. coli,* is detectable in the uterus, peripheral plasma and ovarian follicular fluid of cows with *post partum* uterine infection (Peter *et al.*, 1989; Mateus *et al.*, 2003; Herath *et al.*, 2007; Williams *et al.*, 2007). Administration of intravenous LPS disrupts neuroendocrine activity and results in interference with the oestrous cycle. In heifers infused intrauterine with LPS, the pre-ovulatory LH surge was blocked, resulting in the formation of cystic follicles (Peter *et al.*, 1989). In sheep, hypothalamic GnRH secretion is suppressed, there is a reduction in pituitary responsiveness to GnRH and pulsatile LH secretion from the pituitary is inhibited, following the intravenous infusion of LPS (Williams *et al.*, 2001). However, uterine infection does not affect peripheral plasma FSH concentrations or the consequent emergence of a wave of growing follicles (Sheldon *et al.*, 2002; Williams *et al.*, 2007).

There are also localised effects of LPS in the ovary. Following administration of LPS, an inhibition in peripheral plasma oestradiol concentrations has been observed despite normal plasma LH concentrations (Xiao *et al.*, 1998; Battaglia *et al.*, 2000); and in heifers, LPS delays ovulation by interrupting the preovulatory oestradiol rise, thus delaying the LH surge (Suzuki *et al.*, 2001). These observations are likely to be biologically relevant as we have recently demonstrated the presence of LPS at concentrations of up to 0.8 μg/ml in the follicular fluid of cows with clinical uterine disease. Animals with subclinical disease had mid-range concentrations, while in normal animals, LPS was not detectable in follicular fluid (Herath *et al.*, 2007).

Cellular mechanisms

Besides being responsible for prostaglandins E_2 and $F_{2\alpha}$ (PGE, PGF) production, the uterine mucosa has a role in immunity. The endometrium provides a barrier against infection and an opportunity to detect bacteria by innate immune receptors recognising PAMPs (Beutler, 2004). In the human endometrium, nine TLRs are expressed at the mRNA level including TLR4 (Pioli *et al.*, 2004; Young *et al.*, 2004; Hirata *et al.*, 2005; Schaefer *et al.*, 2005). It is the role of TLR4 to detect LPS, although signalling through TLR4 also requires accessory molecules such as CD14, LBP and MD2 (Beutler, 2003 and 2004). Bovine uterine endometrial and stromal cells express TLR4 and the accessory molecules required for LPS recognition (Herath *et al.*, 2006). Following treatment with LPS, epithelial and stromal cells respond to *E. coli* or LPS treatment by secreting PGE or PGF, respectively, and this effect is abrogated in the presence of Polymixin B, a compound that binds to LPS, thus reducing the amount available to bind to MD2/TLR4. Thus, it appears that besides fulfilling an endocrine role, endometrial cells have an immune role in detecting and responding to bacterial pathogens (Herath *et al.*, 2006). Uterine tissue explants secrete more PGE than PGF in response to LPS (Herath *et al.*, 2006). It is well known that secretion of PGF, in response to endometrial oxytocin receptor activation, induces luteolysis and initiates the follicular phase of the ovarian cycle (Poyser, 1995). On the other hand, PGE has a luteotrophic role to help maintain the CL, particularly during pregnancy. Infection of the uterus with bacteria disrupts luteolysis and, between 1960 and 1980, short luteal phases or an 'inadequate CL' after calving were a substantial area of research interest. *Post partum* uterine infection and the release of PGF was thought to reduce luteal phase length. Although our recent data support the hypothesis that

