CHAPTER 25 - RETAINED FETAL MEMBRANES AND POSTPARTUM MICROBIAL DISEASES OF THE REPRODUCTIVE SYSTEM OF CATTLE

Professor Iain Martin Sheldon BVSc DCHP DBR PhD FRCVS

Swansea University Medical School, Swansea University, Swansea, SA2 8PP

i.m.sheldon@swansea.ac.uk + 44 1792 602709

CHAPTER 25 - RETAINED FETAL MEMBRANES AND POSTPARTUM MICROBIAL DISEASES OF THE REPRODUCTIVE SYSTEM OF CATTLE

1. INTRODUCTION

Microbial infections of the female genital tract often cause disease in cattle after calving. Postpartum microbial disease of the female genital tract is particularly common in *Bos taurus* dairy cattle, whereas disease is less common in beef breeds of *Bos taurus* and in *Bos indicus* cattle. Uterine disease is typified by the accumulation of pus in the genital tract, which often discharges from the vulva (Fig. 25.1).

Uterine disease is important in cattle because the consequences are subfertility and, in some cases, infertility. For example, bacterial infections of the uterus causing metritis lead to delayed insemination, reduced conception rates, and increased culling for failure to conceive. The cost to the dairy industry for treating metritis, reduced milk production, and replacing animals that fail to conceive at the farmer's desired time, is estimated to be EURO 1.4 billion every year in the EU (Sheldon et al 2009). This chapter sets out the definitions, causes, diagnosis, treatment, and management of postpartum non–specific uterine infectious diseases in cattle.

2. DEFINING THE DISEASES OF THE POSTPARTUM UTERUS

The definitions and brief details of non–specific postpartum infectious diseases are presented firstly, to provide a framework for understanding the aetiology, pathogenesis, diagnosis, and treatment of uterine disease. The typical incidence and duration of uterine disease is shown in Figure 25.2.

Retained fetal membranes

Retained fetal membranes (RFM) are defined as the failure of an animal to expel the fetal membranes, within 24 hours of the end of parturition. Retained placenta is an alternative name used for RFM. There is some variation in the literature about the duration of retention that defines the clinical disorder. Some prefer to define retention as being for12 hours, but the timing is arbitrary and most normal cows expel the fetal membranes within a few hours of parturition. The incidence of RFM varies amongst herds, but is typically 5 to 10% of animals. The importance of RFM is that they are associated with reduced milk yield and an increased risk of metritis.

Metritis

Metritis is most common within 10 days of parturition. Metritis is characterized by an enlarged uterus and a watery red-brown fluid, to viscous off-white purulent, uterine discharge, which often has a fetid odour (Sheldon et al 2006). The incidence of metritis varies between breed, country and herd, but in a study of the records from 97,318 cows in the USA, the lactation incidence of metritis, including RFM, was 21% (Zwald et al 2004). However, in some studies the incidence is as high as 40% of the herd. The associated clinical signs are used to classify the severity of disease, which varies from unapparent disease to fatal toxaemic metritis.

Clinical endometritis

Clinical endometritis is defined as the presence of a purulent uterine discharge detectable in the vagina of cattle 21 days or more postpartum, or a mucopurulent discharge detectable in the vagina after 26 days postpartum (Sheldon et al 2006). The incidence of clinical endometritis is around 10 to 20%, with variation between breed, country and herd; a typical study reported that 16.9% of 1,865 dairy cows were affected in Canada (LeBlanc et al

2002a). A simple grading system, based on the character of the vaginal mucus, is readily used to evaluate cows with clinical endometritis, and is prognostic for the likely outcome of treatment (Sheldon et al 2006).

Subclinical endometritis

Subclinical endometritis is characterized by inflammation of the endometrium, in the absence of clinical signs of endometritis, which results in a significant reduction in reproductive performance. The inflammation is presumably associated with recovery of the tissues after metritis and clinical endometritis, trauma, or other non-microbial disease. Definition of subclinical disease is currently dependent on cytological analysis of samples that are collected from the surface of the endometrium by flushing the uterine lumen, or by the use of an endometrial cytobrush. The proportion of animals affected varies widely amongst studies, ranging from about 11% to more than 40% of animals (Wagener et al 2017). Diagnosis relies on the proportion of endometrial cells that contain more than a defined proportion of neutrophils at specific times after parturition.

Pyometra

Pyometra is characterized by the accumulation of purulent or mucopurulent material within the uterine lumen, causing distension of the uterus, in the presence of a closed cervix and a functional corpus luteum. Postpartum pyometra is uncommon, and is thought to be caused by the growth of bacteria within the uterine lumen after the formation of the first corpus luteum (Noakes et al 1990).

Inflammation in other parts of the reproductive system

Oophoritis, cervicitis, and vaginitis are defined as inflammation of the oviduct, cervix, and vagina, respectively. The signs are typical of the cardinal signs of inflammation: redness,

heat, swelling, pain, and loss of function. Whilst the incidence of oophoritis is often unclear, cervicitis and vaginitis are relatively common.

3. THE POSTPARTUM PERIOD

The main reproductive events that are required after parturition to foster optimal future fertility are: prompt expulsion of the fetal membranes, followed by uterine involution; timely return of ovarian cyclical activity; regeneration of the endometrium; and, efficient control of bacteria in the uterus.

3.1 EXPULSION OF THE FETAL MEMBRANES

The primary mechanism by which the caruncle and cotyledon of the placentome anchor to each other is the interdigitating fetal and maternal villi, and the presence of adhesive proteins at the feto-maternal interface. The surrounding and engulfing of the caruncle by the cotyledon provides physical anchoring (Eiler & Fecteau 2007). Detachment of the membranes requires release of the cotyledon from the caruncle by disruption of the physical and cellular arrangements that are present during pregnancy. There appear to be three main factors involved in the separation and expulsion of the fetal membranes:

- Maturation of the placenta, including biochemical and immunological changes at the fetal-maternal junction.
- Exsanguination of the fetal side of the placenta, when the umbilicus ruptures, which causes collapse and shrinkage of the trophectodermal villi and their physical separation from the maternal crypts.
- Uterine contractions, which aid in the exsanguination of the fetal side of the placenta, and aid in the physical separation of the placenta by distorting the shape of the

placentomes, thereby causing ‘unbuttoning’ of the cotyledon from the caruncle, and attempting to expel the dependent and detached parts of the fetal membranes.

Preparatory changes for the release of the fetal membranes are not confined to the peripartum period**,** but begin during the late stage of gestation (Grunert 1986). These changes appear to be largely dependent upon a critical sequence of changes in the concentrations of progesterone and oestrogen**,** and the abundance of their cognate steroid receptors (Agthe & Kolm 1975; Chew et al 1977; Boos et al 2000). For example, the presence of subnormal oestrogen and oestrogen receptor concentrations may lead to RFM.

Physical changes that occur in association with the release of the fetal membranes include: flattening of the maternal crypt epithelium; and, in the last week of gestation, a reduction in the number of binucleate cells in the trophectoderm (Bjorkman 1969; Gross & Williams 1988). Relaxation of the superficial layers of the cotyledon is induced by proteases and collagenase, which break down the type I and III collagen that provides the tensile strength to the caruncular crypts (Boos et al 2003; Eiler & Fecteau 2007). Likewise, collagenases also disrupt the anchoring attachments between the fetal and maternal villi. Failure of proteolysis is considered to be a key factor in failure of membrane detachment although, curiously, there is no difference in the distribution of types I, III or IV collagen in cows that do, or do not, retain their membranes (Sarges et al 1998; Boos et al 2003). Inhibition of collagenase activity by exogenous glucocorticoids appears to be a major contributing factor to the retention of the membranes associated with the premature induction of parturition with glucocorticoids.

Changes in the composition of the glue line adhesive proteins, which are present between the cotyledonary and caruncular epithelium, are of importance in the process of placental detachment. Proteoglycans are intercellular adhesion proteins that are present at the feto-maternal interface: there is evidence that the activity of the enzyme beta-*N*-acetyl-

glucosaminidase, which is responsible for the metabolism of proteoglycans, is reduced in cows with RFM (Kankofer et al 2000).

Changes in synthesis and metabolism of prostaglandins E and F are also associated with the process of detachment. Circulating concentrations of PGFM (13, 14-dihydro, 15-keto PGF_{2alpha}, a stable metabolite of PGF_{2alpha}) are higher, and those of PGEM (a metabolite of PGE_2) are lower, in normal cows than in those with RFM (Wischral et al 2001b). Placentomes from cows with RFM produce less PGF_{2alpha} and more PGE_2 *in vitro*, than do those from normal cows (Gross et al 1987), while there is also interconversion of PGF to PGE in cows with RFM (Kankofer et al 1994). Moreover, PGE secretion continues after parturition, as the retained membranes retain a degree of viability for some days (Eiler & Fecteau 2007). The PGF secretion in placental tissue from normal cows is responsive to stimulation by oxytocin, whereas that from animals with RFM is not (Slama et al 1994). Deficiency of PGF synthesis is likely to be the consequence of a lack of maternal oestrogen synthesis, resulting in a failure of accumulation of prostaglandin precursors, arachidonic and linoleic acids, in placental tissues (Wischral et al 2001a; Wischral et al 2001b).

Failure of oestrogen synthesis in the placenta is associated with inadequate placental or fetal antioxidant activity, in terms of peroxide damage to lipids, protein and nucleic acids (Wischral et al 2001b; Kankofer & Guz 2003; Gupta et al 2005). It is probably for this reason that dietary deficiencies of antioxidants such as selenium and vitamin E predispose to RFM (Bourne et al 2007). Excess PGE synthesis may be a response to maternal stress and oxidative injury, or a response to infection (Wischral et al 2001b; Herath et al 2009a). As PGE is a potent immunosuppressive agent, PGE secretion may adversely affect the immunological environment of the postpartum uterus.

There is increasing evidence that the immune system plays a pivotal role in detachment of the membranes (Heuwieser & Grunert 1987; Peters & Laven 1996). Neutrophil, macrophage and

T lymphocyte invasion of the placenta occurs around the time of detachment, and there is evidence that deficiencies in the activities of each of these cells occurs in cows with RFM (Gunnink 1984; Kimura et al 2002; Miyoshi et al 2002). Cows with RFM also produce reduced quantities of the neutrophil attractant interleukin-8 (IL-8, also known as CXCL8) and other inflammatory mediators (Slama et al 1993; Miyoshi et al 2002; Davies et al 2004). Moreover, in cows with RFM, the activity of the neutrophil anti-endotoxin protein, acyloxyacyl hydrolase, is reduced (Dosogne et al 1999). This may put cows at greater risks from the effects of the endotoxin from bacteria that proliferate in the uterus postpartum. Finally, as leukocytes contribute to the collagenase activity of the parturient uterus, reduced leukocyte activity in cows with RFM may be a contributor to the reduction in collagen proteolysis in these animals.

The role of the cells of the immune system may be due in part to the role of MHC class I antigens in the process of placental detachment. It has been postulated that maternal recognition of fetal MHC antigens provides an initial trigger for placental separation, since MHC class I compatibility between a cow and her fetus has been associated with an increased incidence of placental retention (Davies et al 2004; Eiler & Fecteau 2007). It has been suggested that this indicates that alloreactivity to the fetal membranes by the dam is a significant event in the detachment of the placenta (Joosten et al 1991).

3.2 UTERINE INVOLUTION

Involution is the term used to describe the physical reduction in size of the uterus and cervix after parturition (also see Chapter 7). Involution is thought to be driven by uterine muscular contractions, turnover of the extracellular matrix, necrosis and sloughing of the uterine caruncles, and regeneration of the endometrium (Gier & Marion 1968). Inserting a hand through the cervix is usually difficult in normal animals by 24 hours after parturition, and the

cervix often only admits two fingers by 96 hours postpartum. By about 3 weeks post partum, the entire genital tract is usually palpable per rectum. The previously gravid horn can still be identified because it is wider and longer than the previously non-gravid uterine horn. In parallel with the changes in dimensions, there is a remarkable change in the tissues, with catabolism, repair, and regeneration. In the first 30 days postpartum, the weight of the uterus decreases from about 9 kg at parturition to about 1 kg (Gier & Marion 1968).

The progress of uterine involution can be monitored by repeated estimation of the size of the uterus after parturition (Fig. 25.3). The most practical techniques to evaluate the size of the uterus are transrectal palpation and transrectal ultrasonography (Okano & Tomizuka 1987; Sheldon et al 2000; Sheldon et al 2003). It should be noted that dimensions estimated by transrectal palpation are often about 1 to 2 cm greater than ultrasound measurements; presumably, because operators include the thickness of the rectal wall when using the former to estimate the diameter of the uterus. The size discrepancy between the previously gravid and non-gravid uterine horns is present up to 4 weeks postpartum (Okano & Tomizuka 1987; Tian & Noakes 1991b; Risco et al 1994). However, the changes in uterine horn diameter are almost imperceptible beyond 4 weeks postpartum, and are probably complete by 6 weeks.

The time to completion of uterine involution is often reported in the literature, but this endpoint is rarely used in clinical practice. On the other hand, factors that delay uterine involution are important in clinical practice because delayed involution of the uterus is associated with subsequent impaired fertility (Fonseca et al 1983). The factors that delay involution include dystocia, hypocalcaemia, RFM, metritis, and endometritis.

3.3 REGENERATION OF THE ENDOMETRIUM

The epithelium of the endometrium is often damaged during parturition, and inflammation, remodelling and regeneration of the endometrium is part of the physiology of the postpartum

period (Gier & Marion 1968; Wagner & Hansel 1969; Tian & Noakes 1991a). The caruncular tissue undergoes necrosis of the stratum compactum, and by 12 to 14 days post partum the tissue sloughs as part of the physiological process of the puerperium (Fig. 7.3). After the caruncular tissue is sloughed, there is re-epithelialisation of the caruncles, advancing centripetally from the edges of the caruncle (Wagner & Hansel 1969). Loss of the epithelium is thought to be more likely in animals where there has been damage during parturition or by microbial infection. As re-epithelialisation progresses, the initially flattened epithelial cells develop their columnar appearance, typical of normal endometrium.

Neutrophils and lymphoid aggregates are common in the tissue during the repair of the endometrium, and they are likely to be part of the immune response to tissue damage and infection. Tissue repair is obviously important to counter the negative effects of trauma, and as part of the healing process. Genes involved in extracellular matrix homeostasis, such as the matrix metallopeptidases *MMP1, MMP3, MMP9* and *MMP13*, are differentially regulated in cows with severe negative energy balance compared with more normal animals (Wathes et al 2009). In most cows re-epithelialisation of the caruncles and repair of the endometrial tissue to its normal architecture occurs by about 20 to 30 days post partum (Wagner & Hansel 1969; Archbald et al 1972)

3.4 RETURN OF OVARIAN CYCLIC ACTIVITY

The return of ovarian cyclic activity after parturition requires a coordinated programme of physiological events, involving the hypothalamus, pituitary, ovary, and uterus. Genetic and environmental factors influence these events, and often lead to sub-fertility; for details of return of ovarian cyclic activity consult Chapter X. Briefly, during pregnancy, there are high concentrations of oestradiol and progesterone in the peripheral circulation. Within a few days of parturition, the circulating steroid hormone concentrations decrease to basal values. About

7 days post partum there is an increase in plasma FSH concentration, with subsequent recurrent increases in FSH concentrations every 7 to 10 days (Crowe et al 1998; Duffy et al 2000). The first postpartum dominant follicle, characterised by an internal diameter > 8 mm, is usually selected about 10 days after parturition. This dominant follicle may ovulate to form the first postpartum corpus luteum, or the dominant follicle may undergo atresia, with subsequent emergence of a second dominant follicle, or the dominant follicle may abnormally persist as a follicular cyst (Savio et al 1990; Stagg et al 1995; Beam & Butler 1997). The fate of the first postpartum dominant follicle depends on LH pulse frequency, and failure to ovulate is usually a consequence of inadequate LH pulse frequency and reduced ovarian follicle oestradiol (Beam & Butler 1999; Duffy et al 2000; Cheong et al 2016). In dairy cattle, metabolic stress - most often negative energy balance - is the main cause of reduced LH pulse frequency, although a range of other factors can impact ovarian cyclic activity (Cheong et al 2016).

Of note when examining animals in the postpartum period, is that the first dominant follicle is about twice as likely to be in the ovary contralateral to the previously gravid uterine horn. This imbalance in dominant follicle selection is still evident for the second dominant follicle, but the effect of the uterus on the distribution of ovarian dominant follicles is no longer evident beyond the third dominant follicle (Sheldon et al 2000).

3.5 CONTROL OF MICROBES IN THE UTERUS

Understanding of which microbes are found in the female genital tract has changed markedly over the last 20 years. Previously it was thought that the uterus was sterile during pregnancy, and that non-specific bacteria from the animal and the environment contaminated the uterus, during and after parturition. However, the uterus has a microbiota and is not sterile. Furthermore, whilst non-specific microbes from the environment contaminate the uterine

lumen during and after parturition, specific microbes that are adapted to the endometrium are key to developing postpartum uterine disease. Outside the puerperium, other specific pathogens, such as *Salmonella and Campylobacter* species of bacteria, and Infectious Bovine Rhinotracheitis and Bovine Virus Diarrhoea viruses, cause abortion, endometritis and vaginitis (see Chapter 26).

It is widely accepted that most organs of animals, such as the gut, skin and vagina, have a community of commensal, symbiotic and pathogenic microorganisms; often termed the microbiota. There is emerging evidence that the uterus also has a microbiota, and is not sterile. Studies using fluorescent probes to image bacteria and 16S ribosomal RNA gene sequencing of endometrium, provide evidence that there is a sparse microbiota in the uterus, even during pregnancy (Karstrup et al 2017; Moore et al 2017). The bacteria identified in these studies include *Trueperella, Fusobacteria and Prevotella* species. Whilst the finding of a microbiota in the uterus is important, the abundance of microbes in the uterine microbiota is substantially less than for the gut, skin or vaginal microbiota. Furthermore, the abundance of bacteria in the uterine microbiota, outside the puerperium, is also a small fraction of those present in animals with postpartum uterine disease. Whilst many of the bacteria found in the postpartum uterus likely derive from the vagina, skin, gut and the environment, it is possible that after parturition there is a bloom in the growth of pathogenic bacteria present in the uterus before parturition, which also help establish disease.

3.5.1 Pathogens in the postpartum uterus

Multiple species of bacteria are found in the postpartum uterus of cattle. However, as with most infections, disease depends on the balance amongst the microbes, the environment, and host defences. Most beef breeds and *Bos indicus* cattle are exposed to the same microbes as dairy cattle, yet clinical disease of the uterus is more common in dairy cows.

Postpartum uterine disease is principally associated with the isolation of pathogenic bacteria from the uterus. It is important to note that, unlike many specific clinical diseases, the metritis complex is polymicrobial; rather than that found in other tissues where there is often one-microbe-one-disease. Furthermore, an important concept is that all normal healthy animals have a substantial load of bacteria in the uterus after normal partition. As with many infections, the development of disease depends on the pathogens overcoming host defences. The microbial community in the uterus during the healthy or diseased postpartum period often fluctuates, with cycles of infection, elimination, and re-infection with bacteria. The bacteria most commonly isolated from animals with uterine disease are *Escherichia coli, Trueperella pyogenes, Fusobacterium necrophorum,* and *Prevotella* and *Bacteroides* species (Elliott et al 1968; Griffin et al 1974a; Huszenicza et al 1991; Noakes et al 1991). However, many additional species of bacteria are considered as pathogens, or potential pathogens, of the bovine uterus (Table 25.1).

The importance of pathogens for the postpartum uterus is supported by the ability to develop animal models of endometritis. The role of *E. coli* and *T. pyogenes* is highlighted by infusing *E. coli* and *T. pyogenes* into the uterus of naïve cows to create animal models of endometritis (Ayliffe & Noakes 1982; Amos et al 2014). Infusion of both pathogens more reliably generates an animal model when using naïve heifers, but infusion of either pathogen also generates disease in cows, with often the second pathogen appearing in the uterine discharge within a week of starting the infusion of the first pathogen. When generating the animal model for endometritis, two additional features will help establish the model. The first is the use of exogenous progesterone, which may suppress immune defences. The second is that disease is more likely if the endometrium is gently debrided immediately prior to infusion of the bacterial suspension. Presumably, debridement of the surface of the epithelium disrupts the protective epithelium, allowing the pathogens to adhere and invade the underlying stroma.

The importance of specific pathogens causing metritis is also supported by reports of vaccines containing components of *E. coli*, *F. necrophorum* and/or *T. pyogenes* that protect animals against metritis (Nolte et al 2001; Machado et al 2014). Some of the pathogens appear to be adapted to the endometrium, or are specific to the bovine endometrium. For example, novel strains of *E. coli* have been isolated from the uterus of animals with uterine disease (Sheldon et al 2010). These endometrial pathogenic *E. coli* (EnPEC) are more adherent and invasive for endometrial epithelial and stromal cells than *E. coli* isolated from the uterus of clinically unaffected animals, they stimulate endometrial inflammation, and they establish disease in animal models.

Trueperella pyogenes is the pathogen most associated with the severity of endometrial pathology and clinical disease (Bonnett et al 1991a; Westermann et al 2010). The link between *T. pyogenes* and disease probably depends on the cholesterol-dependent cytolysin pyolysin secreted by *T. pyogenes*. Pyolysin causes cytolysis of a wide range of cells, but endometrial stromal cells are particularly sensitive to pyolysin (Amos et al 2014; Preta et al 2015). Pyolysin binds cholesterol-rich domains in the plasma membrane of endometrial cells to form pores in the plasma membrane, which lead to osmotic cell death (Amos et al 2014; Preta et al 2015). Endometrial stromal cells are probably particularly sensitive to pyolysin because they have abundant cholesterol. The stromal sensitivity to pyolysin may also explain how *T. pyogenes* switches from being a commensal bacterium in the uterus when the epithelium is intact, to causing uterine pathology once the epithelium is breached during and after parturition; thereby allowing bacteria to access the stroma (Amos et al 2014). Although pyolysin does not stimulate inflammatory responses, *T. pyogenes* does stimulate inflammation, presumably via endometrial cells recognizing bacterial lipopeptides (Borges et al 2012; Turner et al 2014).

There is evidence for co-infections that foster disease. *T. pyogenes, F. necrophorum* and *Prevotella* act synergistically to increase the likelihood and the severity of endometritis (Ruder et al 1981; Olson et al 1984). More recent studies, using aerobic and anaerobic culture, confirm the importance of *E. coli*, *T. pyogenes* and anaerobic bacteria (Dohmen et al 2000; Sheldon et al 2002b; Westermann et al 2010). Interestingly there is evidence for an association between *E. coli* and then colonisation by *T. pyogenes* (Williams et al 2007).

Metagenomics techniques have found associations between uterine disease and bacteria that are not readily cultured by standard techniques (Machado et al 2012; Santos & Bicalho 2012; Peng et al 2013; Knudsen et al 2015; Wagener et al 2015). Whilst some of the studies find *E. coli*, *T. pyogenes* and the expected anaerobic bacteria, others report finding *Bacteroidetes* and *Firmicutes*. There remains a gap in understanding about how the latter "unculture able" bacteria contribute to the pathogenesis of uterine disease. However, a consistent finding among most microbiology studies, is that anaerobic bacteria are more abundant in the diseased endometrium than in healthy uteri. Perhaps this is not surprising, as the endometrium is a microaerophilic environment, with tissue damage likely reducing the oxygen tension further. Taken together, the evidence is that *E. coli, T. pyogenes* and anaerobic bacteria are probably the main pathogens causing the clinical signs of uterine disease, but that other pathogens may contribute to postpartum uterine disease.

Viruses and other microbes are rarely associated with non-specific metritis or endometritis in cattle after parturition. One exception is Bovine Herpesvirus 4, which has been linked to sporadic cases or outbreaks of metritis or endometritis in dairy herds in several countries (Frazier et al 2001; Monge et al 2006). The link between Bovine Herpesvirus 4 and uterine disease may be explained by tropism of the virus for endometrial stromal cells, and endometrial inflammation associated with viral infection (Donofrio et al 2007).

3.5.2 Resilience to infections

The ability to counter pathogenic microbes depends on an organism's *immunity* and *resilience* (Schneider & Ayres 2008; Medzhitov et al 2012). *Immunity* is the ability to resist microbes, and limit the pathogen burden by killing the microbes. *Resilience* is the ability to limit the impact of pathogens on health, by tolerating a given microbial burden (Raberg et al 2007). Failure of both immunity and resilience impair health. Much is known about immunity in the postpartum genital tract. However, resilience is increasingly thought to be important for maintaining animal health (Schneider & Ayres 2008; Medzhitov et al 2012).

Fifty years ago, dairy cows tolerated the bloom of bacteria found in the uterus after parturition, and they did not develop disease. Now, 20 to 40% cattle develop uterine disease each year (Sheldon et al 2009) (Fig. 25.2). This increased incidence of disease coincides with intensification of farming, and breeding dairy cattle for increased milk yields over the last 50 years. Modern cows simply cannot consume enough food to meet the metabolic demand of producing > 35 L milk/d. Typical metabolic energy requirements have risen from about 100 MJ/d to > 240 MJ/d (Chagas et al 2007; Kerestes et al 2009). Consequently, these animals have reduced blood glucose, glutamine, and metabolic hormones (Doepel et al 2006; Chagas et al 2007). It is not clear if this increased postpartum uterine disease is a failure of immunity and/or resilience in cows. However, by comparing the health status of organisms with their pathogen load in populations, it is possible to disentangle the relative contributions of immunity (Fig. 25.4A) and resilience (Fig. 25.4B) (Raberg et al 2007). Data from a dairy herd of cows with similar genotype, where milk production, uterine health and bacterial load were recorded (Sheldon et al 2002b), were used to determine if the metabolic stress of high milk yields in early lactation perturbed uterine immunity or resilience. Postpartum cows under the metabolic stress of producing > 35 litres milk/day had worse endometrial health with the same uterine bacterial load (Fig. 25.4C), implying that metabolic stress perturbs

uterine resilience. Thus, factors that affect the resilience of the uterus are likely to be at least as important as immunity. However, it must be remembered that once pathogens overcome the mechanisms of resilience, then immunity and inflammation have a central role in the progression and the resolution of disease.

Physical barriers are an important feature allowing animals to tolerate pathogens (Fig. 25.5). The obvious anatomical barriers include the vulva, vagina and cervix, which physically counter microbial infections ascending the reproductive tract to the uterus. However, during and after parturition, the vulva, vagina and cervix are dilated and so they are not effective barriers to ascending infections of the genital tract, or iatrogenic contamination of the uterus with bacteria.

In humans, *Lactobacilli* species in the vaginal microbiota provide some protection against pathogens, probably by competing for nutrients and generating an acid environment (Ravel et al 2011). However the human is unusual in this respect, with cattle having a fraction of the lactobacilli of humans, and a less acid vaginal environment (Miller et al 2016). Whether manipulating the resident microflora of the vagina, or the pH of the vagina, to counter pathogens and limit uterine disease is in cattle is yet to be verified by clinical trials, but there is evidence that infusion of *Lactobacilli* into the vagina may have a beneficial effect (Deng et al 2014).

Once pathogens enter the uterus, the ability to tolerate them is dependent on the resilience of the uterine tissues and cells. Most mucosal diseases develop after the epithelium is breached, allowing infection of the stroma. The endometrium is the mucosa of the uterus, and most of the pathology of uterine disease is in the stroma. The first line of defence is the physical barrier of the single columnar epithelium of the endometrium. The tight junctions between epithelial cells separate the apical and basolateral components of the endometrium, and this barrier counters microbial invasion from the uterine lumen. Epithelial cells are also more

robust than stromal cells. For example, endometrial epithelial cells are more resistant than stromal cells to pore-formation, and cell death, when exposed to *T. pyogenes* or the cholesterol-dependent cytolysin, pyolysin (Amos et al 2014). This resilience of the epithelial cells may be because they contain less cholesterol in their plasma membrane than stromal cells. Indeed, reducing cellular cholesterol using methyl-beta-cyclodextrins protects stromal cells against pyolysin (Amos et al 2014).

The mucus layer on the apical surface of epithelia provides an additional obstacle to microbes. The secretion and character of mucus in the genital tract is under the control of ovarian steroids, and during oestrus mucus flow increases considerably. In contrast, the cervical mucus plug provides a physical barrier to ascending infections during gestation. The expression of MUC1 is induced, when endometrial epithelial cells are treated with endotoxin from *E. coli* (Davies et al 2008); and in cytobrush samples of the endometrial epithelium of cows with uterine disease (Kasimanickam et al 2014). Lysozyme expression is also increased in the endometrium of cows with uterine inflammation, and lysozyme digests the peptidoglycans found in the cell walls of bacteria (Hoelker et al 2012). Antimicrobial peptides and mucosal glycoproteins cover the mucosa of the vagina, cervix and endometrium, where they also neutralise bacteria and prevent them reaching the plasma membrane of the epithelia. The principal cysteine-rich, cationic, antimicrobial peptides expressed in the bovine endometrium include β-defensins, lingual antimicrobial peptide (LAP) and tracheal antimicrobial peptide (TAP); several gene transcripts for antimicrobial peptides are more abundant in the face of microbial challenge (Davies et al 2008; Chapwanya et al 2009).

Whilst physical barriers and secreted proteins are important for resilience to microbes in the female genital tract, there are some caveats. First, microbes have evolved counter-measures and strategies to avoid many host defences. For example, some bacteria produce enzymes that lyse mucus to penetrate the protective layer, and many bacteria secrete proteases that can

disrupt peptides and proteins produced by the host (Baxt et al 2013). Second, immunity is principally responsible for initiating rapid inflammatory responses to resist microbial infections (Takeuchi & Akira 2010).

3.5.3 Resistance to infections

Once pathogens overcome the resilience of the uterine tissue, innate immunity and adaptive immunity help resist infections by killing pathogens. Furthermore, much of the endometrial pathological change is a consequence of the inflammation associated with the immune response. Innate immunity is an immediate, non-specific defence system against pathogens, which does not depend on prior exposure to microbes. Adaptive immunity is dependent on antigen-specific receptors and the response may take several days, depending on whether there has been prior exposure to the antigen. Several studies have examined the inflammatory response to infection in the endometrium. The expression of several cytokines and chemokines are increased in diseased endometrium, including *IL1A, IL1B, IL6, CXCL8, CXCL5, TLR4* and *PGES* (Table 25.2) (Sheldon 2014). Animals with clinical endometritis also have higher concentrations of IL-1α, IL-1β and IL-6 in uterine fluid than normal animals (Healy et al 2014; Kim et al 2014).

Innate immunity

Innate immunity provides immediate non-specific defence responses against bacteria and tissue damage (Takeuchi & Akira 2010; Moresco et al 2011). The result is uterine inflammation, influx of immune cells, changes in metabolism, induction of acute phase proteins, activation of the complement system and other local defence systems. The aim of these responses is to return the genital tract to a state of homeostasis.

Acute phase proteins are synthesised in the liver, often in response to increased peripheral plasma concentrations of cytokines such as IL-6, and they have important functions in

restoring homeostasis after infection or tissue damage (Baumann & Gauldie 1994). These functions include haemostasis typified by the action of fibrinogen, anti-microbial effects, and the attraction and activation of phagocytes. The severity of bacterial contamination in the postpartum uterus is associated with the concentrations of acute phase proteins in peripheral plasma, including α_1-acid glycoprotein, haptoglobin and ceruloplasmin, particularly in the presence of uterine infections with *E. coli* and *T. pyogenes* (Smith et al 1998b; Sheldon et al 2001). However, the acute phase response is not only initiated by infection**,** but also by trauma (Baumann & Gauldie 1994). Indeed, parturition is a traumatic event**,** and as uterine involution progresses**,** there is a decrease in the concentrations of α_1-acid glycoprotein, haptoglobin and ceruloplasmin (Sheldon et al 2001). The local expression of genes encoding acute phase proteins has also been noted in the uterus and ovary, which may be of interest because they may provide further localized protection (Chapwanya et al 2009; Fischer et al 2010; Lecchi et al 2012). However, it is possible that the more usual and more abundant hepatic production of acute phase proteins will be more relevant *in vivo*.

Antimicrobial S100 calgranulin proteins and cathelicidin are also more abundant in the endometrium of animals with uterine disease (Wathes et al 2009; Swangchan-Uthai et al 2013; Ledgard et al 2015). The proteins are abundant in neutrophils**,** and are associated with antimicrobial activity, attracting neutrophils to sites of inflammation, and stimulating neutrophil activity.

Complement also provides defence against microbial infections. The complement system comprises about 20 proteins, which generate lytic complexes at the surface of pathogens, and provide opsonins such as C1b and C3b, which interact with cell surface receptors to promote phagocytosis by neutrophils and macrophages. To avoid complement actions on tissue cells, the latter possess complement regulatory proteins such as CD46, CD55 and CD59 (Gasque 2004). Components of the complement system, such as the genes *C1QA, C1QB, C1QC, C3*

andC8, are differentially expressed in the endometrium of diseased postpartum cows with more severe negative energy balance (Wathes et al 2009).

Whilst the proteins that counter microbes are important, much of the innate immune defence of the uterus depends on the detection of microbes by cellular- pattern recognition receptors. Host cells possess pattern recognition receptors that bind pathogen-associated molecular patterns (PAMPs). These PAMPs are usually highly conserved molecules found in prokaryotes but not eukaryotes, and include lipopeptides, lipopolysaccharides, and microbial RNA and DNA molecules. The concept is that cellular receptors, such as Toll-like Receptors (TLRs) and NOD-like Receptors (NLRs), bind PAMPs (Takeuchi & Akira 2010; Moresco et al 2011). For example, TLR2 on cells binds to bacterial lipopeptides, whilst TLR4 binds to endotoxin, which is the lipopolysaccharide cell-wall component of Gram-negative bacteria, such as *E. coli*. In general, the TLRs that sense bacteria are in the plasma membrane (TLR1, TLR2, TLR4, TLR5 and TLR6). Binding of PAMPs to TLRs activates intracellular signalling pathways that result in the production of inflammatory mediators IL-1β, IL-6, IL-8 and prostaglandin E_2. These inflammatory mediators attract and activate immune cells such as neutrophils and macrophages to clear the bacteria (Takeuchi & Akira 2010; Moresco et al 2011). Other pattern recognition receptors are intracellular, and principally detect microbes that invade cells. For example, the release of mature IL-1β is dependent on activation of the 'multiprotein inflammasome complex', often containing NLRP3 (nucleotide-binding domain and leucine rich repeat pyrin 3 domain), leading to caspase-1 activation to cleave pro-IL-1β to mature protein (Schroder & Tschopp 2010).

The receptors used by the innate immune system are principally expressed by hematopoietic cells such as macrophages, dendritic cells, and neutrophils (Takeuchi & Akira 2010; Moresco et al 2011). However, bovine endometrial epithelial and stromal cells also express most *TLR* genes (Davies et al 2008). Furthermore, these epithelial and stromal cells respond to bacteria,

endotoxin, and lipopeptides, via TLR1, TLR2, TLR4 and TLR6 by secreting IL-1β, IL-6, IL-8 and prostaglandin E_2 (Herath et al 2006; Cronin et al 2012; Turner et al 2014). Indeed, endotoxin switches endometrial epithelial cell prostaglandin secretion away from the physiological F series to PGE, and this switch is not overcome by administering oxytocin to mimic the luteolytic signal (Herath et al 2009a).

The inflammatory cytokines, such as IL-6, bind their cognate receptors leading to increased permeability of vasculature, and secretion of antimicrobial peptides, eicosanoids and reactive oxygen species. Cytokines in the peripheral plasma drive systemic inflammatory responses distant to the site of infection; these include pyrexia, generalised vasodilation, and release of acute phase proteins from hepatocytes. Chemokines, such as IL-8, attract and activate neutrophils and monocytes to the site of infection. However, excessive inflammation leads to immunopathology or septic shock, and so innate immunity is carefully calibrated. A series of checks and balances are in place to scale inflammation to meet the level of microbial threat during the progression of an infection (Fig. 25.6), and to limit inflammation when infections are cleared (Blander & Sander 2012). One example in the bovine endometrium, is the role of the intracellular signalling molecule STAT3 to regulate the secretion of IL-6 and IL-8 in stromal cells (Cronin et al 2016). Another example is the apical secretion of IL-6 and IL-8 from bovine endometrial epithelial cells, toward the invading pathogens in the uterine lumen and away from the underlying stromal cells (Healy et al 2015).

Trauma to tissues associated with cell destruction by microbes or by physical damage during parturition, may also contribute to inflammation of the endometrium. The ability of pattern recognition receptors to bind damage-associated molecular patterns (DAMPs), may provide an explanation linking tissue trauma to inflammation (Chen & Nunez 2010). Mammalian DAMPs include HMGB1, IL-1α, mitochondrial DNA and ATP, which are released from dying or damaged cells (Chen & Nunez 2010). However, in the scaling of the inflammatory

response, DAMPs are perceived as less of a threat than PAMPs (Blander & Sander 2012). Interestingly, innate immune responses may also be activated by cholesterol-dependent cytolysins, or the ion fluxes that they induce (Gurcel et al 2006; McNeela et al 2010); this may be important for *T. pyogenes* infections because the bacteria secretes pyolysin, which forms pores in the plasma membrane of endometrial cells leading to leakage of potassium ions (Amos et al 2014).

Innate immunity is an ancient evolutionary system, and so it is not surprising that it is integrated with other cellular homeostatic and metabolic pathways (Kotas & Medzhitov 2015). Dairy cattle are under metabolic stress after parturition, with reduced concentrations of nutrients and changes in metabolic hormones, including reduced abundance of glucose, glutamine and insulin-like growth factor 1 (Chagas et al 2007; Kerestes et al 2009). Negative energy balance may impair the inflammatory response and clearance of bacteria from the endometrium, leading to chronic endometritis (Esposito et al 2014). Certainly, the response to pathogen molecules is energetically expensive *in vivo* and *in vitro* (Turner et al 2016; Kvidera et al 2017). A striking example is that animals use > 1 kg of glucose in the first 12 hours after challenge with endotoxin (Kvidera et al 2017). Furthermore, the depletion of the key cellular nutrients, glucose or glutamine, reduces inflammatory responses by endometrial tissues *in vitro* (Turner et al 2016; Noleto et al 2017). If metabolic stress compromises the ability of animals to respond sufficiently to pathogens, this may result in persistence of infections and chronic inflammation.

Neutrophils are the main innate immune cell-type found in the endometrium during the postpartum period. Uterine disease is associated with negative energy balance and reduced neutrophil function (Hammon et al 2006; Galvao et al 2010). Interestingly, the effects of metabolic stress and impaired neutrophil function are even evident prior to parturition (Hammon et al 2006). Cows with a severe negative energy balance have persistent uterine

inflammation, whereas animals with mild negative energy balance had recovered their energy balance and repaired their endometrium by two weeks after parturition (Wathes et al 2009). Although uncommon, it is interesting to note that cows that are too fat at calving are also at risk of reduced neutrophil function, and may develop endometritis (Zerbe et al 2000). Metabolic status is often evaluated by monitoring body condition score during the postpartum period. An ideal body condition score profile has been proposed, with animals calving at score 3 to 3.5 using the 5-point body condition scoring scale (Chagas et al 2007). It is important to minimise body condition score loss in the first seven weeks post partum to less than half a body condition score point.

Adaptive immunity

Some microbes that infect the female genital tract**,** such as *Brucella abortus***,** generate robust protective antibody responses, and effective vaccines are available. For postpartum uterine disease, the role of adaptive immunity in the endometrium is more implied than explicit. Areas rich in T cells and B cells are evident in the postpartum endometrium, often as lymphocytic foci within the stroma (Wagner & Hansel 1969; Bonnett et al 1991b). Adaptive immune responses are also evident in postpartum animals, with increase abundance of antibodies (Dhaliwal et al 2001). Furthermore, adaptive immunity may play a role in countering uterine infections as preliminary data on vaccines for metritis suggests they provide some protection against disease (Nolte et al 2001; Machado et al 2014). However, postpartum endometritis often occurs after successive calvings, and adaptive immunity does not appear to provide long-term protection.

4. DISEASES OF THE POSTPARTUM UTERUS

4.1 RISK FACTORS FOR UTERINE DISEASE

Uterine disease is most common in *Bos taurus* dairy breeds that have high milk yields, such as Holstein-Frisian cows. Genetic selection for milk yield is often associated with the increased incidence of uterine disease after parturition. However, genetic selection is increasingly focussing on health traits, and future genetic selection of dairy cattle is likely to reduce the risk of uterine disease. The heritability of metritis was reported to be 0.19 to 0.26 for primiparous and second lactation cows, respectively (Lin et al 1989); although others have found lower heritability of 0.08 for metritis (Zwald et al 2004).

The environment is an important determinant of susceptibility to several uterine diseases, and many risk factors have been associated with uterine disease. Indeed, environmental factors may be more important than genetic factors. For example, although some polymorphisms in genes associated with immunity have small effects on uterine health in dairy cows, environmental factors such as dystocia, parity, and ketosis are more predictive for uterine disease than the genetic markers evaluated so far (Pinedo et al 2013). The environmental risk factors are associated with trauma to the female genital tract, disorders of metabolism, or problems with hygiene (Table 25.3).

Trauma

The environmental risk factors most likely to cause uterine disease are associated with tissue damage. Why trauma is important is not clear, but trauma may delay uterine involution, keep the cervix open, and allow bacteria to access the underlying stroma below the protective epithelium. Obvious causes of trauma include: dystocia, a large male calf, stillbirths, twins, first parity, and induction of parturition. However, RFM are the most important risk factor for uterine disease (Paisley et al 1986; Kim & Kang 2003; Potter et al 2010). The necrotic

material associated with RFM provides a favourable environment for bacterial growth in the uterine lumen, and the retained membranes obstruct the physical barrier provided by the cervix, and delay uterine involution.

Hygiene

It is intuitive that the hygiene of the calving environment, and the postpartum housing, should be important for development of uterine disease. This might be reflected in the association between uterine disease and when or where cattle are housed, or the angle of the vulva that allows faecal contamination of the vagina. However, direct evidence for the importance of hygiene in the postpartum environment is limited, and some studies have found that the apparent level of hygiene is relatively unimportant (Noakes et al 1991; Potter et al 2010).