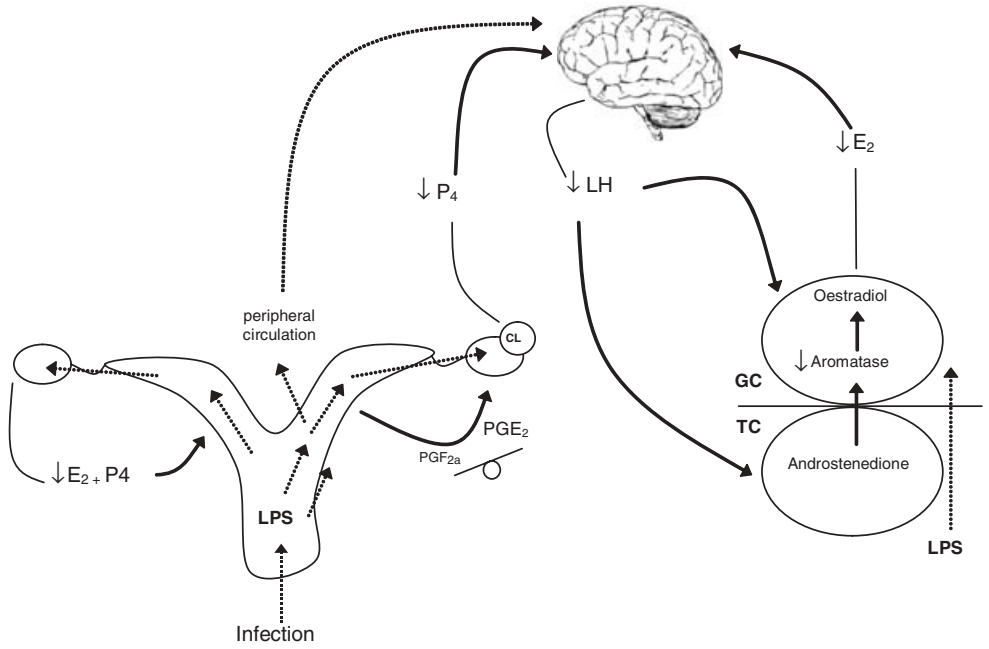

Figure 2 Summary of the effects of *E. coli* lipopolysaccharide (LPS) on the reproductive axis of the cow. *E. coli* ascend the reproductive tract and LPS is recognised by uterine endometrial and stromal cells. The prostaglandin production by these cells is modulated in response to LPS and this in turn may affect luteal phase length. At the ovary, theca cells produce androstenedione, which is aromatised to oestradiol by granulose cells (GC). GC can recognise LPS following which expression of aromatase enzyme is down-regulated and less oestradiol is produced. The corpus luteum produces less progesterone in animals with *E. coli* infection. The disruption of the hormonal milieu will have effects on the hypothalamus and pituitary, and also at the uterus. The presence of LPS in the peripheral circulation also disrupts LH production and release, which may have additional effects on ovarian theca and GC. Together, these observations may explain the infertility in cattle associated with *E. coli* infection of the uterus.

infection results in the formation of an inefficient CL (Williams *et al.*, 2007), it is perhaps now accepted that uterine disease is mainly associated with extended luteal phases. The altered prostaglandin secretion following *E. coli* LPS treatment of uterine cells provides further evidence that *E. coli* may play a role in perturbed ovarian cycles.

In addition to the likely effects on luteal function associated with changes in endometrial cell prostaglandin production, LPS can also directly affect ovarian cell function. Bovine granulosa cells isolated from selected and dominant follicles express the transcripts for TLR4 and the accessory molecules MD2 and CD14 required for LPS recognition (Herath *et al.*, 2007). When treated with LPS, oestradiol production by these cells is inhibited and mRNA for the aromatase enzyme, which catalyses the conversion of androstenedione to oestradiol, is down-regulated. Furthermore, the effect of LPS on oestradiol biosynthesis was not due to the inability of the cells to respond to oestradiol as transcripts for ERα were unchanged by LPS treatment and ERβ was not detected in control or treated granulosa cells, appearing to be down-regulated by culture. Neither was it due to the bioavailability of androstenedione substrate as the LPS treatment of ovarian theca cells did not affect androstenedione production (Herath *et al.*, 2007).