Metabolism

Uterine disease is associated with changes in metabolism after parturition or diseases that disrupt metabolism, such as left displaced abomasum. Dairy cows are often under metabolic stress because they cannot consume enough food to meet the substantial extra demand for nutrients that are required for lactation. At the whole animal level, the metabolisable energy required every day to produce 40 litres of milk is about 200 MJ; three times the 65 MJ needed for normal resting metabolism. Consequently, postpartum dairy cows lose weight as tissues are broken down to satisfy the dietary energy and protein deficits (Chagas et al 2007). The animals also develop insulin resistance; resulting in reduced blood concentrations of insulin-like growth factor (IGF-1), glucose and glutamine, and the mobilization of fat reserves increases the concentration of ketones such as acetoacetate and β-hydroxybutyrate (Doepel et al 2006; Chagas et al 2007; Wathes et al 2011).

Metabolic stress in postpartum dairy cows may compromise their peripheral blood immune cell function (Cai et al 1994; Hammon et al 2006). Various defects in neutrophil function are

reported in cows with diseases and disorders, including metritis, after parturition. For example, superoxide production and chemotaxis by neutrophils is lower before partition in cows that develop metritis than normal animals (Cai et al 1994). Cows with metritis had lower neutrophil myeloperoxidase and cytochrome *c* reduction, than cows with normal uterine health around the time of calving (Hammon et al 2006). Cows that developed uterine disease also had a lower intracellular PMN glycogen level, which was associated with a greater degree of negative energy balance (Galvao et al 2010).

Glucose and glutamine are the major carbon substrates used by most cells for metabolic energy. Cells demand even more metabolic energy and nutrient supply when immune responses are activated. Thus, it is perhaps not surprising that deficits in glucose or glutamine blunt the inflammatory response in *ex vivo* experiments (Turner et al 2016; Noleto et al 2017). Limiting the availability of glucose to *ex vivo* organ cultures of endometrium reduces the secretion of IL-1β, IL-6 and IL-8 in response to endotoxin or lipopeptides (Turner et al 2016). Similarly, depletion of glutamine, in the presence of abundant glucose, reduced the IL-1β, IL-6 and IL-8 response to endotoxin by at least 50% (Noleto et al 2017). Effective innate immune responses in the endometrium depend on glucose availability, glycolysis, and glutamine abundance. As well as the dependence on the abundance of nutrients, hormones and intracellular regulatory pathways regulate cellular energy homeostasis. The principal regulator of cellular energy is AMPK, which senses the ratio of AMP to ATP in the cytosol. A homeostatic level of AMPK activation fosters optimal inflammatory responses (Turner et al 2016).

The impact of ovarian steroid hormones on postpartum uterine function is not clear. Progesterone is considered immuno-suppressive, whilst oestradiol may enhance immunity and was used to treat uterine disease (Lewis 2003). However, there are conflicting data, and exogenous oestradiol infused into the bovine uterus during the postpartum period increased

the abundance of bacteria in the uterus (Sheldon et al 2004). Furthermore, the stage of the oestrous cycle, or exogenous progesterone or oestradiol, did not modulate innate immunity in *ex vivo* organ cultures of bovine endometrium (Saut et al 2014). Similarly, treatment with oestradiol or progesterone, or inhibitors of oestradiol or progesterone nuclear receptors, did not affect gene or protein expression for IL-1β, IL-6 or IL-8 in endometrial cells or macrophages (Saut et al 2014). Apart from ovarian steroid hormones, the metabolic hormones insulin and IGF-1 are obvious candidates that might link postpartum metabolic stress and immunity, as concentrations of IGF-1 are reduced and animals are often insulin-resistant (Wathes et al 2011; De Koster & Opsomer 2013).

When dairy cows mobilize adipose tissue to satisfy the negative energy balance of lactation, there is an increased peripheral plasma concentration of non-esterified fatty acids (Sordillo et al 2009; Wathes et al 2012). These fatty acids are metabolized in tissues to acetyl CoA to provide cellular energy; although, excess fatty acid oxidation leads to increased production of ketones. During an immune response, tissue cells tend to further exploit fatty acid oxidation to supply nutrients, whereas immune cells exposed to pathogens often increase fatty acid synthesis as part of their inflammatory response (O'Neill et al 2016). However, apart from cholesterol, there is limited information about the mechanistic dialogue between lipids and immunity in the bovine endometrium.

Most of the cholesterol in cells is contained within the plasma membrane, and the concentrations of this lipid are tightly regulated by cholesterol uptake, cholesterol efflux transporters, and by cholesterol synthesis (Goldstein & Brown 1990). The first steps in cholesterol synthesis are encompassed by the mevalonate pathway, converting acetyl-CoA to isoprenoids, which are then converted to squalene, and ultimately to cholesterol. Cholesterol is partially responsible for the fluidity of plasma membranes. Inhibition of squalene synthase, can modulate innate immunity in the endometrium, and reduce inflammatory responses to

endotoxin (Healey et al 2016). A discussion of the role of cholesterol in endometritis is incomplete without mentioning cellular resilience, because many bacteria secrete pore-forming toxins that target cholesterol in plasma membranes. Reducing cellular cholesterol, using methyl-β-cyclodextrin or an endocytosis inhibitor, increases the resilience of stromal cells to PLO (Amos et al 2014; Preta et al 2015). Taking the observations together, reducing cellular cholesterol may limit inflammation, but increase stromal cell resilience to pore-forming toxins.

Herd size

It is assumed that uterine disease is not a contagious disease. However, it is notable that larger herds tend to have more uterine disease, and disease is often more common in production systems where animals are confined or managed as large groups. In addition, during experimental infections of naïve animals, disease often develops in the control animals that do not receive an intrauterine infusion of bacteria, if they are not kept separate from the animals infused with bacteria.

4.2 RETAINED FETAL MEMBRANES

Retained fetal membranes (RFM) are a common complication of bovine parturition. The predisposition to infections of the uterus means that RFM are an important contributor to bovine infertility. Occurrence of RFM (Fig. 25.7) is associated with the failure of the normal processes of dehiscence and expulsion. Hence the factors that cause RFM are those that interfere with the detachment of the fetal microvilli from the maternal cotyledons and those that interfere with the patterns of uterine contractility, particularly of third-stage labour. The main aetiological factors associated with RFM are summarized in Table 25.4.

Anything that interferes with the process of maturation of the placentomes, or causes birth to occur before maturation is complete, can result in RFM. Premature birth is very commonly

associated with retention. Cattle twins are usually slightly premature and so 30 to 50% of twin births are followed by retention. Premature removal of the calf by caesarean section and induction of premature calving also cause retention (Gross et al 1986). Likewise, heat stress can reduce gestation length and increase the incidence of retention of the after- birth in dairy cattle. Cows that calved during the warm season in Georgia, USA, where the mean daily temperature was 26°C, had a reduction of 2.8 days in gestation length and an incidence of retention of 24%, compared with 12% for the remainder of the year (DuBois & Williams 1980). The gestation lengths for retaining cows were, on average, 5 days shorter than those of non-retaining cows.

Gross placentitis is also associated with retention of the membranes. Placentitis and RFM occur in cases of abortion due to *Brucella abortus, Salmonella dublin, Campylobacter fetus* and moulds such as *Aspergillus* or *Mucor* species, or genital infections around the time of parturition (Roberts 1986). Retention is also more likely to occur when many cows calve in the same accommodation in quick succession, leading to a build-up of more pathogenic contaminant organisms in the environment. Such outbreaks of retention have also been associated with metritis and calf scour (Roberts 1986). It has been suggested that *T. pyogenes* should only be considered causal of retention, when there is evidence of a significant degree of placentitis (Laven & Peters 1996). Where placentitis results in retention, it is due to inflammatory swelling of the caruncle and cotyledon, impaired endometrial secretory activity and impaired myometrial contractility.

Retention also occurs when there is enlargement of the placentomes in the absence of placentitis. Such enlargement may occur in the presence of oedema of the chorionic villi, hyperaemia of the placentomes, advanced involution of the placentomes in postmature fetuses and prepartum necrosis of the villous tips of the fetal placentome (Paisley et al 1986;

Laven & Peters 1996). These abnormalities may mechanically prevent the separation of fetal and maternal villi.

Uterine inertia, particularly where it results in inadequate uterine contraction during the period of third-stage labour, is variably associated with retention. Early studies associated impaired uterine contractility with retention, especially where secondary uterine inertia occurs as a result of dystocia (Jordan 1952; Venable & McDonald 1958). Not all studies agree with this view, however, while inertia may be associated with a retardation of delivery, the membranes have undergone normal maturation (Zerobin & Spörri 1972; Martin et al 1981; Paisley et al 1986). It is likely that few cases of retention are caused by uterine inertia and that, even when inertia occurs, detachment of the placenta is easily accomplished. On the other hand, dystocia and fetotomy are consistently associated with retention, to which inertia is likely to be a significant contributor (Wehrend et al 2002; Eiler & Fecteau 2007). Uterine inertia caused by gross overstretching of the myometrium, such as in animals with hydrallantois, has been more definitely associated with retention (Arthur & Bee 1986); although the abnormalities of placenta and fetus in such animals suggest that other factors are likely to be involved. Uterine inertia due to hypocalcaemia has also been associated with retention (Arthur & Bee 1986; Gröhn et al 1990; Wilde 2006). However, intervention studies, in which cows have been given calcium as a prophylactic or treatment for RFM, have generally not supported the view that hypocalcaemia has a critical role in the pathogenesis of the disease (Hernandez et al 1999; Morton 2000; Melendez et al 2003).

There is evidence of a higher incidence of RFM when cow diets are deficient in selenium and/or vitamin E (Trinder et al 1973; Julien et al 1976; Aréchiga et al 1994; Allison & Laven 2000; Bourne et al 2007). Correction of dietary deficiencies, or supplementary feeding of selenium or vitamin E, is commonly associated with a reduction of the incidence of retention (Wilde 2006). Nonetheless, some other studies found no reduction in RFM after prepartum

supplementation with selenium (Gwazdauskas et al 1979; Hidiroglou et al 1987). Hence, selenium deficiency may be a cause of a high incidence of RFM in certain deficient areas, but sporadic cases of retention are probably not associated with selenium deficiency.

Finally, a number of other factors have been associated with RFM. There is some evidence of a hereditary predisposition to RFM. Cows of the beef breeds are much less often affected than those of dairy breeds; in the latter the incidence is higher in Ayrshires than in Friesians. Old cows are more commonly affected than young ones. Springtime calving exerts a predisposing influence this might be connected with a vitamin A deficiency, which has been shown to produce retention under experimental conditions. Animals that have previously had RFM are more likely to do so again (Eiler & Fecteau 2007). The incidence of retention is higher in genetically high-yielding dairy cows. In addition, cows on high nutritive planes at parturition are more prone to retention, as are cows with disorders of carbohydrate metabolism, such as fat cow syndrome, ketosis or displaced abomasum, around the time of calving (Whitmore et al 1974; Melendez et al 2003).

Diagnosis and incidence

Surveys of the incidence of RFM usually report an incidence of 4 to 8%, except in animals with dystocia, in which the incidence is higher. The effect of RFM depends largely upon the degree of uterine infection that takes place. Uncomplicated cases may be of little more consequence than the inconvenience of foul smelling membranes interfering with milking, although morbidity, as denoted by some temporary impairment of appetite and reduction of milk yield, has been estimated to occur in 55 - 65% of cases. In an important early investigation of the morbidity of RFM, it was observed that the pathogenicity of retention on appetite in 44 cattle (Palmer 1932) during the 14 days after calving resulted in appetite being good in 31.8%, fair in 54.5% and poor in 13.6% of animals.

Mortality caused by RFM is usually associated with metritis, and applies to 1 - 4% of cases (Roberts 1986). Cows with RFM that have calved spontaneously after a normal length of gestation generally depart little from normal health. On the other hand, when retention follows extensive obstetrical interference for dystocia, a severe metritis and toxaemia can supervene within 2 - 3 days, which can be fatal if untreated. Whether these cases can be directly attributed to the retention is, however, unclear, since similar cases might have been equally ill if the fetal membranes had been expelled at the time of delivery. Clinical signs and treatment of metritis are discussed later in this chapter.

Duration of retention

Cows that fail to expel the membranes within 24 hours or so, are likely to retain them for 7 to 10 days. Myometrial contractions largely cease from 36 hours after the birth of the calf so, if the membranes have not been expelled by this time, freeing of the fetal villi from the maternal crypts eventually occurs as a result of bacterial putrefaction. This process starts within 24 hours of birth, but takes several days to complete. Natural sloughing of the maternal caruncles also contributes to the subsequent dehiscence of the membranes, such that eventual expulsion of the membranes depends upon uterine involution. The duration of retention seems to depend on several factors, such as the extent of the areas of attachment of the fetal membranes, the rate of uterine involution, the amount of uterine exudate and the proportion of the afterbirth that had already passed through the cervix when retention began.

Effects on fertility

RFM is not, of itself, associated with impaired subsequent fertility. This was first demonstrated by comparing the fertility of 44 cows with RFM, with 44 cows in the herd that had cleansed normally, finding that there was no significant difference in the subsequent breeding records of the two groups (Palmer 1932). A consensus has developed, which

supports the idea that uncomplicated retention does not significantly affect the fertility of cows that are mated beyond 60 days from the last calving. The significance of retention is, therefore, dependent upon the degree of metritis that occurs. This aspect of the condition was clarified by means of a retrospective analysis of 652 parturitions of 293 dairy cows in Canada, which revealed that RFM alone did not impair subsequent reproductive performance (Sandals et al 1979). The animals that developed the metritis complex, with or without RFM, did, however, suffer significant increases in days-open, services per conception, calving - first-oestrus interval and days from calving to first service. Various studies have confirmed these findings (Joosten et al 1988; Borsberry & Dobson 1989; Esslemont & Peeler 1993; Kossaibati & Esslemont 1997; Morton 2000). The economic costs of a case of RFM have been estimated as GBP£300 to GBP£ 475 (Joosten et al 1988; Kossaibati & Esslemont 1997).

Treatment and prognosis

The treatment of animals with RFM has long been a contentious subject. A number of approaches have been taken to animals with this condition, including:

- No treatment.
- Manual removal.
- Administration of ecbolic agents.
- Treatment for metritis but no specific treatment of retention itself.

Manual removal

The techniques used for manual removal of RFM range from externally applied gentle traction, through to forced extraction, with manual separation of each cotyledon and caruncle. Manual removal is a superficially attractive method, in that it immediately removes the stinking mass of putrefying afterbirth, thereby improving milking hygiene. However, manual removal may have little benefit, or be detrimental for the animal. In a study that examined

501 cases of RFM, manual removal did not reduce the risk of metritis or endometritis, and did not improve fertility, compared with other treatments (Drillich et al 2006b). The authors recommended that systemic treatment, based on elevated rectal temperature, was the optimal choice for treating RFM.

Forced extraction, once commonly practised, is now generally regarded as not advisable. An exception is where release of the membranes is simply impeded by constriction of the cervix, or where there are only one or two remaining attachments to the maternal caruncles. Forced extractions of extensively attached fetal membranes can result in damage to the endometrium, and more distant parts of the membranes are often left behind (Grunert & Grunert 1990). If fetal cotyledons remain attached after forced traction, they may become detached later and could remain within the uterine lumen as foreign bodies (Roberts 1986). Similarly, the prevalence and severity of uterine infection are often worse after manual removal than after conservative treatment (Roberts 1986; Bolinder et al 1988). Some suggest that the presence of pyrexia was an absolute contraindication to the forced removal of fetal membranes (Roberts 1986). Moreover, forced extraction is commonly associated with impaired subsequent reproductive performance (Bolinder et al 1988).

The current recommendations for the manual removal of fetal membranes are that cows should not be examined until 96 hours after calving and that, if attempted, removal should be gentle, ideally limited to the withdrawal of the membranes from the genital tract after they have become spontaneously detached from the caruncles. While, in many animals, spontaneous detachment may have occurred within 96 hours, it is suggested that it is acceptable to leave membranes for 10 days before removal, if this length of time was needed for their detachment (Roberts 1986). Even with minimal intervention, there appears to be no benefit from removal of the membranes (Laven & Peters 1996; Drillich et al 2006b). In this context, farmers should also be discouraged from attempting to undertake forced removal of

fetal membranes from their own cows, since they are very likely to use too much force and to attempt removal too soon after calving.

Ecbolic agents

Many attempts have been made to reduce the incidence of RFM, or to hasten the release of the membranes once retention has occurred, with ecbolic agents such as oxytocin or PGF_{2alpha}. Oxytocin treatment has little or no beneficial effect (Miller & Lodge 1984; Garcia et al 1992; Stevens & Dinsmore 1997); even when the uterus has been pre-sensitized by the administration of oestrogenic substances (Arthur & Bee 1986). Similarly, a longer-acting form of oxytocin, carbetocin, had no benefit for subsequent fertility when given to dairy cows immediately after parturition (Barrett et al 2009). Prostaglandin F_{2alpha} and its analogues have also been used as ecbolic agents in postpartum cattle. Prostaglandins may assist in detachment of the membranes through direct actions upon the placentomes, rather than just by an ecbolic action (Gross et al 1986).

Treatment for metritis only

Uncomplicated cases of RFM require no immediate treatment. However, those cows with signs of metritis, such as pyrexia, inappetence, or reduced milk yield, need to be treated with antibiotic. A number of subsequent studies have confirmed that this is an appropriate treatment regimen (Drillich et al 2006a; Drillich et al 2006b). The use of parenteral antibiotic for the treatment of cows with metritis is discussed later.

Traditional practice has been that, after forced extraction of RFM or after unsuccessful attempts at extraction, antibiotics were placed into the uterus in an attempt to prevent endometritis. Intrauterine antibiotics reduce odour (Roberts 1986). However, antibiotics also reduce the rate of putrefaction of the membranes and the level of intrauterine phagocytosis, thereby prolonging retention (Paisley et al 1986; Roberts 1986). Administration of

intrauterine antibiotics to cows while they have retained membranes does not reduce the incidence of endometritis or improve fertility (Goshen & Shpigel 2006; Eiler & Fecteau 2007). Furthermore, some of the antibiotics that are present in intrauterine pessaries are inactivated in the presence of the debris that is contained within the uterus (Paisley et al 1986); a problem that is often exacerbated by veterinarians failing to use the recommended dose. Hence, there appears to be little to recommend the use of intrauterine antibiotics while retained membranes are present (Paisley et al 1986; Goshen & Shpigel 2006; Beagley et al 2010). Although, there are potential benefits for cows with metritis (Goshen & Shpigel 2006).

All cows that had RFM require examination and treatment for endometritis before breeding, ideally towards the end of the voluntary wait period (see Chapter 27). Diagnosis and treatment of endometritis is discussed later in the present chapter.

Collagenase

Infusion of collagenase solution into the stumps of the umbilical arteries of the retained membranes has proved an effective means of treatment (Eiler & Fecteau 2007; Beagley et al 2010). Although not licensed for use in cattle, collagenase is used successfully to treat RFM in the mare (see Chapter 26).

4.3 METRITIS

Metritis usually follows an abnormal first or second stage of labour, especially when there has been a severe dystocia that has required prolonged traction or resulted in damage to the vulva and/or birth canal. The condition is also associated with uterine inertia, premature calving including abortion or induced calving, twin births, and RFM. Bacteria colonize and proliferate in the uterus, and may cause pyaemia. Toxins produced by these bacteria are also absorbed from the uterus, resulting in toxaemia.

Diagnosis and incidence

Metritis is more common in dairy cattle than beef breeds of cattle, and usually within 10 days of parturition. Metritis is characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge (Fig. 25.8), which often has a fetid odour. The severity of disease is categorized by the signs of health:

- Grade 1 metritis - Animals with an abnormally enlarged uterus and a purulent uterine discharge, but without any systemic signs of ill health.
- Grade 2 metritis - Animals with an abnormally enlarged uterus and a purulent uterine discharge, with additional signs of systemic illness such as decreased milk yield, dullness, and fever.
- Grade 3 metritis - sometimes called puerperal metritis, or toxic metritis - Animals with an abnormally enlarged uterus and a purulent uterine discharge, with signs of toxaemia such as inappetance, cold extremities, depression, and/or collapse.

Affected animals show both local and general symptoms, and the severity informs the metritis grade. The temperature of affected cows may be elevated to 40 to 41°C initially**,** but is often subnormal by the time veterinary attention is sought. Toxaemia or endotoxic shock, result in clinical signs that may include**:** a fast pulse in the region of 100 beats per minute, rapid respiration, a sluggish capillary refill time, cold extremities, a moderate to severe level of dehydration, and a characteristic toxaemic diarrhoea. The vulva and vagina are typically swollen and deeply congested. The cotyledons are swollen and the fetal membranes often remain firmly attached. The uterus contains a large volume of pus; whereas off-white mucopurulent material is often an encouraging sign, red-brown watery, fetid, reddish, serous exudate that contains fragments of degenerating fetal membranes and other detritus warrants a more guarded prognosis (Fig. 25.8). This fluid is discharged from the vagina accompanied by frequent expulsive straining efforts. Vaginal exploration of an affected case should be cautious, as it causes acute discomfort and is followed by severe and persistent expulsive

efforts. Many animals with grade 3 metritis are recumbent by the time veterinary attention is sought, by which time they are usually anorexic. In these animals it is common for the infection to extend through the uterine wall into the peritoneum, causing a localized or generalized peritonitis. Many animals with grade 3 metritis also develop mastitis, particularly if they are recumbent, and many also have concurrent hypocalcaemia. The main differential diagnoses are**:** metabolic disorders, ruptured uterus, retained fetus, diffuse peritonitis, acute toxic mastitis, salmonellosis and post-calving injuries (e.g. obturator paralysis).

The incidence of metritis varies between breed, country and herd, but in a study of the records from 97,318 dairy cows in the USA, the lactation incidence of metritis, including RFM, was 21% (Zwald et al 2004). In a meta-analysis of records from more than 10,000 animals, there was evidence that postpartum metritis caused subfertility by increasing the time to first insemination by 7.2 days, reducing conception rate to first insemination by 20%, and increasing the calving to conception interval by 18.6 days (Fourichon et al 2000). In some studies metritis is also associated with reduced milk yields (Giuliodori et al 2013).

Treatment and prognosis

Grade 1 cases are often undiagnosed and untreated. Routine examination of postpartum cows is used in some herds to identify these animals. However, whether treatment is warranted is unclear. Parenteral antibiotic may help resolve the clinical signs**,** but probably has little effect on health or subsequent fertility.

Grade 2 cases may require the parenteral administration of broad-spectrum antibiotics, in combination with other supportive and symptomatic treatments, as appropriate to the severity of the condition. These grade 2 metritis cases warrant treatment, not least because metritis is painful (Stojkov et al 2015). However, a review of 17 studies using ceftiofur to treat metritis

found that whilst seven reported clinical improvement, there was no significant improvement in reproductive performance (Haimerl & Heuwieser 2014). Ampicillin trihydrate is also used to treat metritis. Ampicillin was at least as effective as ceftiofur in a study of 528 cows with metritis (Lima et al 2014). If used, parenteral antibiotics should be continued for three to five days. Oestrogens are prohibited in many countries, and even when available oestrogens are contraindicated, as they increase the rate of absorption of endotoxin from the uterus. Oxytocin may be of some benefit, but only within 72 hours of calving. Prostaglandin F_{2alpha} is of little or no benefit despite a short-term ecbolic effect.

If the uterus contains an abundance of pus, uterine lavage and drainage can be attempted (Fig. 25.9). Physiological saline is flushed into the uterus using a wide-bore tube, inserted through the cervix, with the end of the tube guarded by the operator's hand. Relatively small amounts of saline should be infused at a time, as the uterus is too friable to cope with any build-up of pressure. The fluid is then syphoned from the uterus by lowering the tube, and the process repeated several times. Although this procedure is commonly undertaken, it should be noted that it is associated with significant risks of uterine wall damage and toxin absorption as the uterine wall may be extremely friable. Intrauterine antibiotics are inadequate as the sole route of antibiotic administration, although there may be some advantage to the infusion of soluble antibiotics, such as 5 g oxytetracycline in 100 to 500 ml normal saline, once uterine flushing is completed.

Even if animals are not treated, it is important to record the presence of disease**,** because even grade 1 and 2 metritis cows should be examined for clinical endometritis 21 days or more after parturition.

Grade 3 cases of metritis are serious, and the prognosis should be guarded. Prognosis is a poor if the animal is collapsed or if peritonitis is present. In this situation, before initiating treatment, a decision should be made whether the animal has a reasonable chance of recovery

or whether it would be more cost-effective, in some commercial production systems, or from a welfare perspective, to slaughter the animal.

Treatment of grade 3 metritis requires both good nursing care and vigorous medication. As for most cases of sepsis, supporting the animal's physiology is probably more important than trying to kill the pathogens. The cow should be kept warm and made as comfortable as possible by, for example, transferring it to a well-bedded loose box. The animal should be fed and provided with water. The first step in treating these cases is to stabilize the circulatory system by giving fluids, and non-steroidal anti-inflammatory drugs (Smith 2005). Fluids can be given as 2.0 to 2.5 litres of 7% v/w saline intravenously followed by 25 litres of water administered by stomach tube, or 24 to 40 litres of isotonic electrolytes by stomach tube (Frazer 2005). Flunixin meglumine is probably the non-steroidal anti-inflammatory drug of choice, because of its anti-endotoxic effects. Toxaemia is energetically expensive – animals use an extra kilogramme of glucose in the first 12 hours after an intravenous challenge with endotoxin (Kvidera et al 2017). So, encouraging animals to eat, and perhaps intravenous infusion of 500 ml of 50% glucose, are beneficial for survival. Calcium should be given to treat or prevent secondary hypocalcaemia. Fetal membranes should not be removed, and vaginal examination should be very cautious. If the cow is continually straining, caudal epidural anaesthesia can be given, but this only gives transient relief for 1 to 2 hours. However, sometimes an epidural will break the cycle of pain and straining, which is often self-perpetuating and debilitating for the animal.

Parenteral antibiotic therapy should be initiated immediately, as for any cause of sepsis, and continued for three to five days. The choice of antibiotic remains contentious**,** and there are many arguments put forward in favour of one antibiotic over another, with little help from large-scale clinical trials. Some favour bactericidal antibiotics to control systemic infection and pyaemia. Broad-spectrum penicillins and cephalosporins are the most efficacious

choices. Ceftiofur achieves concentrations in uterine tissue and fluid that exceed the minimum inhibitory concentration (MIC) for most of the common metritis pathogens (Drillich et al 2006a), and appears to be effective in treating acute postpartum metritis (Chenault et al 2004). In some studies, there was also an improvement in subsequent fertility (Giuliodori et al 2013). Some clinicians prefer bacteriostatic antibiotics, as they limit the risk of further endotoxic damage during the killing of bacteria. Oxytetracycline has the advantage that it can be given at high dose intravenously, but the MIC of tetracycline for microbes causing intrauterine infections is often high**,** and sufficient concentrations of oxytetracycline are probably not attained in uterine tissue or the lumen (Smith et al 1998a).

Recovery of animals with grade 3 metritis is marked by a return of appetite, cessation of diarrhoea and a change in the contents of the uterus to a less fetid, thicker, more obviously purulent material. The major sequelae of this condition include**:** localized or generalized peritonitis, ascending infection of the urinary tract (cystitis or pyelonephritis), pyosalpinx, and ovariobursal adhesions. Other complications of metritis include**:** pneumonia, polyarthritis and endocarditis. In pyaemic cases, abscesses may develop subsequently in the lungs, liver, kidney or brain. The fertility of cows that recover from the condition is likely to be impaired, so animals should be monitored for subsequent endometritis and ovarian dysfunction.

4.4 ENDOMETRITIS

Endometritis is a common condition of the cow. Unlike metritis, endometritis does not affect the general health of the cow, although it does impair fertility. Most of the specific pathogens causing infertility, such as *Campylobacter fetus* subsp. *venerealis* and *T. fetus*, do so because of the endometritis that they produce (see Chapter 26). However, the most common initiating cause of endometritis is a polymicrobial growth of bacteria in the uterus during the period

around the time of calving, with subsequent overgrowth of pathogens such as *T. pyogenes*, usually in association with *F. necrophorum* and/or *Prevotella* species.

Clinical endometritis increases the interval to first insemination by 11 days, and delays conception by 32 days, compared with animals that did not have endometritis (Borsberry & Dobson 1989). Cows with clinical endometritis between 20 and 33 days post partum are 1.7 times more likely to be culled for reproductive failure, than cows without endometritis (LeBlanc et al 2002a).

Diagnosis and incidence

World-wide figures for the incidence of endometritis are varied, ranging from 43 to 35% in some studies, to other studies that report lower incidences of 6 to 10% (Fonseca et al 1983; Andriamanga et al 1984; Martinez & Thibier 1984; Borsberry & Dobson 1989). Typically, the incidence is 15 to 20% of animals. For example, a survey of 19,870 Holstein cows in Germany found a lactation incidence of 19.2% for endometritis (Gernand et al 2012).

Clinical endometritis is characterized by the presence of a white or whitish yellow mucopurulent vaginal discharge in the postpartum cow; and the condition is also known as leukorrhoea, whites, or purulent vaginal discharge. The volume of the discharge is variable, but frequently increases at the time of oestrus, when the cervix dilates and there is copious vaginal mucus. Affected cows do not show signs of systemic illness. Subclinical endometritis is characterized by inflammation of the endometrium, typically associated with neutrophils in uterine luminal fluid, but without visible purulent material.

The presence of endometritis can be determined by transrectal palpation of the uterus, examination of the vagina and cervix for the presence of purulent discharge and examination of cervical swabs for the presence of neutrophils. The risk of endometritis can also be

inferred from a history of relevant aetiological factors in the peripartum period of individual cows.

Rectal palpation of animals with clinical endometritis frequently reveals delayed uterine involution, and a uterus that has a **'**doughy**'** feel. There are correlations between size and texture of uterus and cervix, the nature of the purulent exudates and the degree of endometritis determined by biopsy, and the nature of the bacterial isolation (Studer & Morrow 1978). Nonetheless, rectal palpation is now regarded as being neither a sensitive**,** nor a specific**,** method for accurate diagnosis of endometritis, as too many other factors interfere with the rate of change of size of the uterine horns over the postpartum period (de Boer et al 2014). Hence, reliance should not be placed upon this method as the primary means of diagnosis.

A simple grading system based on the character of the vaginal mucus is readily used to evaluate cows with clinical endometritis (Fig. 25.10A) (Sheldon & Noakes 1998; Sheldon et al 2009). The endometritis grade correlates with the presence of pathogenic organisms associated with uterine disease (Fig. 25.10B)**,** and is prognostic for the likely outcome of treatment (Fig. 25.10C).

The presence of purulent discharges can be diagnosed in a number of ways. Examination of the vagina with a clean gloved hand is simple, cheap and effective; but some operators may cause discomfort to the cow. Whilst somewhat invasive, manual examination of the vagina carries little risk of further microbial contamination of the uterus in postpartum dairy cattle (Sheldon et al 2002a). Manual examination of the vagina also facilitates collection of fluid from the vagina to evaluate the presence and odour of pus, which can be used to score the severity of disease and predict the likely success of treatment (Sheldon et al 2006). Vaginal examination also allows the operator to detect damage to the wall of the vagina and cervix, indicative of obstetrical injuries, vaginitis, and cervicitis. However, as with any clinical

examination, the evaluation of uterine disease is subjective and there is inter-operator and intra-operator variation (de Boer et al 2014; Sannmann & Heuwieser 2015). Alternatively, a vaginoscope can be used to examine for the presence of discharge around the cervical os or in the cranial vagina. The most recently developed method is a rubber diaphragm on the end of a stainless steel rod (Metricheck), which is inserted into the vagina to collect a sample of discharges from the external cervical os/cranial vagina. This method is as effective as vaginoscopy, but has the advantage of being quick and causing minimal discomfort to the cow. The Metricheck instrument and examples of purulent discharges collected during its use are shown in Figure 25.11.

The absence of pus in the postpartum genital tract does not mean that the tract is normal and healthy. The importance of subclinical endometritis has emerged over the last 15 years, with the realisation that cytological evidence of inflammation of the endometrium is associated with reduced fertility (Kasimanickam et al 2004; Gilbert et al 2005). The cause of subclinical endometritis is not yet clear, and may include resolving bacterial infections, immune-pathology without pathogenic bacteria, or even aberrations of postpartum tissue regeneration and repair. Subclinical endometritis is characterized by inflammation of the endometrium that results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis.

The Australian ‘InCalf’ survey of reproductive performance of dairy cattle (Morton 2000) identified a number of epidemiological factors that were associated with subfertility due to endometritis (Table 25.5). Identification of the cows in which these events occur allows them to be managed and/or treated appropriately before they are mated. The term ‘at risk cows’ has been proposed for these animals.

In the absence of a practical gold standard for the diagnosis of clinical endometritis, there is limited agreement amongst the diagnostic methods (de Boer et al 2014). Beyond the clinical

diagnosis, studies that use a reproductive outcome to determine the diagnostic criteria are most useful on farms.

Effects on fertility

Endometritis reduces fertility by reducing the chances of conception. Consequently, there is an increase in the calving - conception interval, the number of services per pregnancy, and the proportion of cows that fail to conceive. Extension of the calving - conception interval has been shown to be an average of 12 days (Tennant & Peddicord 1968), 10 days (Bretzlaff et al 1982) and 31 days (Borsberry & Dobson 1989). Endometritis also increased services per conception from 1.67 and 2.16, to 2.0 and 2.42, respectively (Tennant & Peddicord 1968; Bretzlaff et al 1982). In two surveys, culling for failure to conceive was 14% and 21%, respectively, in cows with metritis, compared with an average of 6% or 5% for unaffected animals (Tennant & Peddicord 1968; Bretzlaff et al 1982).

The state of the uterus, and its contents, has been used as a prognostic indicator for cows with endometritis. For example, a significant correlation was found between the state of the uterus, as determined by rectal palpation, and the calving - conception interval, especially in relation to the amount of pus in the discharge(Studer & Morrow 1978). Similarly, observations of the nature of purulent vaginal discharges have also been used as a prognostic indicator. The presence of a purulent discharge and cervical diameter > 7.5 cm was also associated with impaired fertility (LeBlanc et al 2002a). There tended to be an increasing impairment of fertility, with increasingly purulent discharge. Mucopurulent discharge tended ($P = 0.09$) to increase time interval to pregnancy. In another study, purulent or foul discharge was consistently associated with a 20% reduction in pregnancy rate (Sheldon & Noakes 1998).

Endometritis therefore reduces the profitability of a dairy enterprise, and the cost can be calculated by relating it to the increase in the calving - conception interval. Losses are mainly

due to an extended calving - conception interval, increased culling rates, reduced milk yield and the cost of treatment (Kossaibati & Esslemont 1997). Most of these losses are preventable by instigating effective treatment of affected animals before the end of the voluntary wait period, or start of the breeding season.

Treatment

Few aspects of theriogenology have attracted more debate than the treatment of endometritis. Two problems that impede the study of its treatment are: spontaneous 'self-cure', which has been estimated to be between 33% (Steffan et al 1984) and 46% (Griffin et al 1974a); and the variable criteria that have been used for its diagnosis, prior to the setting out of agreed definitions (Sheldon et al 2006).

There is, however, consensus on the following:

- There is little merit in performing routine swabbing and bacterial sensitivity tests before treatment.
- Intrauterine infusion of antiseptics is of limited value, and at worst injurious to the uterus.
- Treatment should be delayed until at least 3 weeks post calving (LeBlanc et al 2002b).
- Rational therapy is based upon either hormonal stimulation of uterine defences, or the use of antibiotics.

Stimulating uterine immunity

Three main methods have been used: (1) stimulation of oestrus with PGF_{2alpha} in animals that have an active corpus luteum (CL), (2) administration of low doses of oestradiol and (3) stimulation of oestrus in animals that are anoestrous.

When there is a mature CL on the ovary, PGF_{2alpha} (or an analogue) is an effective means of treatment for clinical endometritis. Administration of PGF_{2alpha} causes luteolysis, thereby

reducing progesterone concentrations and stimulating the return of oestrus. The cow will return to oestrus 3 to 5 days after treatment, often with a mild to moderate purulent vaginal discharge. It is preferable that animals should be treated before the end of the voluntary wait period but, if treatment has been delayed until after the start of the mating period, it is possible to serve or inseminate at the induced oestrus, unless the discharge is severe. Some cases of endometritis present with leukorrhoea when the cow is in oestrus; for these animals, PGF_{2alpha} may need to be given 7 to 10 days later when a responsive CL would be present. The response to PGF_{2alpha} treatment, in terms of conception at the induced oestrus and final pregnancy rate, is generally good (LeBlanc et al 2002b); and markedly better than in untreated controls. Some have advocated intrauterine administration of $PGF_{2alpha,}$ but this appears to confer no particular advantages. Interestingly pathogens switch prostaglandin secretion in the endometrium away from PGF, toward PGE which may also be why PGF_{2alpha} is a successful therapeutic (Herath et al 2009a).Whether PGF_{2alpha} administration to cows without a CL is beneficial is not clear; in some cases there is a positive response, but not in other cases (Steffan et al 1984; LeBlanc et al 2002b).

Historically, intramuscular injection of 3 to 5 mg of oestradiol benzoate to cows without a detectable CL has been used with some clinical success to treat endometritis (Sheldon & Noakes 1998); although fertility was not improved. The rationale for the use of oestrogens is that they increase uterine blood flow and stimulate the immune system, as occurs during oestrus. The withdrawal in the European Union of licensing for the use of oestrogens in food-producing animals means that this treatment is no longer legal in animals that are to supply milk or meat to those countries. High doses of oestrogens are contraindicated as they result in long-term down regulation of the hypothalamic-pituitary axis, resulting in anoestrus and ovarian cysts.

In animals without a CL, it is theoretically possible to induce oestrus with intravaginal progesterone-releasing inserts, but this practice is not recommended as many carry a contraindication for use in animals with vaginal or uterine infection. Induction of oestrus with gonadotrophin-releasing hormone (GnRH) has also been advocated as a means of treating endometritis in acyclic cows, although this is seldom undertaken now. With the use of ovsynch programmes for fixed time AI (see Chapters 8 and 27), many postpartum animals are inadvertently treated for endometritis as part of routine reproductive programmes in many dairy herds. However, studies suggest that ovsynch programmes have little benefit for the treatment of endometritis, and endometritis did not affect the outcome of ovsynch (Hendricks et al 2006; Kasimanickam et al 2006).

Antibiotics

A wide range of antimicrobial agents has been used in the treatment of endometritis. While parenteral antibiotic treatment is needed for metritis, it is generally considered to be preferable to treat endometritis via the intrauterine route. Provided an adequate dose of antibiotic is used, this will result in effective MICs reaching the endometrium, and being established in the intraluminal secretions. The latter point is important for the effective treatment of the disease, since sub-therapeutic dose rates are frequently used. In organic herds antibiotics are not often permitted and so mild antiseptics may be used, such as 2% povidone iodine or a hibitane solution (Mido et al 2016). Some clear principles underlie the choice of antimicrobial and/or antiseptic agents:

- It must be effective against the wide range of aerobic and anaerobic, Gram positive and Gram-negative bacteria that are present.
- It must be effective within the microaerophilic environment of the uterus.

- Whether an effective bactericidal or bacteriostatic concentration can be achieved at the site of infection by the intrauterine route of administration. When the intrauterine route is used, the substance must be evenly and rapidly distributed throughout the uterine lumen with good penetration into the deeper layers of the endometrium.
- It must not inhibit natural uterine defence mechanisms, particularly the cellular component.
- It must not traumatize the endometrium. Several of the vehicles used in the formulation of pharmaceutical preparations can damage the endometrium. Examples include propylene glycol, which can cause a necrotizing endometritis; oils, which can cause granulomata; and, chalky bases, which can cause irritation and blockage of endometrial glands.
- Treatment must not reduce fertility by producing irreversible changes in the reproductive system.
- Treatment must be cost-effective by enhancing fertility.
- Details of its absorption from the uterus and excretion in the milk must be known, so that appropriate withdrawal times can be followed.

In consequence, several antibiotics are inappropriate. Nitrofurazone is irritant, and has an adverse effect on fertility. Aminoglycosides are not effective in the predominantly anaerobic environment of the infected uterus. Field trials have also provided evidence for a lack of effectiveness of these drugs in the treatment of endometritis. Sulphonamides are ineffective because of the presence of para-aminobenzoic acid metabolites in the lumen of the infected uterus. Penicillins are susceptible to degradation by the large numbers of penicillinase-producing bacteria that are present in the diseased uterus.

Antibiotics that have been formulated for intrauterine use include: oxytetracycline as an intrauterine infusion or in the form of pessaries, and cephapirin as an infusion. Parenteral oxytetracycline penetrates the uterine wall and lumen (Ayliffe & Noakes 1978); but the MIC

for many organisms is higher than can be achieved practically (Sheldon et al 2010). Intrauterine oxytetracycline does not penetrate the wall of the uterus well, is not particularly effective against *T. pyogenes* and can have a direct irritant effect upon the endometrium (Cohen et al 1995). The main role for tetracycline is therefore as a prophylactic intrauterine antibiotic after assisted calvings. However, cephapirin has been widely studied as an intrauterine treatment for endometritis, with positive results (LeBlanc et al 2002b; Kasimanickam et al 2005).

Prostaglandin F_{2alpha} versus intrauterine antibiotics

A comparison was made of the effectiveness of intrauterine infusion of 1500 mg oxytetracycline hydrochloride, with intramuscular injection of the PGF_{2alpha} analogue cloprostenol, or 3 mg estradiol benzoate, as treatments for endometritis (Sheldon & Noakes 1998). It was concluded that, provided a CL was present, PGF_{2alpha} was the most successful treatment, both in terms of cure rate and calving to conception interval (Sheldon & Noakes 1998). Oxytetracycline was more effective than oestradiol, but marginally less so than PGF_{2alpha}. Similar results were found comparing a commercial antibiotic preparation with PGF_{2alpha} and oestradiol (Pepper & Dobson 1987). In another study, the effect of intrauterine cephapirin on fertility of cows with endometritis was better than for a prostaglandin analogue, if animals were unselected for the presence of a CL; but when cows with a CL were treated with PGF, the results of the cephapirin and prostaglandin treatments were similar (LeBlanc et al 2002b). In the majority of the foregoing treatments, the severity of the case adversely affected the cure rate. Thus, the severity of endometritis should be recorded at the time of treatment, and should be used for selecting animals for re-examination, and for prognosis (Fig. 25.10C).