Summary and conclusions

The bacterium *E. coli* is the most common uterine pathogen early *post partum*, paving the way for *A. pyogenes* infection and clinical disease. Uterine infections with *E. coli* affect fertility at multiple levels, including the neuroendocrine axis and ovaries as well as in the uterus. At all of these sites, LPS from *E. coli* perturbs the delicate balance of hormone regulation with detrimental consequences for the reproductive function of the animal. Notably, the key endocrine cells of the reproductive tract express the receptor complex for recognition of LPS, and LPS modulates their endocrine function (Figure 2). Thus, treatment regimes tailored to disrupting the actions of *E. coli* in the early *post partum* period may diminish the detrimental effects of this pathogen. This would lead to improved animal welfare and an increase in the economic viability of dairy farming.

Acknowledgements

The authors would like to thank Dave Williams at the National Animal Disease Information Service for kindly providing data. We also thank The Wellcome Trust for funding part of the work. S Herath is supported by the BBSRC (F005129) and IM Sheldon is a BBSRC Research Development Fellow (BB/D02028X/1).

References

Akira S and Takeda K 2004. Toll-like receptor signalling. Nature Reviews Immunology 4, 499–511.

Akira S, Uematsu S and Takeuchi O 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.

Battaglia DF, Krasa HB, Padmanabhan V, Viguie C and Karsch FJ 2000. Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes. Biology of Reproduction 62, 45–53.

Baumann H and Gauldie J 1994. The acute phase response. Immunology Today 15, 74–80.

Beutler B 2003. Innate immune responses to microbial poisons: discovery and function of the Toll-like receptors. Annual Review of Pharmacology and Toxicology 43, 609–628.

Beutler B 2004. Innate immunity: an overview. Molecular Immunology 40, 845–859.

Bonnett B, Martin SW, Gannon VP, Miller RB and Etherington WG 1991. Endometrial biopsy in Holstein-Friesian dairy cows III: bacteriological analysis and correlations with histological findings. Canadian Journal of Veterinary Research 55, 168–173.

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

Dhaliwal GS, Murray RD and Woldehiwet Z 2001. Some aspects of immunology of the bovine uterus related to treatments for endometritis. Animal Reproduction Science 67, 135–152.

Donofrio G, Herath S, Sartori C, Cavirani S, Flammini C and Sheldon IM 2007. Bovine herpesvirus 4 (BoHV-4) is tropic for bovine endometrial cells and modulates endocrine function. Reproduction 134, 183–197.

Elliott L, McMahon KJ, Gier HT and Marion GB 1968. Uterus of the cow after parturition: bacterial content. American Journal of Veterinary Research 29, 77–81.

Farin PW, Ball L, Olson JD, Mortimer RG, Jones RL, Admey WS and McChesney AE 1989. Effect of *Actinomyces pyogenes* and Gram-negative bacteria on the development of bovine pyometra. Theriogenology 31, 979–989.

Griffin JFT, Hartigan PJ and Nunn WR 1974. Non-specific uterine infection and bovine fertility I: infection patterns and endometritis during the first seven weeks postpartum. Theriogenology 1, 91–106.

Herath S, Fischer DP, Werling D, Williams EJ, Lilly ST, Dobson H, Bryant CE and Sheldon IM 2006. Expression and function of Toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology 147, 562–570.

Herath S, Williams EJ, Lilly SR, Gilbert RO, Dobson H, Bryant CE and Sheldon IM 2007. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. Reproduction 134, 683–693.

Hirata T, Osuga Y, Hirota Y, Koga K, Yoshino O, Harada M, Morimoto C, Yano T, Nishii O, Tsutsumi O and Taketani Y 2005. Evidence for the presence of toll-like receptor 4 system in the human endometrium. Journal of Clinical Endocrinology and Metabolism 90, 548–556.

Hussain AMD, Daniel RCW and O'Boyle D 1990. Postpartum uterine flora following normal and abnormal puerperium in cows. Theriogenology 34, 291–302.

Huszenicza G, Fodor M, Gacs M, Kulcsar M, Dohmen MJW and Vamos M 1999. Uterine bacteriology, resumption of cyclic ovarian activity and fertility in postpartum cows kept in large-scale dairy herds. Reproduction in Domestic Animals 34, 237–245.

Laven RA, Biggadike HJ, Proven MJ, Halfacre S and Tickle LR 2000. Changes in vaginal microbiology associated with the use of progesterone-releasing intravaginal devices. The Veterinary Record 146, 760–762.

LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS and Johnson WH 2002a. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. Journal of Dairy Science 85, 2223–2236.

LeBlanc SJD, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS and Johnson WH 2002b. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. Journal of Dairy Science 85, 2237–2249.

Mateus L, Lopes da Costa LL, Bernardo F and Silva JR 2002. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reproduction in Domestic Animals 37, 31–35.

Mateus L, Lopes da Costa LL, Diniz P and Ziecik AJ 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.

Miller AN, Williams EJ, Sibley K, Herath S, Lane EA, Fishwick J, Nash DM, Rycroft AN, Dobson H, Bryant CE and Sheldon IM 2007. The effects of *Arcanobacterium pyogenes* on endometrial function in vitro, and on uterine and ovarian function in vivo. Theriogenology 68, 972–980.

Noakes DE, Till D and Smith GR 1989. Bovine uterine flora postpartum: a comparison of swabbing and biopsy. The Veterinary Record 124, 563–564.

Olson JD, Ball L, Mortimer RG, Farin PW, Adney WS and Huffman EM 1984. Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. American Journal of Veterinary Research 45, 2251–2255.

Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H and de Kruif A 2000. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 53, 841–857.

Peter AT, Bosu WT and DeDecker RJ 1989. Suppression of preovulatory luteinising hormone surges in heifers after intrauterine infusions of *E. coli* endotoxin. American Journal of Veterinary Research 50, 368–373.

Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR and Guyre PM 2004. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infection and Immunity 72, 5799–5806.

Poyser NL 1995. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukotrienes and Essential Fatty Acids 53, 147–195.

Ruder CA, Sasser RG, Williams RJ, Ely JK, Bull RC and Butler JE 1981. Uterine infections in the postpartum cow. II. Possible synergistic effect of *Fusobacterium necrophorum* and *Corynebacterium pyogenes*. Theriogenology 15, 573–580.

Schaefer TM, Fahey JV, Wright JA and Wira CR 2005. Innate immunity in the human female reproductive tract: Antiviral response of uterine epithelial cells to the TLR3 agonist Poly(I:C). Journal of Immunology 174, 992–1002.

Sheldon IM and Dobson H 1999. Postpartum uterine recovery and its influence on ovarian activity – a preliminary report. Cattle Practice 7, 409–410.

Sheldon IM, Noakes DE, Rycroft A and Dobson H 2001. Acute phase protein response to postpartum uterine bacterial contamination in cattle. The Veterinary Record 148, 172–175.

Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU and 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 IM and Dobson H 2004. Postpartum uterine health in cattle. Animal Reproduction Science 82–83, 295–306.

Suzuki C, Yoshioka K, Iwamura S and Hirose H 2001. Endotoxin induces delayed ovulation following endocrine aberration during the proestrous phase in Holstein heifers. Domestic Animal Endocrinology 20, 267–278.

Williams CY, Harris TG, Battaglia DF, Viquie C and Karsch FJ 2001. Endotoxin inhibits pituitary responsiveness to gonadotropin-releasing hormone. Endocrinology 142, 1915–1922.

Williams EJ, Fischer DP, Pfeiffer DU, England GE, Noakes DE, Dobson H and Sheldon IM 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63, 102–117.

Williams EJ, Fischer DP, Noakes DE, England GE, Rycroft A, Dobson H and Sheldon IM 2007. Uterine infection perturbs ovarian function in the postpartum dairy cow. Theriogenology 68, 549–559.

Xiao E, Xia-Zhang L, Barth A, Zhu J and Ferrin M 1998. Stress and the menstrual cycle: relevance of cycle quality in the short and long-term response to a 5-day endotoxin challenge during the follicular phase in the rhesus monkey. Journal of Clinical Endocrinology and Metabolism 83, 2454–2460.

Young SL, Lyddon TD, Jorgenson RL and Misfeldt ML 2004. Expression of Toll-like receptors in human endometrial epithelial cells and cell lines. American Journal of Reproductive Immunology 52, 67–73.