4.5 SUBCLINICAL ENDOMETRITIS

Uterine biopsy has been used to study both the incidence of clinical and subclinical endometritis. Biopsies can be collected using a range of specialised instruments for endometrial biopsy, such as that shown in Fig. 25.12, or by using balloon biopsy forceps. Uterine biopsies collected from infertile cows reveals that many have subclinical endometritis. For example, a study in 1971 reported that 77% of infertile cows had endometritis; bacterial infection was found in 64% of these cows and 80% had lesions of endometritis evident in biopsies (Sagartz & Hardenbrook 1971). Likewise, another study showed that 50% of the genital tracts obtained from an abattoir showed histological evidence of endometritis, yet only 12.5% showed gross lesions (Hartigan et al 1972). Hence, the evidence from such biopsy studies is that many cases of endometritis are subclinical, with no purulent discharge evident.

Although histological analysis of endometrial biopsies had previously shown endometrial inflammation in the absence of clinical signs, the importance of subclinical endometritis was first demonstrated in field studies by collecting samples from the surface of the endometrium using a cytobrush or uterine lavage (Kasimanickam et al 2004; Gilbert et al 2005). These studies found that the presence of polymorphonuclear neutrophils (PMNs) in smears made from the endometrial samples was associated with reduced pregnancy rates. Consequently, subclinical disease is defined by the proportion of PMNs exceeding operator-defined thresholds, usually about 5% of cells in samples collected by flushing the uterine lumen or by endometrial cytobrush, in the absence of clinical endometritis, about 35 to 40 days post partum (Sheldon et al 2006; de Boer et al 2014). In addition, if the cytology samples are analysed for gene expression, there is typically increased expression of inflammatory mediators (Gabler et al 2009; Fischer et al 2010; Gabler et al 2010; Ghasemi et al 2012).

Diagnosis

The cytobrush technique typically uses a cytobrush used for human cervical cytology, which is screwed into a metal rod (Fig. 25.13), which is then protected by a plastic sheath, and inserted through the cervix using a method similar to artificial insemination. The brush is extruded and rolled along the endometrium in the body or horns of the uterus (Bogado Pascottini et al 2016). After removing the instrument from the animal, a cytology smear is prepared on a glass slide. Alternatively, the uterine flush technique uses approximately 20 ml of sterile saline infused into the uterus through a catheter; the uterus is massaged, and the fluid aspirated via the same catheter (Gilbert et al 2005). The suspension is then used to prepare a cytology smear on a glass slide, usually using a specialised centrifuge instrument designed to deposit cells evenly onto a glass slide, such as a **'**Cytospin**'** machine. The slides are stained, and using a light microscope the glass slides are examined, with 100 to 400 cells counted, and the proportion of PMNs recorded. It is now suggested that counting 300 cells provides optimal reproducibility amongst observers (Melcher et al 2014).

There is much debate about the proportion of PMNs that indicate the presence of subclinical endometritis, and the criteria differ with the time after parturition, and vary amongst farms and operators (Sheldon et al 2006; Melcher et al 2014; Wagener et al 2017). Criteria for the diagnosis of subclinical endometritis were proposed for different stages post partum, based upon the proportion of neutrophils present in cytology samples from cervical swabs (Table 25.6). For large herds, it is possible to generate a farm-specific cut-off point for diagnosis of subclinical endometritis that compromises fertility. However, beyond 50 days postpartum the presence of any PMNs in cytology samples is now often regarded as abnormal. Interestingly, cytology does not always correlate well with endometrial biopsy (Westermann et al 2010;

Bogado Pascottini et al 2016). However, both techniques are not practical for commercial dairy farms, and so alternatives are being sought to provide a cow-side test.

There remain many open questions about subclinical endometritis. This may partly be because inflammation is part of the normal repair process of the tissues during the puerperium, as well as infection also stimulating the accumulation of neutrophils in the uterus. The inflammation may also be associated with recovery of the tissues after metritis and clinical endometritis, trauma, or other non-microbial disease. In one study of 528 animals, more animals with metritis subsequently showed evidence of subclinical endometritis, than animals without metritis (Lima et al 2014). However, it is not clear whether subclinical endometritis is absolutely dependent on the presence of pathogens, or may reflect dysregulation of the innate immune response, even in the absence of pathogens.

Treatment

Current ideas for treating subclinical endometritis were reviewed recently (Wagener et al 2017). Unfortunately, there is little agreement about whether, or how, animals with subclinical endometritis should be treated. On the basis that physiological mechanisms usually aim to restore homeostasis, the most rational treatment for subclinical endometritis is to administer PGF_{2alpha} (or analogue) to animals with a CL. Other suggestions are the intrauterine infusion of antimicrobials or antiseptics, or to flush the uterus as if performing an embryo collection.

4.6 PYOMETRA

Pyometra is defined as a progressive accumulation of purulent material within the uterus in the presence of an active CL (Sheldon et al 2006). The condition occurs most commonly when uterine infection is not eliminated during the first follicular phase, and the resulting inflammation means that the uterus ceases to produce PGF. Consequently, the life span of the

CL is prolonged. In most cases, pyometra occurs as a sequel to endometritis during the postpartum period. Venereal infections that cause embryonic death (notably *T. fetus*) can predispose to a high incidence of pyometra in an infected herd after mating, while occasional cases also occur after fetal death, maceration and superinfection with *T. pyogenes*.

Diagnosis

Cows that suffer from pyometra show few or no signs of ill health. Hence, the disease is generally discovered**:** (1) when affected cows are examined for the absence of cyclical activity**,** or (2) at the time of pregnancy diagnosis in a cow that was thought, on the basis of non-return to oestrus, to be pregnant. Because the cervix remains closed, the purulent exudate accumulates within the uterine lumen, although occasionally there is a slight or intermittent purulent discharge. The uterine horns are enlarged and distended (Fig. 25.14), quite often to an unequal degree, owing to incomplete involution of the previously gravid horn or to recent conceptus death. Differentiation of pyometra from a normal pregnancy can sometimes be difficult, but there are a number of distinguishing points:

- The uterine wall is thicker than at pregnancy.
- The uterus has a more ‘doughy’ and less vibrant feel.
- It is not possible to slip the allantochorion (see Chapter 5).
- In some cases of pyometra, no uterine caruncles can be palpated. However, when the infection occurred in a non-involuted uterus, involution of the caruncles is delayed and they may remain palpable for quite a long time.
- Transrectal ultrasonography will demonstrate the absence of a fetus**,** and the presence of a speckled echotexture of the uterine contents compared with the black anechoic appearance of normal fetal fluids.

If there is any doubt about the differentiation between pyometra and pregnancy, the cow should be left untreated and re-examined later for evidence of change. Pyometra associated with *T. fetus* infection presents features that are different from those previously described. Uterine pus is, as a rule, much more copious and may attain a volume of many litres, and the uterus undergoes much greater distension than postpartum pyometra; the uterine pus is often fluid and is greyish-white or white. The mucus occupying the cervix is moist and slippery, rather than sticky and tenacious, and motile trichomonads can generally be found in it.

Treatment

When the presence of pyometra is discovered at the time of pregnancy diagnosis, the decision is often made to cull the cow at the end of the current lactation. Treatment, if undertaken, is with PGF_{2alpha}, which results in regression of the CL, dilation of the cervix, expulsion of the purulent fluid and oestrus 3 to 5 days later. Provided that the condition is not too long-standing and therapy is instituted quickly, there is a reasonable possibility that the cow will eventually conceive again. However, long-standing cases are associated with more severe degeneration of the endometrium, reducing the chances of subsequent conception. There may be advantages to the use of intrauterine cephapirin in conjunction with PGF_{2alpha} (or analogue), and multiple treatments at 11 to 14 day intervals may salvage animals for future breeding.

4.7 CERVICITIS AND VAGINITIS

Cervicitis and vaginitis may be caused by trauma during parturition or during obstetrical manipulation. Tearing of the cervix, vagina and vulva are often self-evident during and after parturition. If these tears become infected they can cause considerable swelling and pain, and animals often strain frequently. Treatment for vaginitis is usually symptomatic with emollient creams applied to the injured tissues and parenteral and/or local antibiotics.

Purulent vaginal discharge 21 days or more post partum, may indicate cervicitis or vaginitis, rather than clinical endometritis. In some cases purulent vaginal discharge may be associated with both vaginitis and endometritis; whereas, in other cases purulent vaginal discharge may represent vaginitis or cervicitis, rather than endometritis (Denis-Robichaud & Dubuc 2015). It is thought that cervicitis, 21 days or more post partum, is associated most often with prior obstetrical trauma and/or RFM. Cervicitis was diagnosed in 61% of animals in a study of 416 cows examined at 42 to 50 days post partum (Hartmann et al 2016). Cervicitis can affect fertility, with fewer animals pregnant by 300 days in milk (Deguillaume et al 2012). Treatment of purulent vaginal discharge that is caused by cervicitis alone is often symptomatic, and so most of these animals are treated as if they had clinical endometritis.

4.8 IMPACT OF UTERINE DISEASE ON OVARIAN FUNCTION

Pathological changes in the endometrium during uterine disease are likely to be detrimental to fertilization and conception. However, postpartum uterine infections also impair fertility after resolution of the clinical disease (Borsberry & Dobson 1989). Thus, one must consider if uterine infections impair the oviduct, ovary, or the hypothalamic-pituitary axis.

The uterine tubes (oviducts) impede bacteria reaching the ovary; consequently, ovarian infections or abscesses are rare. However, extension of infection or inflammation into the oviduct likely disrupts the delicate balance of the immune systems that are required for fertilization (Marey et al 2016).

Beyond the tubular genital tract, uterine infection may impair ovarian function (Sheldon et al 2014a; Bromfield et al 2015). At a practical level, veterinarians should be aware that individual dairy cows with postpartum uterine infections, have a slower growth of the dominant follicle, lower peripheral plasma oestradiol concentrations, and are less likely to ovulate (Sheldon et al 2002b). At the herd level, a history of uterine infection or dystocia are

associated with a delayed return to ovarian cyclic activity, more cystic ovarian disease, and with prolonged luteal phases (Opsomer et al 2000).

One possible mechanism linking uterine disease to ovarian dysfunction is that cytokines and PAMPs perturb the endocrine function of the hypothalamus and the pituitary, reducing the release of GnRH and LH, which impacts ovarian function (Peter et al 1989; Karsch et al 2002). In postpartum cows, endotoxin in the uterus suppresses the luteinising hormone (LH) surge, and prevents ovulation (Peter et al 1989). In addition, during the follicular phase endotoxin decreases LH pulse frequency, decreases oestradiol and interrupts the LH surge and ovulation in cattle (Suzuki et al 2001; Lavon et al 2008). Similar observations have been made in sheep, which is the species most often used to study the effect of endotoxin in the ruminant brain (Karsch et al 2002). Neuroendocrine activity at the hypothalamic level is inhibited by endotoxin suppressing both the frequency and amplitude of GnRH/LH pulses. However, there are also effects within the pituitary gland, as endotoxin lowers LH responses to exogenous GnRH (Karsch et al 2002). Indeed, treatment of ewes with endotoxin suppresses the expression of genes encoding GnRH receptor (GnRHR) and LHβ (Herman et al 2013). However, the peripheral plasma concentrations of FSH are unaffected by uterine disease in cattle, so that recurrent waves of follicles develop in the ovary, even during uterine disease (Sheldon et al 2002b).

A second mechanism is that the cytokines, associated with the host defence response to bacteria in the uterus, may reach the ovary via the localised counter-current mechanism, as used by prostaglandin $F_{2\alpha}$ during luteolysis (see Chapter 1). For example, cytokines such as IL-6 and TNFα perturb bovine ovarian follicular cell steroidogenesis (Alpizar & Spicer 1994; Spicer 1998).

A third mechanism linking infection of the endometrium with ovarian function is that, PAMPs might also reach the ovary from the uterus. The concentrations of endotoxin in

follicular fluid aspirated from dominant follicles is correlated with the severity of uterine disease (Herath et al 2007). Notably, healthy ovarian follicles do not contain hematopoietic immune cells, so ovarian follicle responses to cytokines or PAMPs must rely on the granulosa cells and oocyte (Herath et al 2007; Bromfield & Sheldon 2011). Indeed, endotoxin limits granulosa cell oestradiol production by reducing *CYP19A1* gene expression and aromatase protein levels (Herath et al 2007; Price et al 2013). Interestingly, granulosa cells isolated from growing or dominant ovarian follicles express most of the TLRs (Bromfield & Sheldon 2011; Price et al 2013). Furthermore, PAMPs, such as bacterial endotoxin or bacterial lipopeptides, stimulate an inflammatory response by granulosa cells, with the secretion of IL-1β, IL-6, CXCL1, CXCL2, CXCL3 and IL-8 protein (Bromfield & Sheldon 2011; Price et al 2013). Inhibiting *TLR4* or *TLR2* gene expression in bovine granulosa cells, using siRNA, reduced the secretion of IL-6 in response to their cognate PAMPs (Bromfield & Sheldon 2011; Price et al 2013). So, granulosa cells in antral follicles clearly have roles in innate immunity. Endotoxin also reduces the primordial ovarian follicle pool, with an associated increase in primordial follicle activation, and loss of primordial follicle regulatory proteins (Bromfield & Sheldon 2013). However, not all stages of follicle development are sensitive to PAMPs, and endotoxin did not affect the growth and viability of individually-cultured secondary follicles (Bromfield & Sheldon 2013).

During the later stages of ovarian follicle development, endotoxin stimulates IL-6 secretion from cumulus-oocyte complexes and activates cumulus expansion *in vitro* (Bromfield & Sheldon 2011). Inappropriate timing of cumulus expansion may contribute to infertility, because expansion is normally closely coordinated with ovulation. Furthermore, endotoxin or IL-6 might reach the oocyte via the cytoplasmic trans-zonal projections from granulosa cells that synapse on the oolema. Indeed, endotoxin increases the incidence of meiotic arrest and germinal vesicle breakdown failure in bovine oocytes (Bromfield & Sheldon 2011).

Furthermore, treatment of cumulus-oocyte complexes with endotoxin or PAM perturbed expression of genes such as *GDF9* and *NLRP5*, which are involved in oocyte maturation (Sheldon et al 2014b). Oocyte development takes about120 days, between the primordial follicle stage to ovulation of a cumulus-oocyte complex. Thus, it has been suggested that in cows inseminated 60 to 120 days post partum, the oocytes that are ovulated may have been perturbed, and be less fertile following exposure to metabolic stress (Britt 1992). Therefore, limiting uterine disease is not only important for the affected animals, but also for their offspring.

5. CONCLUSIONS AND OUTLOOK

Whilst there is a clear understanding of the clinical aspects and implication of postpartum uterine disease, and some of the mechanisms of pathology, there are important outstanding questions. The most obvious question is: why are modern high-milk-yield cows so susceptible to metritis and endometritis? Allied to this, is what can be done to prevent uterine disease? Answering these questions is vital for sustainable intensification of the dairy industry over the next 50 years. Genetic selection for more resilient animals that are less susceptible to uterine disease is a priority for the dairy industry. In the meantime, veterinarians should consider that prevention is better than cure, and advise clients about animal nutrition and management to increase the resilience of parturient and postpartum animals so that they are better able to tolerate the presence of uterine pathogens without developing uterine disease.

6. REFERENCES

O Agthe, HP Kolm, 1975 *J Reprod Fertil* **43** 163-166

RD Allison, RA Laven, 2000 *Vet Rec* **147** 703-708

E Alpizar, LJ Spicer, 1994 *Biol Reprod* **49** 38-43

MR Amos, GD Healey, RJ Goldstone, et al., 2014 *Biol Reprod* **90** 54,51-13

S Andriamanga, J Steffan, M Thibier, 1984 *Annal Recherche Veterinaire* **15** 503-508

LF Archbald, RH Schultz, ML Fahning, et al., 1972 *J Reprod Fertil* **29** 133-136

CF Aréchiga, O Ortíz, PJ Hansen, 1994 *Theriogenology* **41** 1251-1258

GH Arthur, D Bee, 1986. 7th edn. Eds GH Arthur, DE Noakes, H Pearson, TJ Parkinson, WB Saunders: London

TR Ayliffe, DE Noakes, 1978 *Vet Rec* **102** 215

TR Ayliffe, DE Noakes, 1982 *Vet Rec* **110** 96-98

AJ Barrett, RD Murray, RM Christley, et al., 2009 *Vet Rec* **165** 623-626

H Baumann, J Gauldie, 1994 *Immunol Today* **15** 74-80

LA Baxt, AC Garza-Mayers, MB Goldberg, 2013 *Science* **340** 697-701

JC Beagley, KJ Whitman, KE Baptiste, et al., 2010 *J Vet Intern Med* **24** 261-268

SW Beam, WR Butler, 1997 *Biol Reprod* **56** 133-142

SW Beam, WR Butler, 1999 *J Reprod Fertil Suppl* **54** 411-424

NH Bjorkman, 1969 *Anat Rec* **163** 17-29

JM Blander, LE Sander, 2012 *Nat Rev Immunol* **12** 215-225

O Bogado Pascottini, M Hostens, P Dini, et al., 2016 *Theriogenology* **86** 1550-1556

A Bolinder, B Seguin, H Kindahl, et al., 1988 *Theriogenology* **30** 45-56

BN Bonnett, SW Martin, VP Gannon, et al., 1991a *Can J Vet Res* **55** 168-173

BN Bonnett, RB Miller, WG Etherington, et al., 1991b *Can J Vet Res* **55** 155-161

A Boos, J Kohtes, A Stelljes, et al., 2000 *J Reprod Fertil* **120** 351-360

A Boos, A Stelljes, J Kohtes, 2003 *Cells Tissues Organs* **174** 170-183

AM Borges, GD Healey, IM Sheldon, 2012 *Am J Reprod Immunol* **67** 526-539

S Borsberry, H Dobson, 1989 *Vet Rec* **124** 217-219

N Bourne, R Laven, DC Wathes, et al., 2007 *Theriogenology* **67** 494-501

KN Bretzlaff, HL Whitmore, SL Sphar, et al., 1982 *Theriogenology* **17** 527-535

JH Britt, 1992 *Bovine Practitioner* **24** 39-43

JJ Bromfield, JE Santos, J Block, et al., 2015 *J Anim Sci* **93** 2021-2033

JJ Bromfield, IM Sheldon, 2011 *Endocrinology* **152** 5029-5040

JJ Bromfield, IM Sheldon, 2013 *Biol Reprod* **88** 98, 91-99

J Bruun, AK Ersboll, L Alban, 2002 *Prev Vet Med* **54** 179-190

T-Q Cai, PG Weston, LA Lund, et al., 1994 *Am J Vet Res* **55** 934-943

LM Chagas, JJ Bass, D Blache, et al., 2007 *J Dairy Sci* **90** 4022-4032

A Chapwanya, KG Meade, ML Doherty, et al., 2009 *Theriogenology* **71** 1396-1407

GY Chen, G Nunez, 2010 *Nat Rev Immunol* **10** 826-837

JR Chenault, JF Mcallister, STJ Chester, et al., 2004 *J Am Vet Med Assoc* **224** 1634-1639

SH Cheong, OG Sa Filho, VA Absalon-Medina, et al., 2016 *Biol Reprod* **94** 18, 11-11

BP Chew, HF Keller, RE Erb, et al., 1977 *J Anim Sci* **44** 1055-1060

RO Cohen, M Bernstein, G Ziv, 1995 *Theriogenology* **43** 1389-1397

JG Cronin, V Kanamarlapudi, CA Thornton, et al., 2016 *Mucosal Immunol* **9** 1125-1136

JG Cronin, ML Turner, L Goetze, et al., 2012 *Biol Reprod* **86** 51, 51-59

MA Crowe, V Padmanabhan, M Mihm, et al., 1998 *Biol Reprod* **58** 1445-1450

CJ Davies, JR Hill, JL Edwards, et al., 2004 *Anim Reprod Sci* **82** 267-280

D Davies, KG Meade, S Herath, et al., 2008 *Reprod Biol Endocrinol* **6** 53

MW De Boer, SJ Leblanc, J Dubuc, et al., 2014 *J Dairy Sci* **97** 1-17

JD De Koster, G Opsomer, 2013 *Vet Clin North Am Food Anim Pract* **29** 299-322

L Deguillaume, A Geffre, L Desquilbet, et al., 2012 *J Dairy Sci* **95** 1776-1783

Q Deng, JF Odhiambo, U Farooq, et al., 2014 *PLoS ONE* **10** e0124167

J Denis-Robichaud, J Dubuc, 2015 *J Dairy Sci* **98** 6848-6855

GS Dhaliwal, RD Murray, Z Woldehiwet, 2001 *Anim Reprod Sci* **67** 135-152

L Doepel, M Lessard, N Gagnon, et al., 2006 *J Dairy Sci* **89** 3107-3121

MJ Dohmen, K Joop, A Sturk, et al., 2000 *Theriogenology* **54** 1019-1032

G Donofrio, S Herath, C Sartori, et al., 2007 *Reproduction* **134** 183-197

H Dosogne, C Burvenich, JaCM Lohuis, 1999 *Theriogenology* **51** 867-874

M Drillich, S Arlt, S Kersting, et al., 2006a *J Dairy Sci* **89** 3431-3438

M Drillich, M Mahlstedt, U Reichert, et al., 2006b *J Dairy Sci* **89** 627-635

PR Dubois, DJ Williams, 1980 *Theriogenology* **13** 115-121

J Dubuc, TF Duffield, KE Leslie, et al., 2010 *J Dairy Sci* **93** 5764-5771

P Duffy, MA Crowe, MP Boland, et al., 2000 *J Reprod Fertil* **118** 9-17

E Eiler, KA Fecteau, 2007 In Current Therapy in Large Animal Theriogenology 2nd edn. Eds RS Youngquist, WR Threlfall, Saunders-Elsevier: St Louis, MO 345–354

L Elliott, KJ Mcmahon, HT Gier, et al., 1968 *Am J Vet Res* **29** 77-81

G Esposito, PC Irons, EC Webb, et al., 2014 *Anim Reprod Sci* **144** 60-71

RJ Esslemont, EJ Peeler, 1993 *Brit Vet J* **149** 537-547

C Fischer, M Drillich, S Odau, et al., 2010 *Reprod Fertil Dev* **22** 818-829

FA Fonseca, JH Britt, BT Mcdaniel, et al., 1983 *J Dairy Sci* **66** 1128-1147

C Fourichon, H Seegers, X Malher, 2000 *Theriogenology* **53** 1729-1759

GS Frazer, 2005 *Vet Clin North Am Food Anim Pract* **21** 523-568

K Frazier, M Pence, MJ Mauel, et al., 2001 *J Vet Diagn Invest* **13** 502-508

C Gabler, M Drillich, C Fischer, et al., 2009 *Theriogenology* **71** 993-1004

C Gabler, C Fischer, M Drillich, et al., 2010 *Reprod Biol Endocrinol* **8** 152

KN Galvao, MJ Flaminio, SB Brittin, et al., 2010 *J Dairy Sci* **93** 2926-2937

KN Galvao, NR Santos, JS Galvao, et al., 2011 *Theriogenology* **76** 290-299

A Garcia, AD Barth, RJ Mapletoft, 1992 *Can Vet J* **33** 175-183

P Gasque, 2004 *Mol Immunol* **41** 1089-1098

E Gernand, P Rehbein, UU Von Borstel, et al., 2012 *J Dairy Sci* **95** 2144-2156

F Ghasemi, P Gonzalez-Cano, PJ Griebel, et al., 2012 *Theriogenology* **78** 1538-1547

HT Gier, GB Marion, 1968 *Am J Vet Res* **29** 83-96

RO Gilbert, ST Shin, CL Guard, et al., 2005 *Theriogenology* **64** 1879-1888

MJ Giuliodori, RP Magnasco, D Becu-Villalobos, et al., 2013 *J Dairy Sci* **96** 3621-3631

JL Goldstein, MS Brown, 1990 *Nature* **343** 425-430

T Goshen, NY Shpigel, 2006 *Theriogenology* **66** 2210-2218

JFT Griffin, PJ Hartigan, WR Nunn, 1974a *Theriogenology* **1** 91-106

JFT Griffin, PJ Hartigan, WR Nunn, 1974b *Theriogenology* **1** 107-114

Y Gröhn, HN Erb, CE Mcculloch, et al., 1990 *Prev Vet Med* **8** 25-39

TS Gross, WF Williams, 1988 *Biol Reprod* **38** 1027-1034

TS Gross, WF Williams, JE Manspeaker, et al., 1987 *Prostaglandins* **34** 903-917

TS Gross, WF Williams, TW Moreland, 1986 *Theriogenology* **26** 365-370

E Grunert, 1986 In Current Therapy in Theriogenology Ed DA Morrow, WB Saunders: Philadelphia

E Grunert, D Grunert, 1990 *Tierarztl Prax* **18** 473-476

JW Gunnink, 1984 *Vet Q* **6** 49-51

S Gupta, HK Gupta, J Soni, 2005 *Theriogenology* **64** 1273-1286

L Gurcel, L Abrami, S Girardin, et al., 2006 *Cell* **126** 1135-1145

FC Gwazdauskas, TL Bibb, ML Mcgilliard, et al., 1979 *J Dairy Sci* **62** 978-981

P Haimerl, W Heuwieser, 2014 *J Dairy Sci* **97** 6649-6661

DS Hammon, IM Evjen, TR Dhiman, et al., 2006 *Vet Immunol Immunopath* **113** 21-29

IK Han, IH Kim, 2005 *J Vet Sci* **6** 53-59

PJ Hartigan, JA Murphy, WR Nunn, et al., 1972 *Ir Vet J* **26** 245-247

PJ Hartigan, WR Nunn, JFT Griffin, 1974 *Br Vet J* **130** 160-163

D Hartmann, J Rohkohl, S Merbach, et al., 2016 *Theriogenology* **85** 247-253

GD Healey, C Collier, S Griffin, et al., 2016 *Journal of Immunology* **196** 823-831

LL Healy, JG Cronin, IM Sheldon, 2014 *Sci Rep* **4** 7060

LL Healy, JG Cronin, IM Sheldon, 2015 *Biol Reprod* **92** 151, 151–112

KEM Hendricks, JA Bartolome, P Melendez, et al., 2006 *Theriogenology* **65** 1454-1464

S Herath, DP Fischer, D Werling, et al., 2006 *Endocrinology* **147** 562-570

S Herath, ST Lilly, DP Fischer, et al., 2009a *Endocrinology* **150** 1912-1920

S Herath, ST Lilly, NR Santos, et al., 2009b *Reprod Biol Endocrinol* **7** 55

S Herath, EJ Williams, ST Lilly, et al., 2007 *Reproduction* **134** 683-693

AP Herman, A Krawczynska, J Bochenek, et al., 2013 *Anim Reprod Sci* **138** 203-212

J Hernandez, CA Risco, JB Elliott, 1999 *J Am Vet Med Assoc* **215** 72-76

W Heuwieser, E Grunert, 1987 *Theriogenology* **27** 907-912

M Hidiroglou, AJ Mcallister, CJ Williams, 1987 *J Dairy Sci* **70** 1281-1288

M Hoelker, D Salilew-Wondim, M Drillich, et al., 2012 *Reprod Fertil Dev* **24** 778-793

G Huszenicza, M Fodor, M Gacs, et al., 1991 *Reprod Dom Anim* **34** 237-245

JM Huzzey, DM Veira, DM Weary, et al., 2007 *J Dairy Sci* **90** 3220-3233

I Joosten, MF Sanders, EJ Hensen, 1991 *Anim Genet* **22** 455-463

I Joosten, J Stelwagen, AA Dijkhuizen, 1988 *Vet Rec* **123** 53-57

WJ Jordan, 1952 *J Comp Pathol Ther* **62** 54-68

WE Julien, HR Conrad, JE Jones, et al., 1976 *J Dairy Sci* **59** 1954-1959

M Kankofer, L Guz, 2003 *Domest Anim Endocrinol* **25** 61-67

M Kankofer, M Hoedemaker, HA Schoon, et al., 1994 *Theriogenology* **42** 1311-1322

M Kankofer, J Wiercinski, H Zerbe, 2000 *Reprod Dom Anim* **35** 97-100

FJ Karsch, DF Battaglia, KM Breen, et al., 2002 *Stress* **5** 101-112

CC Karstrup, K Klitgaard, TK Jensen, et al., 2017 *Theriogenology* **99** 43-47

R Kasimanickam, JM Cornwell, RL Nebel, 2006 *Anim Reprod Sci* **95** 214-223

R Kasimanickam, TF Duffield, RA Foster, et al., 2004 *Theriogenology* **62** 9-23

R Kasimanickam, TF Duffield, RA Foster, et al., 2005 *Theriogenology* **63** 818-830

R Kasimanickam, V Kasimanickam, JP Kastelic, 2014 *Theriogenology* **81** 952-958

M Kerestes, V Faigl, M Kulcsar, et al., 2009 *Domest Anim Endocrinol* **37** 250-261

IH Kim, HG Kang, 2003 *J Reprod Dev* **49** 485-491

IH Kim, HG Kang, JK Jeong, et al., 2014 *Theriogenology* **82** 427-432

K Kimura, JP Goff, ME Kehrli, et al., 2002 *J Dairy Sci* **85** 544-550

LR Knudsen, CC Karstrup, HG Pedersen, et al., 2015 *Vet Microbiol* **175** 319-324

MA Kossaibati, RJ Esslemont, 1997 *Vet J* **154** 41-51

ME Kotas, R Medzhitov, 2015 *Cell* **160** 816-827

SK Kvidera, EA Horst, M Abuajamieh, et al., 2017 *J Dairy Sci* **100** 2360-2374

RA Laven, AR Peters, 1996 *Vet Rec* **139** 465-471

Y Lavon, G Leitner, T Goshen, et al., 2008 *Theriogenology* **70** 956-967

SJ Leblanc, TF Duffield, KE Leslie, et al., 2002a *J Dairy Sci* **85** 2223-2236

SJ Leblanc, TF Duffield, KE Leslie, et al., 2002b *J Dairy Sci* **85** 2237-2249

C Lecchi, F Dilda, P Sartorelli, et al., 2012 *Vet Immunol Immunopath* **145** 556-562

AM Ledgard, GA Smolenski, H Henderson, et al., 2015 *Reprod Fertil Dev* **27** 395-406

GS Lewis, 2003 *Reprod Biol Endocrinol* **1** 117

FS Lima, A Vieira-Neto, GS Vasconcellos, et al., 2014 *J Dairy Sci* **97** 5401-5414

HK Lin, PA Oltenacu, LD Van Vleck, et al., 1989 *J Dairy Sci* **72** 180-186

VS Machado, ML Bicalho, EB Meira Junior, et al., 2014 *PLoS ONE* **9** e91734

VS Machado, G Oikonomou, ML Bicalho, et al., 2012 *Vet Microbiol* **159** 460-469

MA Marey, MS Yousef, R Kowsar, et al., 2016 *Domest Anim Endocrinol* **56 Suppl** S204-211

O Markusfeld, 1984 *Vet Rec* **114** 539-542

LR Martin, WF Williams, E Russek, et al., 1981 *Theriogenology* **15** 513-524

J Martinez, M Thibier, 1984 *Theriogenology* **21** 583-590

EA Mcneela, A Burke, DR Neill, et al., 2010 *PLoS Path* **6** e1001191

R Medzhitov, DS Schneider, MP Soares, 2012 *Science* **335** 936-941

Y Melcher, I Prunner, M Drillich, 2014 *Theriogenology* **82** 57-63

P Melendez, GA Donovan, CA Risco, et al., 2003 *Theriogenology* **60** 843-854

S Mido, N Murata, MS Rawy, et al., 2016 *J Vet Med Sci* **78** 551-556

BJ Miller, JR Lodge, 1984 *Theriogenology* **22** 385-388

E Miller, D Beasley, R Dunn, et al., 2016 *Front Microbiol* **7** 1936

M Miyoshi, Y Sawamukai, T Iwanaga, 2002 *Reprod Dom Anim* **37** 53-56

A Monge, L Elvira, JV Gonzalez, et al., 2006 *Res Vet Sci* **80** 120-125

SG Moore, AC Ericsson, SE Poock, et al., 2017 *J Dairy Sci* **100** 4953–4960

EM Moresco, D Lavine, B Beutler, 2011 *Curr Biol* **21** R488-493

J Morton, 2000 The InCalf Project, Progress Report No. 2. Melbourne, Australia., Dairy Research and Development Corporation

DE Noakes, L Wallace, GR Smith, 1991 *Vet Rec* **128** 440-442

DE Noakes, LM Wallace, GR Smith, 1990 *Vet Rec* **126** 509

PG Noleto, JP Saut, IM Sheldon, 2017 *J Dairy Sci* **100** 2207-2212

O Nolte, J Morscher, HE Weiss, et al., 2001 *Vaccine* **19** 3146-3153

LA O'neill, RJ Kishton, J Rathmell, 2016 *Nat Rev Immunol* **16** 553-565

A Okano, T Tomizuka, 1987 *Theriogenology* **27** 369-376

JD Olson, L Ball, RG Mortimer, et al., 1984 *Am J Vet Res* **45** 2251-2255

G Opsomer, YT Grohn, J Hertl, et al., 2000 *Theriogenology* **53** 841-857

LG Paisley, WD Mickelsen, PB Anderson, 1986 *Theriogenology* **25** 353-381

CC Palmer, 1932 *J Am Vet Med Assoc* **80** 59

Y Peng, Y Wang, S Hang, et al., 2013 *Folia Microbiologica* **58** 593-600

RT Pepper, H Dobson, 1987 *Vet Rec* **120** 53-56

AT Peter, WTK Bosu, RJ Dedecker, 1989 *Am J Vet Res* **50** 368-373

AR Peters, RA Laven, 1996 *Vet Rec* **139** 535-539

PJ Pinedo, KN Galvao, CM Seabury, 2013 *Theriogenology* **80** 384-390

T Potter, J Guitian, J Fishwick, et al., 2010 *Theriogenology* **74** 127-134

G Preta, V Lotti, JG Cronin, et al., 2015 *FASEB J* **29** 1516-1528

JC Price, JJ Bromfield, IM Sheldon, 2013 *Endocrinology* **154** 3377-3386

L Raberg, D Sim, AF Read, 2007 *Science* **318** 812-814

J Ravel, P Gajer, Z Abdo, et al., 2011 *Proc Natl Acad Sci U S A* **108 Suppl. 1** 4680-4687

CA Risco, M Drost, WW Thatcher, et al., 1994 *Theriogenology* **42** 183-203

SJ Roberts, 1986 Veterinary Obstetrics and Genital Diseases (Theriogenology) 3rd Edn., Published by the author: Woodstock, VT

CA Ruder, RG Sasser, RJ Williams, et al., 1981 *Theriogenology* **15** 573-580

JW Sagartz, HJ Hardenbrook, 1971 *J Am Vet Med Assoc* **158** 619-622

WCD Sandals, RA Curtis, JF Cote, et al., 1979 *Can Vet J* **20** 131-135

I Sannmann, W Heuwieser, 2015 *J Dairy Sci* **98** 5460-5466

TM Santos, RC Bicalho, 2012 *PLoS ONE* **7** e53048

J Sarges, W Heuwieser, J Schlüns, et al., 1998 *J Vet Med A* **45** 1-10

JP Saut, GD Healey, AM Borges, et al., 2014 *Reproduction* **148** 593-606

JD Savio, MP Boland, N Hynes, et al., 1990 *J Reprod Fertil* **88** 569-579

DS Schneider, JS Ayres, 2008 *Nat Rev Immunol* **8** 889-895

K Schroder, J Tschopp, 2010 *Cell* **140** 821-832

IM Sheldon, 2014 *Reprod Fertil Dev* **27** 72-81

IM Sheldon, J Cronin, L Goetze, et al., 2009 *Biol Reprod* **81** 1025-1032

IM Sheldon, JG Cronin, GD Healey, et al., 2014a *Reproduction* **148** R41-R51

IM Sheldon, GS Lewis, S Leblanc, et al., 2006 *Theriogenology* **65** 1516-1530

IM Sheldon, DE Noakes, 1998 *Vet Rec* **142** 575-579

IM Sheldon, DE Noakes, H Dobson, 2000 *Theriogenology* **54** 409-419

IM Sheldon, DE Noakes, A Rycroft, et al., 2001 *Vet Rec* **148** 172-175

IM Sheldon, DE Noakes, AN Rycroft, et al., 2002a *Vet Rec* **151** 531-534

IM Sheldon, DE Noakes, AN Rycroft, et al., 2003 *Theriogenology* **59** 1357-1371

IM Sheldon, DE Noakes, AN Rycroft, et al., 2004 *Anim Reprod Sci* **81** 13-23

IM Sheldon, DE Noakes, AN Rycroft, et al., 2002b *Reproduction* **123** 837-845

IM Sheldon, SE Owens, ML Turner, 2017 *J Reprod Immunol* **119** 67-73

IM Sheldon, JC Price, ML Turner, et al., 2014b In Reproduction in Domestic Ruminants VIII. Eds JL Juengel, A Miyamoto, C Price, LP Reynolds, RF Smith, R Webb. Ashby-de la Zouch, Context Products Ltd 415-430

IM Sheldon, AN Rycroft, B Dogan, et al., 2010 *PLoS ONE* **5** e9192

H Slama, D Vaillancourt, AK Goff, 1993 *Can J Vet Res* **57** 293-299

H Slama, D Vaillancourt, AK Goff, 1994 *Domest Anim Endocrinol* **11** 175-185

BI Smith, G Arthur Donovan, C Risco, et al., 1998a *J Dairy Sci* **81** 1555-1562

BI Smith, GA Donovan, CA Risco, et al., 1998b *Vet Rec* **142** 83-85

GW Smith, 2005 *Vet Clin North Am Food Anim Pract* **21** 595-614

LM Sordillo, GA Contreras, SL Aitken, 2009 *Anim Health Res Rev* **10** 53-63

LJ Spicer, 1998 *Endocrine* **8** 109-115

K Stagg, MG Diskin, JM Sreenan, et al., 1995 *Anim Reprod Sci* **38** 49-61

J Steffan, S Adriamanga, M Thibier, 1984 *Am J Vet Res* **45** 1090-1094

RD Stevens, RP Dinsmore, 1997 *J Am Vet Med Assoc* **211** 1280-1284

J Stojkov, MA Von Keyserlingk, JN Marchant-Forde, et al., 2015 *J Dairy Sci* **98** 5352-5361

E Studer, DA Morrow, 1978 *J Am Vet Med Assoc* **172** 489-494

C Suzuki, K Yoshioka, S Iwamura, et al., 2001 *Domest Anim Endocrinol* **20** 267-278

T Swangchan-Uthai, Q Chen, SE Kirton, et al., 2013 *Reproduction* **145** 527-539

O Takeuchi, S Akira, 2010 *Cell* **140** 805-820

B Tennant, RG Peddicord, 1968 *Cornell Vet* **58** 185-192

W Tian, DE Noakes, 1991a *Vet Rec* **128** 109-110

W Tian, DE Noakes, 1991b *Vet Rec* **129** 463-466

N Trinder, R Hall, C Renton, 1973 *Vet Rec* **93** 641-643

ML Turner, JC Cronin, GD Healey, et al., 2014 *Endocrinology* **155** 1453-1465

ML Turner, JG Cronin, PG Noleto, et al., 2016 *PLoS ONE* **11** e0151416

JH Venable, LE Mcdonald, 1958 *Am J Vet Res* **19** 308-313

K Wagener, C Gabler, M Drillich, 2017 *Theriogenology* **94** 21-30

K Wagener, I Prunner, H Pothmann, et al., 2015 *Vet Microbiol* **175** 286-293

WC Wagner, W Hansel, 1969 *J Reprod Fertil* **18** 493-500

DC Wathes, Z Cheng, W Chowdhury, et al., 2009 *Physiol Genomics* **39** 1-13

DC Wathes, Z Cheng, MA Fenwick, et al., 2011 *Reproduction* **141** 269-281

DC Wathes, AM Clempson, GE Pollott, 2012 *Reprod Fertil Dev* **25** 48-61

A Wehrend, T Reinle, K Herfen, et al., 2002 *Dtsch Tierarztl Wochenschr* **109** 56-61

S Westermann, M Drillich, TB Kaufmann, et al., 2010 *Theriogenology* **74** 1248-1255

LC Whiteford, IM Sheldon, 2005 *Vet Rec* **I157** 202-204

HL Whitmore, WJ Tyler, LE Casida, 1974 *J Am Vet Med Assoc* **165** 693-694

D Wilde, 2006 *Anim Reprod Sci* **96** 240-249

EJ Williams, DP Fischer, DE Noakes, et al., 2007 *Theriogenology* **68** 549-559

EJ Williams, IM Sheldon, 2003 *Reprod Abstr Ser* **30** 91

A Wischral, A Nishiyama-Naruke, R Curi, et al., 2001a *Prostaglandins Other Lipid Mediat* **65** 117-124

A Wischral, ITN Verreschi, SB Lima, et al., 2001b *Anim Reprod Sci* **67** 181-188

H Zerbe, N Schneider, W Leibold, et al., 2000 *Theriogenology* **54** 771-786

K Zerobin, H Spörri, 1972 *Adv Vet Sci Comp Med* **16** 303-354

NR Zwald, KA Weigel, YM Chang, et al., 2004 *J Dairy Sci* **87** 4287-4294

FIGURE LEGENDS

Figure 25.1. A typical case of postpartum metritis in a dairy cow, with accumulation of pus in the uterus, which is discharging from the vulva.

Figure 25.2. The incidence of uterine bacterial infection and disease in postpartum dairy cattle (Sheldon et al 2009). Bacteria can be isolated from the uterus of most cows during the postpartum period; each marker (circle) indicates the percentage of animals with bacteria isolated from the uterine lumen in four studies (Elliott et al 1968; Griffin et al 1974b; Bonnett et al 1991a; Sheldon et al 2002b). The shaded areas represent estimates of the proportion of animals with metritis (red), clinical endometritis (orange), or a normal uterus (blue); the remainder of animals have subclinical endometritis (Gilbert et al 2005).

Figure 25.3. Uterine involution monitored in 24 dairy cattle, using transrectal ultrasonography to measure the diameter of the previously gravid and non-gravid uterine horns at the level of the attachment of the inter-cornual ligament (Sheldon et al 2003).

Figure 25.4. (A) Schematic "reaction norm" of health status against pathogen load for two equally resilient groups of animals. The blue group has *impaired immunity*, with reduced health status and more pathogens, compared with the red group (Raberg et al 2007). (B) Two groups with similar immunity, but the red group is *less resilient*, with reduced health at the same pathogen load as the blue group (Raberg et al 2007). (C) The reaction norm of postpartum uterine clinical health score (Sheldon et al 2002b), against uterine bacterial load for dairy cows, where a group of metabolically-stressed cows producing > 35 litres/day (x,red

line, n = 56) were less resilient to infection than a group of animals producing < 35 L milk/day (o, blue line, n = 34).

Figure 25.5. Outline of factors contributing to uterine resilience and immunity. After parturition the anatomical barriers of the vulva, vagina and cervix are breached, allowing the introduction of bacteria into the uterus, including pathogens, along with bacteria that constitute the uterine microbiome. However, tissue factors such as mucus, glycoproteins, the pH of the genital tract, and antimicrobial peptides, help counter bacterial invasion. If bacteria or their pathogen-associated molecules, such as endotoxin (lipopolysaccharide, LPS), are sensed by the innate or adaptive immune systems then an inflammatory response ensues; including increased expression of complement, calgranulins and acute phase proteins, and chemotaxis of neutrophils and macrophages to the site of infection. As well as inflammation, uterine disease is characterised by tissue damage, including cytolysis caused by the cholesterol-dependent cytolysin, pyolysin (PLO).

Figure 25.6. A schematic outline of the course of an infection (Sheldon et al 2017). During an infection, microbes must first overcome the resilience of the endometrial tissue and cells (A). Sensing of PAMPs and vita-PAMPs leads to inflammation, with the secretion of cytokines such as IL-6, and chemokines such as IL-8 (B). The inflammatory response to PAMPs is further increased when host cells additionally sense microbial virulence factors (C), such as pore-forming toxins and bacterial secretion systems. The inflammation is scaled by autocrine and paracrine signalling (D); for example, via IL-6 receptor and STAT3 signalling to endometrial cells. Finally, cell damage and sensing of DAMPs also enhances the inflammatory response (E). Innate immunity also helps direct the adaptive immune response

to infection (F). The consequences of inflammation include the immigration of phagocytes to help clear the microbes; phagocytes are attracted to the site of infection along a chemokine gradient, and regulated by cytokines such as IL-6 (G). Once the microbes are controlled, there is active resolution of the inflammation, and restoration of endometrial tissue homeostasis (H). All these stages are modulated by hormones, particularly by the ovarian steroid hormones oestradiol and progesterone (I).

Figure 25.7. Retained fetal membranes.

Figure 25.8. Animals with metritis have pus in the uterus that ranges from to red-brown watery exudate that contains fragments of degenerating fetal membranes**,** to off-white mucopurulent material.

Figure 25.9. Uterine lavage involves repeated cycles of infusing saline into the uterus (A), followed by syphoning the purulent suspension from the uterus (B).

Figure 25.10. Grading scheme for clinical endometritis (Sheldon et al 2009). (A) Vaginal mucus character is graded as 0 (clear or translucent mucus), 1 (mucus containing flecks of white or off-white pus), 2 (exudate containing < 50% white or off-white mucopurulent material), or 3 (exudate containing > 50% purulent material, usually white or yellow but occasionally sanguineous) (Sheldon et al 2006). (B) Endometritis grades reflect the number of pathogenic (black bars) but not opportunist non-pathogenic (white bars) bacteria isolated from the uterus of cattle (Williams & Sheldon 2003); data are presented as semi-quantitative

scores of the number of colony-forming units (CFU) from uterine swabs, where CFU is scored as 0 (no growth), 1 (< 10 CFUs), 2 (10–100 CFUs), 3 (101–500 CFUs), or 4 (> 500 CFUs). Values differ from endometritis grade 0, **P < 0.01 and ***P < 0.001. (C) Endometritis grade is prognostic for treatment success (Sheldon & Noakes 1998); treatment success rates were determined as the percentage of animals (n = 300) with normal vaginal mucus 2 weeks after initial endometritis grading and treatment 21 to 28 days postpartum. Values differ between endometritis grades, *P < 0.05 and **P < 0.01.

Figure 25.11. The vaginal mucus can be sampled and pus (A) or mucopurulent material (B) detected using a Metricheck instrument (C).

Figure 25.12. Uterine biopsy instrument (Hartigan et al 1974). (A) Whole instrument showing window with cutting edge. (B) Close-up with edge partially withdrawn (arrowed). Close-up views (C1, C2) show the cutting edge (arrowed) and interchangeable tip (C2).

Figure 25.13 Subclinical endometritis evaluation (Bogado Pascottini et al 2016). (A) Image showing how the endometrial biopsies and cytology samples were taken next to each other with an 8.0-mm punch biopsy and a cytobrush, respectively. (B) Endometrial histopathology sample stained with Naphthol-AS-D-chloroacetate-esterase. (C) Endometrial cytology sample stained with Wright-Giemsa Reproduced (Bogado Pascottini et al 2016).

Figure 25.14. Uterus with pyometra, with evidence of a CL (arrow) with distended uterine horns.

TABLES

Table 25.1. Categorization of bacteria isolated from the postpartum uterus of cattle, according to their potential pathogenicity (Sheldon et al 2002b). The categories are: (1) pathogens known to cause endometrial lesions; (2) potential uterine pathogens; and, (3) bacteria not recognized as uterine pathogens that are likely contaminants of the uterine lumen (Elliott et al 1968; Griffin et al 1974a; Ruder et al 1981; Olson et al 1984; Huszenicza et al 1991; Noakes et al 1991; Dohmen et al 2000; Sheldon et al 2002b; Westermann et al 2010).

Pathogens	Potential pathogens	Contaminants
Escherichia coli	*Acinetobacter* spp.	*Aerococcus viridans*
Trueperella pyogenes	*Bacillus licheniformis*	*Clostridium butyricum*
Prevotella spp.	*Enterococcus faecalis*	*Clostridium perfringens*
Fusobacterium necrophorum	*Haemophilus somnus*	*Corynebacterium* spp.
Fusobacterium nucleatum	*Mannhiemia haemolytica*	*Enterobacter aerogenes*
	Pasteurella multocida	*Klebsiella pneumoniae*
	Peptostreptococcus spp.	*Micrococcus* spp.
	Staphylococcus aureus (coagulase +)	*Providencia rettgeri*
		Providencia stuartii
	Streptococcus uberis	*Proteus* spp.
	Bacteroidetes species	*Proprionobacterium granulosa*
	Firmicutes species	*Staphylococcus* species
	Fusobacteria species	α-haemolyic Stretococci
		Streptococcus acidominimus

Table 25.2. Differentially expressed genes in the endometrium or endometrial cytology samples between normal animals and postpartum cows with uterine disease (Sheldon 2014).

Ontology	**Differentially expressed Genes**	**Supporting references**
Cytokines	*IL1A*, *IL1B*, *IL6*, *TNF*, *IL12A*, *IL1R1*, *IL1R2*	(Chapwanya et al 2009; Gabler et al 2009; Herath et al 2009b; Fischer et al 2010; Gabler et al 2010; Galvao et al 2011; Ghasemi et al 2012; Kasimanickam et al 2014)
Chemokines	*CXCL5*, *CXCXL8*	(Fischer et al 2010; Gabler et al 2010; Galvao et al 2011; Ghasemi et al 2012; Kasimanickam et al 2014)
Prostaglandins	*PTGS1*, *PTGS2*, *PTGDS*	(Gabler et al 2009; Gabler et al 2010)
Innate Immunity	*TLR4*, *NFKB1*	(Chapwanya et al 2009; Herath et al 2009b; Kasimanickam et al 2014)
Mucins	*MUC1*	(Kasimanickam et al 2014)
Antimicrobial peptides	*TAP*, *DEFB5*, *DEFB1*	(Chapwanya et al 2009)
Acute phase proteins	*HP*, *SAA3*	(Chapwanya et al 2009)
Metabolism	*IGF1*	(Kasimanickam et al 2014)

Table 25.3. The environment and risk factors for uterine disease (Sheldon 2014).

Environment	Risk factor	Example references
Trauma and tissue damage	Retained fetal membranes	(Paisley et al 1986; Bruun et al 2002; Kim & Kang 2003; Han & Kim 2005; Dubuc et al 2010; Potter et al 2010)
	Male calf	(Potter et al 2010)
	Stillbirth	(Markusfeld 1984; Potter et al 2010)
	Twins	(Markusfeld 1984; Dubuc et al 2010; Potter et al 2010)
	Dystocia	(Dubuc et al 2010; Potter et al 2010; Pinedo et al 2013)
	Induction of parturition	(Markusfeld 1984)
	Parity	(Markusfeld 1984; Kim & Kang 2003)
Metabolism	Milk fever	(Bruun et al 2002; Whiteford & Sheldon 2005)
	Reduced feed intake ante partum	(Huzzey et al 2007)
	Ketosis	(Markusfeld 1984; Bruun et al 2002; Dubuc et al 2010; Pinedo et al 2013)
	Left displaced abomasum	(Markusfeld 1984)
	Metabolic disorder	(Kim & Kang 2003)
Hygiene	Calving season	(Markusfeld 1984; Bruun et al 2002)
	Angle of vulva	(Potter et al 2010)

Table 25.4. Factors which predispose to retained fetal membranes

Abortion, especially where the cause is associated with placentitis
Abnormal gestation length; either prolonged or shortened
Dystocia, primary uterine inertia, delivery by caesarean section
Fatty liver, possibly because it predisposes to uterine atony
Deficiencies of selenium/vitamin E or possibly, vitamin A
Failure of placental maturation Twin births and induced-calvings are commonly associated with retention of fetal membranes, as the placenta had not completed the maturational changes that are required for normal separation. Heat stress, which often results in shortened gestations, can also result in increased incidences of retained fetal membranes.
Abnormalities of oestrogen; progesterone ratio in late gestation
Secondary inertia caused by hypocalcaemia is weakly associated with RFM, which is probably because of the association of both with dystocia

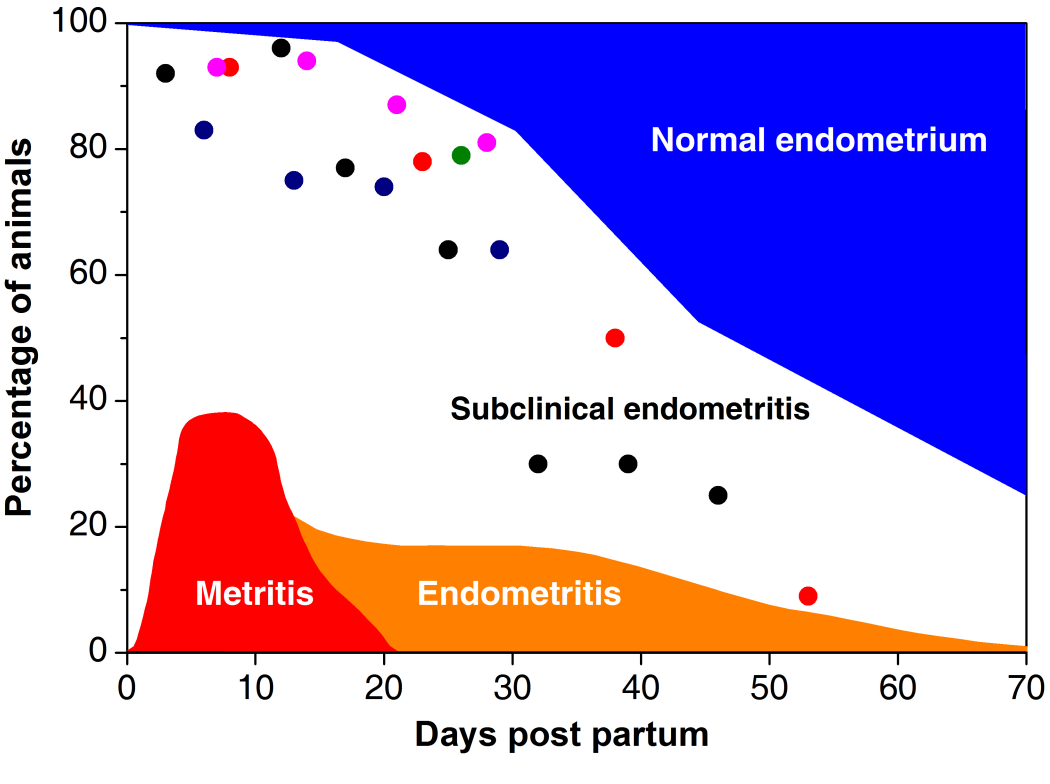
Table 25.5. Factors associated with subfertility due to endometritis (Morton 2000)

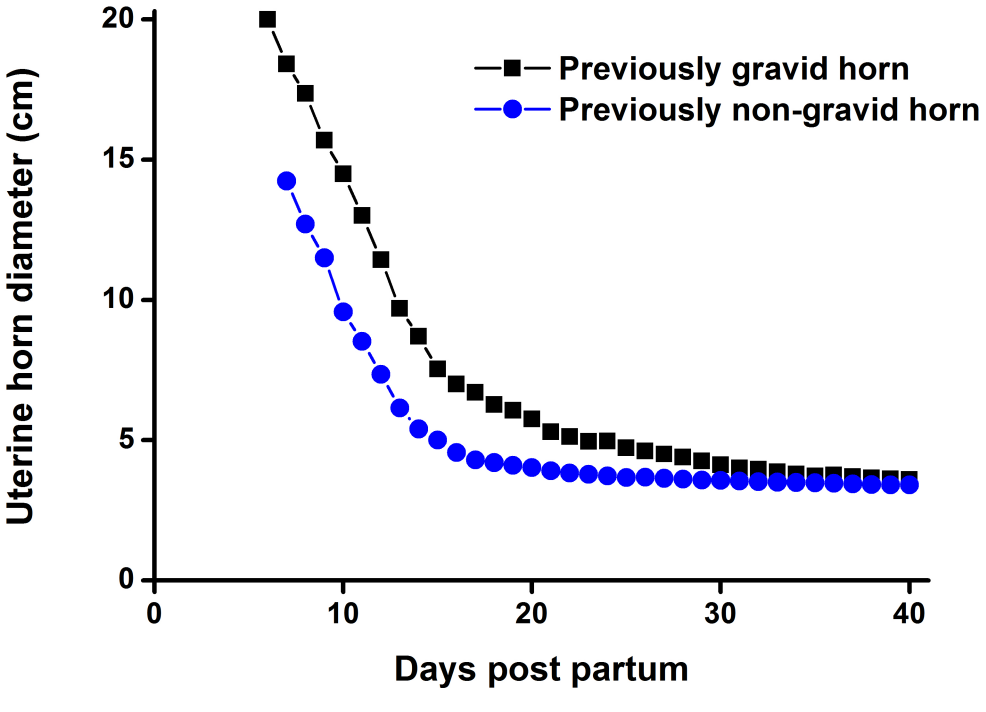
Assisted calvings, especially where internal manipulation is required
Retained fetal membranes
Purulent vaginal discharges in the interval between calving and the start of mating
Premature calving (including twins, abortion) Induction of calving before term has adverse effects on fertility in some, but not all studies
BUT NOT uncomplicated cases of hypocalcaemia

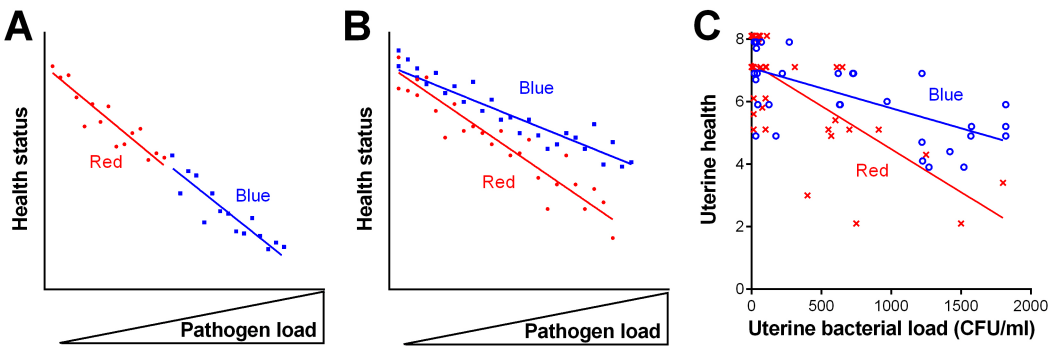
Table 25.6. Criteria for the diagnosis of subclinical endometritis from cervical swabs (Sheldon et al 2006)

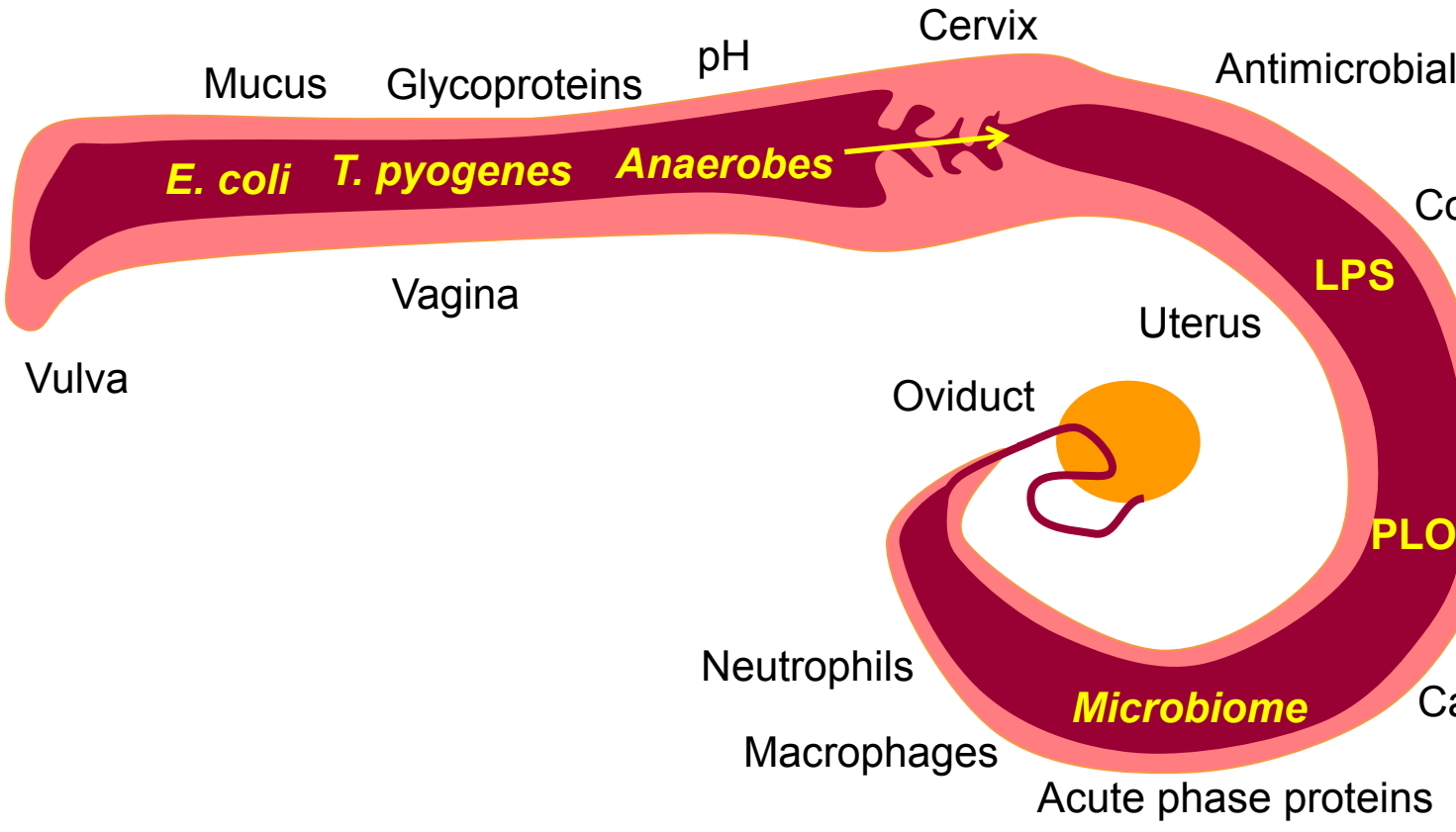
Days after calving	**% PMN in cytology samples**
20 - 33	> 18
34 - 49	> 10
> 50	> 5

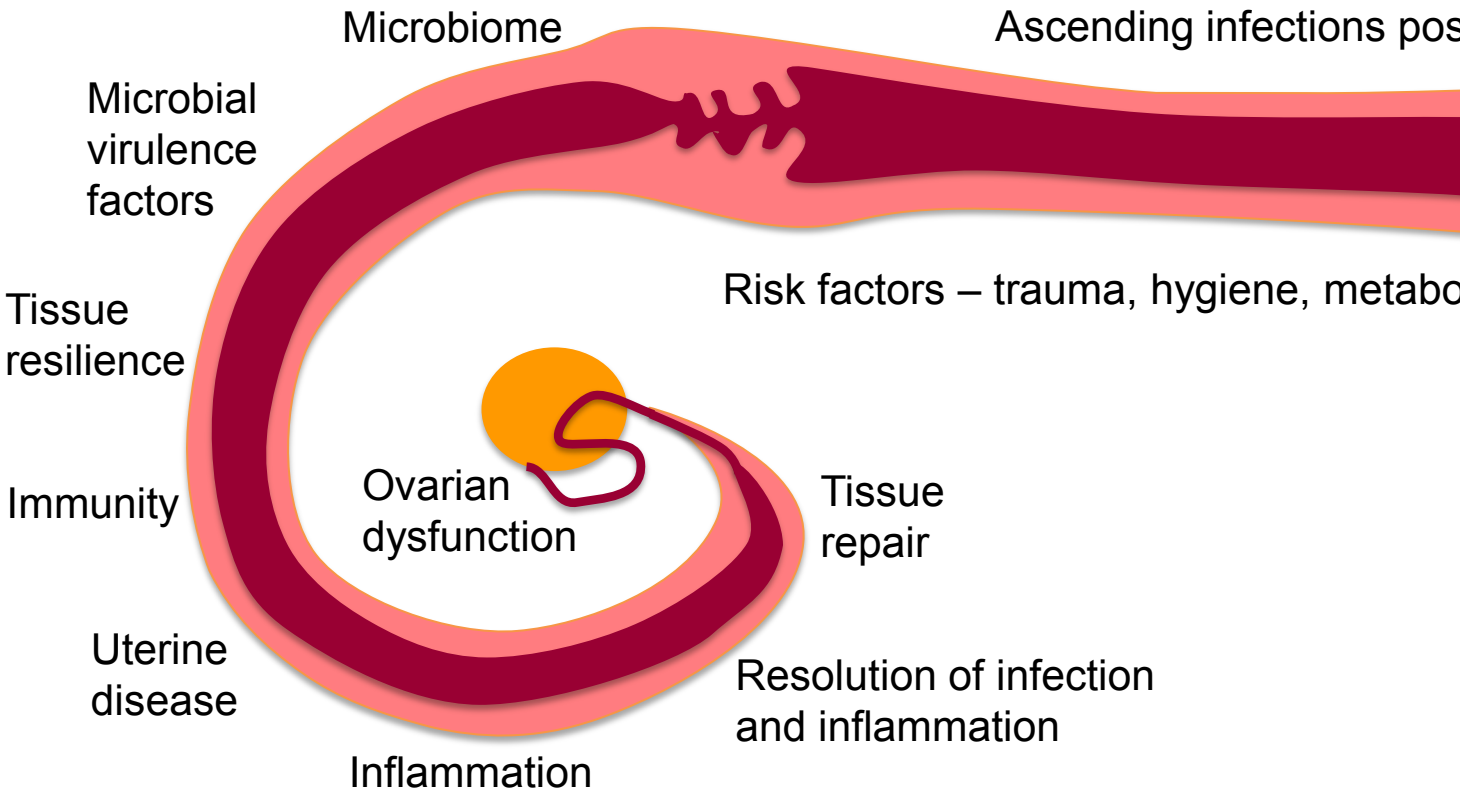
PMN, polymorphonuclear leukocytes.

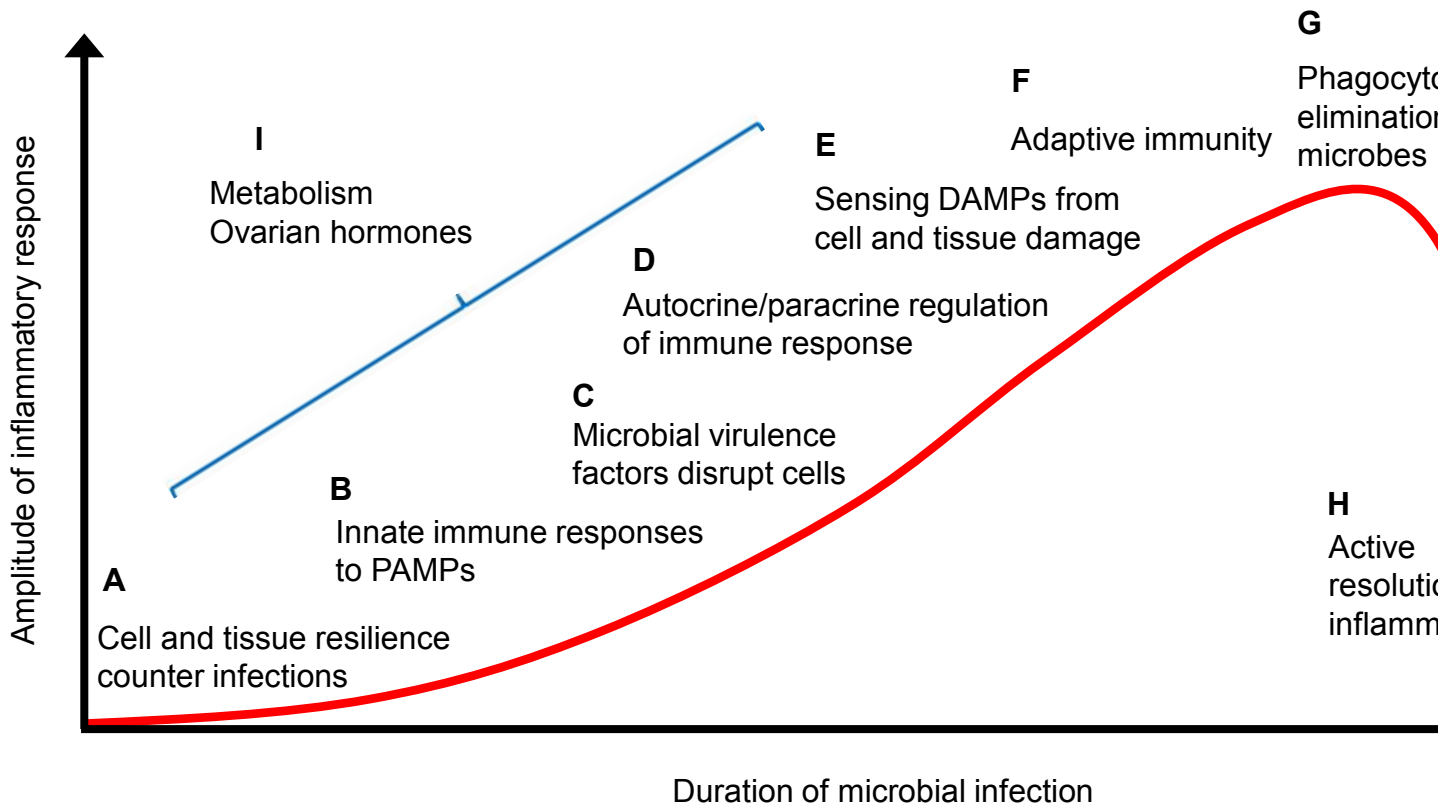

Percentage of animals
Days post partum
Normal endometrium
Subclinical endometritis
Metritis
Endometritis
0
20
40
60
80
100
0
10
20
30
40
50
60
70

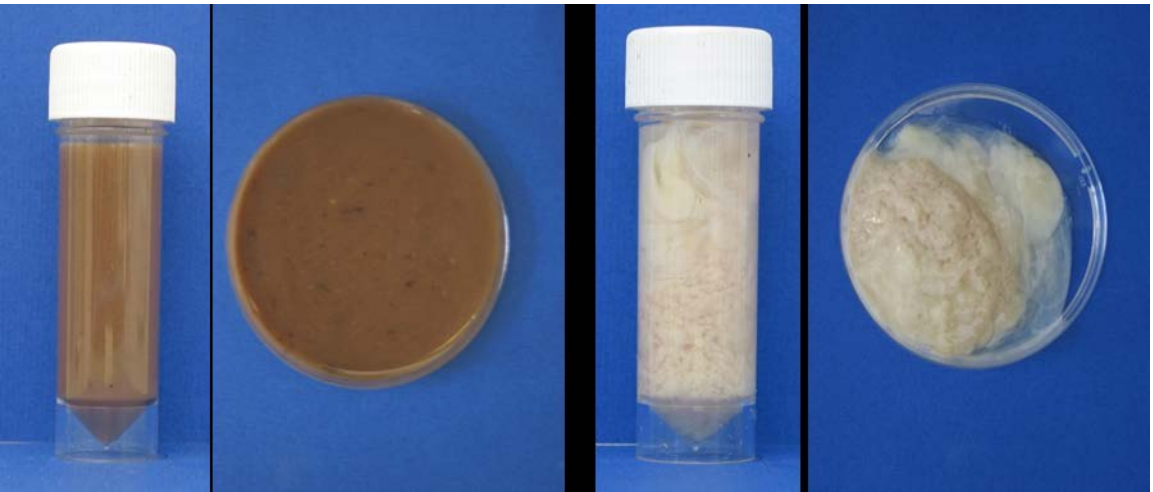

Uterine horn diameter (cm)
20
15
10
5
0
0
10
20
30
40
Days post partum
Previously gravid horn
Previously non-gravid horn


A
Health status
Red
Blue
Pathogen load
B
Health status
Blue
Red
Pathogen load
C
Uterine health
Blue
Red
0
2
4
6
8
0
500
1000
1500
2000
Uterine bacterial load (CFU/ml)


Cervix
pH
Mucus
Glycoproteins
Antimicrobial
E. coli
T. pyogenes
Anaerobes
LPS
Vagina
Uterus
Vulva
Oviduct
PLO
Neutrophils
Microbiome
Macrophages
Acute phase proteins

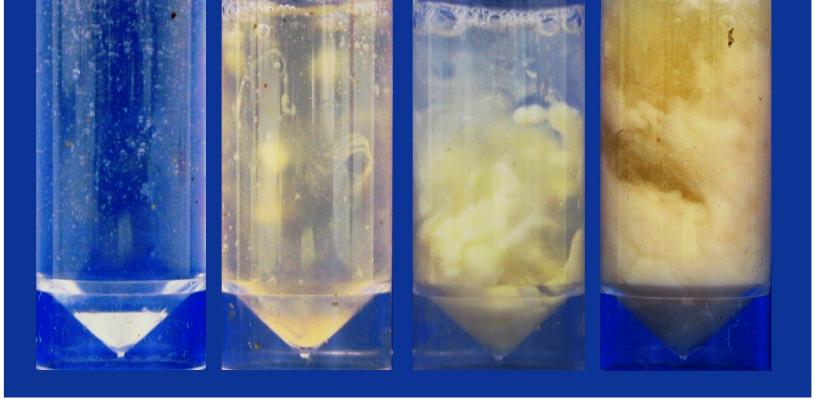

Microbiome
Ascending infections pos
Microbial virulence factors
Risk factors – trauma, hygiene, metabo
Tissue resilience
Immunity
Ovarian dysfunction
Tissue repair
Uterine disease
Resolution of infection and inflammation
Inflammation


Amplitude of inflammatory response
A
Cell and tissue resilience
counter infections
B
Innate immune responses
to PAMPs
C
Microbial virulence
factors disrupt cells
D
Autocrine/paracrine regulation
of immune response
E
Sensing DAMPs from
cell and tissue damage
F
Adaptive immunity
G
Phagocyto
eliminatio
microbes
H
Active
resoluti
inflamm
I
Metabolism
Ovarian hormones
Duration of microbial infection

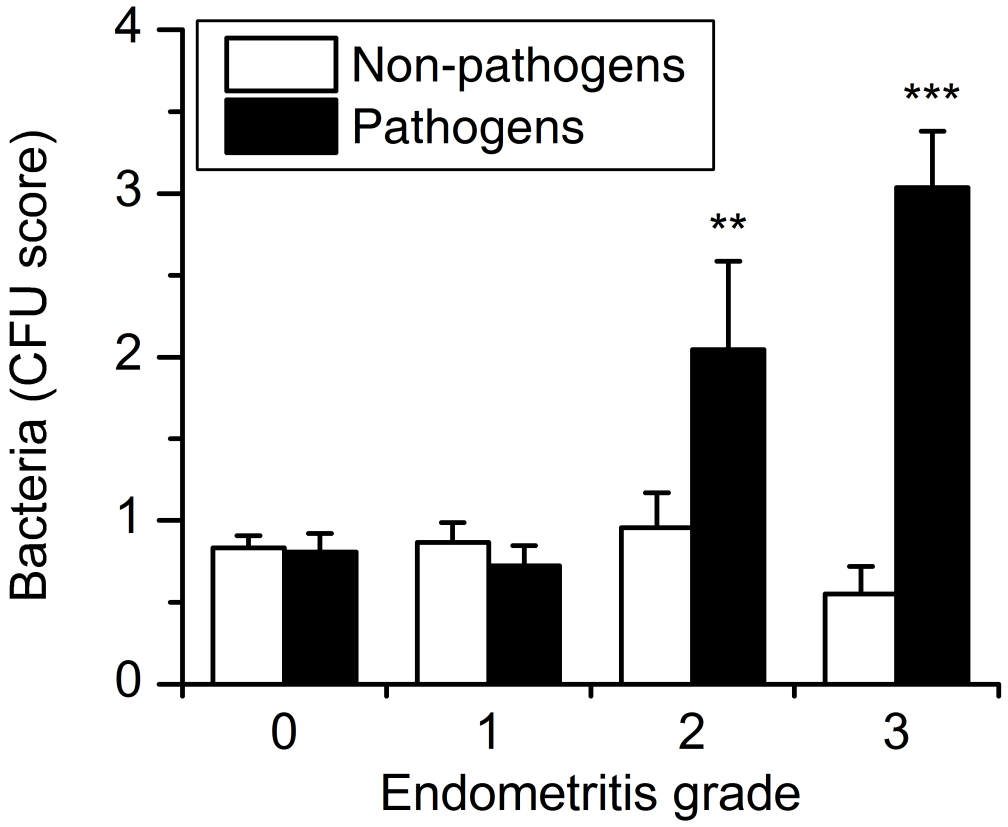



A

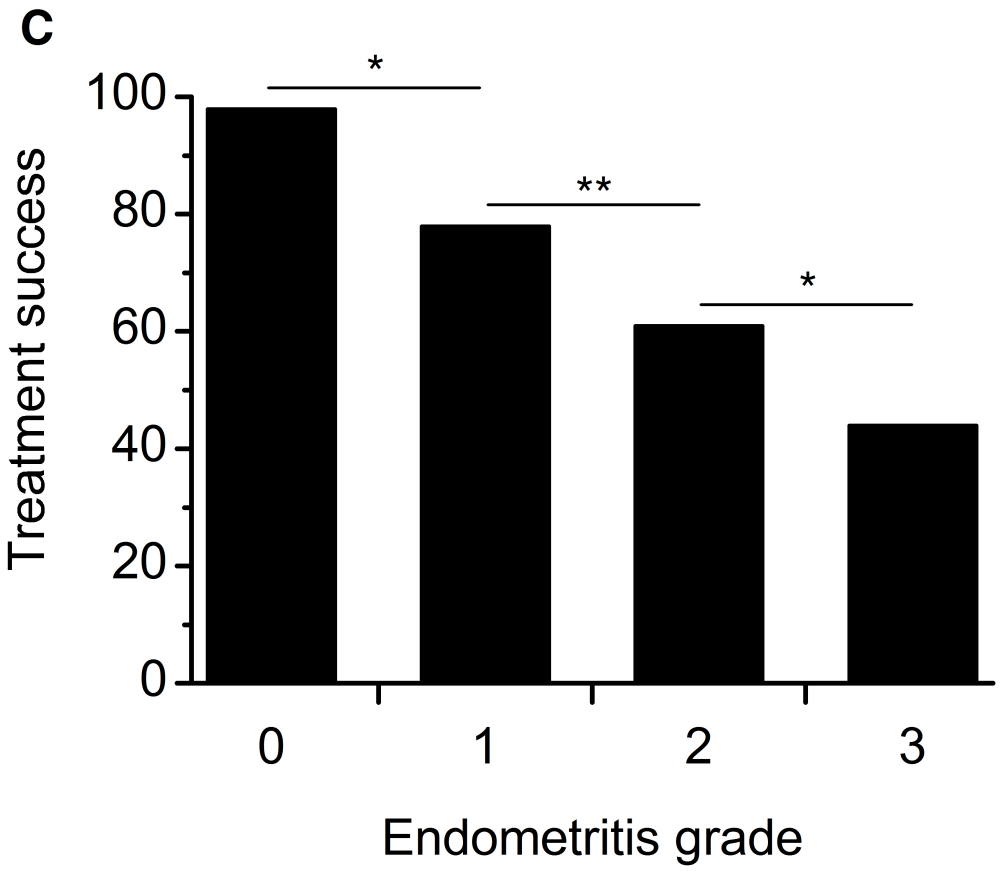

B

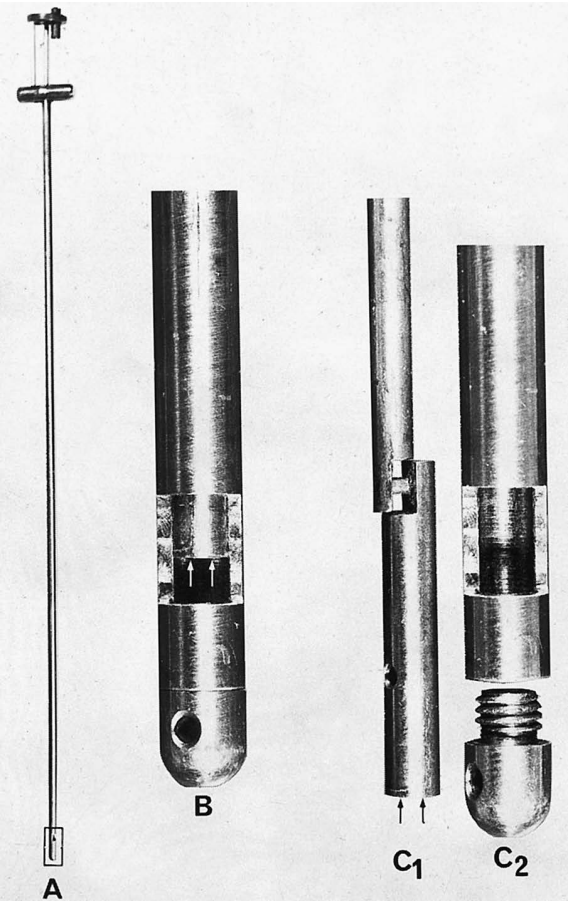


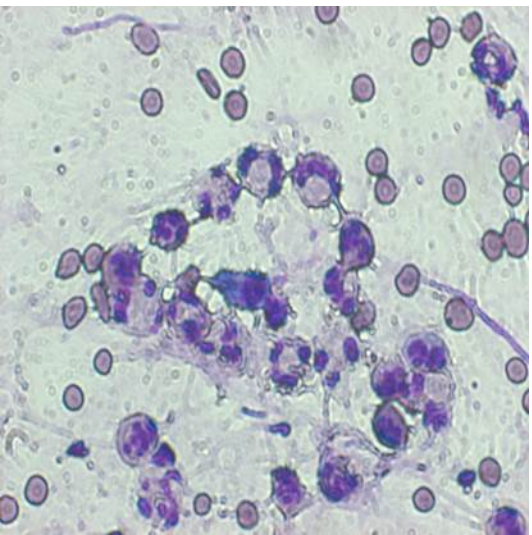
B

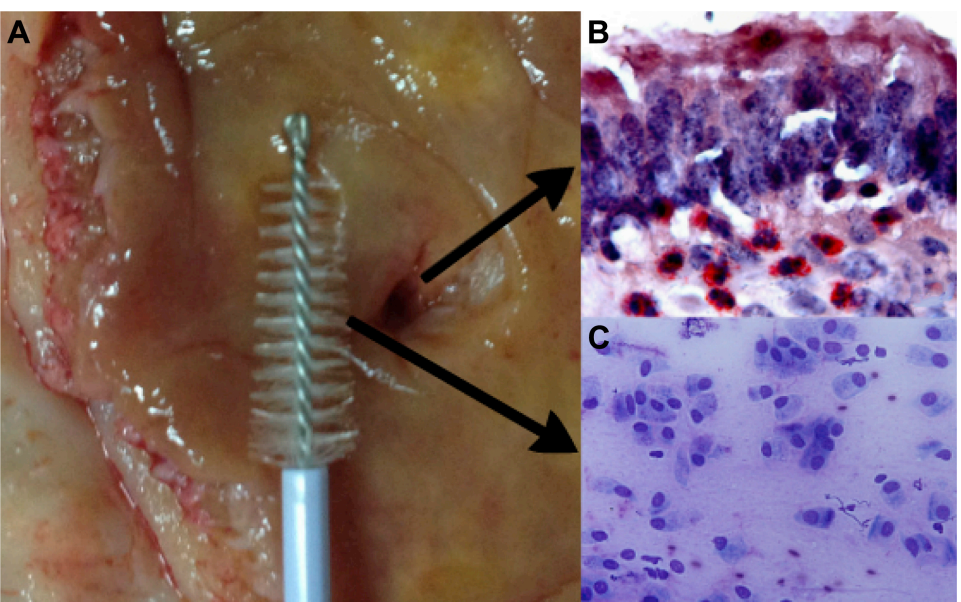

Non-pathogens
Pathogens
Bacteria (CFU score)
0
1
2
3
4
**
***
0
1
2
3
Endometritis grade

C


Treatment success
0
20
40
60
80
100
*
**
*
0
1
2
3
Endometritis grade


A
B
C1
C2




A
B
C